International Journal of Environment and Climate Change

Volume 14, Issue 1, Page 338-348, 2024; Article no.IJECC.111691
ISSN: 2581-8627
(Past name: British Journal of Environment & Climate Change, Past ISSN: 2231–4784)

Partial Chemical Characterization of Drought-Related Metabolites in Maize Under Drought Stress Conditions

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJECC/2024/v14i13839

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/111691

Original Research Article

Received: 03/11/2023
Accepted: 08/01/2024
Published: 13/01/2024

ABSTRACT

This study investigates the metabolic response of maize genotypes UASBM13 (drought-tolerant) and UASBM10 (drought-sensitive) to drought stress using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Drought stress significantly impacts crop productivity, with potential consequences for global food security. Secondary metabolites produced by maize under drought conditions, provide insights into molecular response. The identified metabolites include sinapic acid, sinapoyl malate, coumaroyl shikimate, caffeoyl shikimate, syriogenin, sinapaldehyde, hydroxy ferulate, naringenin chalcone, and resveratrol. These compounds play crucial roles in antioxidant defence, lignification, and stress-related pathways. The results suggest that UASBM13 exhibits a more robust metabolic response to drought, with higher levels of key metabolites associated with stress tolerance. The findings contribute to our understanding of plant adaptation to environmental stress and provide valuable information for developing drought-tolerant crop varieties to ensure global food security in the context of a changing climate and growing population.

Keywords: Drought tolerant; drought sensitive; LCMS/MS and corn CYNC.

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1. INTRODUCTION

The global challenge of ensuring food security for an ever-expanding human population is compounded by the pervasive impact of both biotic and abiotic stresses on agricultural productivity. Among the array of abiotic stresses, drought stands out as one of the most formidable threats, exerting a profound negative influence on crop yields worldwide. Drought stress has been documented to cause a staggering decline in crop productivity, ranging from 50% to 70% [1]. The World Health Organization (WHO) underscores the severity of this issue, reporting that approximately 55 million people worldwide face livelihood disruptions annually due to drought. Furthermore, a staggering 40% of the global population is affected by drought, with projections suggesting that as many as 700 million people may face displacement by 2030 due to this pervasive environmental stress [2].

At the physiological level, drought stress induces the production of reactive oxygen species (ROS), encompassing both free radicals (such as superoxide radicals, alkoxy radicals, and hydroxyl radicals) and non-radicals (including singlet oxygen and hydrogen peroxide). These ROS, characterized by their high toxicity and reactivity, wreak havoc on cellular homeostasis by causing damage to crucial biomolecules such as proteins, carbohydrates, lipids, and DNA [3]. The repercussions of drought extend beyond molecular damage, affecting fundamental aspects of plant growth and development. Drought stress adversely impacts plant height, canopy structure, root development, and leaf area index. Additionally, it profoundly influences essential physiological processes, including alterations in osmotic potential, stomatal conductance, carboxylation efficiency, photosynthesis rate, pressure potential, and transpiration rates [4]. In light of these multifaceted effects, understanding and mitigating the impact of drought stress on crops is paramount for sustaining global food security in the face of a changing climate and growing population.

In response to drought stress, plants initiate biochemical adaptations, leading to the production of secondary metabolites [5]. These metabolites play a pivotal role in either positively or negatively influencing plant growth and survival under adverse conditions. However, the identification and characterization of these metabolites' present challenges, given their abundance, structural diversity, and the complexity of the matrix [6]. The advancement of analytical techniques, notably liquid chromatography coupled with mass spectrometry (LC-MS/MS) [7], has addressed these challenges by offering excellent resolution, mass accuracy, and improved analytical methods for complex sample matrices. This study aims to partially characterize the secondary metabolites produced by maize during drought stress, focusing on varieties with differing levels of drought tolerance and susceptibility. Leveraging LC-MS/MS and Tandem Mass Spectrometry, the research provides detailed structural information about the identified metabolites, enhancing our understanding of the molecular responses of maize to drought. This knowledge is crucial for the development of more resilient crop varieties capable of withstanding environmental stresses and ensuring food security in the future.

2. MATERIALS AND METHODS

2.1 Plant Materials

The biosample for the presences study was homozygous inbreed lines of maize with contrasting characters of drought tolerance at reproductive stage. The seeds of reproductive stage drought tolerant (UASBM13) and reproductive stage drought sensitive (UASBM10) lines were obtained from the department of biotechnology. The seed of these genotypes were separately packed and store at 4°C, in sealed box, until the experiment was conducted. Drought induction studies were conducted in pots under greenhouse condition. All pots were watered first 20 days to maintain soil moisture in the range of 30 to 32%. During this time, evaporation loss of water was supplemented on the basis of calculated value form soil dehydration dynamics studies. On completion of 20^{th} day, water supply was withdrawn to both groups unwatered condition to reduce the mean soil moisture level to 15 per cent. This moisture level (15%) was maintained for 30 days. The evaporation water loss after attaining 15 percent soil moisture was correlated by supplementing water based on calculated value from soil dehydration dynamics study.

2.2 Sample Extraction

The leaf sample (0.5 g) of UASBM10 and UASBM13 collected after different drought

induction and stored in -20, were chopped into fine pieces and extracted using HPLC grade methanol. Extraction was performed with mechanical disruption using mortar and pestle. Each sample extract was then made up to 25 ml using standard volumetric flask. An aliquots of 1 ml from each of this extract was taken and subjected to centrifugation at 10000 RPM. After removal of tissue debris, the supernatant was appropriately diluted and injected into LC-MS system for further study [8].

2.3 Liquid Chromatograph

Liquid chromatography was performed in reverse phase mode using the binary gradient solvent manager (waters) with following conditions; solvent A: methanol with 0.01% formic acid; solvent B: water with 2% acetic acid and 0.01% formic acid; totalflow rate: 1ml/min; gradient conditions:0-10 100% B, 10-140: 100% A (linear gradient); 140-165 mim: 100% A; detector wavelength range (waters 2998 PDA) : 220 to 400 nm, detector sampling rate 5 points per sec, chromatogram display: 280 nm; column: C18, 250 x 2.1 mm, 3 microm particle size [8].

2.4 Mass Spectrometry

Tandem mass spectrometery was performed with the following conditions; Instrument: Waters, Xevo TQD; Mass data acquisition format: continuum; Ionization mode: Electrospray ionization; ionization mode: negative; capillary voltage:3.5 kv; cone voltage: 30 v; desolvation temperature: 500^{o}C; source temperature: 150^{o}C; cone gas:50 L/h; desolvation gas flow: 1000 L/h; mass scan range: 150 to 1000; mass scan time: 165 min., Parent ions were captured and subjected to daughter ion fragmentation for fragmentation pattern-based identification [8].

2.5 Partial chemical characterization

The chromatographic peaks that are responding to different levels of drought were subjected to compound identification based on fragmentation pattern and searching the same with tandem mass spectrum database. From the aligned view of PDA at 280 nm over total ion chromatogram and molecular ion peaks were selected. The molecular ion peak over corresponding daughter ions peaks were further selected and the same were searched in ms-ms spectral database and putative identity of drought responsive peaks were made. The putative compounds identified using the molecular ion peaks as well as daughter ion peaks from the instrument spectral database were searched in CornCys database to confirm the identity.

3. RESULTS

Chemical profiling of drought tolerant (UASBM13) and drought sensitive (UASBM10) maize inbred lines subjected to drought stress is given in Fig. 1.

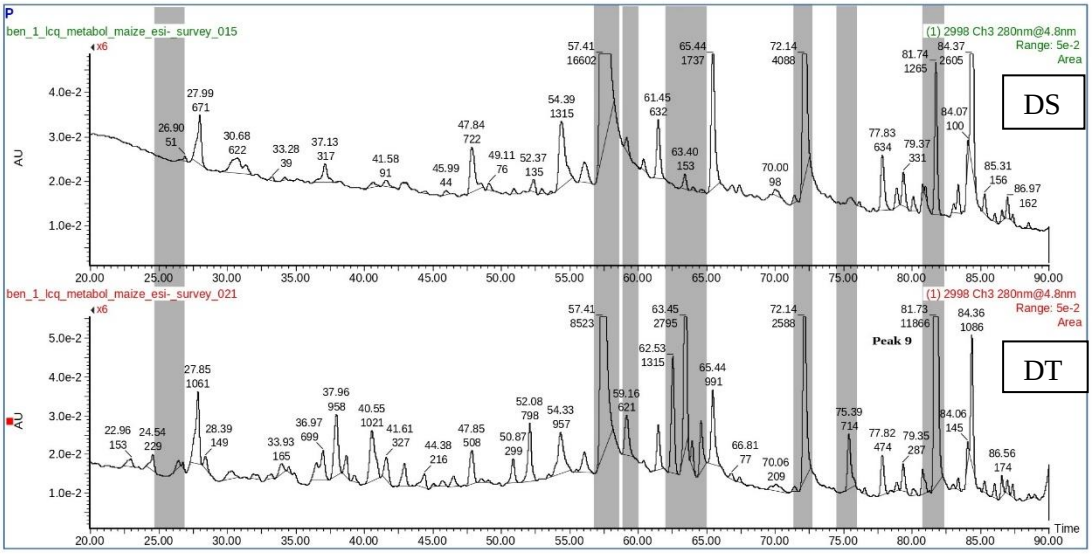

Fig. 1. Metabolic profile comparison between sensitive and tolerant maize inbred line during drought stress. DS represent the Drought Sensitive and DT represent the Drought Tolerant.

Based on the metabolite profiling by the PDA detector of liquid chromatography module, several peaks were identified that respond to drought stress. The drought responsive peaks in the UV absorption chromatogram at 280 nm was analyzed and putative molecules were identified based on the UV absorption chromatogram corresponding total ion chromatogram and by drawing the respective molecular ion mass spectrum. The LC-MS-MS used in the study was configured with a photodiode array detector as the first detector followed by mass detector. Hence, there was a minor time delay between the PDA detector and mass detector. In order to align the total ion chromatogram and UV absorption chromatogram, the instrument derived correction factor was used. From the aligned chromatogram pair, the molecular ion mass (m/z -1) corresponding to the drought responsive peaks in the UV absorption chromatogram was taken by using the Waters™ data analysis software. On comparison of UV absorption chromatogram of PDA detector and total ion chromatogram of mass detector, it was found that certain molecules with high UV responsiveness at 280 nm was not ionized in the negative electrospray ionization mode. Such unionized peaks were ignored from further studies.

As the mass detector used in this study is a triple quadruple, and the unit mass resolution is 0.1, identification of respective compounds using the molecular ion peak without a reference standard is difficult. Therefore, mass fragmentation and MS-MS data base search was followed to identify the well ionized total ion chromatogram peaks corresponding to the absorption peaks of chromatogram at 280 nm. The identified peaks thus obtained was further searched in the “CornCyc” database to identify the respective physical, chemical and biological properties such compound listed in Table 1 .

CornCys 4.0 [9] was developed and maintain by plant metabolite network and genome, based on maize reference line version B73_v4, which is a key tool for searching and mapping maize metabolic pathway. The availability of genome-wide metabolic pathway resources provide a systems-level view of the chemical interactions in a cell, which creates phenotypes of interest. CornCys provides more than 500 metabolic pathways and 3000 enzymatic reactions.

Based on the MS-MS spectral database search results and MW obtained from the low resolution triple quadruple mass detector, [(m/z)-1 made to m/z] search queries were customized to find structural analogues and putative compounds from CornCys database. Among 2617 metabolites reported in the CornCyc database, highly probable structural analogues were identified and the same is listed in the Table 1.

Table 1. Compound of mass spectrum and mass extract, [M+H]+ m/z mass in spectrum

Compound number	[M+H]+ m/z	Extract mass	Compound name	Change in concentration	Affected inbred line
1	207.2	208.213	Sinapaldehyde	Increase	Tolerant
2	208.17	209.178	5-hydroxyferulate	Increase	Tolerant
3	222.2	223.205	Sinapate	Decrease	Tolerant
4	227.24	228.247	Resveratrol	Increase	Tolerant
5	271.25	272.257	naringenin chalcone	Increase	Tolerant
6	318.29	319.29	4-coumaroylshikimate	Increase	Sensitive
7	334.29	335.29	Caffeoylshikimate	Increase	Sensitive
8	337.27	338.27	sinapoyl-(S)-malate	Increase	Tolerant
9	344.28	345.285	Syringetin	Decrease	Tolerant

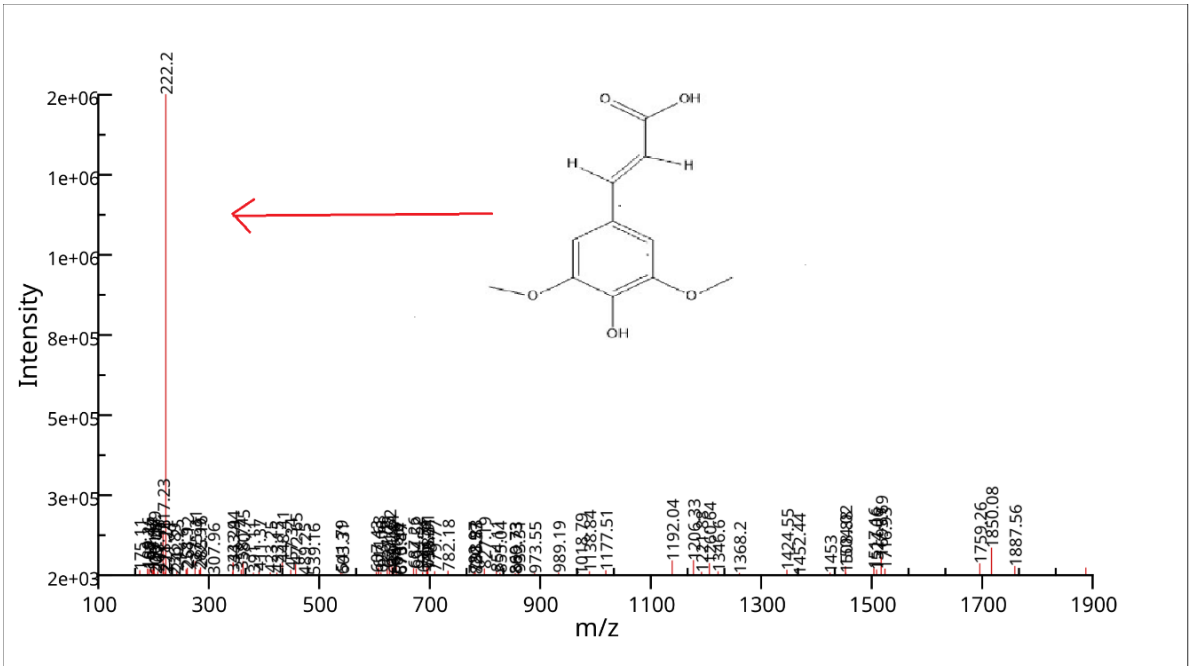

Fig. 2. Mass spectrum of Sinapic acid

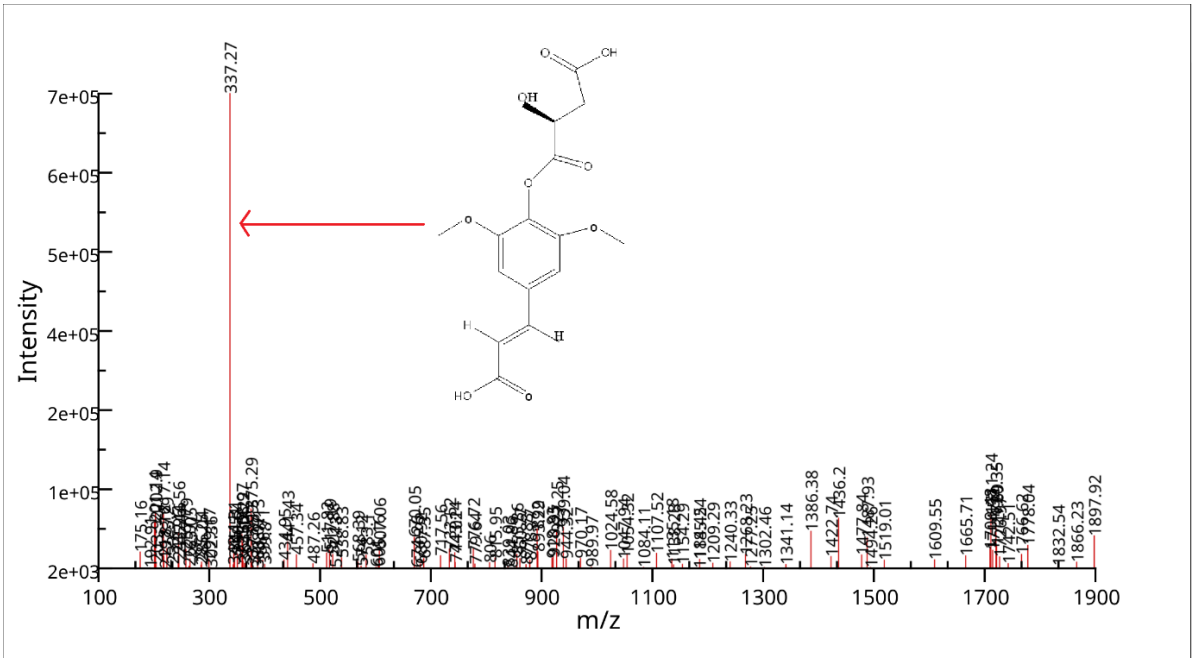

Fig. 3. Mass spectrum of Sinapoyl malate

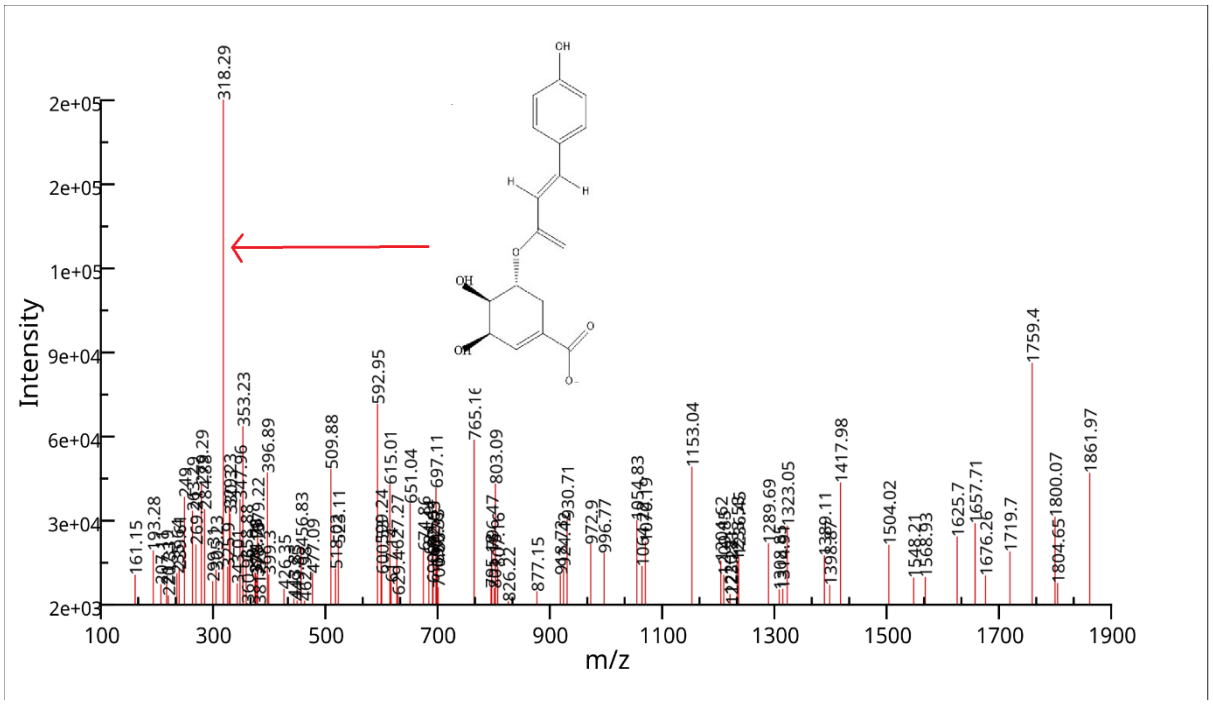

Fig. 4. Mass spectrum of p-Coumaroyl shikimate

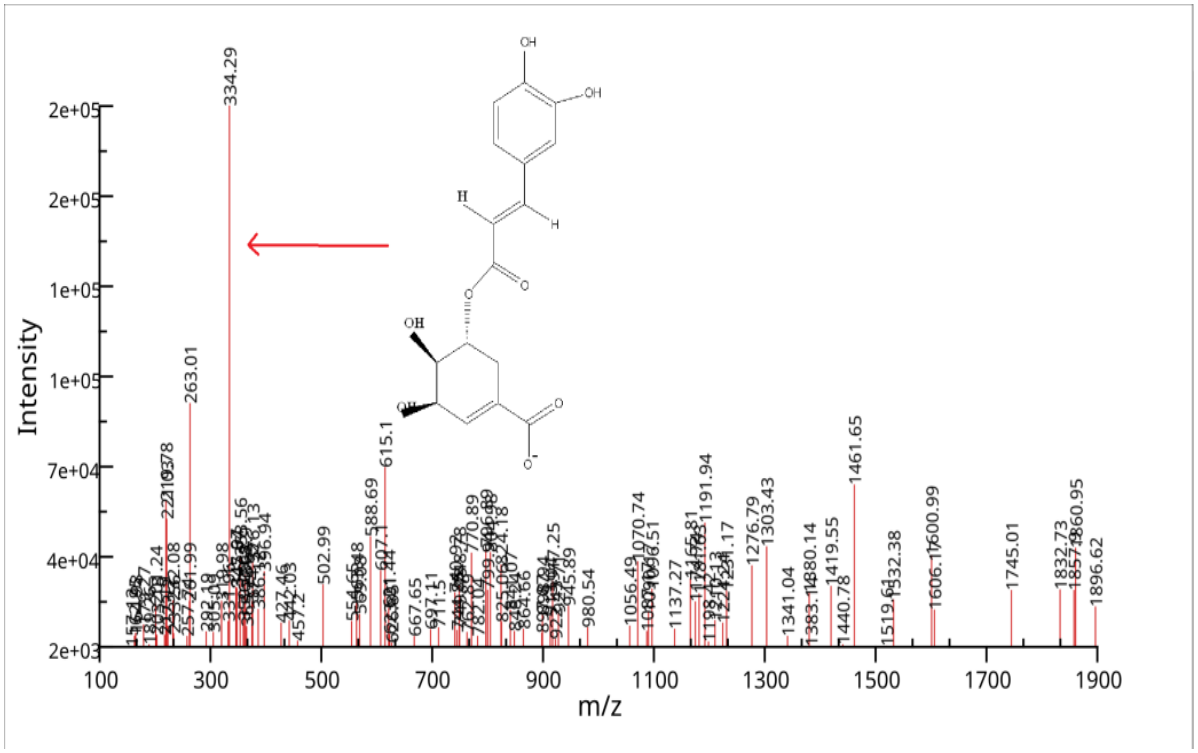

Fig. 5. Mass spectrum of Caffeoyl shikimate

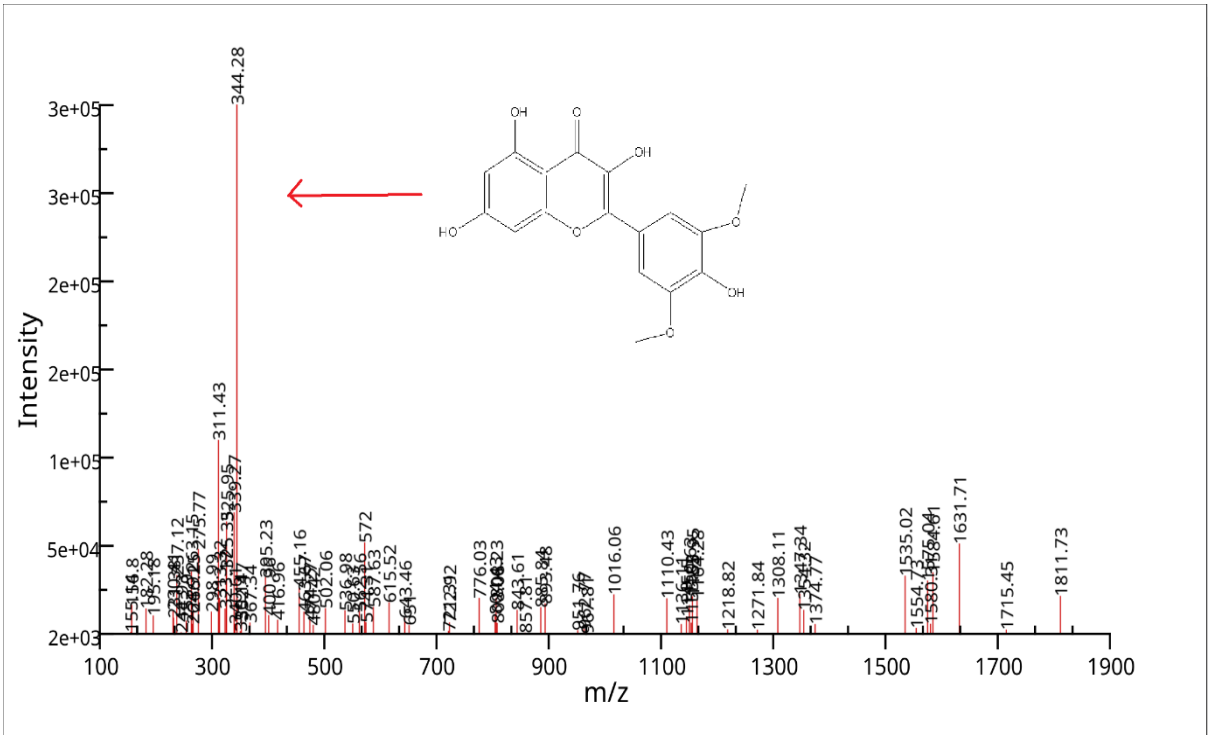

Fig. 6. Mass spectrum of Syringentin

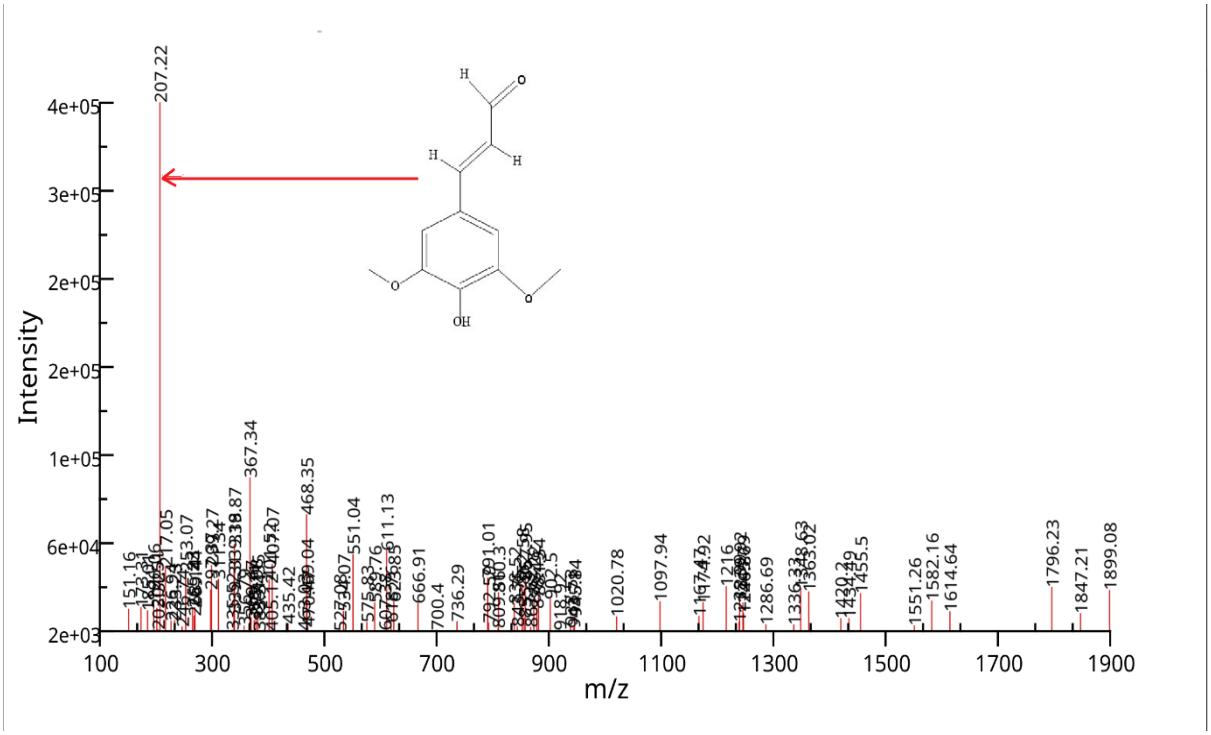

Fig. 7. Mass spectrum of 5-Hydroxy ferulate.

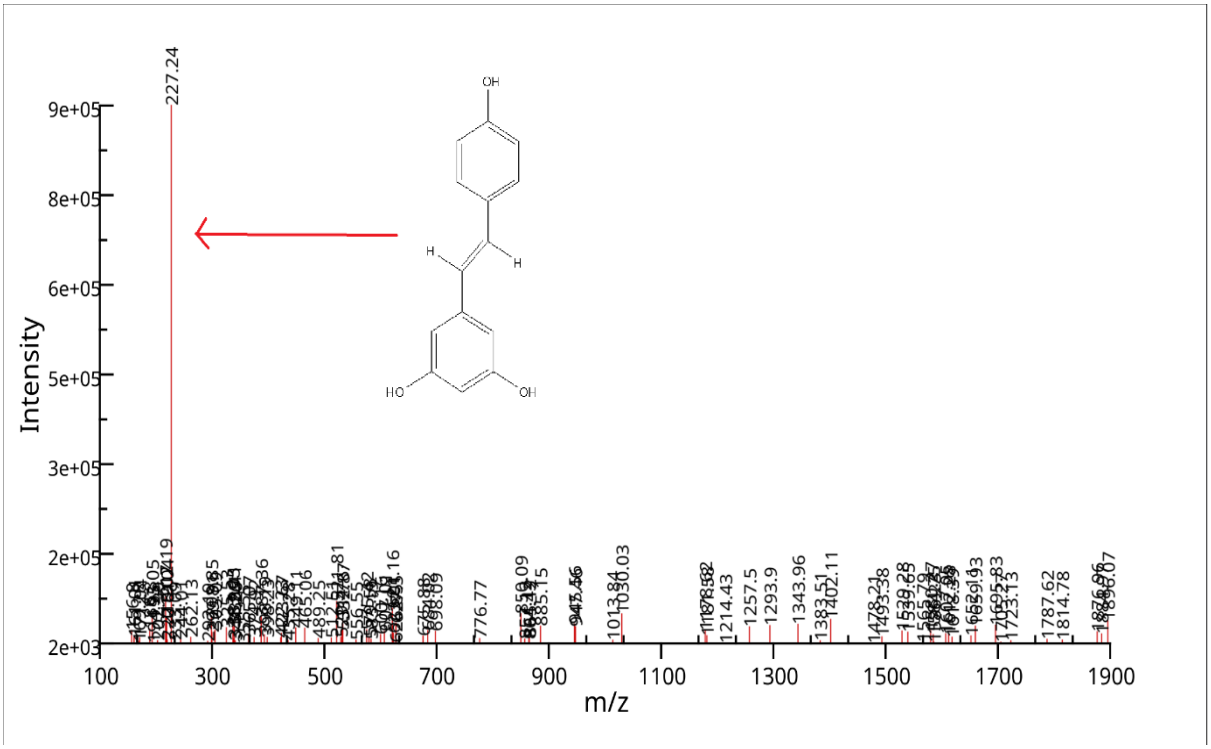

Fig. 8. Mass spectrum of Resveratrol

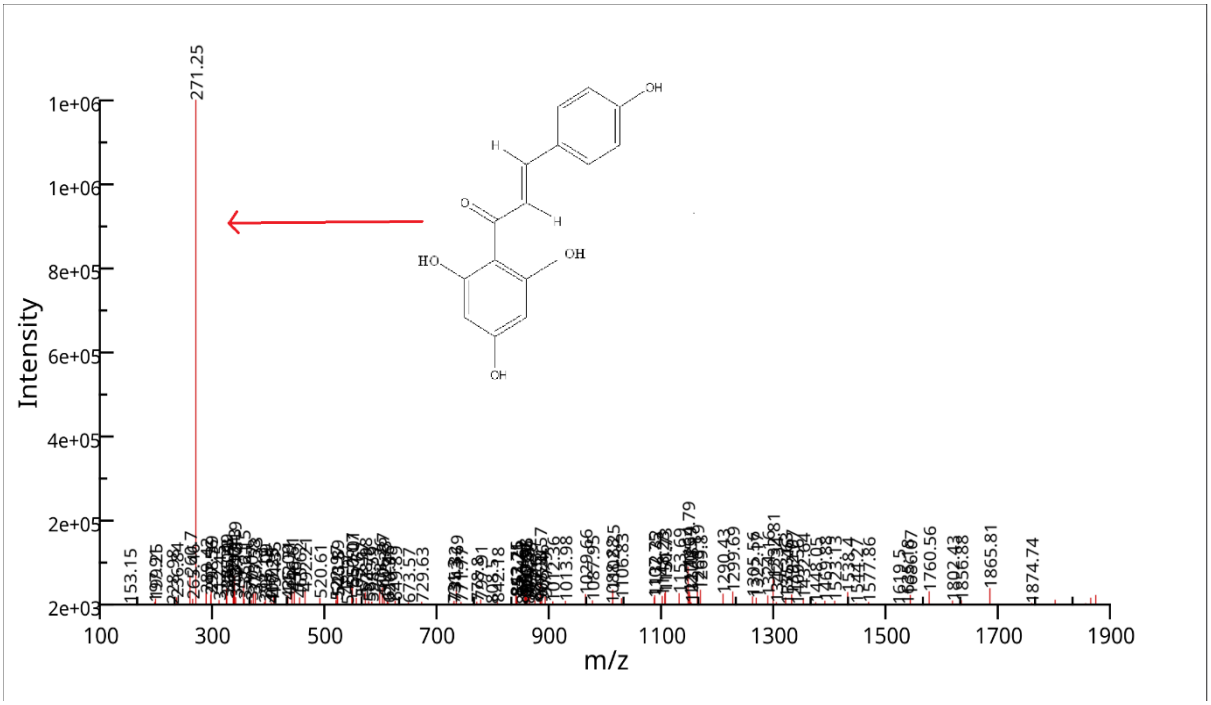

Fig. 9. Mass spectrum of Naringenin chalcone.

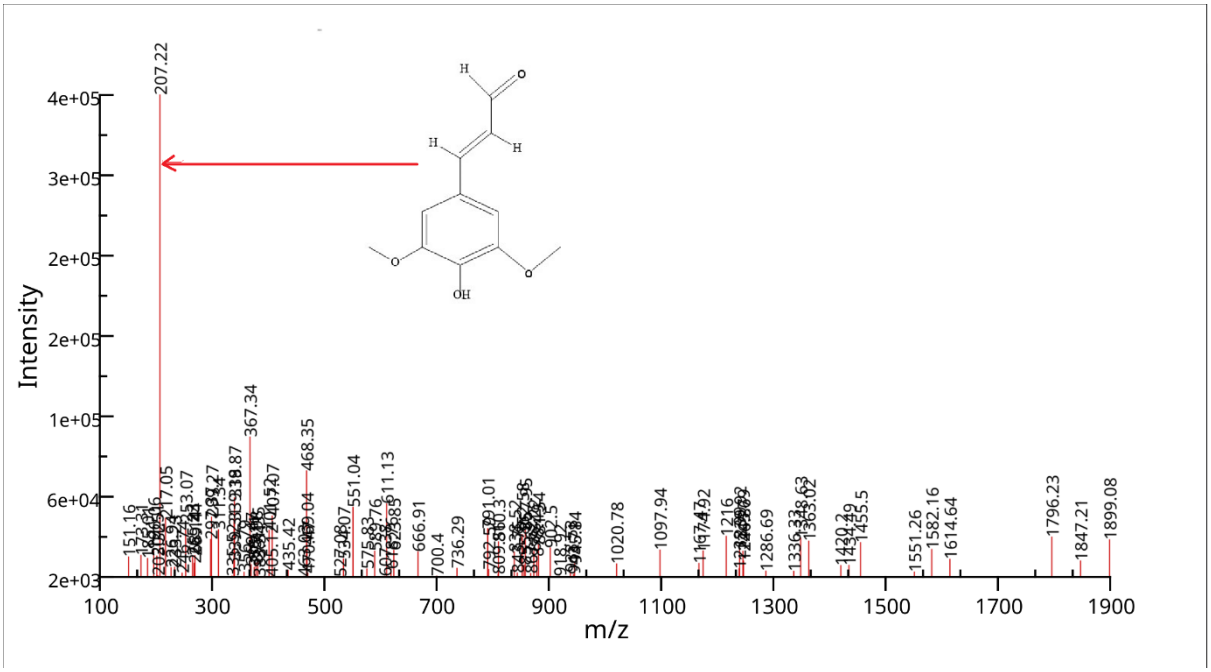

Fig. 10. Mass spectrum of Sinapaldehyde

4. DISCUSSION

In a study investigating the response of the maize genotype UASBM13 to drought stress, metabolite profiling and MS-MS identification revealed a distinctive pattern. Mild drought stress (15% water holding capacity for 10 days) induced the synthesis of syringetin, while higher stress levels resulted in decreasing amounts. This observation suggests that UASBM13 does not inherently contain syringentin but biosynthesizes it in response to mild drought stress.

Superoxide production, a consequence of drought stress, is mediated by abscisic acid, leading to stomatal closure and incomplete electron transport from NADPH to O2. Plants, however, possess endogenous enzymatic defense systems to scavenge these ROS. Syringentin, identified as a potential component of the non-enzymatic antioxidant defense system, may contribute significantly to a plant's overall antioxidant capacity, especially in species inherently equipped with superior antioxidant mechanisms.

Among the myriad consequences of drought on plant metabolism, the synthesis of syringentin emerges as a noteworthy aspect, particularly as it relates to the phenylpropanoid pathway. Syringentin, a terminal metabolite synthesized by various plant species, has its precursor in laricitrin, an intermediate with an unknown metabolic fate [10]. The phenylpropanoid pathway encompasses critical intermediates like naringenin, which serves as a branching point for flavone and flavonol biosynthesis through kaempferol. The connection between kaempferol and laricitrin involves quercetin and myricetin.

Upregulation of naringenin chalcone, a key intermediate metabolite leading to naringenin, a precursor for various flavonoids. Surprisingly, naringenin chalcone was detected only under drought stress, indicating its involvement in the plant's response to stress. The upregulation of naringenin chalcone and its downstream metabolites is proposed to enhance drought tolerance by scavenging free radicals and contributing to cell wall integrity through the synthesis of lignification metabolites [11].

The study also uncovered the drought-induced upregulation of metabolites involved in cell wall lignification, such as ferulic acid, sinapic acid, sinapoyl malate, and resveratrol. These metabolites play a role in cellular protection against drought-induced oxidative stress and reinforce cell wall integrity by participating in lignification processes [12,13].Notably, the tolerant genotype, UASBM13, exhibited higher levels of these metabolites compared to the sensitive genotype, suggesting a potential

correlation between the ability to synthesize lignification metabolites and drought tolerance.

The investigation extended to the analysis of metabolites like sinapaldehyde, coumaroyl shikimate, and caffeoyl shikimate, which showed distinct responses to drought stress in both sensitive and tolerant genotypes. Sinapaldehyde, involved in lignin synthesis, was upregulated in the tolerant genotype under drought stress [14,15]. Coumaroyl shikimate, an intermediate in ferulic acid synthesis, was induced in both genotypes, but more prominently in the tolerant one [16]. Wang *et al* also reported increase in coumaryl alcohol production in response to drought conditions in *Poa crymophila Keng* [16]. On the other hand, caffeoyl shikimate levels were higher in the sensitive genotype, potentially due to increased turnover for subsequent metabolite synthesis downstream of ferulic acid [17].

The comprehensive metabolomic analysis sheds light on the multifaceted responses of UASBM13 to drought stress. The upregulation of metabolites involved in antioxidant defense, lignification, and other stress-related pathways suggests a concerted effort by the plant to enhance its resilience to drought. The findings underscore the intricate metabolic adjustments plants undergo to mitigate the impact of abiotic stresses, providing valuable insights for developing crops with improved drought tolerance and sustainable food production in the face of climate challenges.

5. CONCLUSION

In conclusion, the metabolomic analysis of drought-tolerant maize genotype UASBM13 reveals a dynamic response to drought stress, unveiling intricate metabolic adjustments. The upregulation of antioxidant compounds, involvement in lignification processes, and activation of stress-related pathways showcase the plant's resilience strategy. Notably, UASBM13 demonstrates a superior capacity to synthesize lignification metabolites compared to the sensitive genotype, suggesting a potential correlation between this ability and drought tolerance. These insights into the plant's multifaceted response provide valuable knowledge for developing crops resilient to abiotic stresses, crucial for sustainable food production amid evolving climate challenges.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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