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Floral malformation in pearl millet due to downy mildew infection leads to changes in the metabolite profiles in inflorescence florets

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ABSTRACT

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is an important cereal and forage crop in arid and subtropical regions of the Indian subcontinent as well as several African regions. One of the major constraints for its production is the 'green ear' or 'downy mildew' disease caused by the oomycetous, biotrophic, obligate pathogen *Sclerospora graminicola* (Sacc.) Schroet. wherein extensive floral malformation leads to conversion of inflorescence to vegetative structures. To understand the mechanism of this malformation in the present study the comparative metabolite profiling was carried out. The metabolites from florets of normal and malformed inflorescence were extracted and subjected to HRLC-MS/MS and HRGC-MS/MS analysis to identify the different metabolites present. The results indicate several secondary metabolites as well as phytohormones such as salicylic acid and gibberellic acid to be present in different levels in these two florets indicating their possible roles in the process of floral malformation in pearl millet.

Keywords: Plant hormones, metabolites, Gibberellic acid, Salicylic acid, metabolites, *Sclerospora graminicola,* inflorescence.

INTRODUCTION

Pearl millet is one of the major food crops in arid and semi-arid tropical regions of Asia and Africa. It is an important crop towards addressing the food security concerns due to its progressive nature towards acidic and drought conditions. One of the major drawbacks for pearl millet production is the 'green ear' or 'downy mildew' disease caused by the oomycetous, biotrophic, and obligate pathogen *Sclerospora graminicola* (Yadhav *et al*., 2002). The disease includes early chlorosis at the base of the leaf (half leaf or partial leaf symptoms), which extends to the whole leaf. At later stages, a white powdery growth of the asexual sporangia is observed on the abaxial side of infected leaves, hence the name downy mildew. Systemically infected host plants show stunted growth and

modified panicles with leafy growth instead of seed setting (phyllody) and hence the name 'green ear' disease (Semisi and Ball, 1989).

Some pathogens, including *S. graminicola,* have the ability to change the branching architecture of plant inflorescence due to infection leading to the development of vegetative structures (Raghavendra & Safeeulla, 1979). Phytohormones are an important part of the plant signaling system and play various roles in controlling growth, development, and biotic and abiotic alterations. It is thought that alteration in the levels of phytohormones and other metabolism in infected plants may lead to floral malformations (Matheussen *et al*., 1991). It is well known that pathogen infection results in altered secondary metabolism in plants due to modulation of gene expression. In recent studies, it has been reported that hormones like salicylic acid (SA), jasmonic acid (JA) and abscisic acid (ABA) have an involvement in plant responses to biotic and abiotic stress, while Auxins, cytokinins (CKs) and strigolactones (SL) are involved in apical dominance (Verma *et al*., 2016). Gibberellic acid (GA) has been shown to play an important role in symptom formation during *Sporisorium reilianum* infection in sorghum. Fungus presence leads to decrease in GA concentration and this could be the reason for the increased tillering in infected plants. The presence of GA also did not lead to phyllody formation in maize inflorescences when tested in vitro (Santner *et al*., 2009). The way hormones contribute to development and growth of various organs is widely studied in Arabidopsis. It is observed that the stamen development is dependent on all hormones, petal development is largely affected by GAs and JAs, and carpel development can be regulated by auxins (Ghareeb *et al*., 2011). In the last two decades, Mass spectrometry (MS) coupled to liquid chromatography (LC) and gas chromatography (GC) has remained the method of choice for the detection and quantification of phytohormones (Chandler, 2011). In recent studies, multiple research have been carried out on phytohormones profiling, but only a few studies have explored on the analysis of primary and secondary metabolites and phytohormones from a single sample (Denoroy *et al*., 2013). As a step towards unravelling the mechanism of floral malformation in pearl millet due to *S. graminicola* infection, the present study was taken up to analyse the metabolite profiles of the normal and malformed florets of pearl millet. The metabolites were extracted and subjected to LC-MS/MS and GC-MS/MS for profiling. The present study provides information on the various metabolite compounds from normal and malformed pearl millet floret samples and the putative role of GAs and SAs in the floral malformation.

MATERIALS AND METHODS

Chemicals and reagents

All the solvents used for the extraction of floret samples were of HPLC grade and were procured from Sisco Research Laboratory (Mumbai, India). Solvents used for high-resolution liquid chromatography-mass spectrometer (HR-LCMS) were of Spectroscopic grade obtained from SD Fine Chemicals Limited (SDFCL; Mumbai, India).

Plant sample collection

Pearl millet plants (Cv 7042S, highly susceptible to downy mildew) infected with *S. graminicola* and uninfected (normal control) maintained under greenhouse conditions were used in the study. The normal and infected (green ear) inflorescences were collected from the 48 day old plants and the individual florets were separated from them (Fig.1). These florets (malformed and normal) were used for metabolite extraction and analyses.

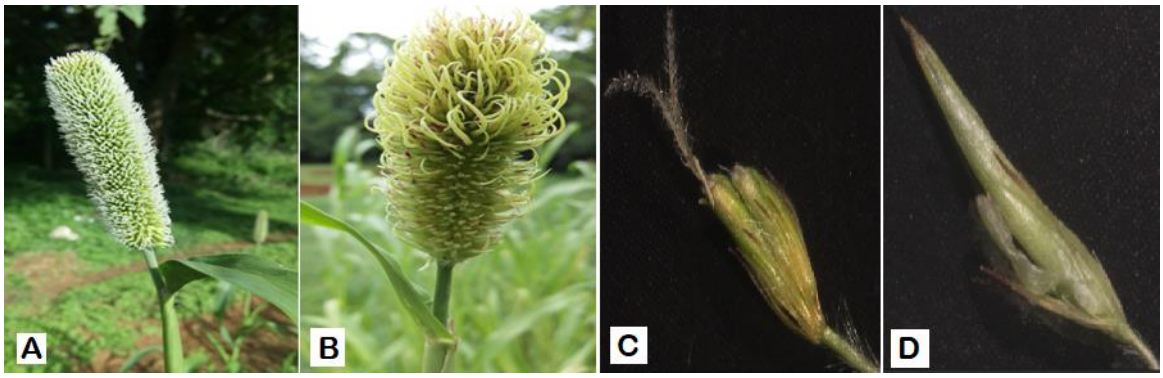

Fig 1.A: Normal inflorescence; 1.B: Malformed inflorescence; C: Normal floret; D: Malformed floret

Extraction of hormones, metabolites from a single sample

Homogenized floret samples (1g fresh weight) were extracted by incubation with 1 ml of precooled (-20°C) Methyl tert- butyl ether (MTBE): MeOH (3:1, v:v) mixture (Schäfer *et al*., 2016). The extracts were incubated for 30 min on an orbital shaker at 4°C before subjecting to sonication under chilled conditions. The samples were centrifuged for 10 min at 10,000 g at 4°C. The supernatant was transferred to two new microcentrifuge 2-ml tubes, each with 0.5 ml of the supernatant: one for hormone extraction and the other for metabolites extraction (Salem *et al*., 2016). To the first supernatant (tubes for hormone extraction), a volume of 0.5 ml of acidified water (0.1% HCl) was added and the samples were thoroughly vortexed for 1 min. The samples were kept on an orbital shaker for an additional 30 min at 4°C and were centrifuged at 10,000 g for 10 min at 4°C. The upper supernatant phase (MTBE phase) was collected and transferred in a 1.5- ml microcentrifuge tube and dried down using a Speedvac concentrator at RT (sample is take up to 2 h at 30°C). The dried pellets were resuspended in 50 µl water: methanol (50:50) solution and the resuspended samples were immediately subjected to UHPLC-ESIMS /MS hormonal and metabolic analysis. To the second supernatant (tubes for metabolite extraction), a volume 0.5 ml of water: methanol (3:1, V:V) was added and the samples were quickly vortexed and finally centrifuged for 10 min at 10,000 g at 4°C. The lower phase (300µl) was collected for GC/MS analysis of primary and secondary metabolites. Samples were dried in a speed-vacuum concentrator (Salem *et al*., 2017).

Separation of metabolites and identification by HRLCMS and HRGCMS

The metabolites were separated in 1290 Infinity UHPLC System, 1260 infinity Nano HPLC with Chipcube, 6550 iFunnel Q-TOFs with the solvent system: Mobile phase A: 0.1% formic acid in water (+ve mode) or 10mM ammonium acetate (for –ve mode) and 100% methanol, Mobile phase B: 0.1% formic acid in ACN (+ve mode) or 10mM Ammonium acetate (-ve mode) and 100% ACN. ZORBX Eclipse Plus C18 column with a Narrow Bore 2.18150mm, 5micron was used. The flow rate was 0.4ml/min with gradient elution of mobile phase A and B. The separated metabolic fractions were ionized through ESI, and CID mode of fragmentation, which were further separated (QTOF) and detected based on m/z values, carried out in positive and negative modes. The metabolites in the peaks identified based on the database search in NIST (National Institute of Standards and Technology) library.

The derivative compounds were subjected to GCMS based separation and identification using a gas-chromatography coupled with mass spectrometer (GC-HRMS). The GC was carried out in the Agilent 7890 instrument fitted with an FID detector system, further separation was done in MS of AccuTOF GCV with EI ionizer source. The compounds were identified based on NIST library source.

The structural and spectral information of metabolites were retrieved from different online metabolite databases like Metlin (https://metlin.scripps. edu/) PubChem (https://pubchem.ncbi.nlm.nih.gov/), Chem Spider (http://www.chemspider.com/), CHEMEBI (https://www.ebi.ac.uk/chebi/) and ChEMBL (https://www.ebi.ac.uk/chembl/) in ".sdf" and ".mol" file formats.

RESULTS AND DISCUSSIONS

High-Resolution Liquid Chromatography Mass Spectrometry (HR-LC-ESI-MS/MS) Analysis of metabolites from malformed and normal samples

floret sample (Table 2a and 2b). A few important classes of organic compounds, such as flavonoids, phenolics, terpenoids, steroids, alkaloids, and oligopeptides were detected in the extract of the floret samples. The compounds identified in the floret sample MTBE extract have different roles and functions. The two phytohormones were identified: salicylic acid and gibberellic acid, which are responsible for their growth and development. The LC chromatograms of both malformed and normal samples showed a difference in the concentration of metabolites present in MTBE extract, with the normal floret sample showing higher metabolite content compared to the malformed florets. This may be due to the alteration in the production of metabolites during downy mildew infection. The abundance profile of representative compounds (Fig. 2a and 2b) both in normal and malformed samples also support the observation that the normal floret sample have higher metabolite content.

Table 1(a): Representing the metabolites compounds identified in malformed inflorescence (M) by HRLCMS analysis; ESI: positive mode

Sr No	Compound name	Formula	Mass	R.Time (min)	Abund.	Class/Type
1	10-Hydroxy-8-nor-2-fenchanone glucoside	$C_{15}H_{24}O_7$	316.15	5.559		terpene glycoside
2	Maritimetin	$C_{15}H_{10}O_6$	286.04	8.689	72865	flavonoids
3	4R,5R,6S-Trihydroxy-2-hydroxymethyl-2-cyclohexen-1-one 6- (-hydroxy-6-methylbenzoate)	$C_{15}H_{16}O_7$	308.08	9.711		It is functionally related to a salicylic acid.
4	Cajanin	$C_{16}H_{12}O_6$	300.05	9.837		Plant metabolite and a anti melanogenic agent
5	Coriandrone E	$C_{13}H_{12}O_5$	248.06	10.355	26987	Antioxidant
6	Gibberellin A75	$C_{19}H_{24}O_8$	380.14	11.775		A plant growth hormone
7	12-Hydroxy-8,10-octadecadienoic acid	$C_{18}H_{32}O_3$	296.23	12.383	34725	lineolic acids derivatives
8	6-Methyl-2-methylene-6-octene-1,3,8-triol	$C_{10}H_{18}O_3$	186.12	12.734	53239	An aliphatic alcohol.
9	4,4'-Methylenedianiline	$C_{13}H_{14}N_2$	198.11	12.962	24504	Carcinogenic agent and an allergen
10	Glycylprolylhydroxyproline	$C_{12}H_{19}N_3O_5$	285.13	13.016	41676	An oligopeptide
11	Miraxanthin-II	$C_{13}H_{14}N_2O_8$	326.07	13.091	29249	An organooxygen compound.
12	Nigakilactone B	$C_{22}H_{32}O_6$	392.21	13.536		A triterpenoid.
13	2,5-Heptadien-1-ol	$C_7H_{12}O$	112.08	13.689		An aliphatic alcohol
14	Nigakilactone B	$C_{22}H_{32}O_6$	392.21	13.907		A triterpenoid
15	Homocapsaicin	$C_{19}H_{29}NO_3$	319.21	16.034	27515	A compound found in chili peppers and responsible for their burning and irritant effect.
16	Cichoriin	$C_{15}H_{16}O_9$	340.08	16.352	52983	It has a role as an anti-diabetic activity.
17	Moracin E	$C_{19}H_{16}O_4$	308.10	18.595	40016	It has an anti-inflammatory, antimicrobial, antiparasitic, and antitumor activities.
18	Spectinomycin	$C_{14}H_{24}N_2O_7$	332.15	19.031		It has a role as an antimicrobial agent, a bacterial metabolite and an antibacterial drug.
19	2-Undecyl-4(1H)-quinolinone N-oxide	$C_{20}H_{28}NO_2$	314.20	19.58		Antimalarial drug

Table 1(b): Representing the metabolites compounds identified in malformed inflorescence (M) by HRLCMS analysis; ESI: negative mode

Sl No	Compound name	Formula	Mass	RT	Abund.	Class/Type
1	Aromadendrin	$C_{15}H_{12}O_6$	288.06	8.411	13930	Flavanonol
2	N-Desmethylclobazam	$C_{15}H_{11}ClN_2O_2$	286.05	8.688	21211	Phenolic compound
3	Northienamycin	$C_{10}H_{14}N_2O_4S$	258.06	12.177	40877	An antibiotic.
4	RU-0211	$C_{20}H_{32}F_2O_5$	390.22	14.42	37699	Fatty acid & chlorine channel opener

5	27-Nor-5b-cholestane-3a,7a,12a,24,25-pentol	$C_{26}H_{46}O_5$	438.33	15.837	29604	steriod
6	9-HOTE	$C_{18}H_{30}O_3$	294.22	16.552	63417	Lineolic acid derivate
7	3-keto stearic acid	$C_{18}H_{34}O_3$	298.25	16.799	24440	intermediate compound during Type 2 fatty acid biosynthesis
8	Sambutoxin	$C_{28}H_{39}NO_4$	453.29	16.898	150335	Mycotoxin from *Fusarium sambucinum*
9	Methyl 3b,24-dihydroxy-11,13(18)-oleanadien-30-oate	$C_{31}H_{48}O_4$	484.34	17.134	18903	triterpenoids
10	Iriomoteolide 1a	$C_{29}H_{46}O_7$	506.31	17.961	35680	Antibacterial compound
11	Tsangane L 3-glucoside	$C_{19}H_{34}O_7$	374.22	20.572	145544	It is a terpene glycoside.
12	(3beta,22R,23R,24S)-3,22,23-Trihydroxystigmastan-6-one	$C_{29}H_{50}O_4$	462.37	22.962	67242	It is a steroid. a stigmastanes derivate
13	1-O-Octadec-9-enyl glycerol	$C_{21}H_{42}O_3$	342.31	23.856	30141	Selachyl alcohol; a bulidling block for synthesis of complex compounds
14	6-Epikarpoxanthin	$C_{40}H_{58}O_4$	602.43	24.285	35640	It is a xanthophyll and have antioxidant property.
15	2-Octaprenyl-3-methyl-6-methoxy-1,4-benzoquinol	$C_{38}H_{60}O_3$	564.44	24.307	16974	It has an anti-inflammatory activity.
16	PE(20:2(11Z,14Z)/18:2(9Z,12Z))	$C_{43}H_{78}NO_8P$	767.53	24.424	38209	It is a human metabolite.
17	Cucurbitachrome 1	$C_{40}H_{56}O_4$	600.42	24.913	30165	It is a xanthophyll.

Table 2(a): Representing the metabolites identified in normal inflorescence (N) by HRLCMS analysis; ESI: positive mode

Sl No.	Compound name	Formula	Mol. wt	RT	Abund.	Functions
1	Orotidine	$C_{10}H_{12}N_2O_8$	288.06	8.421	33436	It has a role as a bacterial metabolite, a fungal metabolite and a plant metabolite.
2	Citrinin	C13 H14 O5	250.08	9.449	146746	A secondary metabolite produced by fungi that contaminates long-stored food and it causes different toxic effects, like nephrotoxic, hepatotoxic and cytotoxic effects.
3	4R,5R,6S-Trihydroxy-2-hydroxymethyl-2-cyclohexen-1-one 6- (-hydroxy-6-methylbenzoate)	$C_{15}H_{16}O_7$	308.08	10.08	218673	It is functionally related to a salicylic acid.
4	3-Hydroxynonyl acetate	$C_{11}H_{22}O_3$	202.15	11.245	52337	It is a secondary alcohol and an acetate ester.
5	Methyl 7-epi-12-hydroxyjasmonate glucoside	$C_{19}H_{30}O_9$	402.18	11.681	56817	It is a jasmonic acid metabolite.
6	Gibberellin A75	$C_{19}H_{24}O_8$	380.14	12.092		A plant growth hormone
7	12-Hydroxy-8,10-octadecadienoic acid	$C_{18}H_{32}O_3$	296.23	12.38	69251	A plant metabolite
8	N-(Heptan-4-yl)benzo[d][1,3]dioxole-5-carboxamide	$C_{15}H_{21}NO_3$	263.15	12.667	92040	A member of benzodioxoles which having antitumor, antibacterial, antifungal and antioxidant properties.

9	6-Methyl-2-methylene-6-octene-1,3,8-triol	$C_{10}H_{18}O_3$	186.12	12.67	146490	An aliphatic alcohol.
10	Nigakilactone B	$C_{22}H_{32}O_6$	392.21	13.476		A triterpenoid.
11	16-Hydroxy hexadecanoic acid	$C_{16}H_{32}O_3$	272.23	13.48	228942	It has a role as a plant metabolite.
12	4,4'-Methylenedianiline	$C_{13}H_{14}N_2$	198.11	15.333	147144	It has a role as a carcinogenic agent and an allergen
13	Homocapsaicin	$C_{19}H_{29}NO_3$	319.21	15.898	36957	A compound found in chili peppers and responsible for their burning and irritant effect.
14	N-Acetyl-b-glucosaminylamine	$C_8H_{16}N_2O_5$	220.10	15.899	75999	It has a role in the treatment for osteoarthritis.
15	Cichoriin	$C_{15}H_{16}O_9$	340.08	16.624	164314	It has a role as an anti-diabetic activity.

Table 2(b): Representing the metabolites identified in normal inflorescence (M) by HRLCMS analysis; ESI: negative mode

Sl No.	Compund name	Formula	Mol. wt	RT	Abund.	Functions
1	Myricitrin	$C_{21}H_{20}O_{12}$	464.10	6.436	10589	Glycosyloxyflavonoid
2	N-Desmethylclobazam	$C_{15}H_{11}ClN_2O_2$	286.05	8.689		Phenolic compound
3	6alpha-Fluoro-11beta,17-dihydroxypregn-4-ene-3,20-dione	$C_{21}H_{29}FO_4$	364.20	9.886	13265	Corticosteroid hormone
4	(-)-menthyl beta-D-glucoside	$C_{16}H_{30}O_6$	318.20	11.69	12190	Glucopyranoside; a natural compound *Pedicularisplicata*
5	6alpha-Fluoro-17-hydroxy pregn-4-ene-3,20-dione acetate	$C_{23}H_{31}FO_4$	390.22	13.32	15498	Corticosteroid hormone
6	12-Hydroxy-8,10-octadecadienoic acid	$C_{18}H_{32}O_3$	296.24	15.752	111719	Derivative of lineolic acids
7	12-Hydroxy-8,10-octadecadienoic acid	$C_{18}H_{32}O_3$	296.24	16.13	238150	Derivative of lineolic acids
8	12R-hydroxy-9Z-octadecenoic acid	$C_{18}H_{34}O_3$	298.25	16.76	20371	LCF acid
9	Sambutoxin	$C_{28}H_{39}NO_4$	453.29	16.878	76166	Mycotoxin from *Fusarium sambucinum*
10	DG(18:3(6Z,9Z,12Z)/14:0/0:0)	$C_{35}H_{62}O_5$	562.46	19.673	14301	DG; supporting the biosynthesis (and degradation) of glycerolipids, and regulating PKC activity.
11	Staphidine	$C_{42}H_{58}N_2O$	606.46	20.114	15485	Bis-diterpene alkaloid
12	MG(24:1(15Z)/0:0/0:0)	$C_{25}H_{48}O_4$	440.39	24.523	129941	Monoacylglyceride
13	PI(16:0/18:0)	$C_{43}H_{83}O_{13}P$	838.54	28.373	25312	Phosphatidylinositols are important lipids; membrane-bound signaling molecules

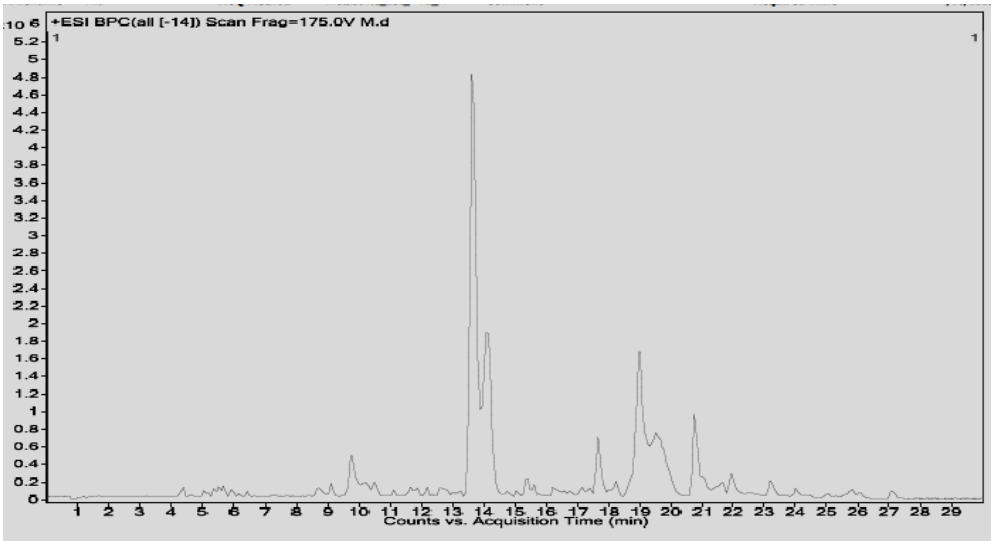

Fig. 2a: LC chromatogram of MTBE extract Malformed sample chromatogram

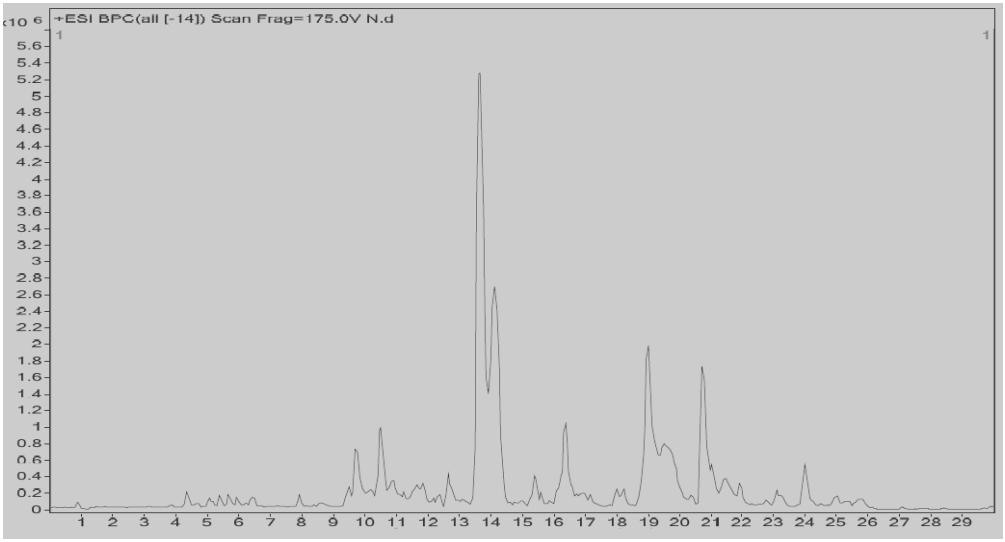

Fig. 2b: LC chromatogram of MTBE extract Normal sample chromatogram

In addition, there are a significant number of metabolites present both in normal and malformed extracts, only a few metabolites have been focused in the present study. Seven compounds were detected to be present in both samples and showing significant differences based on their abundance values and chromatogram peak. These include 4R,5R,6S-Trihydroxy-2-hydroxymethyl-2-cyclohexen-1-one 6- (-hydroxy-6-methylbenzoate) (Salicylic acid), Gibberellin A75, 12-Hydroxy-8,10-octadecadienoic acid, 6-Methyl-2-methylene-6-octene-1,3,8-triol, 4,4'-Methylenedianiline, Homocapsaicin, and Cichoriin. Salicylic acid plays a crucial role in regulating cell division and cell expansion, which are the key processes that determine the final stature of plants (Li *et al*., 2022). Gibberellins play significant roles in almost every aspect of plant growth regulation and development, including cell elongation, leaf expansion, seed germination and leafy head formation (Ritonga *et al*., 2023). The differential levels of these hormones in the normal and malformed pearl millet florets indicate their possible involvement in the process of floral malformation due to pathogen infection. 12-Hydroxy-8,10-octadecadienoic acid is a derivate of lineolic acid. 6-Methyl-2-methylene-6-octene-1,3,8-triol and 2,5-Heptadien-1-ol are aliphatic alcohols, which play a key role in the regulation of carbohydrate metabolism (Thind, 1991). These compounds can be further examined for identification of their roles in floral malformation in pearl millet due to infection.

3.2. High-Resolution Gas Chromatography Mass Spectrometry (HR-GC-ESI-MS/MS) Analysis of MTBE extracts of malformed and normal samples

The MTBE extract of malformed and normal samples were subjected to HRGCMS analysis to screen volatile metabolites that may be involved in the floral malformation.. Based on the retention time of the chromatogram peaks and MS spectra, a total of 100 compounds were identified in the MTBE extract of malformed floret sample (Fig. 3a) (Table 3a).

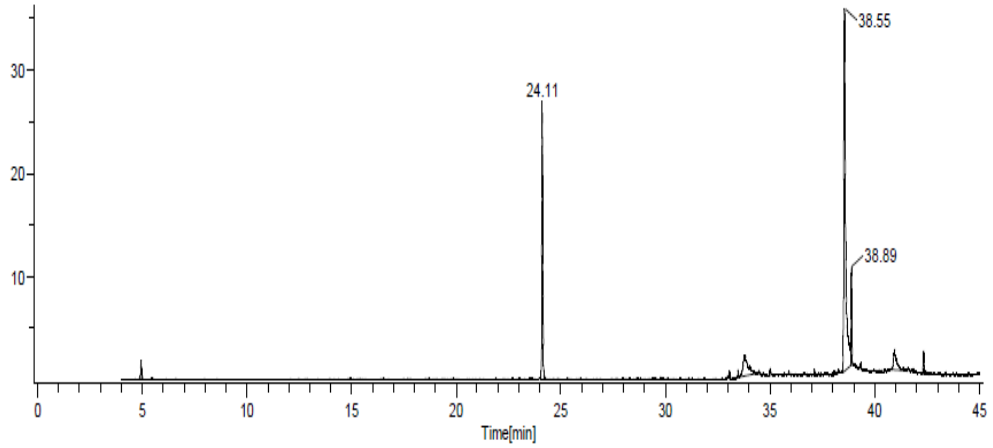

Fig. 3a: GC chromatogram of MTBE extract Malformed sample chromatogram

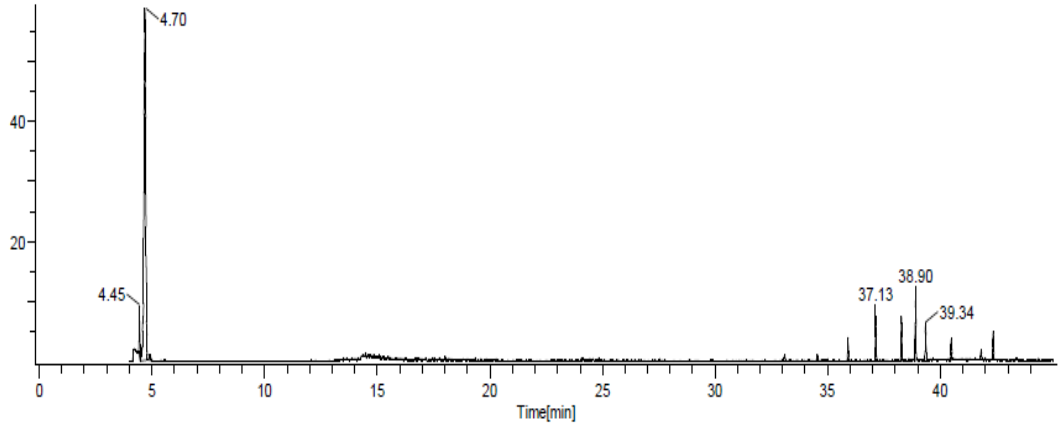

Fig. 3b: GC chromatogram of MTBE extract Normal sample chromatogram

Table 3: Representing the metabolites identified in normal and malformed inflorescence (M) by HRGCMS analysis.

Sl. No	Compound Name	Formula	MW	Functions
	3a. Malformed sample			
01	10-(Methoxycarbonyl)-N-acetylcolchinol	$C_{22}H_{25}NO_7$	415	An alkaloid, play a key role as plant defences Abiotic and biotic stress condition. A mitotic inhibitor, know to induce polyploidy, slow growth, flowering and fruit setting.
02	1,16-Cyclocorynan-16-carboxylic acid, 17-(acetyloxy)-19,20-didehydro-10-methoxy-, methyl ester, (16.xi.,19E)	$C_{24}H_{28}N_2O_5$	424	An Indole alkaloid, know to protect the plants from predators and helps in regulation of growth. Acts as a Nitrogen reservoir and helps to get through the environmental stress
03	1,2,3,4-Tetrahydroisoquinolin, 2-acetyl-6,7-dimethoxy-1-phenmethylene-	$C_{20}H_{21}NO_3$	323	An Alkaloids; with a chemical defence mechanism in plants and regulating the chaperon activity. Promotes the antioxidant, antifungal enzyme activity in plants.

04	3β-Stigmast-5-en-3ol, flophemesyl ether	$C_{37}H_{55}F_5OSi$	638	A type of Beta-sitosterols, (classs of phytosterol); provides resistance to biotic and abiotic stress; regulation of water permeability; stability of membrane proteins; plays a role in embryonic development of seeds.
05	3β,22E-Stigmasta-5,22-dien-3-ol, flophemesyl ether	$C_{37}H_{53}F_5OSi$	636	A Sterol which acts as a precursor of phytohormones production and acts as defensive compound against pathogens
06	Echinenone	$C_{40}H_{54}O$	550	A type of beta carotene, an antioxidant which protects the cell from photoxidative damages/ ROS.
07	Docosanoic acid, 1,2,3-propanetriyl ester	$C_{69}H_{134}O_6$	1058	A derivative of dodecanoic acid, provides a structural integrity and as signal transduction mediators.
08	Pregna-3,5-dien-20-one, 6-methyl-3,17,21-tris[(trimethylsilyl)oxy]-	$C_{31}H_{56}O_4Si_3$	576	A Steroid saponin. Increased production is observed during up regulation of MeJA pathway in plants.
09	Vitexin xyloside	$C_{26}H_{28}O_{15}$	580	A flavonoid, especially seen in petals, trigger for the production of defense related enzyme like PAL under stress condition.
10	Octadecanoic acid, decyl ester	$C_{28}H_{56}O_2$	424	Defense molecule against fungal pathogens in plants.
	3b. Normal sample			
11	Pregn-5-en-20-one, 11-(acetyloxy)-3,14-dihydroxy-12-(2-hydroxy-3-methyl-1-oxobutoxy)-, (3β,11α, 12β, 14β)	$C_{28}H_{42}O_8$	506	A Steroid saponin. Increased production is observed during up regulation of MeJA pathway in plants.
12	D-Homo-24-nor-17-oxachola-1,20,22-triene-3,16-dione, 7-(acetyloxy)-14,15:21,23-diepoxy-4,4,8-trimethyl-, (5α, 7α, 13α, 14β,15β,17aα)	$C_{28}H_{34}O_7$	482	Gedunin is a plant secondary metabolite and has a various activities such as antimalarial, an antineoplastic agent, an Hsp90 inhibitor, antimicrobial activity. Antifungal activity especially against phytopathogens such as *Fusarium oxysporum*, *Magnaportheoryzae*, *Sclerotiumrolfsii*, *Rhizoctoni asolani*, *Alternaria* spp.,and *Botrytis cinerea*, and three oomycetes *Phytophthora* species.
13	Fluazinam	$C_{13}H_4Cl_2F_6N_4O_4$	464	A fungicide used to control grey mould, downy mildew and other fungal pathogens
14	[5-(3-Methoxymethoxy-10,13-dimethyl-2,3,4,9,10,11,12,13,14, 15,16,17-dodecahydro-1H-cyclopenta[a] Phenanthrene-17-yl)-hex-1-ynyl]-trime	$C_{30}H_{48}O_2Si$	468	An antimicrobial compound
15	Syrosingopine	$C_{35}H_{42}N_2O_{11}$	666	An alkaloid defensive against insect-pest and pathogens
16	Olean-12-en-28-al, 3β,16α,22α-trihydroxy-, 16-acetate	$C_{32}H_{50}O_5$	514	A plant defensive metabolite under abiotic stress.
17	Pentacontanoic acid, propyl ester	$C_{53}H_{106}O_2$	774	An antibacterial compound against ***P.atrosepticum.***
18	D-Glucopyranoside, (3β,22α,25S)-22,25-epoxy-3-methoxyfurost-5-en-26-yl 2,3,4,6-tetra-O-methyl-	$C_{38}H_{62}O_9$	662	A chemical defensive molecule regulated by the levels of JA in plants during stress
19	1,4:5,8-Dimethanonaphthalene-2,3-diol, 5,6,7,8,9,9-hexachloro-1,2,3,4,4a,5,8,8a-octahydro-, (1α,2α,3β,4α,4aβ,5α,8α,8aβ)	$C_{12}H_{10}Cl_6O_2$	396	Aldrin-transdiol is a metabolite of dieldrin. having insecticidal property

A total of 130 compounds were identified in the MTBE extract of normal floret sample (Fig. 3b; Table 3b). The identified compounds from malformed sample such as 10-(Methoxycarbonyl)-N-acetylcolchinol, an alkaloid was reported to play a key role in plant defences during abiotic and biotic stress condition. This compound is also a mitotic inhibitor, known to induce polyploidy, slows growth, flowering and fruit setting (Kumar *et al*., 2019). The compound 1,16-Cyclocorynan-16-carboxylic acid, 17-(acetyloxy)-19,20-didehydro-10-methoxy-, methyl ester, (16.xi.,19E) is an indole alkaloid, known to protect the plants from predators and helps in regulation of growth. It also acts as a Nitrogen reservoir and helps to get through the environmental stress (Heinrich *et al*., 2021). 1,2,3,4-Tetrahydroisoquinolin, 2-acetyl-6,7-dimethoxy-1-phenmethylene is an alkaloid act as a defence mechanism in plants and regulating the chaperon activity. Promotes the antioxidant, antifungal enzyme activity in plants (Matsuoka *et al*., 2016), 3β-Stigmast-5-en-3ol, flophemesyl ether is a classs of phytosterol; provides a resistance to biotic and abiotic stress, regulates water permeability, plays a role in embryonic development of seeds (Valitova *et al*., 2016), 3β,22E-Stigmasta-5,22-dien-3-ol, flophemesyl ether is an sterol which acts as a precursor of phytohormones production and acts as a defensive compound against pathogens (Mailafiya *et al*., 2018), Echinenone is a type of beta carotene, and act as an antioxidant which protects the cell from photoxidative damages/ROS (Chaudhary *et al*., 2015), Pregna-3,5-dien-20-one, 6-methyl-3,17,21-tris [(trimethylsilyl) oxy] is an steroid saponin which increase production during up regulation of MeJA pathway in plants. (Pagassini *et al*., 2021). Vitexin xyloside is a flavonoid, especially seen in petals, and triggers the production of defense related enzyme like PAL under stress condition (Upchurch RG 2008), Octadecanoic acid, decyl ester is an important compound act as a defence molecule against fungal pathogens in plants (Tan *et al*., 2021), Some compounds identified in the normal sample such as Pregn-5-en-20-one, 11-(acetyloxy)-3,14-dihydroxy-12-(2-hydroxy-3-methyl-1-oxobutoxy)-, (3β,11α,12β,14β) were earlier reported to have function of steroid saponin and their increased production was observed during up regulation of MeJA pathway in plants (Chaudhary *et al*., 2015). The GC-MS analysis was performed to identify the volatile compounds that could not be detected in LC-MS/MS analysis. In this context, there are significant number of bioactive metabolites present both in normal and malformed extract and only a few metabolites have been focused in the present study.

CONCLUSION

Scelerospora graminicola infection of pearl millet leads to the malformation. The present study is a first report to the best of authors' knowledge, on listing the important metabolite compounds present in malformed and normal MTBE extracts of pearl millet inflorescence, and indicates chemical difference between malformed and normal samples. The study also focus on new recent analytical tools in cataloging chemical constituents within a genus of monocot and dicot plants, which may possibly help in the development of a chemo-taxonomical database to authenticate and identify the plant species.

Further investigation on increased concentration of metabolite study will help in development of chemical and biological based combating strategy by inducing resistance in pearl millet against pathogen (*S. graminicola*). Further, the present investigation gives us an insight into the importance of constructing a closely related monocot species based chemical library which can be useful for future work.

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Author Contributions
KRK and SS conceptualized and designed the study. JSK carried out the experiment and collected the data. JSK, KRK and SS analysed the data and finalized the manuscript. All authors have read and approved the final manuscript.

Conflict of interest
The Authors declare that they have no conflict of interest.

Ethical statement
This article does not contain any studies with human participants or animal performed by any of the authors

REFERENCES

Chandler JW (2011) The hormonal regulation of flower development. *Journal of Plant Growth Regulation*, *30*(2), 242-254.

Chaudhary S, Chikara SK, Sharma MC, Chaudhary A, AlamSyed , Chaudhary PS & Iriti M (2015) Elicitation of diosgenin production in *Trigonella foenum-graecum* (fenugreek) seedlings by methyl jasmonate. *International journal of molecular sciences*, *16*(12), 29889-29899.

Denoroy L, Zimmer L, Renaud B & Parrot S (2013) Ultra high performance liquid chromatography as a tool for the discovery and the analysis of biomarkers of diseases: a review. *Journal of Chromatography B*, *927*, 37-53.

Ghareeb H, Becker A, Iven T, Feussner I & Schirawski J (2011) *Sporisorium reilianum* infection changes inflorescence and branching architectures of maize. *Plant Physiology*, *156*(4), 2037-2052.

Heinrich M, Mah J & Amirkia V (2021) Alkaloids used as medicines: Structural phytochemistry meets biodiversity—An update and forward look. *Molecules*, *26*(7), 1836.

Kumar R, Jha KK, Sengupta S, Misra S, Mahto CS, Chakravarty MK, Yadav M (2019) Effect of colchicine treatment on plant growth and floral behaviour in cape gooseberry (*Physalis peruviana* L.). *Journal of Pharmacognosy and Phytochemistry*, *8*(5), 405-411.

Li A, Sun X & Liu L (2022) Action of salicylic acid on plant growth. *Frontiers in Plant Science*, *13*, 878076.

Mailafiya MM, Yusuf AJ, Abdullahi MI, Aleku G A, Ibrahim IA, Yahaya M &Alebiosu CO (2018) Antimicrobial activity of stigmasterol from the stem bark of *Neocarya macrophylla*. *Journal of Medicinal Plants for Economic Development*, *2*(1), 1-5.

Matheussen AM, Morgan PW, & Frederiksen RA (1991) Implication of gibberellins in head smut (*Sporisorium reilianum*) of Sorghum bicolor. *Plant physiology*, *96*(2), 537-544.

Matsuoka E, Matsubara T, Takahashi I, Murano H & Hara M (2016) The isoquinoline alkaloid sanguinarine which inhibits chaperone activity enhances the production of heat shock proteins in Arabidopsis. *Plant Biotechnology*, *33*(5), 409-413.

Pagassini JAV, de Godoy LJG, Campos FG, Barzotto GR, Vieira MAR &Boaro CSF (2021) Silicon and mechanical damage increase polyphenols and vitexin in *Passiflora incarnata* L. *Scientific Reports*, *11*(1), 22064.

Raghavendra S & Safeeulla KM (1979) Histopathological studies on ragi (*Eleusine coracana* (L.) Gaertn.) infected by *Sclerophthora macrospora* (Sacc.) Thirum. Shaw and Naras. *Proceedings of the Indian Academy of Sciences-Section B. Part 2, Plant Sciences*, *88*, 19-24.

Ritonga FN, Zhou D, Zhang Y, Song R, Li C, Li J & Gao J (2023) The Roles of Gibberellins in Regulating Leaf Development. *Plants*, *12*(6), 1243.

Salem M, Bernach M, Bajdzienko K & Giavalisco P (2017) A simple fractionated extraction method for the comprehensive analysis of metabolites, lipids, and proteins from a single sample. *JoVE (Journal of Visualized Experiments)*, (124), e55802.

Salem MA, Jüppner J, Bajdzienko K & Giavalisco P (2016) Protocol: a fast, comprehensive and reproducible one-step extraction method for the rapid preparation of polar and semi-polar metabolites, lipids, proteins, starch and cell wall polymers from a single sample. *Plant Methods*, *12*(1), 1-15.

Santner A, Calderon-Villalobos LIA, & Estelle M (2009) Plant hormones are versatile chemical regulators of plant growth. *Nature chemical biology*, *5*(5), 301-307.

Schäfer M, Brütting C, Baldwin IT & Kallenbach M (2016) High-throughput quantification of more than 100 primary-and secondary-metabolites, and phytohormones by a single solid-phase extraction based sample preparation with analysis by UHPLC–HESI–MS/MS. *Plant Methods*, *12*(1), 1-18.

Semisi ST, Ball SFL (1989) Infection of the pearl millet (*Pennisetum americanum*) inflorescence by the downy mildew fungus (*Sclerospora graminicola*). *Plant Pathol* 38, 571–576.

Tan TN, Trung HT, Le Dang Q, Thi HV, Vu HD, Ngoc TN & Tran Dinh T (2021) Characterization and antifungal activity of limonoid constituents isolated from Meliaceae plants *Melia dubia, Aphanamixis polystachya, and Swietenia macrophylla* against plant pathogenic fungi in vitro. *Journal of Chemistry*, *2021*, 1-12.

Thind SK (1991) Effects of a long chain aliphatic alcohol mixture on growth and solute accumulation in water stressed wheat seedlings under laboratory conditions. *Plant growth regulation*, *10*, 223-234.

Upchurch RG (2008) Fatty acid unsaturation, mobilization, and regulation in the response of plants to stress. *Biotechnology letters*, *30*, 967-977.

Valitova JN, Sulkarnayeva AG & Minibayeva FV (2016) Plant sterols: diversity, biosynthesis, and physiological functions. *Biochemistry (Moscow)*, *81*, 819-834.

Verma V, Ravindran P & Kumar PP (2016) Plant hormone-mediated regulation of stress responses. *BMC plant biology*, *16*, 1-10.

Yadav RS, Hash CT, Bidinger FR, Cavan GP & Howarth CJ (2002) Quantitative trait loci associated with traits determining grain and stover yield in pearl millet under terminal drought-stress conditions. *Theoretical and Applied Genetics*, *104*, 67-83.