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RESEARCH ARTICLE

Unravelling the disease associated metabolites in mutant lines of spanish jasmine (*Jasminum grandiflorum*)

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Abstract

Jasminum grandiflorum cv. Pink pitchi is a novel type in Spanish jasmine which differs from the common cultivars owing to its flower buds which has pink bottom and pure white colour on its upper side. Mutations were made to create variations in plant type and flower bud through physical and chemical mutagens which was done three years ago. Those plants were examined for the current study and among the mutant population, two promising mutants, PP-LBR-2 and PP-LBR-3 were selected based on their yield performance. Of these two mutant lines, PP-LBR-2 was found to be free of Alternaria leaf blight (*Alternaria alternata*) symptoms, while PP-LBR-3 expressed blight symptoms under field conditions. Leaf samples were collected from these two mutant lines and subjected to Gas chromatography-mass spectrometry (GC-MS) analysis for identifying the metabolites present in the samples, which correlate with the disease. The metabolite profiling resulted in identification of various compounds as shown in heat map. PP-LBR-3 contained metabolites that induced susceptibility and pathogen invasion, PP-LBR-2 accumulated chemicals associated with antimicrobial and defensive properties. Interestingly, n-hexadecanoic acid, squalene, phytol and tetradecanoic acid were among the shared molecules with pharmacological activity verified in the previous literature that were found in both lines. The research highlights how varying responses to illness in pink pitchi jasmine are brought about by metabolic heterogeneity caused by mutations. These findings stress the therapeutic value of new mutant lines and provide valuable information for breeding disease-resistant jasmine.

Keywords: *Alternaria alternata*; disease; *Jasminum grandiflorum*; metabolite profiling; mutants

Introduction

Jasmine belongs to the family Oleaceae which is one of the most important fragrant flowers that is majorly used as loose flower in the southern regions since ancient days. The name originated from Arabic Yasmin or yasmn (1). Jasmine is said to be originated from tropical countries such as Africa and southeast Asia (2). Jasmine is a well-known glabrous twinning shrub which is majorly found in Indian regions where it is widely used as a loose flower and garden plant. Besides these uses, the plant also possesses significant medicinal values as it possesses anti- bacterial and anti- inflammatory properties. The leaves are ternate and its typically white blossoms have a bitter and caustic flavour (3). In jasmine, mutation breeding is an efficient method to improve genetic variability and enhance economic traits such as flower yield, plant architecture and improve disease resistance (4). Mutation breeding results in phenotypic alteration which causes permanent heritable modifications in pattern of genetic material (5). Mutagenic exposure when given at higher doses than the recommended, destroys the growth promoters and induces growth inhibitors besides causing chromosomal destruction. On the other hand, lower dosage exposure will not support potential useful mutants due to the lower frequency and hence low effectiveness. Hence, optimization of the dosage to be exposed should be determined, where the researcher chooses the optimal dose based on plant material and intended result because plant response to mutagens is species specific and varies even among genotypes of the same species (4). GC-MS is a reliable tool for screening volatile compounds. It is also an easier and cheaper method to analyse small amounts of sample. To be more accurate, GC-MS analysis promotes screening of compounds less than 1 g pure compounds (6). Antibacterial, antioxidant and antifungal properties are present in active compounds. For standardization of herbal product and their formulations of active constituents in plants is GC-MS, the best method is to identify the bioactive compounds of long chain, ethers, acids, esters, alcohols, etc. (7). Due to mutation, the resistance is induced in plants and through GC-MS the

difference in compounds that are responsible for disease infestation and susceptible compounds between mutated plants could be identified.

Materials and Methods

The study was carried out in the Department of Floriculture and Landscape Architecture, Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu during 2024-2025 (Latitude 11° 00' N, Longitude 77°00' E, elevation 412 m above mean sea level). Two promising mutants of *J. grandiflorum* (Pink Pitchi) designated as PP-LBR-2 (disease free) and PP-LBR-3 (diseased) derived through mutation which was done 5 years ago was induced with gamma rays and EMS were involved in this study for unveiling the metabolites associated with disease infection and resistance in the current year plants.

Metabolite extraction in leaves

Leaves weighing about 80 g were collected, cleaned and were pulverized into fine powder using liquid nitrogen. The leaf powder was then transferred into conical flask and mixed with equal volume of ethyl acetate. This mixture was stirred at 100 rpm in an orbital shaker for 96 hr at 28 °C. The mixture was later filtered using Whatman No. 3 filter paper. The filtrate was later let to concentrate in 55 °C and in 80 rpm using rotary flash evaporator. The extract which was concentrated was dissolved in 1mL of HPLC grade methanol and filtered using PVDF hydrophilic membrane which is of 0.22 mm pore size. The GC-MS system, which included a Thermal Desorber turbo matrix 150 (Perkin Elmer, USA) was operated with 10:1 split ratio using helium as carrier gas at 20 psi. The oven temperature was set to increase from 50 °C to 250 °C at the rate of 10 °C/min. Mass spectrometry was performed in positive ion mode with electron impact ionization at 70 eV, employing a DB-5 column with 30 m × 0.25 mm, 0.25 µm film thickness. Individual component produces a specific spectral peak and was recorded on a paper chart electronically. The time of retention is the amount of time that passes between elution and injection of sample. The retention time was used to distinguish the various substances. The peak value was measured from the base to the tip of the peak.

Working principle of GC-MS

The GC-MS principle is that the mixture will separate into individual substances when heated. Then the sample is injected into the GC inlet port in which the sample vaporizes and it is further moved into chromatographic column by the carrier gas which is helium. The sample passes through the column and the compounds are separated because they interact with the carrier gas (mobile phase) and the column coating (stationary phase). Compounds that elute from the column are transformed into ions at the ion source entrance after the latter portion of the column passes through a heated transfer line. The sample molecules are ionized by a beam of electrons which results in formation of molecular ion and smaller ions with characters that provide a "fingerprint" for the molecular structure. The analyser separates the ions and then detects the spectrum of the compounds that are known and are stored in the inbuilt library.

Statistical analysis

To identify the variations in the metabolite compounds between the disease-free sample (PP-LBR-2) and diseased sample (PP-LBR-3) of *J. grandiflorum* mutants, the peak area analysis was performed with Metaboanalyst software and a heat map was performed (Fig. 1 and 2).

A Venn diagram which shows the significant metabolites of interactions of metabolites was created using j Venn software (Fig. 3). The Venn diagram shows the various compounds present in both the samples individually and the common compounds that are present in both the samples as interaction compounds.

Results

GC-MS analysis was used to examine the presence of secondary

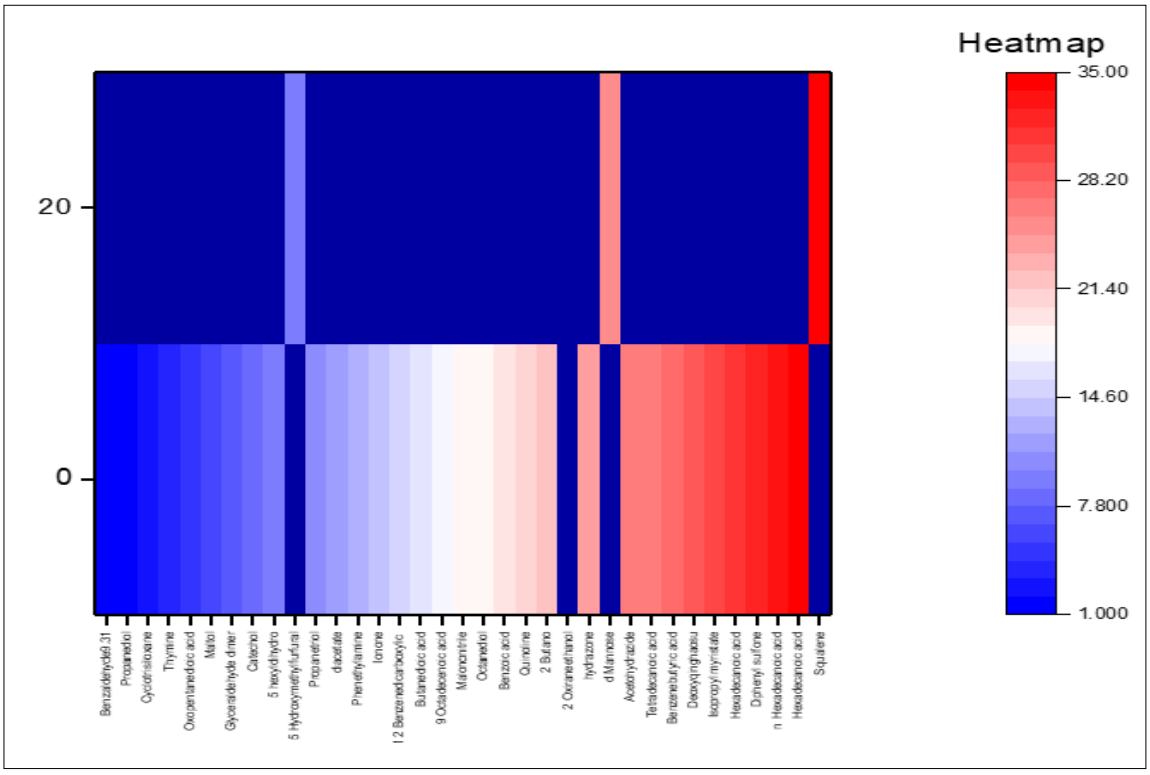

Fig. 1. Heat map analysis of metabolites peak area (%) in PP-LBR-2 of *Jasminum grandiflorum* (disease free).

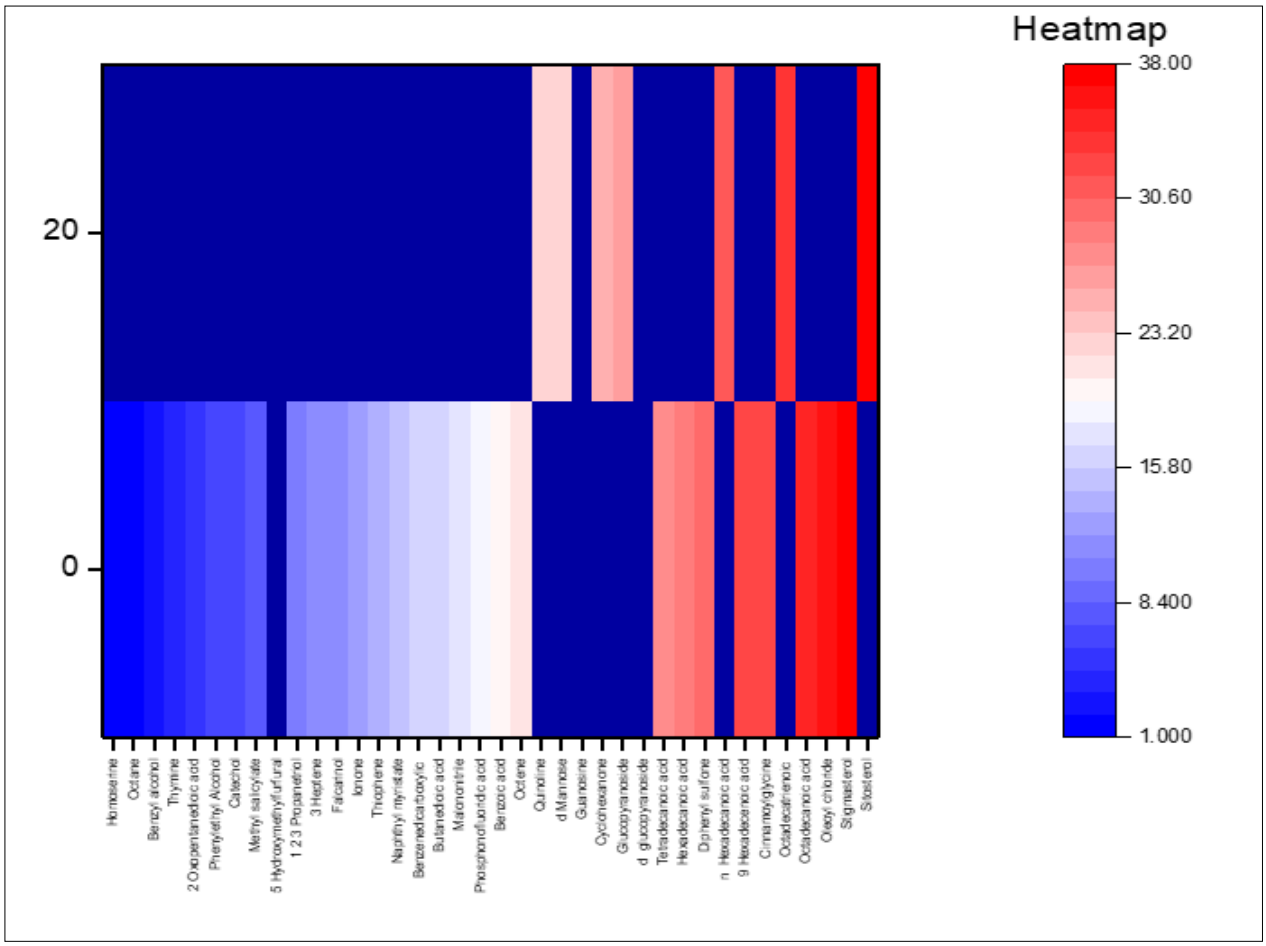

Fig. 2. Heat map analysis of metabolites peak area (%) in PP-LBR-3 of *Jasminum grandiflorum* (diseased).

metabolites from the leaf extracts of mutant lines of *Jasminum grandiflorum* Pink pitchi, (PP-LBR-2) disease free and (PP-LBR-3) diseased samples. The identification of the compounds was determined through the examination of peak area and the metabolite properties. Further, the metabolites were confirmed with the help of NIST (National Institute of Standards and Technology) database. The metabolites present in the leaf extracts are shown in the Fig. 4 and 5. The GC-MS analysis of leaf extracts revealed the presence of diverse compounds with important biological properties. The study shows that the PP-LBR-2 (diseases free) has more compounds that exhibit major antimicrobial, antifungal and antioxidant properties which could be further explored in terms of phytochemical studies. The common compounds present in both the mutants were n-hexadecanoic acid, octadecanoic acid, squalene, phytol, methyl palmitate, oleic acid etc. (Table 1). The additional compounds present in the disease-free mutant PP-LBR-2 were d-mannose (22.4 %), 9,12,15-Octadecatrienoic acid (12.8 %), (1,5,5,8-Tetramethyl-bicyclo [4.2.1] non-9-yl)-acetic acid (18.8 %), 2,2-Dimethyl-5-(3-methyl-2-oxiranyl) cyclohexanone (13.8 %), Benzaldehyde (9.31 %) etc. (Table 2). The compounds in PP-LBR-3 are 5-Hydroxymethylfurfural(39.60), d-Mannose (19.5), Guanosine (68.73), Cyclohexanone (24.2 %), Methyl beta-D-galactoside (26.71 %), Ethyl hexopyranoside (100 %), Linolenic acid (24.27 %), Squalene (72.74 %), 3-Hydroxy-2,3-dihydromaltol (17.17 %), Quinoline (11.02) etc. (Table 3, Fig. 6).

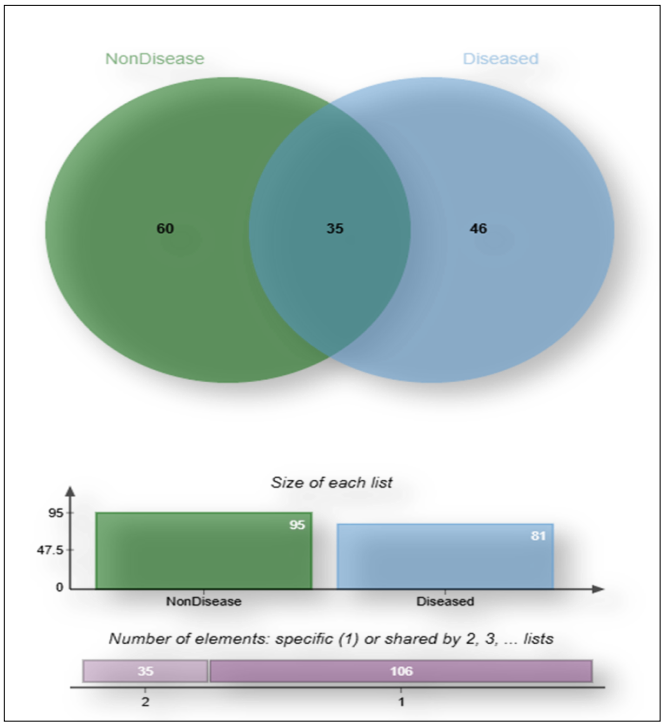

Fig. 3. Metabolite divergence: Individual and shared metabolites between disease free (PP-LBR-2) and diseased sample (PP-LBR-3) of *Jasminum grandiflorum* mutants.

Discussion

The main purpose of this study was to identify the compounds in both disease free (PP-LBR-2) and diseased samples (PP-LBR-3) within the mutant population. The compounds identified in the mutants of *J. grandiflorum* are known to contain various potential pharmacological properties. The common compounds in both the samples PP-LBR-2 and PP-LBR-3 include n-Hexadecanoic acid which has been reported to have antioxidant activity (8), squalene

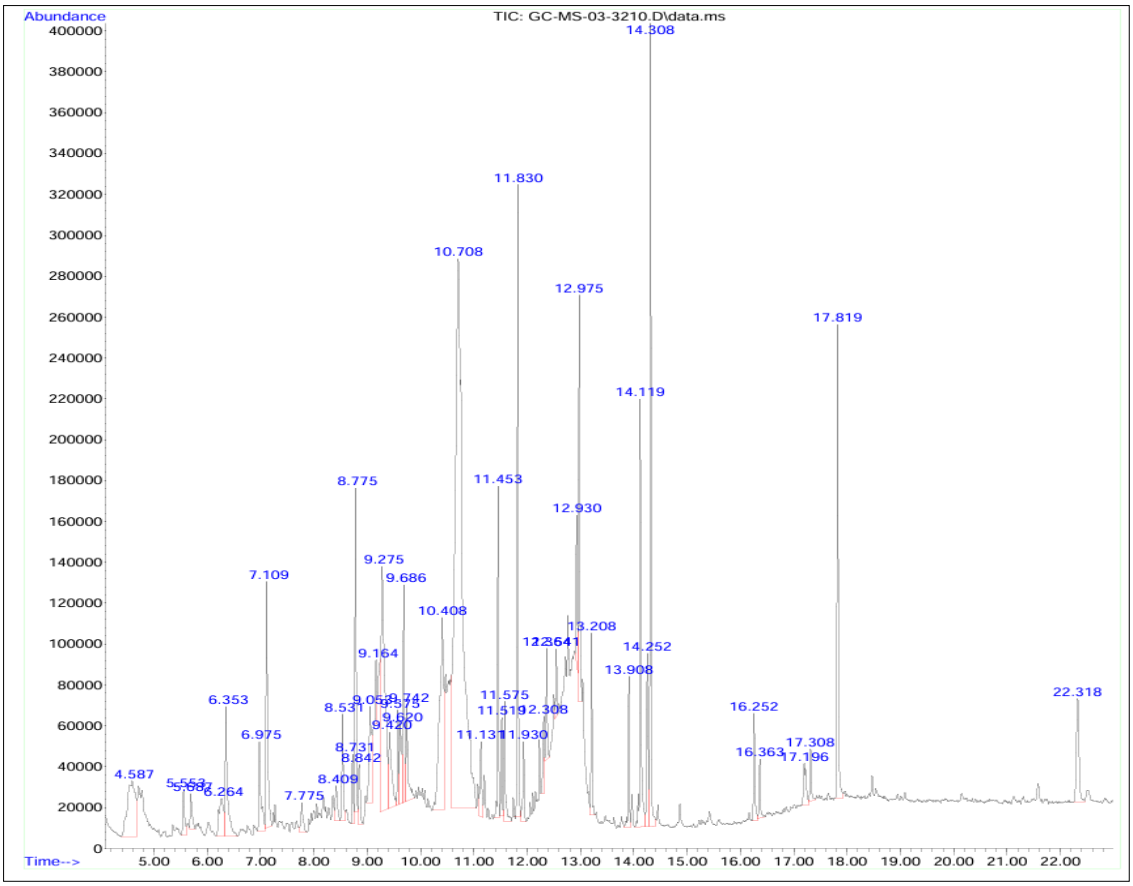

Fig. 4. Metabolite profiling of VOCs in leaves of *J. grandiflorum* mutant PP-LBR-2 (disease free).

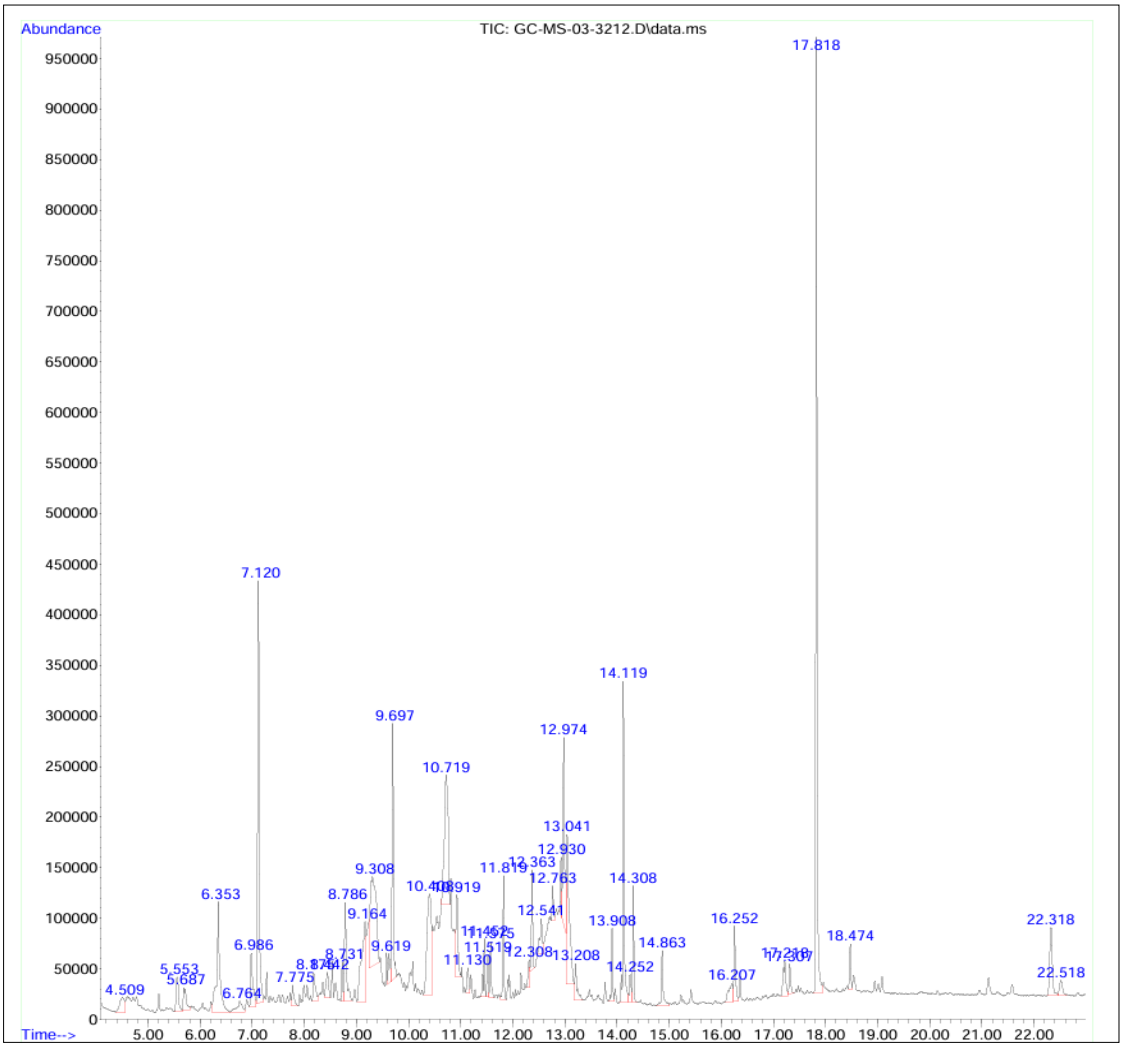

Fig. 5. Metabolite profiling of VOCs in leaves of *J. grandiflorum* mutants PP-LBR-3 (diseased).

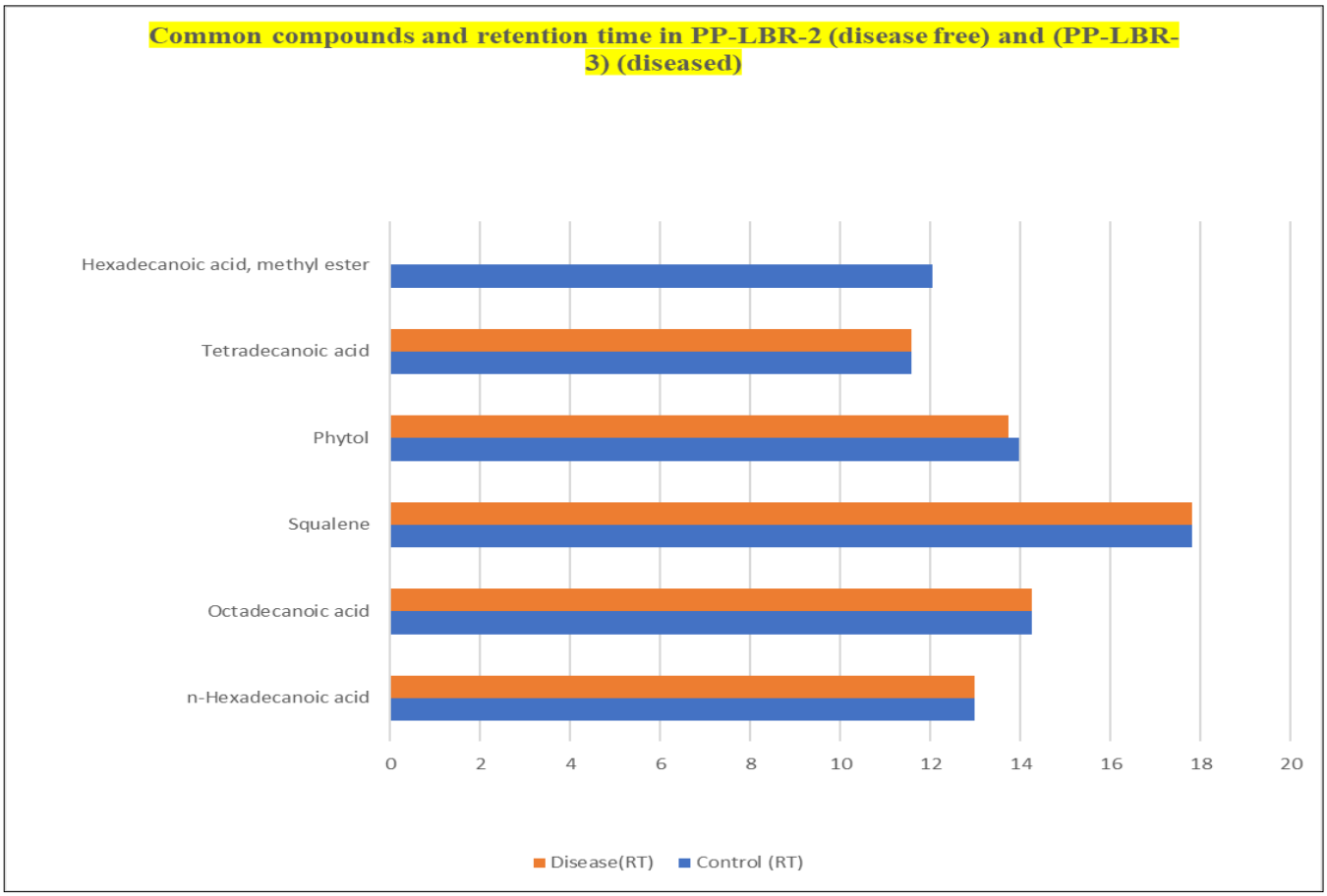

Fig. 6. Common compounds and retention time in PP-LBR-2 (disease free) and PP-LBR-3 (diseased).

Table 1. Common metabolite compounds identified in the leaf extracts of PP-LBR-2 and PP-LBR-3

S. No.	Metabolites	RT		Common name	Molecular formula	Molecular weight (%)	Area (%)		Functions	Reference
		Control	Disease				Control	Disease		
1	n-Hexadecanoic acid	12.9747	12.9745	Palmitic acid	$C_{16}H_{32}O_2$	256.42 g/mol	6.41	13.16	Signalling & stress responses	(8)
2	Octadecanoic acid	14.2523	14.2521	Stearic acid, Octadecanoic acid	$C_{18}H_{36}O_2$	284.5 g/mol	5.94	2.80	Signal transduction and defence responses, antimicrobial properties	(22)
3	Squalene	17.8185	17.8183	Spinacene	$C_{30}H_{50}$	410.7 g/mol	14.57	72.74	Defence against oxidative stress, drought resilience	(23)
4	Phytol	13.9635	13.724	trans-phytol	$C_{20}H_{40}O$	296.5 g/mol	0.79	0.56	Defence signalling & stress responses, abiotic stress tolerance, antimicrobial & herbivore deterrent	(24)
5	Tetradecanoic acid	11.5749	11.5747	Myristic acid	$C_{14}H_{28}O_2$	228.37 g/mol	2.77	3.28	Defence against pathogens, signalling and stress responses, membrane lipid composition & stability	(25)
6	Hexadecanoic acid, methyl ester	12.0526	12.7634	Methyl palmitate	$C_{17}H_{34}O_2$	270.5 g/mol	0.37	1.57	Plant defence & antimicrobial activity, herbivore deterrence & indirect defence, cuticular wax component, signalling & stress responses	(26)

Table 2. Additional metabolite compounds identified in Pink Pitchi (PP-LBR-2) disease free mutant

S. No	RT	Metabolites	Common name	Molecular formula	Molecular weight	Area (%)	Functions	Reference
1	4.5869	Benzaldehyde	Benzoic aldehyde	C_6H_5CHO	106.12 g/mol	9.31	pollinator attractant and antifungal compound	(27)
2	4.7091	1,3-Propanediol	Trimethylene glycol	$C_3H_8O_2$	76.09 g/mol	7.42	Upregulate specific resistance genes and priming defence pathways against pathogens	(28)
3	7.1088	5-Hydroxymethylfurfural	5-Hydroxymethyl-2-furaldehyde	$C_6H_6O_3$	126.11 g/mol	10.18	Stress response biomarker, defence signalling molecule, growth modulator, metabolic intermediate	(29)
4	8.7753	Quinoline, 2-methyl-	Chinaldine	$C_{10}H_9N$	143.18 g/mol	9.84	anti-inflammatory and antimicrobial effects	(30)
5	9.6862	Cyclohexanone, 2,2-dimethyl-5-(3-methyloxiranyl) [2. alpha. (R*),3. alpha.]- (. +-.)-	2,2-Dimethyl-5-(3-methyl-2-oxiranyl) cyclohexanone #	$C_{11}H_{18}O_2$	182.26 g/mol	13.88	Defence & allelopathy, precursor for phytohormones, stress signalling	(31)
6	10.4084	d-Mannose	D-Mannopyranose	$C_6H_{12}O_6$	180.16 g/mol	22.42	Stress response, defence mechanisms, antioxidant and anti-inflammatory properties	(32)
7	10.7083	Ethyl. alpha. -d-glucopyranoside	Ethyl hexopyranoside #	$C_8H_{16}O_6$	208.21 g/mol	100.00	Carbohydrate storage & transport, osmo protection, defence signalling	(33)
8	14.1190	9,12,15-Octadecatrienoic acid, (Z, Z, Z)-	linolenic acid, alpha-Linolenic acid	$C_{18}H_{30}O_2$	278.4 g/mol	12.80	Jasmonic acid (JA) biosynthesis, stress responses, seed development	(34)
9	14.3079	9,12,15-Octadecatrienoic acid, ethyl ester, (Z, Z, Z)-	Ethyl linolenate, Linolenic acid ethyl ester	$C_{20}H_{34}O_2$	306.5 g/mol	19.56	Lipid storage & mobilization, stress protection, precursor for signalling molecules	(35)
10	17.8185	Squalene	Spinacene, trans-squalene	$C_{30}H_{50}$	410.7 g/mol	14.57	Defence against oxidative stress, cuticular wax & surface protection, drought resilience, higher photosynthetic efficiency	(23)
11	11.8304	(1,5,5,8-Tetramethyl-bicyclo [4.2.1] non-9-yl)-acetic acid	(1,5,5,8-Tetramethyl-bicyclo [4.2.1] non-9-yl)-acetic acid	$C_{15}H_{26}O_2$	238.37 g/mol	18.85	Defence mechanisms, signalling, secondary metabolism, increased resistance, Jasmonate pathway activation.	(36)

Table 3. Metabolite compounds identified in the leaf extracts of Pink Pitchi (PP-LBR-3) Diseased mutant

S.NO	RT	METABOLITES	COMMON NAME	MOLECULAR FORMULA	MOLECULAR WEIGHT	AREA (%)	FUNCTIONS	REFERENCE
1	6.3531	4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-	3-Hydroxy-2,3-dihydromaltol	$C_6H_8O_4$	144.12 g/mol	17.17	Flavour and aroma contribution, antioxidant properties, potential antimicrobial effects	(37)
2	7.1197	5-Hydroxymethylfurfural	5-(hydroxymethyl) furan-2-carbaldehyde	$C_6H_6O_3$	126.11 g/mol	39.60	Stress response, moderate antioxidant, potential allelochemical	(29)
3	8.7861	Quinoline, 2-methyl-	Khinaldin, Chinaldine	$C_{10}H_9N$	143.18 g/mol	11.02	Antimicrobial properties, UV protection & signalling	(38)
4	9.1638	d-Mannose	Carubinose, Seminose	$C_6H_{12}O_6$	180.16 g/mol	19.54	Cell wall biosynthesis, energy & metabolism, stress response, defence mechanisms, antioxidant and anti-inflammatory properties	(32)
5	9.3083	Guanosine	Vernine, Guanozin	$C_{10}H_{13}N_5O_5$	283.24 g/mol	68.73	Nucleotide metabolism, RNA synthesis, energy & signalling, growth & development	(39)
6	9.6971	Cyclohexanone, 2,2-dimethyl-5-(3-methyloxiranyl)-, [2. alpha. (R*),3. alpha.]- (. +-.)-	Cyclohexanone	$C_{11}H_{18}O_2$	182.26 g/mol	24.23	Defence & allelopathy, precursor for phytohormones, stress signalling	(31)
7	10.4081	Methyl beta. -D-glucopyranoside	Methyl beta-D-galactoside	$C_7H_{14}O_6$	194.18 g/mol	26.71	Carbohydrate storage & transport, Stress response, metabolic intermediate	(40)
8	10.5415	2-(Hydroxyimino) propanol oxime	Methyl glyoxime	$C_3H_6N_2O_2$	102.09 g/mol	22.92	Nitrogen metabolism & detoxification, defence against herbivores & pathogens, phytohormone biosynthesis, abiotic stress response	(41)
9	10.7192	Ethyl alpha -d-glucopyranoside	Ethyl hexopyranoside	$C_8H_{16}O_6$	208.21 g/mol	100.00	Carbohydrate storage & transport, stress metabolite, fermentation byproduct	(42)
10	10.9192	2-Butanone	Dihydro-beta-ionone	$C_{13}H_{22}O$	194.31 g/mol	20.04	Attraction of pollinators/predators, stress response, microbial interactions	(43)
11	12.9745	n-Hexadecanoic acid	palmitic acid	$C_{16}H_{32}O_2$	256.42 g/mol	13.16	Signalling & stress responses	(8)
12	14.1188	9,12,15-Octadecatrienoic acid, (Z, Z, Z)	linolenic acid alpha-Linolenic acid	$C_{18}H_{30}O_2$	278.4 g/mol	24.27	Lipid storage & mobilization, stress protection, precursor for signalling molecules	(35)
13	17.8183	Squalene	Spinacene	$C_{30}H_{50}$	410.7 g/mol	72.74	Phytosterol biosynthesis, defence against oxidative stress, cuticular wax & surface protection, drought resilience, higher photosynthetic efficiency	(23)

which is said to have antioxidant property because it could capture oxygen singlets during autohydrolytic reaction pathways and oxidation products (9). Phytol has antiquorum-sensing and antibacterial properties and it is the precursor of tocopherols (vitamin E) which is a diterpene alcohol (10). Tetradeconic acid shows excellent antioxidant activity and hence it can be used in agriculture (11).

The compounds present in PP-LBR-2 (disease free) include Benzaldehyde which is said to have insecticidal activity against *G. mellonella* and possesses antimicrobial, antioxidant activities (12). 5-Hydroxymethylfurfural with antioxidant property (13); quinoline with antifungal properties in plants (14); cyclohexanone with antifungal activity against certain phytopathogenic fungi in plants that are used majorly in agrochemicals (15); D-Mannose with antioxidant defence and also involved in oxidative process in wheat coleoptiles (16); Ethyl α-d-glucopyranoside found in plants like *Rumex hastatus* D and *Kazakhstan Sedum ewersii Lebed* possessing antioxidant properties and is likely to be used in food, human health and cosmetics (17).

The compounds present in PP-LBR-3 (diseased) contain various molecules. A study on *Poa annua* revealed that Heptadecane present in oil of *Onopodium acanthium* majorly inhibited root elongation when present in sufficient amounts in the plant environment (18). 8-Hydroxyquinoline (8-HQ) is a quinoline derivative and is said to inhibit root growth of *Brassica campestris* (mustard) and *Phalaris minor* (grass weed) (19). 1,2-Benzene dicarboxylic is said to reduce seed germination rates, inhibit root growth, alter the antioxidant enzyme activity and interfere in mineral nutrients uptake in watermelon (20). Malononitrile acts as a potent toxin causing rapid lethality due to its ability to release cyanide (HCN) in metabolic breakdown (21).

Conclusion

The metabolite profiling of the two mutant lines of *J. grandiflorum* has revealed significant differences in biochemical composition between PP-LBR-2 (disease free) and PP-LBR-3 (diseased). Particularly, PP-LBR-2 showed a higher abundance of bioactive metabolites such as trimethyl glycol, nonadecane, dodecanoic acid, octadecanoic acid and squalene which are proven to have antimicrobial, antioxidant and defence signalling properties. These compounds are likely to play a crucial role in plant defence to pathogens and stress. On the other hand, the diseased mutant PP-LBR-3 showed increased level of the compounds heptadecane, ethanamine, quinoline, 1,2- Benzene dicarboxylic, malononitrile which are associated with oxidative stress, disruption of signalling and increased susceptibility to pathogens. This study highlights the potential of the disease-free induced mutants as a valuable source of phytochemicals for therapeutic and agricultural applications. Additionally, this work focuses on the functional analysis of these metabolites which will further elucidate their role in plant health and open avenues for their exploitation in disease management strategies.

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Authors' contributions

SMV has written the whole manuscript. GM guided in providing technical support to write the manuscript in a proper format and approved the final manuscript. TSP guided in providing technical support. GS and MLP guided to write the manuscript. All authors were involved in research work, literature review, compiled the research findings, drafted the manuscript, revised and finalized the article. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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