

Colchicine-induced *Jasminum sambac* Polyploids Secured Altered Metabolic Profile with Unique Antifungal Compounds

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Abstract

Cercospora leaf spot is one of the critical foliar fungal diseases of jasmine, which causes considerable yield losses. There are no cultivars or germplasm accessions that are resistant to this infection and hence fungicide usage is the mainstay of management of this disease, which poses a hazard to the environment. Polyploidization is one of the key breeding methods in jasmine as newly obtained polyploids may have increased resistance/tolerance to diseases owing to altered metabolite production. In the present study, seventeen putative polyploids were obtained from *Jasminum sambac* Cv. Ramanathapuram Gundumalli by treating with the polyploidizing agent, colchicine. Among them, two mutant plants (generated from the wild parent following treatment with 0.25% colchicine) exhibited no symptoms for *Cercospora* leaf spot, whereas the control had the highest mean PDI (63.65%). Non-targeted metabolite profiling experiment indicated that couple of antifungal compounds (such as 1H-1,2,4-Triazole-1-ethanol, α -butyl- α -(2,4-dichlorophenyl) and Dasycarpidan-1-methanol, acetate (ester)) were present only in the two newly evolved mutants of *J. sambac* and absent in wild parent. Molecular docking results have shown that the compound Dasycarpidan-1-methanol, acetate (ester) binds effectively with two fungal proteins 7EUS and 7EUT, which are actively involved in biosynthesis of cercosporin (which is a photoactivated toxin that uses reactive oxygen species (ROS) in host cells and damage cell components such as membranes, proteins and lipids). Thus, this study enabled the development of a natural and eco-friendly method of evolving and characterizing novel lines in jasmine, which would have potential applications in flower industry.

Introduction

Jasmine (*Jasminum sambac*) is one of the Indian plants domesticated from time immemorial for its elegant and fragrant flowers. Due to its popularity and strong demand for its loose flowers and floral decorations, jasmine is valued as a highly prized ornamental plant (Saripalle, 2016). The flowers are used for making garlands, for ceremonial offerings, hair adornment by women and production of “attar” and “concrete” which are used in the cosmetic and perfumery industry. Two hundred species of *Jasminum* have been reported to exist in the tropical and sub-tropical regions throughout the globe, of which, 40 are found in the Indian sub-continent (Green and Miller, 2009).

In India, Tamil Nadu state is the leading producer of *J. sambac* with an annual production of 128963.05 MT from the cultivated area of 12893.31 ha during the year 2021–2022 (Department of Horticulture and Plantation Crops, Government of Tamil Nadu). India is the largest producer of jasmine and the flower is exported to the countries like Singapore, the United Arab Emirates, and the United States

Commercial cultivation of *J. sambac* is largely affected by many pests and diseases and leaf spot disease caused by *Cercospora jasminicola* is one among them. There are no cultivars or germplasm accessions that are resistant to this disease and hence fungicide usage is the mainstay of management of this disease, which poses a hazard to the environment and hence it is essential to evolve a leaf spot-resistant variety. Improved cultivars of economically important ornamental crops have been developed earlier by artificial polyploidization. Ornamental traits such as leaf size and thickness (Dunn and Lindstrom, 2007), inflorescence size and number (Allum et al., 2007; Kermani et al., 2003), internode length and plant height (Oates et al., 2012) have all been reported to be influenced by polyploidization. It has been reported that newly formed polyploid lineages are more pathogen-resistant than their progenitors (Levin, 1983). It has been found that many characteristics of polyploids' morphology and/or developments are associated to differences in their resistance/tolerance to biotic stress. Artificial polyploidization can alter a plant's anatomy and physiology to make it more resilient to biotic and abiotic challenges, which could help the plant growth and improve its net metabolite production (Tossi et al., 2022). For instance, triploid citrus has shown more tolerance to natural chilling temperatures when compared to diploids and parental cultivars. Triploids were observed to possess higher amounts of E-cimene and linalool, which might assist them adapt more readily to cold stress and better photosynthetic abilities under low natural temperatures (Lourkisti et al., 2020).

During polyploidization, the genotype remains stable while the genetic material is multiplied in autopolyploids due to direct genomic duplication. Reduced membrane to chromatin ratio caused by induced polyploidy results in more chromatin in contact with the nuclear membrane and more gene activity in each cell. This leads to increased activity of previously weakly expressed genes in a target biosynthetic pathway and enhanced active metabolite production (Madani et al., 2021). Remarkably, an altered chemical profile of the metabolic products is often encountered (Levin, 2002).

Metabolic differences produced by polyploidization can have profound effects for the development and ecological interactions of plant neopolyploids such as altered resistance/ tolerance mechanism to biotic and abiotic stress (Vergara et al., 2016). Alkaloids, terpenes, saponins and other classes of secondary metabolites may confer resistance to fungal pathogens (Levin, 1983). Hence, attempts were made in the present study using the polyploidizing agent colchicine to induce desirable traits particularly disease resistance. in *J. sambac*.

Materials and Methods

Treatments with polyploidizing agents

Uniform sized rooted cuttings of *J. sambac* Cv. Ramanathapuram Gundumalli kept under 50% shade net were used for the study. Colchicine solutions of 0.25, 0.50, 0.75, and 1.00 per cent concentration were used for inducing polyploidy. The plants were taken from polybags and washed with running tap water to remove the adhering soil particles and other impurities. The plants were subjected to complete submergence in colchicine solution for six hours in the laboratory and then taken out, washed with running tap water thoroughly and planted in poly bags with growing media composed of red soil: FYM: sand (1:1:1 ratio) and placed in shade-net house. The experiment trial was laid out in non-replicated design comprising of 25 plants in all the treatments including control (wild parent).

Screening for resistance against *Cercospora* leaf spot under natural conditions

After five months of planting, *Cercospora* leaf spot symptoms (circular to irregular reddish-brown spot of 2-8mm diameter on the upper surface of the leaves) were observed in both wild parent and treated plants (Fig. 1a). The plants were examined and scored for disease severity with 0–5 scale as described by (Kumar et al., 2011). Scoring for disease severity was done by following a 0–5 scale as detailed below.

Score	Symptoms
0	No disease symptoms
1	A few spots towards tip covering 10 per cent leaf area
2	Several dark brown patches covering up to 20 per cent leaf area
3	Several patches with paler outer zone covering up to 40 per cent leaf area
4	Leaf spot covering up to 75 per cent leaf area or breaking of the leaves from centre
5	Complete drying of the leaves or breaking of the leaves from centre

Per cent disease index was calculated using the following formula (Wheeler, 1969):

$$PDI = \left[\frac{\text{Sum of numerical ratings}}{\text{Total number of leaves examined}} \right] \times \left[\frac{100}{\text{Maximum grade}} \right].$$

Selection and isolation of mutants

Among the putative polyploids, two mutants derived from 0.25% colchicine treatment of wild parent were observed to be free from *Cercospora* leaf spot symptoms (Fig. 1b and 1c). These two mutants were selected, labelled as RG-CLSR-1 and RG-CLSR-2 (Ramanathapuram Gundumalli- *Cercospora* Leaf Spot Resistant) and subjected to laboratory analysis.

Extraction of secondary metabolites from wild parent and mutants

Secondary metabolites were extracted from the leaves and stems of wild parent and the two mutants, RG-CLSR-1 and RG-CLSR-2. Five grams of leaves along with stem were collected, cleaned and ground into fine powder using liquid nitrogen. The powder was transferred to conical flasks and added with equal volume of ethyl acetate. The mixture was stirred at 100 rpm in an orbital

shaker for 96 hours at 28 °C and filtered through Whatman No. 3 filter paper. The filtrate was concentrated in a rotary flash evaporator at 55 °C at 80 rpm. The concentrated extract was dissolved in 1 ml HPLC grade methanol and filtered through PVDF hydrophilic membrane (0.22 mm pore size, Himedia) and used for GC/MS analysis.

Gas Chromatography and Mass Spectrometry

The volatile compounds of leaf and stem samples of parent and mutants were analysed through GC-MS customized with Thermal Desorber turbomatrix 150 (Perkin Elmer, USA). The GC conditions were 10:1 split, helium carrier at 20 psi, and oven temperature from 50 to 250 °C at 10 °C per min. The MS conditions were positive ion mode, and electron impact spectra at 70 eV and the analyses were done using DB-5 MS capillary non-polar column was used whose dimensions were 0.25mm OD x 0.25µm ID x 30 meters length. The MS was set to scan from 50 to 550 Da. The GC instrument vaporizes the sample and then separates the various components for analysis. Each component was ideally produced a specific spectral peak that was recorded on a paper chart electronically. The detected compounds were identified by mass spectral database (NIST 2014). Only ten metabolites pertinent to plant defence mechanism which were selected based on earlier scientific reports were investigated in this study.

Molecular Docking

Molecular docking with two protein targets, 7EUS (2-oxoglutarate dependent dioxygenase CTB9) and 7EUT (2-oxoglutarate dependent dioxygenase CTB9) (which are actively involved in biosynthesis of cercosporin, a photoactivated toxin that uses reactive oxygen species (ROS) of host cells and damage cell components such as membranes, proteins, lipids, etc) (Liu et al., 2022) was done using AutoDock vina module of PyRx 0.8 (Dallakyan and Olson, 2015). Target proteins were uploaded and converted to macromolecules by using the “make macromolecule” option in the PyRx program. The Computed Atlas of Surface Topography of Proteins (CASTp) was used to identify the binding regions of protein targets (Tian et al., 2018). Besides, the grid configuration and docking were done using parameter files about AutoDock4 and Autogrid4. Further, visualization of the docked complexes was done using BIOVIA Discovery Studio and docking scores were calculated and identified the least energy (as it helped to indicate the effective binding/interaction of ligand and target).

Results

Disease incidence/index is the number or proportions of plant parts that are diseased. In the present study, two mutant plants generated from the wild parent following treatment with 0.25% colchicine exhibited no symptom for *Cercospora* leaf spot, whereas the control (wild parent) had the highest mean PDI (63.65%).

Metabolic profiling is the measurement of low molecular weight metabolites and their intermediates in biological systems that reflects the dynamic response to genetic modification and physiological, pathophysiological, and/or developmental stimuli. The quantification and interpretation of the endogenous metabolite profile from a biological sample (biological tissue extract) provide many prospects to explore the variations induced by external stimuli (Clarke and Haselden, 2008). Metabolites were extracted from the leaves and stems of wild parent and mutants RG-CLSR-1 and RG-CLSR-2, and analysed through GC-MS. Results of the non-targeted metabolite profiling through GC-MS analysis are presented in Table 1. The respective chromatogram images of the GC-MS analysis are presented in the Fig. 2. The volatile organic compounds in leaves and stems of wild parent detected through GC-MS analysis are 2- propenoic acid, 3-(3- hydroxy-2,6,6-trimethyl- 1- cyclohexen- 1-yl), methyl ester; n- hexadecanoic acid; oleic acid, phenol and benzoic acid, 4-formyl-, methyl ester. The volatile organic compounds in the leaves and stems of the mutant RG-CLSR-1 are phenol; benzoic acid, 4- formyl-, methyl ester; 2- propenoic acid, 3-(3- hydroxy-2,6,6-trimethyl-1- cyclohexen- 1-yl), methyl ester; oleic acid; L- ascorbic acid, 6-octa decanoate; 2- nonadecanone 2,4- dinitrophenylhydrazine; n-hexadecanoic acid; 1- docosene; dasycarpidan-1-methanol, acetate (ester) and 1H-1,2,4-triazole-1-ethanol, α-butyl-α-(2,4-dichlorophenyl). The volatile organic compounds present in leaves and stems of RG-CLSR-2 are phenol; benzoic acid, 4- formyl-, methyl ester; 2- propenoic acid, 3-(3- hydroxy-2,6,6-trimethyl-1- cyclohexen- 1-yl), methyl ester; oleic acid; 1H-1,2,4-triazole-1-ethanol, α-butyl-α-(2,4-dichlorophenyl); dasycarpidan-1-methanol, acetate (ester); n-hexadecanoic acid and 1-docosene .

Table 1

Selected bioactive compounds with plant defence properties identified through GC-MS analysis in the leaves and stems of parent and mutants

S. No.	Name of the compound	Peak Area %						Nature of the compound	Property
		Wild Parent		RG-CLSR-1		RG-CLSR-2			
		Leaves	Stems	Leaves	Stems	Leaves	Stems		
1	Phenol	0.676	1.323	1.310	2.512	2.164	0.896	Carbolic acid	Pre infection inhibitors
2	Benzoic acid, 4-formyl-, methyl ester	1.736	3.882	3.131	7.094	2.904	2.741	Acid ester	Fungistatic compound
3	Oleic acid	3.270	3.524	38.863	0	0	24.237	Fatty acid	Antifungal, antioxidant,
4	2- Propenoic acid, 3-(3-hydroxy-2,6,6-Trimethyl- 1-cyclohexen- 1-yl)-, Methyl ester	1.116	0	1.604	0	1.970	1.381	Methyl ester	Antioxidant
5	n- hexadecenoic acid	1.057	0.356	0	3.876	0	4.821	Palmitic acid	Antifungal, antioxidant and antimicrobial
6	L- Ascorbic acid, 6-Octa decanoate	0	0	11.203	0	0	0	Vitamin C monostearate	Antioxidant
7	2- Nonadecanone 2,4-dinitrophenylhydrazine	0	0	2.169	0	0	0	Hydrazine	Antimicrobial
8	1-Docosene	0	0.607	0	2.809	0	5.205	Alkene	Antioxidant
9	1H-1,2,4-Triazole-1-ethanol, α -butyl- α -(2,4-dichlorophenyl)	0	0	0.187	4.332	2.645	12.888	Heterocyclic Azoles	Antifungal
10	Dasycarpidan-1-methanol, acetate (ester)	0	0	0.318	0	1.236	0	Alkaloid	Antifungal

Docking study was carried out for the bioactive compounds identified from the GC–MS analysis of mutants RG-CLSR-1 and RG-CLSR-2, against two fungal proteins (7EUS and 7EUT). 7EUS (2-oxoglutarate dependent dioxygenase CTB9) and 7EUT (2-oxoglutarate dependent dioxygenase CTB9 in complex with N-oxalylglycine). The results of the molecular docking studies are presented in Table 2 and Figs. 3 & 4. The binding score, amino acids involved in the conventional H bond, alkyl, and pi-alkyl and other forms of interactions were noted. Among the investigated putative *Cercospora* leaf spot resistant metabolites, Dasycarpidan-1-methanol, acetate (ester) interacted well with 7EUS and showed a binding score of – 7.6 K Cal/mol. Conventional H bonds were formed with GOL:402; alkyl and pi-alkyl bonds were formed with the amino acids VAL:236 and MSE:238 of 7EUS. The same compound when docked with the protein 7EUT showed a binding affinity of – 7.4 K Cal/mol and formed conventional H bonds with HIS:234.

Table 2
Molecular docking studies of the bioactive compounds against two fungal proteins

Ligand	Docking details	7EUS	7EUT
Phenol	Binding score	-4.9	-5.8
	Conventional H bond	THR: 212	ASP:302
	Alkyl and pi-alkyl	PRO:215	PRO:215
Benzoic acid, 4-formyl-, methyl ester	Binding score	-5.3	-5.4
	Conventional H bond	GLN:176, ARG:45	ASN: 203, HIS:234, ASN:248
	Alkyl and pi-alkyl	PRO:48	LEU:138
Oleic acid	Binding score	-4.8	-5.1
	Conventional H bond	ARG:45, THR:232, GLU:54	ARG:140, GLN:163
	Alkyl and pi-alkyl	LEU:233,	LEU:138, ARG:313, VAL:236
2- Propenoic acid, 3-(3- hydroxy-2,6,6-Trimethyl- 1- cyclohexen- 1-yl)-, Methyl ester	Binding score	-6.1	-6.5
	Conventional H bond	ARG:45,	EDO:403, GLU:311
	Alkyl and pi-alkyl	-	-
n-hexadecenoic acid	Binding score	-5	-5.9
	Conventional H bond	TRP:199, GLU:195	-
	Alkyl and pi-alkyl	PRO:48, VAL:179, PHE:281	LEU:138, VAL:236
L- Ascorbic acid, 6-Octa decanoate	Binding score	-6.3	-6.6
	Conventional H bond	GLU:54, GLN:176, ASN:231	ARG: 313, GLN:173, EDO:403, HIS:234, ASN:248
	Alkyl and pi-alkyl	PRO:48, VAL:179	LEU:138, VAL:236
2- Nonadecanone 2,4- dinitrophenylhydrazine	Binding score	-5.6	-4.9
	Conventional H bond	HIS:139	ASN:248, HIS:234, ASN:203
	Alkyl and pi-alkyl	ARG:313, HIS:234, MSE:238	TYR:107, VAL:236, LEU:138
1-Docosene	Binding score	-4.8	-4.1

Ligand	Docking details	7EUS	7EUT
	Conventional H bond	-	-
	Alkyl and pi-alkyl	LEU:233, PHE:230, ARG:180, VAL:179, PRO:48, ARG:49	VAL:236, MET: 238, LEU: 138, HIS:234
1H-1,2,4-Triazole-1-ethanol, α -butyl- α -(2,4-dichlorophenyl)	Binding score	-6.1	-4.8
	Conventional H bond	-	GLU:311, ARG:313
	Alkyl and pi-alkyl	PHE:230	VAL:236, HIS:234
Dasycarpidan-1-methanol, acetate (ester)	Binding score	-7.6	-7.4
	Conventional H bond	GOL:402	HIS:234
	Alkyl and pi-alkyl	VAL:236, MSE:238	-

Discussion

The results obtained from per cent disease incidence are in accordance with earlier reports suggested that neopolyploids may exhibit greater disease resistance than their parents, initially (Oswald and Nuismer, 2007). Altered pathogen resistance is induced by changes in disease resistance genes between polyploids and diploids (Innes et al., 2008). Comparisons between diploids and tetraploids of several crops shown higher resistance of polyploids to fungal pathogens (Burdon and Marshall, 1981; Kulkarni and Ravindra, 1988; Vestad, 1960).

Putative mechanism of resistance in RG-CLSR-1 and RG-CLSR-2

When plant tissues are exposed to stress as a consequence of pathogen attack, phenols are frequently generated and deposited in the subepidermal layers of the tissues. When plant pattern recognition receptors sense potential pathogens by conserved pathogen-associated molecular patterns (PAMPs), phenols are produced leading to PAMP-triggered immunity (Cavaco et al., 2021). As a result, the infection does not spread as quickly before the disease completely takes over the plant (Bhattacharya et al., 2010). Benzoic acid, 4-formyl, methyl ester is a phytoalexin produced in plants during biotic stress and it has antifungal properties (Farooq and Ngaini, 2018; Franich et al., 1986). An oleic acid is a fatty acid produced in plants and it induces constitutive defence signalling during stress and enhances resistance to multiple pathogens (Kachroo et al., 2008) and (Walters et al., 2004). Hexadecanoic acid, commonly termed as palmitate found in plants, has been reported for its antifungal property (Chandrasekaran et al., 2011).

The above-mentioned compounds *viz.*, phenol; benzoic acid, 4-formyl, methyl ester; oleic acid and n-hexadecenoic acid were found both in wild parent and the newly identified mutants (RG-CLSR-1 and RG-CLSR-2) (Table 1). However, these compounds were present in higher levels in the mutants than the wild parent as evident from the peaks in the chromatogram. The peak area is proportional to the amount of the components present in the analyte (Douglas, 2004) and hence it is speculated that presence of these compounds in significant levels might have helped in providing resistance mechanism(s) in the mutants RG-CLSR-1 and RG-CLSR-2.

Among the compounds that were detected only in the mutants RG-CLSR-1 and RG-CLSR-2 but were absent in the wild parent, there were few potential antifungal compounds namely 1H-1,2,4-triazole-1-ethanol, α -butyl- α -(2,4-dichlorophenyl) (Kazeminejad et al., 2022) and dasycarpidan-1-methanol, acetate (ester) (Al-Rubaye et al., 2017; Moni et al., 2021). Hence, it is suggested that

these antifungal compounds might be responsible for conferring specific resistance/tolerance to *Cercospora* leaf spot in the mutants RG-CLSR-1 and RG-CLSR-2.

Thus, the GC-MS analysis in the present study led to identification of several antioxidant compounds in the mutant RG-CLSR-1. The compounds namely L- ascorbic acid, 6-octa decanoate (Bilska et al., 2019), 1-docosene (Cheng et al., 2015) and 2-nonadecanone 2,4- dinitrophenylhydrazine (Muthulakshmi and Mohan, 2012) have been reported for their antioxidant/ antimicrobial activity in plants. Similarly, in the mutant RG-CLSR-2, the antioxidant compounds viz., 2- propenoic acid, 3-(3-hydroxy-2,6,6-trimethyl-1- cyclohexen- 1-yl), methyl ester (Mujeeb et al., 2014) ; n-hexadecanoic acid (Sermakkani and Thangapandian, 2012) and 1-docosene (Cheng et al., 2015) were identified.

Biotic stress causes many physiological and biochemical changes in the plant due to the production and accumulation of many cytotoxic reactive oxygen species (ROS) in the plant cell (oxidative stress) which makes the plant susceptible to apoptosis. The coordinated action of the various antioxidant compounds present in the two mutants might have contributed to the ROS quenching mechanism facilitating detoxification of the toxic products produced by the fungal pathogen, ultimately leading to. an effective antioxidative defense system against the fungal attack.

Cercospora is an ubiquitous and invasive genus of Ascomycetous fungi characterized by production of the phytotoxin cercosporin. Cercosporin is a red-pigmented perylenequinone product and it is the most predominant virulence factor of *Cercospora*, as it plays a key role in its pathogenicity. Cercosporin being a photosensitizer, can absorb light energy and transfer it to other molecules, leading to the production of highly reactive oxygen species (ROS) (Mander and Liu, 2010). This leads to peroxidation of lipids in the plant cell membranes. The subsequent membrane damage allows for leakage of nutrients into the intercellular spaces, that are used by the fungus to colonize the leaf, leading to necrosis and eventual cell death. The production of cercosporin by *Cercospora* is supposed to be a mechanism by which the organism can overcome the plant's natural defenses and establish infection (Newman and Townsend, 2016). Catalase deoxygenase (*CTB*) are the cluster of genes involved in the biosynthesis of cercosporin. *CTB* catalyses the conversion of the molecule deoxyerythrose-4-phosphate (DEP) to erythrose-4-phosphate (E4P) (an essential precursor for the synthesis of the polyketide backbone of cercosporin) in the pentose phosphate pathway, which is a critical step in the production of aromatic amino acids and secondary metabolites like cercosporin. Therefore, *CTB* is essential for cercosporin production, and mutants lacking *CTB* are unable to produce the pigment (Daub and Chung, 2007).

In the present study, the output of the molecular docking simulation predicted the ligand-protein complexes and their binding scores and it could be inferred that Dasycarpidan-1-methanol, acetate (ester) binds to target protein 7EUS and 7EUT (*CTB*) with high affinity. The compound Dasycarpidan-1-methanol, acetate (ester) present in the mutants (RG-CLSR-1 AND RG-CLSR-2) may inhibit the activity of the target proteins (*CTB*) by binding to their active sites and preventing them from carrying out their normal functions such as biosynthesis of cercosporin or by affecting the protein structure which may alter its activity or stability. This may lead to the disruption of the *Cercospora* fungal mechanism against its host and ultimately reduces pathogenicity in the plants. The results of the present study are in accordance with earlier findings reported in tobacco (Daub and Chung, 2007) wherein, the gene (*CTB1*) encoding the polyketide synthase responsible for cercosporin production in *C. nicotianae* was disrupted and consequently the mutants produced no cercosporin. When inoculated onto tobacco, the *ctb1* mutants produced fewer lesions and did not cause the necrotic blighting symptom characteristic of wild type infection.

Using methods like X-ray crystallography or NMR spectroscopy, experimental validation of the predicted binding affinity and antifungal activity of the newly identified compounds is necessary to prove their effectiveness as antifungal drugs. For more precise predictions of ligand-protein interactions, molecular docking should also be used in conjunction with other techniques such as molecular dynamics simulations and free energy calculations.

Conclusion

Cercosporin is essential for the pathogenicity of *Cercospora* sp. in a variety of crop species including *Jasminum* spp. Therefore, any strategy for reducing cercosporin toxicity is likely to be useful for controlling *Cercospora* leaf spot diseases in the field. The present study reveals that polyploidization in jasmine enhances the crop's fungal pathogen resistance through altered metabolite

production. *Cercospora* leaf spot resistant mutants can be exploited as a genetic stock for further breeding programmes to evolve *Cercospora* leaf spot resistant genotypes. Possibly, the identified novel compound Dasycarpidan-1-methanol, acetate (ester) requires to be studied in detail with respect to its biosynthetic pathway in the plant biological system and may be used to design a potential antifungal drug.

Declarations

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Competing Interest

The authors have no relevant financial or non-financial interests to disclose.

Authors contributions

All the authors have made significant contribution to the present study.

- Execution of research, data collection and analysis were performed by Vishnupandi S.
- Ganga M, Rajamani K, Manonmani S and Kannan R helped in execution of research.
- Manikanda Boopathi N supported in characterization of the mutant and drafting the manuscript.
- Suhail Ashraf assisted in molecular docking.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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References

1. Allum JF, Bringloe DH, Roberts AV (2007) Chromosome doubling in a *Rosa rugosa* Thunb. hybrid by exposure of in vitro nodes to oryzalin: the effects of node length, oryzalin concentration and exposure time. *Plant Cell Rep* 26:1977–1984
2. Al-Rubaye A, Fauzi AF, Kaizal, Imad Hadi Hameed (2017) Phytochemical screening of methanolic leaves extract of *Malva sylvestris*. *Int J Pharmacognosy Phytochemical Res* 9:537–552
3. Bhattacharya A, Sood P, Citovsky V (2010) The roles of plant phenolics in defence and communication during *Agrobacterium* and *Rhizobium* infection. *Mol Plant Pathol* 11:705–719
4. Biliska K, Wojciechowska N, Alipour S, Ewa Marzena Kalembe (2019) Ascorbic acid—The little-known antioxidant in woody plants. *Antioxidants* 8:645
5. Burdon JJ, DR Marshall (1981) Inter-and intra-specific diversity in the disease-response of *Glycine* species to the leaf-rust fungus *Phakopsora pachyrhizi*. *The Journal of Ecology*:381–390
6. Cavaco A, Rita, Ana Rita Matos and Andreia Figueiredo (2021) Speaking the language of lipids: the cross-talk between plants and pathogens in defence and disease. *Cell Mol Life Sci* 78:4399–4415
7. Chandrasekaran M, Senthilkumar A, V Venkatesalu (2011) Antibacterial and antifungal efficacy of fatty acid methyl esters from the leaves of *Sesuvium portulacastrum* L. *Eur Rev Med Pharmacol Sci* 15:775–780

8. Cheng M-C, Chang W-H, Chen C-W, Li W-W, Tseng C-Y, Song T-Y (2015) Antioxidant Properties of Essential Oil Extracted from *Pinus morrisonicola* Hay Needles by Supercritical Fluid and Identification of Possible Active Compounds by GC/MS. *Molecules* 20:19051–19065
9. Clarke CJ, John N, Haselden (2008) Metabolic profiling as a tool for understanding mechanisms of toxicity. *Toxicol Pathol* 36:140–147
10. Dallakyan S, Arthur J, Olson (2015) Small-molecule library screening by docking with PyRx. *Chemical biology: methods and protocols*:243–250
11. Daub ME, Kuang-Ren Chung (2007) Cercosporin: a photoactivated toxin in plant disease. *APSnet Feature*: 1–12
12. Department of Horticulture and Plantation Crops, Govt. of Tamil Nadu (2022) *Horticulture*. Available at: <https://tnhorticulture.tn.gov.in/statistics>
13. Douglas F (2004) Gc/ms analysis. *Scientific Testimony-An Online Journal*
14. Dunn BL, Jon T, Lindstrom (2007) Oryzalin-induced chromosome doubling in *Buddleja* to facilitate interspecific hybridization. *HortScience* 42:1326–1328
15. Farooq S, Zainab Ngaini (2018) Methyl-2-formyl benzoate: A Review of Synthesis and Applications. *Curr Organocatalysis* 5:205–209
16. Franich RA, Michael J, Carson, Susan D, Carson (1986) Synthesis and accumulation of benzoic acid in *Pinus radiata* needles in response to tissue injury by dothistromin, and correlation with resistance of *P. radiata* families to *Dothistroma pini*. *Physiological and Molecular Plant Pathology* 28: 267–286
17. Green P, Miller D (2009) The genus *Jasminum* in cultivation. Kew Publishing, Royal Botanic Gardens, Kew, pp 110–113
18. Innes RW, Carine Ameline-Torregrosa T, Ashfield E, Cannon SB, Cannon B, Chacko, Nicolas WG, Chen A, Couloux A, Dalwani, Roxanne Denny (2008) Differential accumulation of retroelements and diversification of NB-LRR disease resistance genes in duplicated regions following polyploidy in the ancestor of soybean. *Plant Physiol* 148:1740–1759
19. Kachroo A, Fu D-Q, Havens W, Navarre DuRoy, Kachroo P, Said A, Ghabrial (2008) An Oleic Acid–Mediated Pathway Induces Constitutive Defense Signaling and Enhanced Resistance to Multiple Pathogens in Soybean. *Mol Plant-Microbe Interactions®* 21:564–575. 10.1094/mpmi-21-5-0564
20. Kazeminejad Z, Marzi M, Shiroudi A, Kouhpayeh SA, Farjam M, Zarenezhad E (2022) Novel 1, 2, 4-Triazoles as Antifungal Agents. *Biomed Res Int* 2022: 4584846. doi: 10.1155/2022/4584846
21. Kermani MJ, Sarasan V, Roberts AV, Yokoya K, Wentworth J, Sieber VK (2003) Oryzalin-induced chromosome doubling in *Rosa* and its effect on plant morphology and pollen viability. *Theor Appl Genet* 107:1195–1200
22. Kulkarni RN, NS Ravindra (1988) Resistance to *Pythium aphanidermatum* in diploids and induced autotetraploids of *Catharanthus roseus*. *Planta Med* 54:356–359
23. Kumar GBS, Kamanna BC, Benagi VI (2011) Management of chrysanthemum leaf blight caused by *Alternaria alternata* (Fr.) Keissler under field condition. *Plant Archives* 11:553–555
24. Levin DA (1983) Polyploidy and novelty in flowering plants. *Am Nat* 122:1–25
25. Levin DA (2002) The role of chromosomal change in plant evolution. Oxford University Press, USA
26. Liu X, Yuan Z, Su H, Hou X, Deng Z, Xu H, Guo B, Yin D, Sheng X, Yijian Rao (2022) Molecular basis of the unusual seven-membered methylenedioxy bridge formation catalyzed by Fe (II)/ α -KG-dependent oxygenase CTB9. *ACS Catal* 12:3689–3699
27. Lourkisti R, Froelicher Y, Herbette S, Morillon R, Tomi F, Gibernau M, Giannettini J, Berti L (2020) Jérémie Santini Triploid citrus genotypes have a better tolerance to natural chilling conditions of photosynthetic capacities and specific leaf volatile organic compounds. *Frontiers in plant science* 11: 330
28. Madani H, Escrich A, Hosseini B, Sanchez-Muñoz R, Khojasteh A, Javier Palazon (2021) Effect of polyploidy induction on natural metabolite production in medicinal plants. *Biomolecules* 11:899
29. Mander L, Hung-Wen Liu (2010) *Comprehensive Natural Products II*. Elsevier Science

30. Moni SS, Alam MF, Sultan MH, Makeen HA, Alhazmi HA, Mohan S, Alam MdS, Ur Rehman Z, Jabeen A, Sanobar S (2021) Spectral analysis, in vitro cytotoxicity and antibacterial studies of bioactive principles from the leaves of *Conocarpus lancifolius*, a common tree of Jazan, Saudi Arabia. *Brazilian Journal of Biology* 83
31. Mujeeb F, Bajpai P, Pathak N (2014) Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of *Aegle marmelos*. *Biomed Res Int* 2014:497606. 10.1155/2014/497606
32. Muthulakshmi ARJM, VR Mohan (2012) GC-MS analysis of bioactive components of *Feronia elephantum Correa* (Rutaceae). *Journal of Applied Pharmaceutical Science*:69–74
33. Newman AG, Craig A, Townsend (2016) Molecular characterization of the cercosporin biosynthetic pathway in the fungal plant pathogen *Cercospora nicotianae*. *J Am Chem Soc* 138:4219–4228
34. Oates KM, Thomas G, Ranney, Darren H, Touchell (2012) Influence of induced polyploidy on fertility and morphology of *Rudbeckia* species and hybrids. *HortScience* 47:1217–1221
35. Saripalle M (2016) Jasmine cultivation in Tamil Nadu: Market structure and pricing. *World Dev Perspect* 1:12–14
36. Oswald BP, Scott L, Nuismer (2007) Neopolyploidy and pathogen resistance. *Proceedings of the Royal Society B: Biological Sciences* 274: 2393–2397
37. Sermakkani M, V Thangapandian (2012) GC-MS analysis of *Cassia italica* leaf methanol extract. *Asian J Pharm Clin Res* 5:90–94
38. Tian W, Chen C, Lei X, Zhao J, Liang J (2018) CASTp 3.0: computed atlas of surface topography of proteins. *Nucleic Acids Res* 46:W363–W367
39. Tossi VE, Leandro J, Martínez Tosar LE, Laino Jesica Iannicelli, José Javier Regalado, Alejandro Salvio Escandón, Irene Baroli, Humberto Fabio Causin, Sandra Irene Pitta-Álvarez (2022) Impact of polyploidy on plant tolerance to abiotic and biotic stresses. *Frontiers in Plant Science* 13. doi: 10.3389/fpls.2022.869423
40. Vergara F, Kikuchi J, Breuer C (2016) Artificial Autopolyploidization Modifies the Tricarboxylic Acid Cycle and GABA Shunt in *Arabidopsis thaliana* Col-0. *Sci Rep* 6:26515. 10.1038/srep26515
41. Vestad R (1960) The effect of induced autotetraploidy on resistance to clover rot (*Sclerotinia trifoliorum* Erikss.) in red clover. *Euphytica* 9:35–38
42. Walters D, Raynor L, Mitchell A, Walker R, Kerr Walker (2004) Antifungal activities of four fatty acids against plant pathogenic fungi. *Mycopathologia* 157:87–90
43. Wheeler BE (1969) John An introduction to plant diseases. An introduction to plant diseases

Figures



A



B



C

Figure 1

Cercospora leaf spot symptoms present in wild parent and absent in the mutants (RG-CLSR-1 and RG-CLSR-2) on 150 days after treatment with Colchicine

(a) Parent (b) Mutant RG-CLSR-1 (c) Mutant RG-CLSR-2

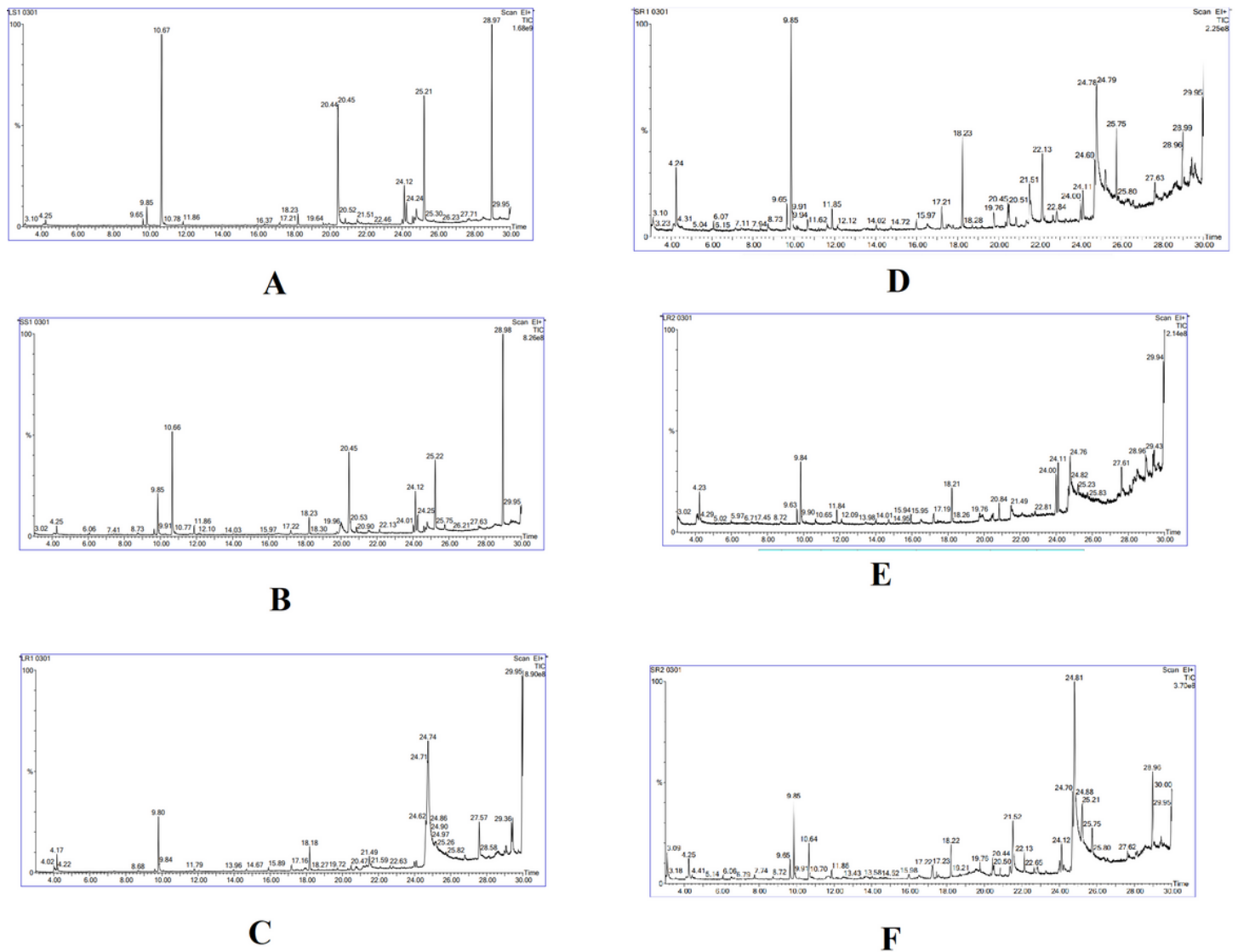


Figure 2

GC-MS total ion chromatogram of volatile organic compounds in leaves and stems of the wild parent and mutants

(a) Leaves of wild parent (b) Stems of wild parent (c) Leaves of Mutant RG-CLSR-1 (d) Stems of Mutant RG-CLSR-1 (e) Leaves of Mutant RG-CLSR-2 (f) Stems of Mutant RG-CLSR-2

7EUS 2-oxoglutarate (2-OG)-dependent dioxygenase AND
Dasycarpidan-1-methanol, acetate (ester)

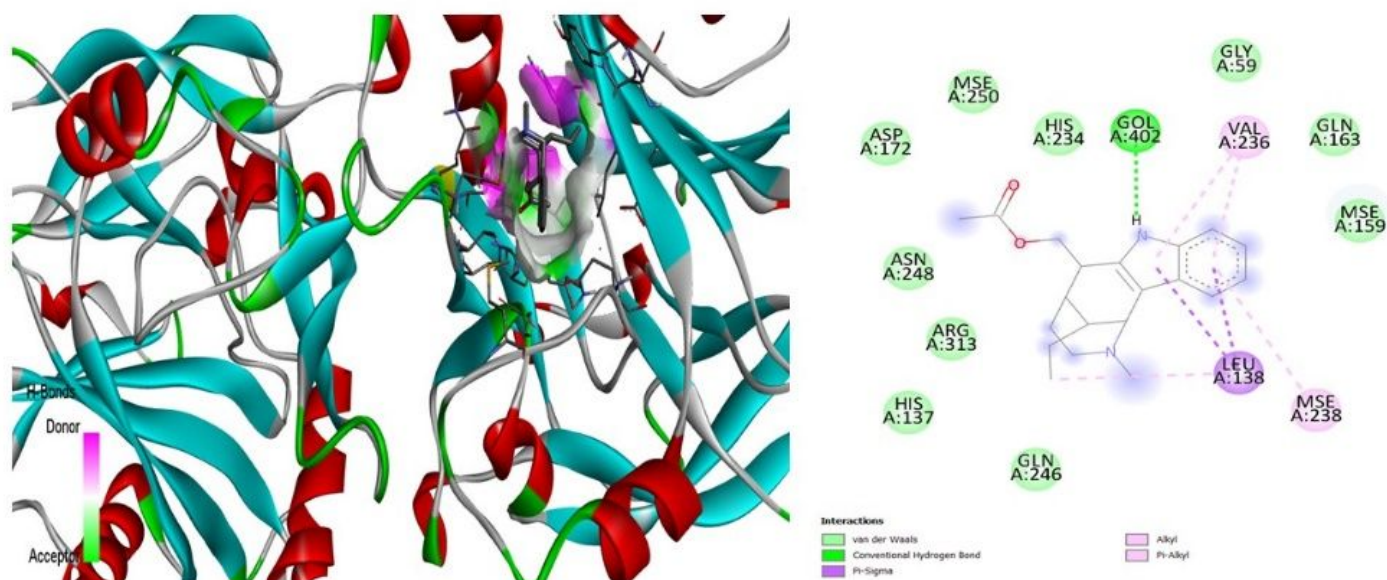


Figure 3

Binding of Dasycarpidan-1-methanol, acetate (ester) with 7EUS 2-oxoglutarate (2-OG)-dependent dioxygenase

7EUT 2-oxoglutarate (2-OG)-dependent dioxygenase AND
Dasycarpidan-1-methanol, acetate (ester)

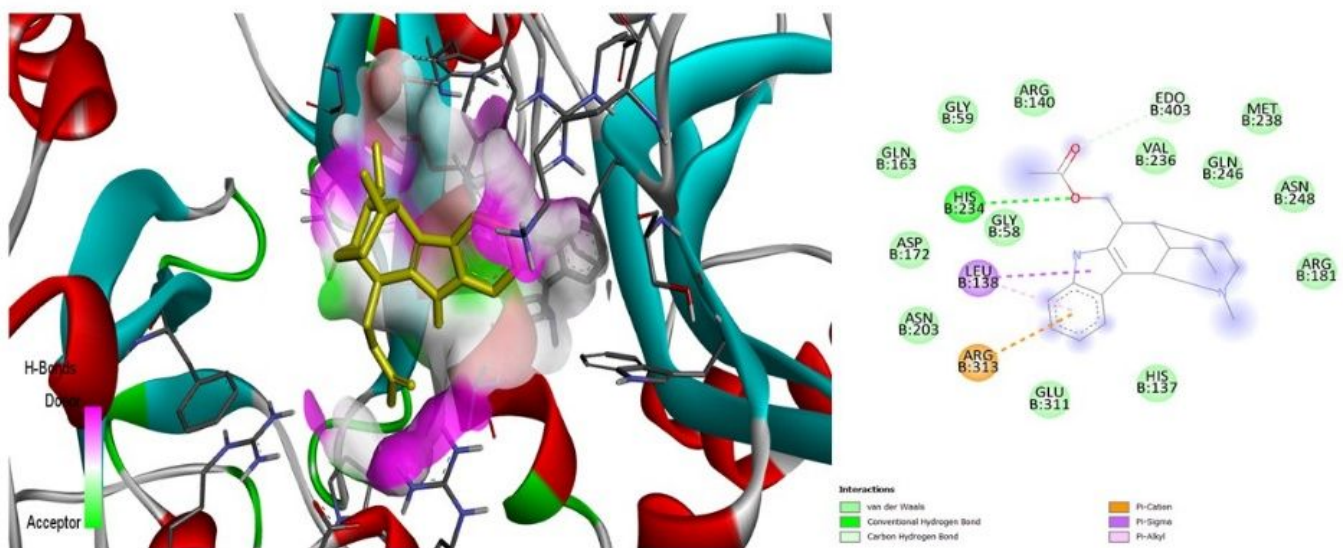


Figure 4

