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Phenotypic Characterization and Biochemical analysis in Mutant Genotypes of Pink Pitchi (*Jasminum grandiflorum,* L.)

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ABSTRACT: Jasmine *(Jasminum grandiflorum* L.) is one of the important flower crop commercially cultivated for their attractive fragrant flowers and is highly valued for its essential oil in India. Essential oil extracted from the fragrant flowers has great demand in the international market for perfumery industry. The aesthetic and economic value of Jasmine is determined by the shape, colour and size of the flower, aroma and flower yield. Since jasmine is normally propagated by asexual means, there exists only limited variability in this species. Creation of variability is the major strategy for varietal diversification and evolving novel varieties in Jasmine. The unique variability for various economic traits can be obtained by employing several methods such as hybridisation, induced mutations, somaclonal variations and combination of several other methods. In this study, the derived mutant genotypes of cultivated Pink Pitchi that are developed by inducing mutation through physical (Gamma) and chemical mutagens (EMS) were assessed for 35 phenotypic traits including vegetative and floral characters. Variations in vegetative and floral characters were recorded both in cultivated as well as in mutant genotypes and resulted in the identification of four promising mutant genotypes of Pink Pitchi for further utilization in jasmine improvement for developing elite varieties in jasmine. In addition, the selected mutant genotypes *viz.,* PPM-12, PPM-72, PPM-96, PPM-115 and non mutated Pink Pitchi genotypes were subjected to GCMS analysis for identifying the biochemical phytoconstituents and its respective biosynthetic pathways. The metabolite profiling resulted in the identification of various biochemical compounds such as Hexanoic acid, an aromatic hydrocarbon, ethyl ester and a saturated hydrocarbon Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S)- were found to be present only in Pink Pitchi whereas these compounds are absent in all the derived mutants of Pink Pitchi. Three compounds *viz.,* Boldione, an organooxygen compound, a organochloride compound Caryophyllene and a branched alkane Nonane, 4,5-dimethyl- were present only in the gamma ray derived mutants PPM-96 and PPM-115 whereas they are absent in pink Pitchi and EMS derived mutants. Toluenes, Benzenoids and Polycyclic hydrocarbons such as 1-(p-Tolyl)butan-1-one, 2-Heptanone and Bicyclo [7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-, [1R-1R*,4Z,9S*)]- were found to be present only in the two EMS derived mutants PPM-12 and PPM-72 respectively. The morphological characterization for various phenotypic traits and identification of various metabolites in the mutant accessions of *Jasminum* species will have potential applications in jasmine improvement programme. Identification of biochemical compounds may ultimately help in industrial utility to identify the perfect stage of the flowers for higher concrete recovery and for unique fragrance of jasmine.

Keywords: *Jasminum grandiflorum*, Pink pitchi mutants, phenotypic traits, GCMS profiling.

INTRODUCTION

Jasmines (*Jasminum sp.*) are commercially important flower crop cultivated for its attractive and fragrant flowers in the Southern and Eastern parts of India. A native of tropical and subtropical region, jasmine is esteemed for its attractive fragrant flowers and is highly valued for the essential oil. Indo-Malayan region being the centre of origin, the diversity existing in jasmine is enormous in India. The distribution of *Jasminum* genus is pan-tropical but a large number of species are centered around India, China and Malaya (Anon, 2014 and Nirmala *et al.,* 2017). Jasmine contributes to India's national economy. Tamil Nadu is the leading producer of jasmine in India and the flowers produced are being exported to the neighbouring countries *viz.,* Singapore, Malaysia, Sri Lanka and the Middle East countries and also to distant nations including the United State. Apart from their medicinal uses which has very good demand in India as well as in many developed countries, jasmine flowers are preferred for making special type of flower strings called veni, garlands, floral decorations, extraction of essential oil which is used in preparing high grade perfumes, colognes and flavouring the beverages etc.

Essential oil is extracted from the fragrant flowers of jasmine that has great demand in the international market for perfumery industry. The natural oils of jasmine are used in high-grade perfume and invariably most of the superior perfumes contain at least a small quantity of jasmine oil. The odour of jasmine flowers cannot be imitated by any known synthetic aromatic chemical or natural isolates thus giving it a unique status in the perfume world. Among Indian jasmines, scented species such as *J. grandiflorum, J. auriculatum, J. sambac, J. angustifolium, J. officinale, J. humile and J. pubescense* were found useful. The Indian *J. grandiflorum* is compared favourably with that of Spanish jasmine both in yield and quality of oil (Bose *et al.,* 2003).

Although jasmine is under cultivation from centuries, identification of species, varieties and common types is enigmatic, as this has been done mainly based on morphological characters. The classification is inadequate and misleading due to existence of large number of varieties and cultivars with synonyms. Though Dickey (1970) has stated that *Jasminum* is a genus of more than 200 species, many of these species are however synonyms and 90 species are considered to be true in existence (Muthukrishna and Pappiah 1980). Of the 40 species that have been identified in India, 20 are cultivated in South India (Bhattacharjee, 1980). Collection of jasmine germplasm and its morphological evaluation will provide an idea about the relatedness among the genotypes. Genetic improvement in jasmine through hybridization is limited due to non-fruit setting in most of the species (Ramanatha Raoand Toby Hodgkin, 2002). Therefore to create variability in Pink Pitchi, the terminal cuttings of Pink Pitchi CO 1 variety were irradiated with gamma rays and EMS for inducing variability and the best mutant identified for high flower yield were characterized for various morphological and floral traits.

In addition to this, GCMS analysis was also performed in the Pink Pitchi CO 1 variety and its mutant genotypes to identify and compare the various biochemical metabolites present in the pink flowers of *Jasminum grandiflorum*. Gas Chromatography-Mass Spectrometry (GCMS) process combines the features of gas-chromatography and mass spectrometry to identify different organic compounds presents in the organic matter, which includes Alkanes, Fatty acids, Alkenones, Sterols etc. GC-MS is becoming the tool of choice for tracking organic compounds derived from variety of plants and identification of unknown samples (Bramer, 1998). Therefore the purpose of this study is to characterize the phenotypic traits for various growth, leaf and floral traits and to identify the volatile biochemical compounds and the biochemical pathways involved in the production of those metabolites in pink pitchi and its mutant genotypes of *Jasminum grandiflorum.*

MATERIALS AND METHODS

The experiment was undertaken at the Department of Floriculture and Landscaping, Tamil Nadu Agricultural University, Coimbatore during the year 2020. A total of 128 mutant genotypes of CO 1 Pink Pitchi derived by inducing gamma rays and EMS were used as the study material. *J. grandiflorum* cv. CO.1 Pitchi is a variety developed through clonal selection at TNAU, Coimbatore during 1980. The plant is a woody bush having pinnate leaves with 3-5 leaflets of equal size. Flowers are white with pinkish tinge beneath, delightfully fragrant and are borne in lax, axillary or terminal cymes. The period of flowering extends for 6 months with a peak flowering season during July-September.

Morphological characteristics were recorded for all the 128 mutants by selecting the current season shoots called primary lateral shoots. Observations were recorded for 35 morphological traits such as Plant habit (bushy, spreading or climbing), leaf character (leaf type -simple or compound, leaf texture –pubescent or glabrous, phyllotaxy of the leaves, leaf shape, leaf colour and shape of the leaf base, floral characters of the fully matured buds in peak season of flowering, shape of the flower bud (ovate to acute or ovate to elliptic or round etc.). From this four best performing mutants PPM-12, PPM-72, PPM-96 and PPM-115 for various traits *viz.,* Early flowering, Long corolla tube, High yield, Profuse branching mutants were selected and subjected to GC-MS analysis to identify any biochemical compounds other than those in the CO 1 Pink Pitchi are present in the derived mutants.

Freshly opened blossoms were collected before 9.30 a.m., from the research plots raised at University Botanical garden, Department of Floriculture and Landscape Architecture, HC&RI, Tamil Nadu Agricultural University, Coimbatore during the year 2021. The fully blossomed flowers are weighed and subjected to extraction of oil and concrete. A non-polar solvent such as hexane is used to extract the aromatic

compounds from the flowers. In nature all the volatile compounds are fixed in the flowers along with fibrous materials. At the end of the process, the hexane is evaporated leaving behind a waxy, semisolid substance known as concrete and used for GCMS analysis.

The concrete extracted from the flowers of *J. grandiflorum* genotype Pink Pitchi and its four mutants (PPM-12, PPM-72, PPM-96, PPM-115) was dissolved in hexane and directly injected into the injection port of gas chromatograph (Agilent Technologies 7890A GC system) coupled with a mass spectrometer The Clarus SQ 8C Gas Chromatography - Mass Spectrometer from Perkin Elmer, available at Department of Agricultural Microbiology, Tamil Nadu Agricultural University and Coimbatore.

The instrument was set as follows: Injector port temperature set to 220°C, Interface temperature set as 250°C, source kept at 220°C. The oven temperature programmed as 75°C for 2 mins, 150^0 C @ 10°C/min, up to 250°C @ 10°C/min. Split ratio set as 1:12 and the injector used was splitless mode. The DB-5 MS capillary standard –non-polar column was used whose dimensions were 0.25mm OD × 0.25µm ID × 30 meters length procured from Agilent Co., USA. Helium was used as the carrier gas at a constant flow of 1 ml/minute. The MS was set to scan from 50 to 550 Da. The source was maintained at 220°C and 4.5e -6 motor vacuum pressure. The ionization energy was -70eV. The MS was also having inbuilt pre-filter which reduced the neutral particles. 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 retention time is the amount of time that passes between elution and injection. The retention time was used to distinguish between various substances. The peak value was measured from the base to the tip of the peak.

The data system has inbuilt libraries for detecting and matching the spectrum. NIST MS Search 2.2v contains more than five lakh references. Interpretation of mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST14). The resulting data of the three flower samples in spectrum was compared with known spectrum available in NIST, PubChem and Human Metabolome Databases. The important credentials *viz.*, molecular weight, molecular formula and molecular structures of the constituents in the flowers were ascertained. The spectrum of the known component was compared with the spectrum of the known components stored in the inbuilt library.

RESULTS AND DISCUSSION

Phenotypic characterization in mutant genotypes of Pink Pitchi

The present study resulted in identification of morphological variations at both vegetative as well as reproductive characters in promising jasmine mutants for growth and flower quality characters (Table 1 and Fig. 1). The plant growth habit and plant height at flowering was found to be spreading and medium in Pink Pitchi whereas it was strongly spreading and tall in the mutant genotypes. Except PPM-12 and PPM-115 mutants, young shoot anthocyanin colouration was present in all other genotypes. The terminal leaflet of the compound leaf was medium in Pink Pitchi and PPM-12 whereas it was large in PPM-72 and PPM-96 and small in PPM-115. The Intensity of green colour was dark in two mutants *viz.*, PPM-12 and PPM-96 whereas it was medium in all other genotypes. Leaf anthocyanin colouration in young leaves was present in all the genotypes except PPM-115. Leaf pubescence and Leaf glossiness on upper surface of mature leaf was absent in all the genotypes.

Flowers are borne in clusters and the flowering cymes are borne both axillary and terminal in all the genotypes. Flower bud length was long in all the genotypes whereas the flower bud shape was pointed and short in Pink Pitchi whereas it was pointed and long in all the mutant accessions. Boldness of flower bud was found to be medium in Pink Pitchi whereas it was Bold in all the mutant accessions. The flower bud colour was off white in all entries but the flower colour on opening was found to be white in all the genotypes. Shape of open corolla was round in Pink Pitchi whereas it was star shaped in the mutant accessions. Shape of corolla lobe was rounded in Pink Pitchi whereas it was lanceolate in the mutant accessions. Phenotypic variations with respect to various characters in different genotypes of *Jasminum* sp has been reported earlier by Mukundan *et al.* (2008); Champa, (2012); Safeena *et al.* (2017); Malik Abid Mahamood *et al.* (2013); Shekhar *et al.* (2013); Nirmala *et al.* (2017); Patel *et al.* (2018).

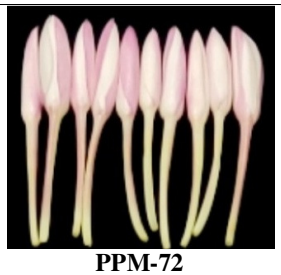
Table 1: Phenotypic traits recorded in CO 1 Pink Pitchi and its mutant accessions

Sr. No	Characteristics /Genotypes	Pink Pitchi	PPM-12	PPM-72	PPM-96	PPM-115
1.	Plant growth type	Shrub	Climber	Climber	Climber	Climber
2.	Plant growth habit	Spreading	Strongly spreading	Strongly spreading	Strongly spreading	Spreading
3.	Plant height (at flowering)	Medium	Tall	Tall	Tall	Tall
4.	Young shoot anthocyanin colouration (Shoots up to 30 cm from growing tip)	Present	Absent	Present	Present	Absent
5.	Young shoot intensity of anthocyanin colouration	Weak	Weak	Weak	Weak	Weak
6.	Ridges on the stem	Present	Present	Present	Present	Present
7.	Leaf Phyllotaxy	Opposite	Opposite	Opposite	Opposite	Opposite
8.	Leaf type	Compound	Compound	Compound	Compound	Compound
9.	Leaf size - Terminal leaflet of compound leaf	Medium	Medium	Large	Large	Small

10.	Leaf size - Other leaflets of compound leaf	Small	Medium	Medium	Medium	Small
11.	Intensity of green colour (upper surface of mature leaf)	Medium	Dark	Medium	Dark	Medium
12.	Leaf anthocyanin colouration (young leaf)	Present	Present	Present	Present	Absent
13.	Leaf pubescence	Absent	Absent	Absent	Absent	Absent
14.	Leaf glossiness on upper surface (mature leaf)	Absent	Absent	Absent	Absent	Absent
15.	Shape of leaf blade- Terminal leaflet of compound leaf	Lanceolate	Elliptic	Lanceolate	Lanceolate	Elliptic
16.	Shape of leaf blade- Other leaflets of compound leaf	Ovate	Ovate	Elliptic	Ovate	Elliptic
17.	Leaf tip: Terminal leaflet of compound leaf	Sharp	Sharp	Sharp	Sharp	Sharp
18.	Leaf tip: Other leaflets of compound leaf	Medium	Medium	Medium	Medium	Medium
19.	Shape of base of leaf blade: Terminal leaflet of compound leaf	Acute	Obtuse	Obtuse	Acute	Acute
20.	Shape of base of leaf blade: Other leaflets of compound leaf	Obtuse	Obtuse	Obtuse	Acute	Acute
21.	Flower bearing habit	Cluster	Cluster	Cluster	Cluster	Cluster
22.	Flower bearing position (axillary /terminal/Both)	Both	Both	Both	Both	Both
23.	Flower bud length	Long	Long	Long	Long	Long
24.	Boldness of flower bud	Medium	Bold	Bold	Bold	Bold
25.	Flower bud shape	Pointed and short	Pointed and long	Pointed and long	Pointed and long	Pointed and long
26.	Flower bud colour	Off white	Off white	Off white	Off white	Off white
27.	Tinge on flower bud	Present	Present	Present	Present	Present
28.	Flower colour on opening	Pure white	Pure white	Pure white	Pure white	Pure white
29.	Corolla tube length	Medium	Long	Long	Long	Long
30.	Shape of open corolla Round/star shaped	Round	Star	Star	Star	Star
31.	Shape of corolla lobe	Rounded	Lanceolate	Lanceolate	Lanceolate	Lanceolate
32.	Flower petal tip	Blunt	Blunt	Blunt	Blunt	Blunt
33.	Reflexing of flower	Present	Present	Present	Present	Present
34.	Flower type	Single	Single	Single	Single	Single
35.	Seed setting	Absent	Absent	Absent	Absent	Absent

Pink Pitchi CO1

PPM-12



PPM-72

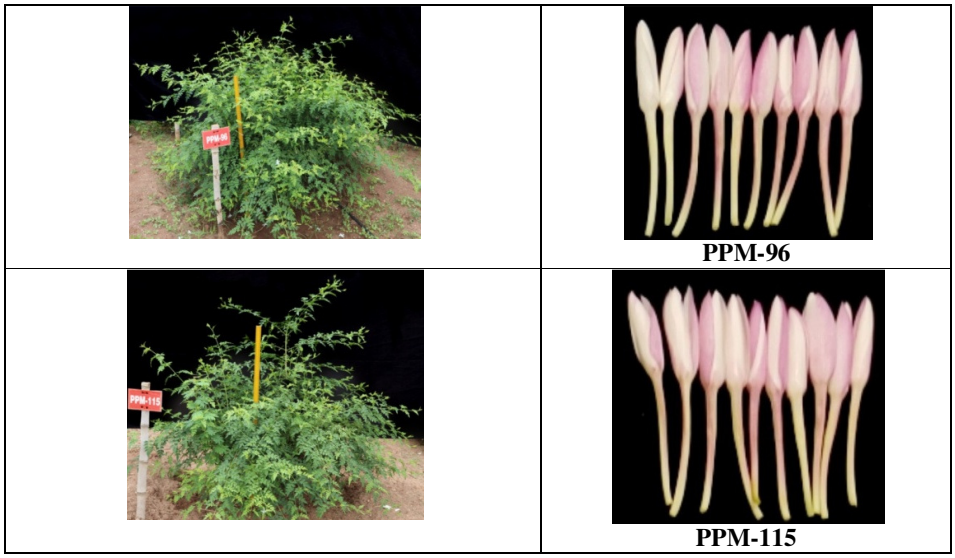


Fig. 1. Plant growth, Leaf and Flower characteristics recorded in the mutant accessions of Jasmine.

GC-MS analysis in mutant genotypes of Pink Pitchi. The concrete extracted from the flowers of *Jasminum grandiflorum* cultivar – Pink pitchi, and its mutants PPM-12, PPM-72, PPM-96 and PPM-115 were subjected to GC-MS analysis to identify and compare the biochemical compounds present in the CO1 variety Pink Pitchi and its mutants. The chromatogram generated by gas chromatography showed the composition of the various biochemical compounds present in *Pink Pitchi* and their mutants are given in Tables and Figures (Tables 2-7 and Fig. 2-7).

Five major compounds *viz.,* Benzene, Ethyl Acetate, O-Cymene and Phenylethyl Alcohol and α-Phellandrene were found to be present in all the genotypes indicating that all these compounds are common to Pink Pitchi (Table 2 and Fig. 2) and its derived mutants PPM-12 (Table 3 and Fig. 3), PPM-72 (Table 3 and Fig. 3), PPM-96 (Table 4 and Fig. 4) and PPM-115 (Table 5 and Fig. 5). Five aromatic hydrocarbons *viz.,* 1-Bromo-3, 7- dimethyl- 2, 6-octadiene, 4, 5-Dihydrooxazole-5-one, 4-chloromethylene- 2-phenyl-, Acetic acid, methyl ester, Butanoic acid, butyl ester and Trichloromethane; two organic oxides Nifuroxazide and Nonanal and one fatty acyl compound Cyclopentasiloxane, decamethyl- were found to be present in CO1 Pink Pitchi and its two EMS derived mutants PPM-12 and PPM-72 respectively whereas these compounds are absent in gamma ray derived mutants PPM-96 and PPM-115. Similarly Toluenes, Benzenoids and Polycyclic hydrocarbons such as 1-(p-Tolyl) butan-1-one, 2-Heptanone and Bicyclo [7.2.0] undec-4-ene, 4,11,11-trimethyl-8-methylene-, [1R-1R*,4Z,9S*)]- were found to be present only in the two EMS derived mutants PPM-12 and PPM-72 respectively. The peak area for each biochemical compound identified in Pink Pitchi and its mutant genotypes are presented in Table 6 and heat map (Fig. 6).

Another aromatic hydrocarbon called Hexanoic acid, ethyl ester and a saturated hydrocarbon Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S)- were present only in Pink Pitchi whereas these compounds are absent in all the derived mutants of Pink Pitchi. Three compounds *viz.,* Boldione, an organooxygen compound, a organochloride compound Caryophyllene and a branched alkane Nonane, 4,5-dimethyl- were present only in the gamma ray derived mutants PPM-96 and PPM-115 whereas they are absent in control and EMS derived mutants. The presence of various biochemical compounds in *Jasminum grandiflorum* was also reported by Anac Olcay (1986); Younis (2008); Feng Huan Wei *et al.* (2015); Ranchana *et al.* (2017); Hesham Hussein Rassem (2018); Bharathi *et al.* (2020) ; Sanchita Ghosh (2020) in their earlier studies carried out through Gas Chromatographic analysis.

Table 2: GC-MS for identification of the chemical constituents in Pink Pitchi.

Sr. No.	Biochemical compound	MW	Retention Time	Area (%)
1.	Acetic acid, methyl ester	74	1.749	21.333
2.	Ethyl Acetate	88	1.959	2.327
3.	Trichloromethane	119	2.099	7.868
4.	Benzene	78	2.259	2.663
5.	1-Butanol, 2-methyl-, (S)-	88	2.774	1.628
6.	Cyclotrisiloxane, hexamethyl-	222	3.489	1.309
7.	1-Butanol, 3-methyl-, acetate	130	4.370	0.460
8.	Bestatin	308	4.570	5.797
9.	L-Serine	105	4.715	1.304
10.	Nifuroxazide	275	4.975	0.470
11.	Bestatin	308	5.105	2.771
12.	Galactonic phenylhydrazide	286	5.200	0.619

13.	1,2-Ethanediol, monoformate	90	5.260	0.752
14.	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	136	5.370	1.565
15.	4,5-Dihydrooxazole-5-one, 4-chloromethylene-2-phenyl-	207	5.945	0.718
16.	Cyclotetrasiloxane, octamethyl-	356	6.170	0.841
17.	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	136	6.255	0.444
18.	1-Bromo-3,7-dimethyl-2,6-octadiene	217	6.416	0.770
19.	Butanoic acid, butyl ester	144	6.521	0.557
20.	Hexanoic acid, ethyl ester	144	6.566	0.813
21.	3-Amino-2-oxazolidinone	102	7.006	2.651
22.	O-Cymene	134	7.141	4.676
23.	Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S)-	138	7.236	2.429
24.	α-Phellandrene	136	7.281	9.736
25.	α-Pinene	136	7.521	0.580
26.	γ-Terpinene	136	7.776	0.423
27.	1,3,5-Trioxepane	90	8.081	1.894
28.	Nonanal	142	8.621	0.469
29.	Phenylethyl Alcohol	122	8.837	1.440
30.	Benzenamine, N-hydroxy-	123	8.962	1.086
31.	Cyclopentasiloxane, decamethyl-	370	9.102	0.636
32.	4[h]-Pyridone, 1-benzyl-3,5-dichloro-2,6-dimethyl-	195	9.197	1.318
33.	1,3-Dioxolane-2-methanol	104	9.512	1.769
34.	1,3:2,4-Di-O-methylene-dl-xylitol	328	14.604	0.567
35.	α-acorenol	222	15.039	0.585
36.	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2α,4aα,8aβ)]-	204	15.124	0.510
37.	Tetradecylphosphonate	278	17.220	0.567
38.	Butylaldehyde, 4-benzyloxy-4-[2,2,-dimethyl-4-dioxolanyl]-	278	17.410	0.465
39.	Pentaethylene glycol	238	17.995	0.640
40.	n-Hexadecanoic acid	256	19.411	0.399

Table 3: GC-MS for identification of the chemical constituents in mutant PPM-12.

Sr. No.	Biochemical Compound	MW	Retention Time	Area (%)
1.	Acetic acid, methyl ester	74	1.729	22.342
2.	Butanal	72	1.799	4.732
3.	Ethyl Acetate	88	1.969	6.867
4.	Trichloromethane	119	2.044	0.800
5.	Benzene	78	2.244	3.978
6.	1-(p-Tolyl)butan-1-one	162	2.669	0.258
7.	1-Pentanol	88	2.759	0.488
8.	L-Threonine	119	2.794	0.570
9.	Cyclobutene, 2-propenylidene-	92	3.069	0.517
10.	Hexanal	100	3.404	0.450
11.	Cyclotrisiloxane, hexamethyl-	222	3.514	3.101
12.	Isopropylamine	59	4.425	3.583
13.	2-Heptanone	114	4.600	0.298
14.	Isonicotinic acid, 2-phenylethyl ester	227	4.670	0.641
15.	Nifuroxazide	275	5.035	2.237
16.	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	136	5.410	0.710
17.	4,5-Dihydrooxazole-5-one, 4-chloromethylene-2-phenyl-	207	5.995	0.453
18.	1-Butanol, 3-methyl-, propanoate	144	6.055	0.462
19.	Dimethyl trisulfide	126	6.145	0.681
20.	Cyclotetrasiloxane, octamethyl-	356	6.215	1.831
21.	1-Octen-3-ol	128	6.280	0.413
22.	1-Bromo-3,7-dimethyl-2,6-octadiene	217	6.461	0.578
23.	Butanoic acid, butyl ester	144	6.561	0.689
24.	α-Phellandrene	136	6.836	0.575
25.	Ethyldiethanolamine	133	6.941	0.314
26.	O-Cymene	134	7.186	2.003
27.	α-Phellandrene	136	7.326	14.235
28.	α-Ocimene	136	7.561	7.604
29.	Dodecane	170	7.716	0.696
30.	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1α,2α,5α)-	154	7.826	0.374
31.	Acetophenone	120	7.991	0.881
32.	Ketone, isopropylidenecyclopropyl methyl	124	8.396	0.673
33.	Undecane, 4,7-dimethyl-	184	8.566	0.489
34.	Nonanal	142	8.671	0.381
35.	Phenylethyl Alcohol	122	9.027	1.967
36.	Cyclopentasiloxane, decamethyl-	370	9.142	1.781
37.	1,3-Dioxolane	74	9.482	0.349
38.	1H-Indene, 1-methylene-	128	10.212	0.362
39.	Tetrasulfide, dimethyl	158	10.747	0.660
40.	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*, 4Z,9S*)]-	204	14.094	0.850

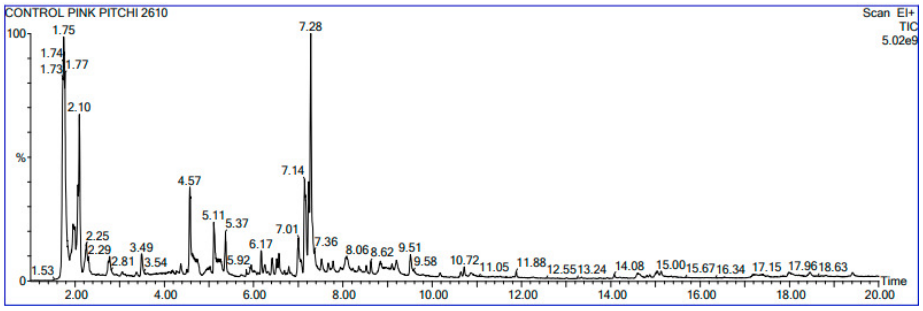


Fig. 2. Gas Chromatography Mass Spectrometry (GC-MS) for Pink Pitchi.

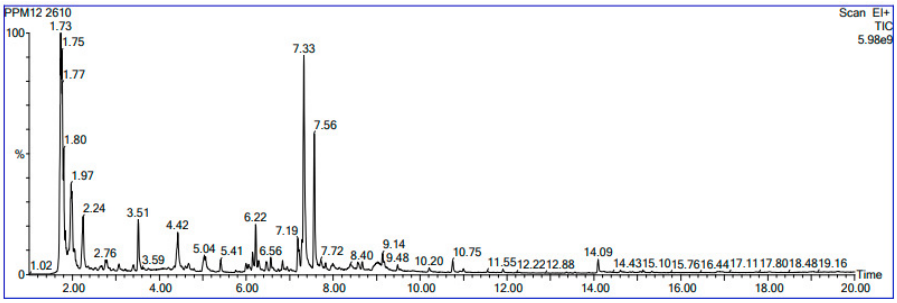


Fig. 3. Gas Chromatography Mass Spectrometry (GC-MS) for mutant PPM-12.

Table 4: GC-MS for identification of the chemical constituents in mutant PPM72.

Sr. No.	Biochemical Compound	MW	Retention Time	Area (%)
1.	Bicalutamide	430	1.769	26.938
2.	Ethyl Acetate	88	1.984	10.964
3.	Benzene	78	2.259	0.636
4.	1-Butanol, 2-methyl-, (S)-	88	2.799	1.219
5.	Butanoic acid, 2-methyl-, methyl ester	116	3.154	0.364
6.	Hexanal	100	3.414	0.435
7.	Cyclotrisiloxane, hexamethyl-	222	3.539	2.218
8.	Tetrapropylammonium cation	130	3.950	0.406
9.	O-Acetyl-L-serine	147	4.025	0.548
10.	3-Hexen-1-ol, (Z)-	100	4.145	1.003
11.	Carbonic acid, prop-1-en-2-yl undecyl ester	256	4.225	0.755
12.	1-Butanol, 3-methyl-, acetate	130	4.405	1.401
13.	2-Heptanone	114	4.605	0.443
14.	Nifuroxazide	275	5.025	0.977
15.	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	136	5.420	1.230
16.	Benzaldehyde	106	5.975	0.360
17.	Cyclotetrasiloxane, octamethyl-	356	6.195	0.529
18.	1-Bromo-3,7-dimethyl-2,6-octadiene	217	6.446	0.745
19.	Butanoic acid, butyl ester	144	6.541	0.610
20.	Hexanoic acid, ethyl ester	144	6.596	0.850
21.	α-Phellandrene	136	6.821	0.425
22.	O-Cymene	134	7.171	3.763
23.	D-Limonene	136	7.261	1.710
24.	α-Phellandrene	136	7.311	10.032
25.	Bestatin	308	7.466	2.785
26.	α-Ocimene	136	7.541	4.470
27.	Undecane	156	7.696	0.722
28.	Tetrapropylammonium cation	130	7.781	2.922
29.	Acetophenone	120	7.971	0.466
30.	2-Nonanone	142	8.376	0.426
31.	Undecane	156	8.546	0.796
32.	Nonanal	142	8.646	0.654
33.	l-Alanine, N-methoxycarbonyl-, ethyl ester	189	8.781	0.789
34.	Phenylethyl Alcohol	122	8.982	0.427
35.	Cyclopentasiloxane, decamethyl-	370	9.112	0.583
36.	1,3,5-Trioxepane	90	9.372	0.876
37.	Phenylethyl Alcohol	122	10.162	1.617
38.	1-(p-Tolyl)butan-1-one	162	10.302	1.214
39.	Tetrasulfide, dimethyl	158	10.732	0.630
40.	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*, 4Z,9S*)]-	204	14.084	0.609

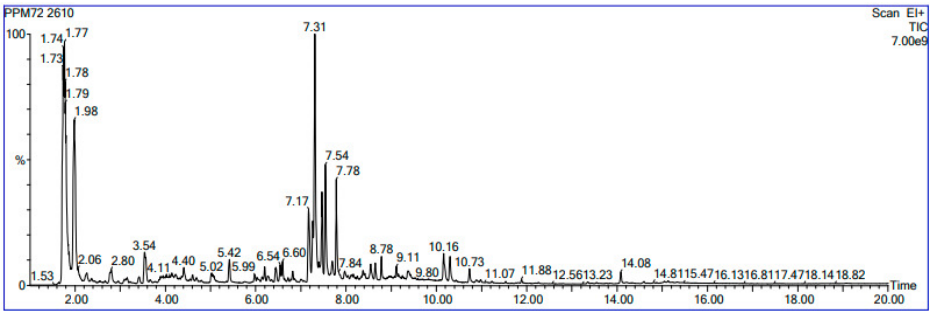


Fig. 4. Gas Chromatography Mass Spectrometry (GC-MS) for mutant PPM-72.

Table 5: GC-MS for identification of the chemical constituents in mutant PPM-96.

Sr. No.	Biochemical Compound	MW	Retention Time	Area (%)
1.	DL-Homocysteine, S-ethyl-	163	1.749	31.397
2.	Ethyl Acetate	88	2.004	21.356
3.	Benzene	78	2.264	5.635
4.	Isopropylamine	59	2.524	5.136
5.	n-Propyl acetate	102	2.564	0.212
6.	1-Pentanol	88	2.784	0.707
7.	Tris(hydroxymethyl)aminomethane	121	2.824	0.918
8.	sec-Butyl acetate	116	2.979	0.243
9.	Cyclobutene, 2-propenylidene-	92	3.109	0.572
10.	Tetrapropylammonium cation	130	3.444	1.173
11.	Cyclotrisiloxane, hexamethyl-	222	3.564	2.214
12.	1,2-Ethanediol, diformate	118	3.900	0.568
13.	3-Hexanol, 3,4-diethyl-,	158	4.000	0.525
14.	Hexanoic acid, 4-methyl-	116	4.100	0.297
15.	1-Butanol, 3-methyl-, acetate	130	4.445	1.161
16.	1,4-Dioxan-2-ol	104	5.030	0.315
17.	α-Pinene	136	5.470	0.518
18.	Arsenous acid, tris(trimethylsilyl) ester	342	6.035	0.234
19.	Dimethyl trisulfide	126	6.200	0.562
20.	Cyclotetrasiloxane, octamethyl-	296	6.265	0.655
21.	1-Octen-3-ol	128	6.315	0.274
22.	Bicyclo[4.1.0]heptane, 3,7,7-trimethyl-, [1S-(1α,3α,6α)]-	138	6.511	0.529
23.	α-Phellandrene	136	6.876	0.206
24.	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	138	7.081	0.300
25.	O-Cymene	134	7.226	3.715
26.	Limonene	136	7.316	0.559
27.	α-Phellandrene	136	7.361	4.543
28.	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	136	7.606	0.485
29.	Nonane, 4,5-dimethyl-	156	7.751	0.284
30.	γ-Terpinene	136	7.861	3.120
31.	Acetophenone	120	8.031	0.267
32.	2-Nonanone	142	8.421	0.459
33.	Octane, 3,5-dimethyl-	142	8.591	0.376
34.	Nonanal	142	8.691	0.384
35.	Phenylethyl Alcohol	122	8.922	1.904
36.	1,3-Dioxolane	74	9.302	0.661
37.	Boldione	284	10.232	0.524
38.	Tetrasulfide, dimethyl	158	10.762	0.367
39.	[1,1'-Bicyclopentyl]-2-one	152	10.962	0.285
40.	Caryophyllene	204	14.099	0.213

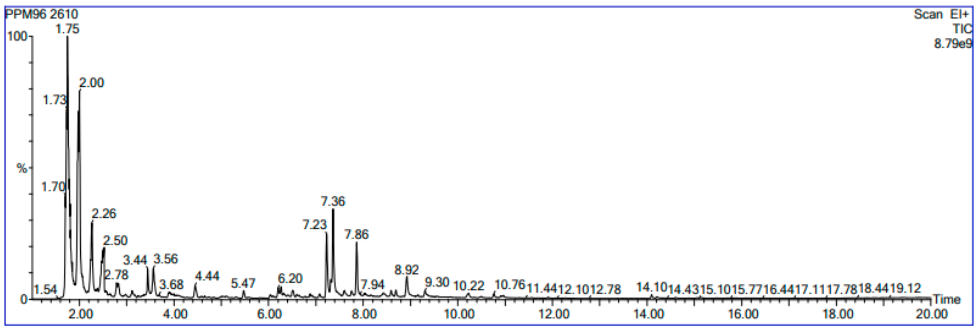


Fig. 5. Gas Chromatography Mass Spectrometry (GC-MS) for mutant PPM-96.

Table 6: GC-MS for identification of the chemical constituents in mutant PPM-115

Sr. No.	Biochemical Compound	MW	Retention Time	Area (%)
1.	Ethyl ether	74	1.739	16.845
2.	Ethyl Acetate	88	1.964	10.138
3.	Benzene	78	2.249	2.305
4.	Butane, 1-methoxy-3-methyl-	102	2.409	3.730
5.	1-Pentanol	88	2.779	0.373
6.	Aziridine, 2-methyl-	57	2.814	0.338
7.	Isopropylamine	59	2.914	3.236
8.	Tetrapropylammonium cation	130	2.944	2.296
9.	Toluene	92	3.099	0.518
10.	Hexanal	100	3.439	0.751
11.	Cyclotrisiloxane, hexamethyl-	222	3.549	1.236
12.	Acetic acid	60	3.739	0.705
13.	Isobutyl nitrite	103	3.870	0.381
14.	2,3-Butanediol, [R-(R*,R*)]-	90	3.955	0.337
15.	(R)-3-Hydroxybutyric acid	104	4.100	1.579
16.	3-Hexen-1-ol	100	4.175	1.112
17.	Cyclobutane, ethyl-	84	4.340	0.533
18.	1-Butanol, 3-methyl-, acetate	130	4.440	0.459
19.	2-Butanol, 3-chloro-, acetate, (R*,R*)-	150	4.730	0.758
20.	α-Pinene	136	5.465	2.600
21.	Dimethyl trisulfide	126	6.185	0.502
22.	Cyclotetrasiloxane, octamethyl-	296	6.250	0.412
23.	N-Methyl-N-(2-hydroxyethyl)carbamic acid,phenyl ester	195	6.325	1.090
24.	α-Myrcene	136	6.491	1.470
25.	Butanoic acid, butyl ester	144	6.591	1.454
26.	α-Phellandrene	136	6.866	1.818
27.	O-Cymene	134	7.216	2.799
28.	D-Limonene	136	7.316	3.607
29.	γ-Terpinene	136	7.361	18.479
30.	α-Ocimene	136	7.596	6.934
31.	Dodecane	170	7.741	0.388
32.	γ-Terpinene	136	7.851	0.548
33.	Acetophenone	120	8.016	0.401
34.	2-Methyl-1-undecanol	186	8.441	0.341
35.	Nonane, 4,5-dimethyl-	156	8.586	0.416
36.	Phenylethyl Alcohol	122	8.936	0.599
37.	Boldione	284	10.232	0.306
38.	Tetrasulfide, dimethyl	158	10.762	0.392
39.	Benzothiazole	135	10.917	0.503
40.	Caryophyllene	204	14.104	0.972

Table 7: Peak area of biochemical compounds in *J. grandiflorum* Pink Pitchi and its mutants.

Sr. No.	Biochemical compounds	Peak Area (%)					Classification
		Pink Pitchi	PPM-12	PPM-72	PPM -96	PPM-115	
1.	Benzene	2.663	2.244	0.636	5.635	2.305	Benzoids
2.	Ethyl Acetate	2.327	1.969	10.964	21.356	10.138	Carboxylic acid
3.	O-Cymene	4.676	7.186	3.763	3.715	2.799	Terpenoid
4.	Phenylethyl Alcohol	1.44	9.027	1.617	1.904	0.599	Sulfenyl compounds
5.	α-Phellandrene	9.736	6.836	10.032	4.543	1.818	Terpenoid
6.	(1R)-2,6,6-Trimethylbicyclo [3.1.1] hept-2-ene	1.565	5.41	1.23	0.485	0	Polycyclic hydrocarbons
7.	1,3-Dioxolane	1.769	9.482	0	0.661	0	Sulfenyl compounds
8.	1-Bromo-3,7-dimethyl-2,6-octadiene	0.77	6.461	0.745	0	0	Aromatic hydrocarbons
9.	4,5-Dihydrooxazole-5-one, 4-chloromethylene-2-phenyl-	0.718	5.995	0.560	0	0	Aromatic hydrocarbons
10.	Acetic acid, methyl ester	21.333	1.729	0.458	0	0	Aromatic hydrocarbons
11.	Butanoic acid, butyl ester	0.557	6.561	0.610	0	0	Aromatic hydrocarbons
12.	Cyclopentasiloxane, decamethyl-	0.636	9.142	0.583	0	0	Fatty Acyls
13.	Nifuroxazide	0.47	5.035	0.977	0	0	Organic oxides
14.	Nonanal	0.469	8.671	0.654	0	0	Organic oxides
15.	Trichloromethane	7.868	2.044	0.384	0	0	Aromatic hydrocarbons
16.	1-Butanol, 3-methyl-, acetate	0.46	0	1.401	1.161	0.459	Organic oxides
17.	Hexanoic acid, ethyl ester	0.813	0	0	0	0	Aromatic hydrocarbons
18.	α-Pinene	0.58	0	0	0.518	2.6	Terpenoid
19.	γ-Terpinene	0.423	0	0	3.12	18.479	Sesquiterpenoids

20.	Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S)-	2.429	0	0	0	0	Saturated hydrocarbons
21.	1,3,5-Trioxepane	1.894	0	0.876	0	0	Folic acid
22.	1-Butanol, 2-methyl-, (S)-	1.628	0	1.219	0	0	Organic oxides
23.	Bestatin	5.797	0	2.785	0	0	Benzoids
24.	Hexanal	0	3.404	0.435	0	0.751	Aldehydes
25.	α-Ocimene	0	7.561	4.47	0	6.934	Saturated hydrocarbons
26.	1-(p-Tolyl)butan-1-one	0	2.669	1.214	0	0	Toluenes
27.	2-Heptanone	0	4.6	0.443	0	0	Benzenoids
28.	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-1R*,4Z,9S*)]-	0	14.094	0.609	0	0	Polycyclic hydrocarbons
29.	1-Pentanol	0	2.759	0	0.707	0.373	Alkyl alcohol
30.	Dimethyl trisulfide	0	6.145	0	0.562	0.502	Sulfenyl compounds
31.	Isopropylamine	0	4.425	0	5.136	3.236	Amines
32.	1-Octen-3-ol	0	6.28	0	0.274	0	Fatty alcohols
33.	Cyclobutene, 2-propenylidene-	0	3.069	0	0.572	0	Cyclic ketones
34.	Dodecane	0	7.716	0	0	0.388	Fatty Acyls
35.	Tetrapropylammonium	0	0	0.636	1.173	2.296	Amines
36.	Boldione	0	0	0	0.524	0.306	Organooxygen
37.	Caryophyllene	0	0	0	0.213	0.972	Organochlorides
38.	Nonane, 4,5-dimethyl-	0	0	0	0.284	0.416	Branched Alkanes
39.	3-Hexen-1-ol	0	0	1.003	0	1.112	Prenol lipids
40.	D-Limonene	0	0	1.71	0	3.607	Monocyclic monoterpenoid
41.	2-Nonanone	0	0	0.426	0.459	0	Ketones

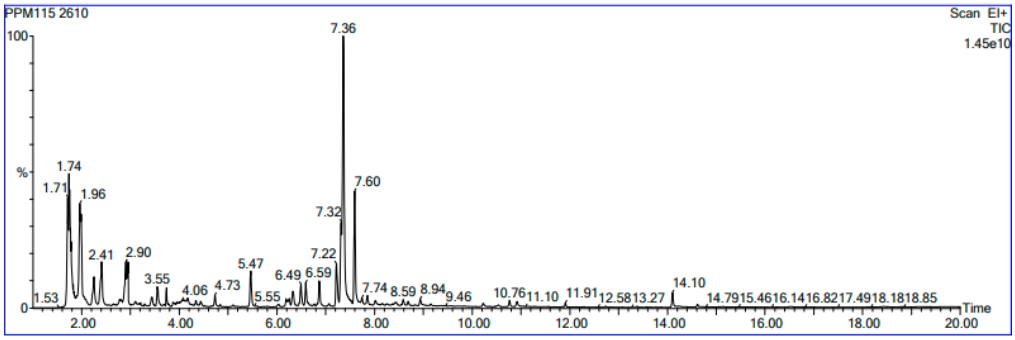


Fig. 6. Gas Chromatography Mass Spectrometry (GC-MS) for mutant PPM-115.

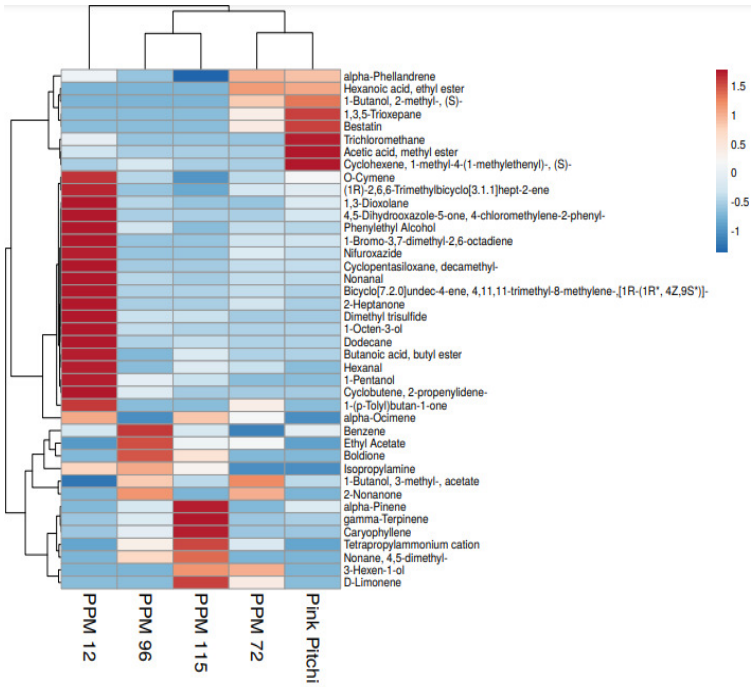


Fig. 7. Heat Map for the biochemical compounds in Pink Pitchi and its mutant accessions.

CONCLUSION

This study has documented various morphological and flowering traits along with various biochemical compounds present in different mutant genotypes of *J. grandiflorum* to represent the variability existing in Pink Pitchi mutants of jasmine. The jasmine mutants exhibited an incredible range of variations for various morphological and floral quality traits. Variations in vegetative and floral characters were recorded both in cultivated as well as in mutant genotypes. Such morphological variations resulted in the identification of four promising mutant genotypes of Pink Pitchi for further utilization in crop improvement programmes for development of elite varieties in jasmine. Data thus generated would be helpful in future crop improvement programmes in jasmine to cater to the needs of floriculture industry. Identification of biochemical compounds may ultimately help in industrial utility to identify the perfect stage of the flowers for higher concrete recovery and can assume that the identified compounds possesses a response for unique fragrance exclusive for jasmine species.

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