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Olfactory evaluation and untargeted profiling of floral volatiles of fragrant rose cultivars *Pusa Mahak* and its seed parent *Century Two* by HS-SPME-GC × GC-TOFMS

Shephalika Amrapali[*], Ahammad Shabeer TP[**], Bappa Ghosh[**], Namita[***], M. K. Singh[***] and Sunil Archak[****]

Division of Plant Genetic Resources, ICAR-National Bureau of Plant Genetic Resources, Pusa Complex, New Delhi 110012

ABSTRACT

Pusa Mahak is a recurrent flowering and floriferous rose cultivar bearing attractive dark pink coloured flowers with strong outstanding fragrance. Pusa Mahak, a hybrid tea rose, is ideal for garden display and floral arrangements. It was developed by selection from the open-pollinated seedlings of cv. Century Two, a bold and pink coloured blooming rose used for garden display purpose. This study was undertaken to evaluate intensity and likeability of fragrance of the two cultivars based on olfactory evaluation by an untrained panel and to profile the floral volatiles using headspace solid phase microextraction – 2D gas chromatography time of flight mass spectrometry. The fragrance profiles of cvs. Pusa Mahak and Century Two were found to be distinct in the study. Sensory evaluation indicated that cv. Pusa Mahak had stronger fragrance and greater likeability with sweet woody and mild spicy nuances in comparison to the damask floral and green herb like nuances of Century Two. Biochemical evaluation detected 82 compounds (55% terpenoids) in cv. Pusa Mahak and 66 compounds (37% phenylpropanoids/benzenoids) in Century Two. Major components (≥5%) identified in cv. Pusa Mahak, were β-pinene (20%), β-citronellol (14%), 2- phenyl ethyl acetate (9.5%), 2-phenyl ethanol (8.3%), 3, 5-Dimethoxytoluene (7.23%), geranial (7.23%) and α-myrcene (7%). Major constituents of headspace volatile of Century Two were 3, 5-dimethoxytoluene (67%), 2-phenyl ethanol (19%) and theaspirane (4.5%). Difference in fragrance quality and intensity was attributed to the variation in the composition of headspace volatiles and different aroma impressions of specific compounds.

Key words: *Rosa* hybrida, floral volatiles, headspace volatiles, HS-SPME, GCxGC-TOFMS.

INTRODUCTION

Roses are synonymous with fragrance since antiquity. According to an online survey of rose researchers, fragrance is an important horticultural trait for roses second only to disease resistance (Byrne *et al.,* 5). However, breeders find it difficult to make improvement in fragrance especially in varieties bred for garden and cut flower purpose. Most of the modern rose varieties are characterized by negligible fragrance. To enable recurrence of the fragrance as trait of interest in the rose breeding necessitates documentation of olfactory and biochemical profiles of the well-known fragrant rose cultivars. Cv. Pusa Mahak is a hybrid tea rose cultivar developed by ICAR-Indian Agricultural Research Institute, New Delhi. It bears dark pink coloured flowers that possess strong outstanding fragrance. It was developed as a selection from the open-pollinated seedlings of Century Two, a famous rose cultivar of 1970s (AICRP Floriculture), Annual Report 2014-15, Pune-411005, Maharashtra, India). The plants are tall and vigorous with a height of 100 - 120 cm. The flowering starts in 40-45 days after pruning. Flowers are large and semi-double with 22-23 petals. It is a recurrent flowering and floriferous cultivar and each plant produce on an average 50-60 flowering shoots in a season. The cultivar is ideal for garden display and the fragrant flowers can be used for floral arrangements (IARI News Vol 31 (1) : page 2). The seed parent cv. Century Two is a medium sized bush with light pink coloured, high centred blooms and moderate fragrance (Roses Database).

Fragrance of roses is attributed to various volatile organic compounds. The compounds are mainly grouped into three categories based on their biosynthetic pathways. These are (i) Terpenoids (germacrene D, ketones, monoterpene alcohol and aldehydes like geraniol and its derivatives, nerol, citronellol *etc.*); (ii) Phenylpropanoids/ benzenoids (3,5-dimithoxytoulene, 1,3,5-trimithoxy benzene, eugenol, isoeugenol *etc.*); and (iii) Fatty acid derivatives (hexenal, hexenol, methyl jasmonate

[*]Corresponding author's E-mail: shephalikaamrapali@gmail.com; namitabanyaliari@gmail.com
[**]ICAR-National Research Centre on Grapes, Pune, Maharashtra
[***]Division of Floriculture and Landscaping, ICAR-IARI, New Delhi
[****]ICAR-National Bureau of Plant Genetic Resources, New Delhi

etc.). Other than these, there are some carotenoid derivatives such as β- ionone and β-demascone (Baldermann *et al*.3). Emission of these compounds in different combinations and their proportion define the characteristic fragrance of a particular rose cultivar. As a result, fragrance follows a complex inheritance pattern and selection of donor parents based on presence and absence of fragrance is not effective. However, selection based on individual compounds rather than overall fragrance is known to be more effective (Kuanprasert *et al*., 12; Kuanprasert *et al*., 13; Cherri-Martin *et al.,* 6; Suzuki *et al.,* 16; Kishimoto *et al.,* 11; Aros *et al.* 1 and Aros *et al.,* 2). Rose varieties rich in monoterpenes are considered to be better parents as these compounds show direct inheritance (Cherri-martin *et al.,* 6). Interest in the identification of scent molecule is attracting scientific community for their commercial value in various industrial products (Guterman *et al*., 7).

Olfactory and biochemical analysis have been used for the evaluation of fragrance. The preference for certain type of rose fragrance and the difference in their intensity as detected by the human senses can be determined by scent panel tests. Until now, several headspace solid phase micro-extraction (HS-SPME) techniques such as fiber, stirrer, tube, vessel walls are widely used for volatile isolation. Among these, automated headspace solid phase micro-extraction (automated HS-SPME) fiber is a simple, sensitive and solvent free extraction technique that enables the extraction and the concentration steps to be performed simultaneously. HS-SPME coupled with two dimensional gas-chromatography-time of flight mass spectrometry (GCxGC-TOFMS) offers unique possibilities for fast and high-sensitive metabolite profiling and fingerprinting of volatile compounds of complex samples (Marriott *et al.,* 14*;* Karami *et al.,* 9 and Kim *et al*., 10). This study was undertaken to evaluate intensity and likability of fragrance of Cv. Pusa Mahak and its seed parent Century Two based on olfactory evaluation by an untrained panel and to profile the floral volatiles using headspace solid phase microextraction – 2D gas chromatography time of flight mass spectrometry.

MATERIALS AND METHODS

Two rose cultivars, cv. Pusa Mahak and cv. Century Two (Figure 1), maintained in the field of Division of Floriculture and Landscaping of ICAR-Indian Agricultural Research Institute, New Delhi were used for the study. Flowers were harvested during 4th–6th developmental stages as these stages exhibit maximum scent emission (Guterman *et al.,* 7), during morning hours between 8 to 10 a.m. in the month of January, 2018. Fresh flowers were taken

Fig. 1. Flowers of rose cultivars Pusa Mahak (L) and Century Two (R)

to the laboratory and kept in flower vase with water for olfactory analysis. The flowers were evaluated solely for fragrance. Fresh coded samples of cv. Pusa Mahak and Century Two were presented to a group of the untrained panelists (n=94) consisting of students and faculty of ICAR- IARI, New Delhi and ICAR-NBPGR, New Delhi. The panelists were asked to rate the intensity and their likability for fragrance. Each panelist was provided coffee to sniff before evaluating each cultivar in order to avoid the saturation caused by the fragrance of the previously evaluated cultivar. Scoring for intensity was done on a linear scale of 0 (no) to 10 (extremely fragrant) and likability was detected using a 5-point hedonic scale as 1; Liked very much, 2; Liked, 3; Neutral, 4; Disliked and 5. Disliked very much. Panelists were also asked to give remarks about the fragrance of each sample.

Biochemical analyses were carried out at National Referral Lab at NRC Grapes, Pune. Fresh samples of rose petals were harvested and transported to the lab same day and stored at -20°C till the samples were analysed (Sharma & Kumar, 15). Normal alkane standards, n-tetradecane (99.9% Purity), n-pentadecane (99.8% Purity) and n-heptadecane (99.5% Purity) from Sigma-Aldrich, Germany (now MERCK) were used for confirmation of retention time (RT) and calculation of retention index (RI)**.**

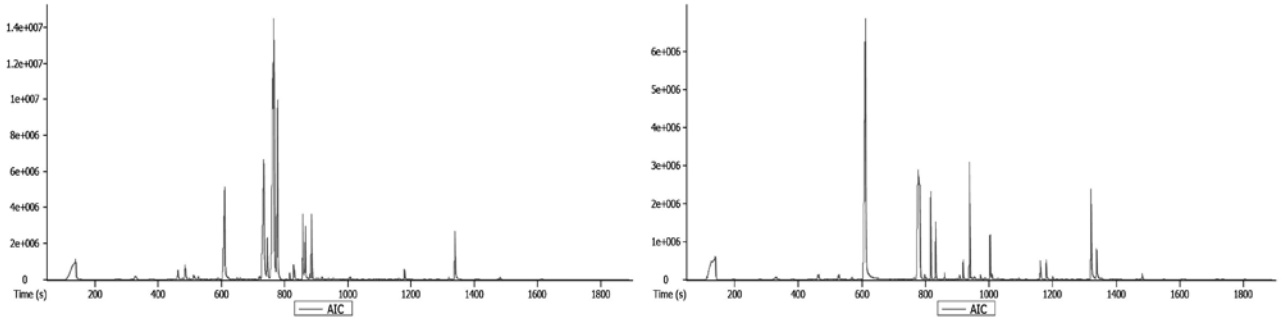
Extraction of the volatile compound was carried out through Headspace Solid Phase Micro Extraction (SPME). The petals were gently plucked, squeezed and mixed thoroughly. Approximately 2 g petal sample along with internal standards (30 µl each of n-tetradecane, n-pentadecane n-heptadecane) were immediately placed into 20 ml headspace vials sealed with a polypropylene screw-on cap with a PTFE/silicone septum (Supelco, Bellefonte, PA, USA). The vial was heated to 50°C for 30 min while being agitated. Sample incubation was done at 70ºC for 30 min. Volatile compounds were absorbed on 50 µm DVB/CAR/PDMS SPME fibre. Fibre was then inserted through the septum of headspace vial and exposed therein for 30 min. for the absorption of the volatile compound.

Gas chromatography analysis was carried out on Agilent 7890A GC × GC (Agilent Technologies, Palo Alto, CA, USA) coupled with a Pegasus 4D time-of-flight mass detector (TOF-MS) (Leco Corp., MI, USA). Helium was used as a carrier gas with a flow rate of 1.5 mL/min. A 50 µm DVB/CAR/PDMS fibre was used for absorption and injection of the compounds. The SPME fibre was introduced into the injector port of the gas chromatograph for 40 min in split-less mode, set at 240°C, in order to desorb the volatile compounds. The desorbed components were analysed in a polar to semi-polar manner, DB-5 capillary column (i.d. 0.25 mm, length: 30 m, film thickness = 0.25 µm) in the first dimension and coupled to a semi-polar Rxi-17Sil MS capillary column (i.d. 0.1 mm, length: 0.69 m, film thickness = 0.1 µm) as the second dimension. The primary GC oven temperature was programmed at 50°C isothermally for 1 minute, with ramping rate of 5°C/minup to 250°C (3 minute hold), whereas the temperature of the secondary oven was programmed from 60°C (1 minute hold), with a ramping rate of 5°C/ min, up to 270°C (3 minute hold). Finally, the temperature was increased to 220ºC at the rate of 3ºC /min and held for 3 min at the final temperature. Temperature modulation was carried out using a 60ºC offset temperature held for 1 minute and ramped to 300ºC at the rate of 3ºC / min (hold 3 min) and a 5 seconds modulation time (hot pulse 0.5 seconds and cold pulse 2.0 seconds) and -80ºC chiller temperature. The MS transfer line temperature was 250ºC. The detector voltage was set at -1750 eV. Data acquisition was carried out within the mass range of 50–650 m/z at the rate of 200 spectra/ seconds in 2-D mode with 70 eV electron ionization at 220ºC. LECO ChromaTOF™ software version 3.30 was used to operate the GC × GC-TOFMS system as well as for instrument control, data acquisition and processing. The components of headspace volatiles were identified as different peaks of the chromatogram (Fig. 2a, and 2b). The compounds with different mass to charge (m/z) ratio have different retention times and were identified based on the National Institute of Standards and Technology (NIST 2005) mass spectra library v2.0 and authentic standard compounds. The compound identity was confirmed based on the retention time, retention index (RI) with NIST (2005) library and the compounds with spectral matching value of >700 were taken for analysis.

RESULT AND DISCUSSION

Olfactory evaluation of cv. Pusa Mahak and its seed parent Century Two was carried out in order to understand the variations in the scent phenotype and differential preferences of the consumers. The judgment of the 94 panelists in terms of description of fragrance is summarized in Table 1. The scores for intensity as well as likability for two cultivars indicated that they differed in their fragrance profile. New cultivar Pusa Mahak was found to be more fragrant. The likability scores of 1.64 for cv. Pusa Mahak and 2.17 for Century Two indicated that the fragrance quality of cv. Pusa Mahak is preferred over its seed parent cv.Century Two.

The information on the aroma impressions of different scent molecules was obtained from published literature (Jirovetz *et al.,* 8) and the information system of the good scent company (www.thegoodscentcompany.com) as well as the chemical database of National Centre for Biotechnology Information (https://pubchem.ncbi.nlm.nih.gov). β-pinene is responsible for pine-like woody scent. Citronellol has an intense floral old rose-like fragrance with citrus nuances. 2-phenyl ethanol (2PE) is responsible for typical rose water like fragrance. Whereas 3, 5-dimethoxy toluene (3,5-DMT) gives typical tea aroma of hybrid tea roses (Baudino *et al.,* 4). 2- phenyl ethyl acetate has a weak floral rose-like fragrance. Geraniol has a mild sweet fruity rose-like fragrance. Theaspirane has herbal tea



a. Pusa Mahak **b. Century Two**

Fig. 2. Chromatogram showing headspace volatiles in (a) cv. Pusa Mahak and (b) cv. Century Two

Table 1. Mean values of olfactory attributes of cvs. Pusa Mahak and Century Two

S. No.	Cultivar	Pedigree	Intensity		Likeability		Type
			Score	Category	Score	Category	
1	Pusa Mahak	OPV of Century two (ICAR-IARI, 2015)	7.94	High to Very high fragrance	1.64	Liked	Sweet with woody and mild spicy nuances
2	Century Two	Charlotte Armstrong' × 'Duet' (David Armstrong, 1971)	5.36	Moderate fragrance	2.17	Liked	Damask/ rosy/ floral green herb like nuances

like fragrance with spicy nuances. Other than these major components, there are certain other minor compounds which contribute significantly despite being present in very small quantity. Of these minor compounds, the most important is rose oxide. Rose oxide gives floral rose-like fragrance with a geranium note.

Components of floral scents of the two cultivars were identified through gas chromatography and mass spectrometry (Figure 2) which detected a total of 114 volatile compounds (Table 2). Volatile organic compounds of rose fragrance are broadly classified into three groups based on their biosynthetic pathways *viz.,* terpenoids, phenylpropanoid/benzenoids and fatty acid derivatives. Groups of volatile compounds detected in Cv. Pusa Mahak and Century (Figure 3) indicated very distinct profiles of the cultivars.

Cv. Pusa Mahak exhibited differences within each goup of volatiles in comparison to cv. Century Two. Figure 4 depicts the comparison of major volatile compounds present in two cultivars. Total 82 compounds were identified in cv. Pusa Mahak. Out of these 82 compounds, 45 were terpenoid derivatives (55%), 16 were phenylpropanoid/benzenoid derivatives (20%) and 20 were fatty acid derivatives (25%). Major components with more than 5% of peak area response were β-pinene (20%), β-citronellol (14%), 2- phenyl ethyl acetate (9.5%), 2-phenyl ethanol (8.3%), 3, 5-Dimethoxytoluene (7.23%), geranial (7.23%) and α-myrcene (7%). Other than these, there were certain other compounds which were present in traces, which also contributed significantly in the fragrance quality of a rose flower. These were rose oxide and ionone. Rose oxide was detected but ionone was not detected in cv. Pusa Mahak. Cv. Pusa Mahak was found to be rich in terpenoid contents which may be responsible for the higher intensity and specificity of the fragrance

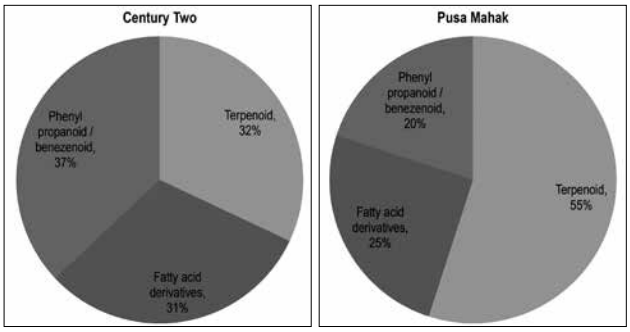


Fig. 3. Groupwise composition of fragrance compounds

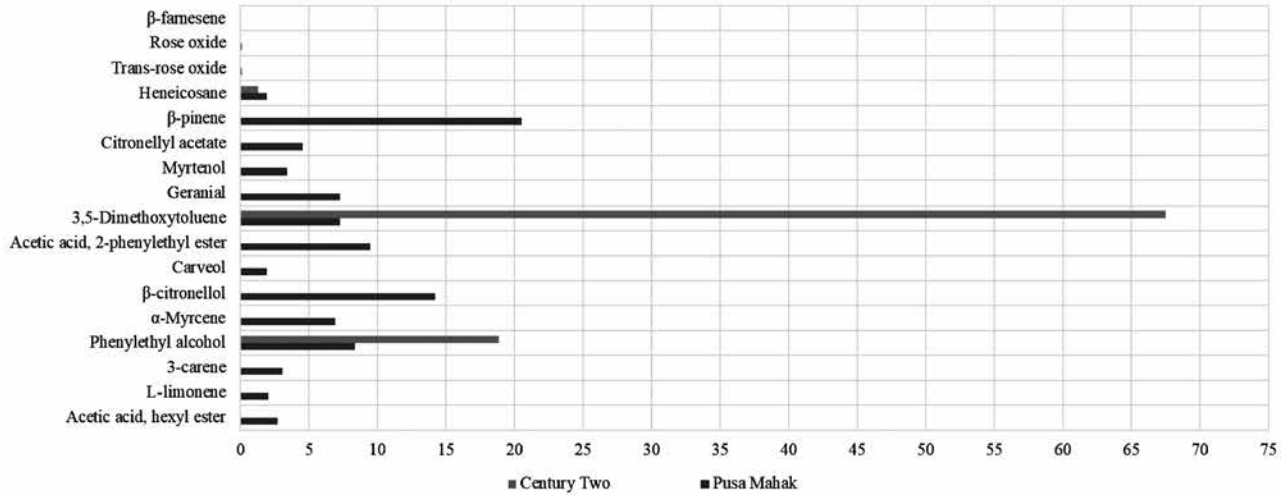


Fig. 4. Comparison of major volatile compounds identified in two rose cv. Pusa Mahak and its seed parent cv. Century Two

Table 2. Compounds identified in Headspace volatile of rose cv. Pusa Mahak and its seed parent cv. Century two

S. No.	Compound Name	Category	RI*	RT*	Per cent peak area detected	
					Pusa Mahak	Century Two
1	Hexanal	Fatty acid derivatives	704.37	270.5	0.0055	nd
2	2-Hexenal	Fatty acid derivatives	756.39	318.1	0.1741	nd
3	2-Hexen-1-ol, (E)-	Fatty acid derivatives	767.21	328	0.4426	nd
4	1-Hexanol	Fatty acid derivatives	770.93	331.4	nd	0.3955
5	Styrene	Phenylpropanoids/benzenoids	800.98	358.9	0.0010	nd
6	Amyl acetate	Fatty acid derivatives	820.22	376.5	0.0088	nd
7	Benzene, methoxy-	Phenylpropanoids/benzenoids	827.54	383.2	nd	0.0105
8	Benzene, (methoxymethyl)-	Phenylpropanoids/benzenoids	914.32	462.6	nd	0.2038
9	Furan, 2-pentyl-	Furon	915.08	463.3	0.3600	0.0859
10	Decane	Fatty acid derivatives	927.10	474.3	nd	0.0030
11	Acetic acid, hexyl ester	Fatty acid derivatives	940.11	486.2	2.7198	0.0125
12	2-Hexen-1-ol, acetate, (Z)-	Fatty acid derivatives	942.51	488.4	0.5247	nd
13	α-Phellandrene	Terpenoid	952.79	497.8	0.0154	nd
14	L-limonene	Terpenoid	968.31	512	2.0372	nd
15	Benzyl Alcohol	Phenylpropanoids/benzenoids	972.13	515.5	0.1508	0.0072
16	Benzeneacetaldehyde	Phenylpropanoids/benzenoids	985.25	527.5	0.1101	0.0629
17	Chrysanthemum oxide	Phenylpropanoids/benzenoids	1030.27	568.7	0.0008	0.0292
18	Terpinolene	Terpenoid	1038.25	576	0.0297	nd
19	γ-terpinene	Terpenoid	1038.36	576.1	0.0203	nd
20	Verbenone	Terpenoid	1041.75	579.2	0.0467	nd
21	Perillene	Terpenoid	1051.58	588.2	0.1235	0.0059
22	3-Carene	Terpenoid	1053.99	590.4	3.0574	nd
23	Pelargonaldehyde	Fatty acid derivatives	1058.91	594.9	0.0017	0.0011
24	Trans-Rose oxide	Terpenoid	1066.67	602	0.0761	nd
25	Rose oxide	Terpenoid	1070.49	605.5	0.0810	nd
26	Pentane, 2,2,3,4-tetramethyl-	Fatty acid derivatives	1072.46	607.3	nd	0.0088
27	Phenylethyl Alcohol	Phenylpropanoids/benzenoids	1079.45	613.7	8.3105	18.8588
28	m- cymene	Terpenoid	1082.08	616.1	0.0072	nd
29	Allo ocimene	Terpenoid	1087.21	620.8	0.0213	nd
30	Citronellal	Terpenoid	1116.28	647.4	0.0495	nd
31	Nerol oxide	Terpenoid	1117.60	648.6	0.0498	0.0034
32	4 Methoxy styrene	Phenylpropanoids/benzenoids	1121.09	651.8	0.0501	0.0130
33	2-Nonenal, (E)-	Fatty acid derivatives	1126.34	656.6	nd	0.0019
34	Benzyl acetate	Phenylpropanoids/benzenoids	1130.16	660.1	0.1173	nd
35	Ethyl benzoate	Phenylpropanoids/benzenoids	1141.86	670.8	0.0018	0.0008
36	Verbenol	Terpenoid	1149.62	677.9	0.0230	nd
37	Diisoamylene	Fatty acid derivatives	1164.37	691.4	nd	1.8081
38	Octanoic acid, ethyl ester	Fatty acid derivatives	1167.32	694.1	0.0149	0.0184
39	Dodecane	Fatty acid derivatives	1174.10	700.3	nd	0.0052
40	Cis-Isogeraniol	Terpenoid	1194.86	719.3	0.1993	nd

Contd...

Table 2 contd...

S. No.	Compound Name	Category	RI*	RT*	Per cent peak area detected	
					Pusa Mahak	Century Two
41	α-Myrcene	Terpenoid	1206.89	730.3	6.8971	nd
42	Citronellol	Terpenoid	1207.76	731.1	nd	0.0107
43	β-Citronellol	Terpenoid	1209.07	732.3	14.1672	nd
44	Carveol	Terpenoid	1221.97	744.1	1.8916	nd
45	β-ocimene(Z)	Terpenoid	1224.70	746.6	0.2165	nd
46	Geraniol	Terpenoid	1225.90	747.7	0.5931	nd
47	α-ionone	Terpenoid	1228.09	749.7	nd	0.0105
48	α-Pinene	Terpenoid	1235.41	756.4	0.3614	nd
49	Acetic acid, 2-phenylethyl ester	Phenylpropanoids/benzenoids	1243.39	763.7	9.4867	0.0535
50	Pelargonic acid	Fatty acid derivatives	1246.67	766.7	0.1286	nd
51	3,5-Dimethoxytoluene	Phenylpropanoids/benzenoids	1254.86	774.2	7.2322	67.4494
52	Geranial(E)	Terpenoid	1255.30	774.6	0.0117	nd
53	Geranial	Terpenoid	1259.13	778.1	7.2363	nd
54	Myrtenol	Terpenoid	1262.08	780.8	3.4000	nd
55	Ionone	Terpenoid	1278.14	795.5	nd	0.1031
56	2-Octanone	Fatty acid derivatives	1282.51	799.5	0.0035	nd
57	Tridecane	Fatty acid derivatives	1290.82	807.1	0.0148	0.0024
58	Theaspirane	Terpenoid	1299.89	815.4	0.5833	4.4629
59	Methyl geranate	Terpenoid	1314.10	828.4	0.3806	0.0442
60	α-Farnesene, (Z,E)-	Terpenoid	1325.14	838.5	0.0235	nd
61	P-Menth-1-en-9-al/ carvomentenal	Terpenoid	1339.02	851.2	nd	0.0021
62	Geranic acid	Terpenoid	1339.67	851.8	0.0025	nd
63	Citronellyl acetate	Terpenoid	1344.26	856	4.5148	nd
64	β-Pinene	Terpenoid	1353.66	864.6	20.4835	nd
65	Linalool	Terpenoid	1370.93	880.4	0.0843	nd
66	α-ocimene	Terpenoid	1374.86	884	0.1990	nd
67	Copaene	Terpenoid	1384.70	893	nd	0.0016
68	Biphenyl	Phenylpropanoids/benzenoids	1388.74	896.7	0.0014	0.0023
69	Ethyl geranate	Terpenoid	1392.35	900	0.0323	0.0193
70	2,6-Nonadienal, (E,Z)-	Fatty acid derivatives	1392.79	900.4	0.0127	nd
71	β-elemene	Terpenoid	1397.16	904.4	0.0030	nd
72	Germacrene A	Terpenoid	1397.60	904.8	0.0032	nd
73	Methyl eugenol	Phenylpropanoids/benzenoids	1399.89	906.9	0.1054	0.1494
74	3,4,5-trimethoxytoluene	Phenylpropanoids/benzenoids	1407.81	914.5	nd	0.0177
75	Diphenyl ether	Phenylpropanoids/benzenoids	1409.90	916.5	0.1029	0.0197
76	Phloroglucinol trimethyl ether	Phenylpropanoids/benzenoids	1411.56	918.1	0.1029	0.4229
77	α-Gurjunene	Terpenoid	1419.27	925.5	0.0065	nd
78	Caryophyllene	Terpenoid	1432.92	938.6	0.1038	1.9391
79	Dihydro-β- ionone	Terpenoid	1439.58	945	0.0107	0.0702

Contd...

Table 2 contd...

S. No.	Compound Name	Category	RI*	RT*	Per cent peak area detected	
					Pusa Mahak	Century Two
80	trans-à-Bergamotene	Terpenoid	1440.31	945.7	nd	0.0252
81	Isoamyl benzoate	Phenylpropanoids/benzenoids	1443.54	948.8	nd	0.0088
82	Dihydro-β-ionol	Terpenoid	1448.65	953.7	0.1260	0.0632
83	Patchoulane	Terpenoid	1448.75	953.8	0.0624	nd
84	β-Farnesene (Z)	Terpenoid	1454.69	959.5	nd	0.0509
85	β-cubebene	Terpenoid	1456.56	961.3	nd	0.0072
86	α-Caryophyllene	Terpenoid	1468.85	973.1	0.0154	0.0692
87	Rose butanoate	Phenylpropanoids/benzenoids	1489.90	993.3	nd	0.0009
88	Germacrene D	Terpenoid	1493.23	996.5	nd	0.0150
89	Phenethyl isovalerate	Phenylpropanoids/benzenoids	1494.79	998	nd	0.0022
90	Pentadecane	Fatty acid derivatives	1500.00	1003.3	0.0493	0.4061
91	Benzyl tiglate	Phenylpropanoids/benzenoids	1500.79	1004	0.0493	0.4071
92	α-Farnesene	Terpenoid	1505.11	1007.8	0.1281	0.0042
93	2,4-di-tert-butyl phenol	Phenylpropanoids/benzenoids	1506.02	1008.6	0.0769	nd
94	α- Cadinene	Phenylpropanoids/benzenoids	1506.24	1008.8	nd	0.0910
95	Ledene oxide-(II)	Terpenoid	1518.84	1019.9	0.0075	nd
96	Cis-Calamenene	Phenylpropanoids/benzenoids	1531.90	1031.4	nd	0.0007
97	1,4-Cadinadiene	Phenylpropanoids/benzenoids	1543.47	1041.6	nd	0.0034
98	Sesquirosefuran	Terpenoid	1550.17	1047.5	0.0047	nd
99	Carvenone	Terpenoid	1574.69	1069.1	nd	0.0019
100	6-Tetradecene, (E)-	Fatty acid derivatives	1582.29	1075.8	0.0028	nd
101	Benzoic acid, hexyl ester	Phenylpropanoids/benzenoids	1585.47	1078.6	nd	0.0047
102	Ethyl pergonate/wine ether	Fatty acid derivatives	1593.98	1086.1	0.0022	nd
103	Cetane	Fatty acid derivatives	1602.38	1093.5	nd	0.0422
104	Cyclodecene, (Z)-	Fatty acid derivatives	1671.62	1154.5	nd	0.0653
105	β-farnesene	Terpenoid	1694.78	1174.9	0.0020	0.0363
106	Heptadecane	Fatty acid derivatives	1700.00	1179.5	0.3001	0.8516
107	1,8-Nonadiene	Fatty acid derivatives	1739.95	1214.7	nd	0.0001
108	Phenethyl benzoate	Phenylpropanoids/benzenoids	1849.83	1311.5	nd	0.0001
109	Heneicosane	Fatty acid derivatives	1879.91	1338	1.9076	1.3154
110	Eicosane	Fatty acid derivatives	1963.45	1411.6	nd	0.1060
111	Octacosane	Fatty acid derivatives	2043.36	1482	0.0040	nd
112	Heptacosane	Fatty acid derivatives	2043.59	1482.2	0.0081	0.0181
113	Cycloundecene(Z)	Fatty acid derivatives	1898.07	1354	0.0242	0.0770
114	10-Heneicosene (c,t)	Fatty acid derivatives	2035.64	1475.2	nd	0.0096
					82	66

* RI: Retention Index, **RT: Retention time, nd: not detected

type of this cultivar. Olfactory evaluation of this cultivar indicated that it has sweet spicy aroma with woody nuances. This could be because of presence of monoterpene derivatives β pinene which is responsible for pine like aroma with woody nuances, β citronellol for sweet old rose fragarance, geraniol for spicy nuances. Other major compounds such as 2PE, 3, 5-DMT *etc.* and the presence of

rose oxide add to the sweet floral rose like aroma of this cultivar and making it more intense. For cv. Century Two, 66 compounds were identified with 21 terpenoid derivatives (32%), 24 phenylpropanoid/ benzenoid derivatives (37%) and 20 fatty acid derivatives (31%). Headspace volatile of Century Two was rich in phenylpropanoid/benzenoid derivatives. Major components with approximately 5% of peak area response were 3, 5-Dimethoxytoluene (67%), 2-phenyl ethanol (19%) and theaspirane (4.5%). Ionone was present in trace amount, however, rose oxide was not detected. As indicated earlier, 3 5-DMT is responsible for tea like aroma of roses. Its proportion was highest in Century Two. Another major compound theaspirane is also responsible for herbal tea like aroma with spicy nuances.

The olfactory evaluation indicated that the cultivar has rosy and herb like fragrance which could be attributed to the two major compounds *i.e.* 3 5-DMT and theaspirane while rosy impression can be attributed to 2PE and the ionone. Olfactory evaluation showed that cv. Pusa Mahak posseses an intense fragrance that the panellists found relatively more likeable compared to cv. Century Two. Volatile profiles of the two cultivars were also found to be contrasting. As many as 48 compounds present in cv. Pusa Mahak were completely undetected in cv. Century Two. Top ones included mainly Terpenoids viz. β-Pinene (signature), β-Citronellol, Geranial, α-Myrcene, Citronellyl acetate, Myrtenol, 3-Carene, L-limonene and Carveol. Similarly, profile of Century Two showed 32 unique compounds that were not detected in cv. Pusa Mahak. Top volatiles among them were Fatty acid derivatives viz. Diisoamylene (signature), 1-Hexanol and Eicosane. Further, out of top 10 volatiles (based on percent peak area) of cv. Pusa Mahak seven were completely absent in cv. Century Two. On the other hand, there was only one volatile among the top 10 compounds of cv. Century Two that was undetected in cv. Pusa Mahak. Two Phenylpropanoids/benzenoids 3,5-Dimethoxytoluene and 2-pheny ethanol were present in very high quantity (~10x) in Century Two compared to cv. Pusa Mahak. Olfactory results were supported by the observations in volatile profiles. Cv. Pusa Mahak was mostly rich in compounds with floral, pine-like woody, citrus and balsamic nuances with high odour strength. Contrastingly, Century Two was found to be rich in 3,5-DMT as the major component (67%), which is responsible for the herbal tea-like aroma. It also contains ionone in traces which may contribute to the fragrance quality of Century Two.

Fragrance is an outcome of the number and relative abundance of the floral volatiles. An untargeted assay may not be able to pinpoint the exact volatiles, individually and in combination, responsible for the fragrance but the results in this experiment identified definitite candidate molecules for further research. For instance, a thorough quantitative analysis of essential oil extracted from cv. Pusa Mahak can validate its potential in the fragrance industry.

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