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Original Research Paper

Biochemical characterization of gamma-ray induced mutants in mango

Perveen N.[1], Dinesh M.R.[1], Sankaran M.[1*] and Shivashankara K.S.[2]
[1]Division of Fruit Crops; [2]Division of Basic Sciences
ICAR-Indian Institute of Horticultural Research, Bengaluru - 560089, India.
*Corresponding author Email : m.sankaran@icar.gov.in

ABSTRACT

The volatile compound (VC) compositions of putative mutants were estimated and compared with the untreated seedlings and mother plants of mango genotype Bappakkai. Sesquiterpenes were the major VC detected in mother plants, control plants and putative mutant samples *viz.*, BM_4, BM_5 and BM_6, while, monoterpenes dominated the volatile fractions of other three putative mutant samples. A positive and significantly high correlation between all the mother plants, between mother plants and control seedlings as well as between the control seedlings, suggests a high level of similarity or lack of variability between mother plants and control seedlings, indicating that they might be of nucellar origin. Hence, we conclude that volatile profiling can be used as a biochemical marker for characterization and validation of putative mutants in polyembryonic mango genotypes.

Keywords : Gamma irradiation, HS-SPME-GC/MS, mango, monoterpenes, sesquiterpenes, volatile profiling

INTRODUCTION

Headspace solid-phase microextraction coupled with gas chromatography/mass spectrometry (HS-SPME-GC/MS) is a method of choice for comprehensive analysis of volatile compounds (Shimizu et al., 2021). Leaf or fruits volatile organic compounds has extensively been used for investigating varietal differences in fig (Oliveira et al., 2010), mango (Shimizu et al., 2021), avocado (Ali et al., 2020) and apple (Roberts & Spadafora, 2020). The aromatic volatile profile of mango is dominated by monoterpenes, sesquiterpenes, esters, lactones, alcohols, aldehydes, ketones, volatile fatty acids and some carotenoid compounds (Pandit et al., 2009; Shimizu et al., 2021). Polyembryony is a peculiar trait in certain mango genotypes wherein multiple apomictic embryos develop from the maternal nucellar tissues along with a single zygotic embryo with the plants originating from apomictic embryos being identical to the mother plants.

Induced mutations have widely been used for widening the genetic base of mango (Rime et al., 2019; Perveen et al., 2022). Gamma irradiation has been reported to alter the concentration and composition of volatile compounds in rose (Ryu et al., 2020) and pistachio nut (Alinezhad et al., 2021) etc., and is emerging as a useful technique in characterization and validation of putative mutants. In the present study, an attempt was made to use volatile organic compounds as biochemical marker for validation of putative mutant progenies of polyembryonic mango genotype Bappakkai developed through gamma irradiation. This study was based on the hypothesis that any variation observed in the seedlings emerging from gamma irradiation treated kernels could be considered to have been resulted from mutation, while, the seedlings emerging from untreated kernels (control) should be similar to the mother plants owing to the nucellar origin of these plants.

MATERIALS AND METHODS

Generation of putative mutants

Fully matured fruits were collected from 30 years old genotype Bappakkai mother plants (BMP) being maintained in the field genebank of ICAR-Indian Institute of Horticultural Research, Bengaluru, for imposing irradiation treatment. The seed kernels were gamma irradiated with five doses (Gy) *viz.*, 15, 20, 25, 30 and 35. After 2-3 months of germination, seedlings emerging from each seed kernel were separated and transplanted in new polybags. Six months after germination, only one to two seedlings survived per seed, which were considered to be nucellar in origin due to the vigour taking cognisance of the previous study (Srivastava et al., 1988). On the basis of coefficient of variability, 30 Gy irradiation treatment was found to result in maximum morphological diversity (Perveen, 2022) which were used for the present study.

Extraction and analysis of organic volatile compounds

Recently matured leaves from mother plants, selected putative mutants (10 months old) and untreated (control) plants (10 months old) of polyembryonic mango genotype Bappakkai were collected, flash frozen in liquid nitrogen and brought to laboratory for further analysis. A total of 18 control seedlings were divided into three samples, *viz.*, BC_1, BC_2, and BC_3, each sample consisting of leaves from six seedlings. A total of 18 putative mutants were divided into six samples, each consisting of leaves from three putative mutant seedlings (BM_1, BM_2, BM_3, BM_4, BM_5 and BM_6). Volatile organic compounds were extracted using headspace-solid phase micro-extraction (HS-SPME) technique and identified with gas chromatography–mass spectrometry (GC-MS). Five-gram powdered leaf sample was transferred to 150 mL conical flask having a magnetic stirrer and the mouth of conical flask was sealed with a silicon stopper. A pre-conditioned SPME fibre was exposed to the sample headspace for 2 hours for adsorption of volatiles. The volatile compounds were desorbed from the fibre for 10 minutes in the injector (250°C) of a gas chromatograph, Varian-3800 gas chromatograph coupled with Varian 4000 GC-MS-MS ion trap mass selective detector.

Statistical analysis

The total chromatogram for each sample was obtained by adding all the GC peak areas and each volatile compound was expressed as relative per cent area. The volatile compounds were identified by their retention times with references to standard compounds and the obtained mass spectra were compared with the spectra available in the Wiley and NIST-2007 libraries. After applying squared Euclidian cluster analysis to all of the characters' means, Ward's approach was used to create a dendrogram (Rencher, 1995). Principal components analysis (PCA) was then performed on the correlation matrix (SAS, 2012).

RESULTS AND DISCUSSION

Volatile compounds are low-molecular-weight, organic compounds, involved in plant defence against insects, adaption to abiotic stress and confers aroma and flavour to fruits (Vivaldo et al., 2017). In mango, volatile compounds have been used a biochemical marker for germplasm characterisation (Li et al., 2017; Shimizu et al., 2021) and investigating susceptibility to mango gall fly (Augustyn *et al.,* 2010). Analysis of leaf volatile compounds of Bappakkai allowed to differentiate between gamma irradiated and untreated plants. In the present study, monoterpenes and sesquiterpenes were found to be the major volatile compounds in all the samples. Earlier reports have also confirmed terpenes to be the most abundant volatile constituent of mango (Pino et al., 2005; Li et al., 2017). Sesquiterpenes were the major volatile compounds detected in mother plants as well as control seedlings, while, monoterpenes dominated the volatile fractions of three putative mutant samples (BM_1, BM_2 and BM_3) and sesquiterpenes were the most abundant volatile constituent in the remaining three putative mutants BM_4, BM_5 and BM_6 (Table 1). In general, the concentration of monoterpenes was more than sesquiterpene in all the samples except putative mutant seedling BM_4, where sesquiterpenes dominated the overall volatile profile being 59.14%. Beta-Selinene, was the most abundant volatile constituent (>30%) in the leaf samples of mother plants as well as control seedlings (Table 1). β-Pinene was detected in all the mother plants and control seedling while among the putative mutants it was present only in BM_4.

Among all the samples, BM_4 had the highest amount of sesquiterpenes (86.70%). Pandit et al. (2009) for the first time reported sesquiterpene dominated mango cultivars all of which were of Indian origin. In the selected putative mutant seedlings, the number of monoterpenes and sesquiterpenes was more as compared to mother plants and control seedlings. An increase in the number of volatile compounds with gamma irradiation has previously been reported in rose (Ryu et al., 2020) and pistachio nut (Alinezhad et al., 2021).

Correlation analysis

A positive and significantly high correlation was observed between all the mother plants, between mother plants and control seedlings as well as between the control seedlings. This suggests a high level of similarity or lack of variability between mother plants and control seedlings, indicating that they might be of nucellar origin. Further, considerable amount of variability was observed among the selected putative mutants (Table 2).

Table 1 : Volatile composition (relative percentage) of leaf samples of mother plants, control seedlings and selected putative mutants of genotype Bappakkai

Compound	Mother plant			Control			Mutants					
	BMP$_1$	BMP$_2$	BMP$_3$	BC$_1$	BC$_2$	BC$_3$	BM$_1$	BM$_2$	BM$_3$	BM$_4$	BM$_5$	BM$_6$
Alcohols												
1-Hexyn-3-ol	ND	ND	ND	ND	ND	ND	0.25	ND	0.04	ND	ND	ND
Aldehydes												
trans-2-Hexenal	0.54	0.44	0.85	0.44	0.53	0.33	ND	ND	ND	ND	0.99	ND
Mono-terpenoids												
α-Thujene	ND	ND	ND	ND	ND	ND	0.21	ND	0.14	ND	ND	ND
α-Pinene	6.19	4.98	5.84	7.72	13.17	10.47	31.31	8.75	34.14	0.94	2.17	4.01
Camphene	ND	ND	ND	ND	ND	ND	0.45	ND	ND	ND	ND	ND
Sabinene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.72	0.26
trans-Ocimene	0.83	0.56	0.33	0.25	1.00	0.32	1.02	0.71	3.17	0.00	1.04	0.11
beta-Ocimene	ND	ND	ND	ND	ND	ND	2.45	1.10	2.38	ND	ND	ND
β-Pinene	0.90	0.61	0.57	0.41	1.50	4.35	ND	ND	ND	0.25	ND	ND
I-Phellandrene	7.11	17.66	20.43	11.76	9.18	10.22	21.25	18.82	31.22	0.00	0.00	5.34
α-Terpinene	ND	ND	ND	ND	ND	ND	1.63	ND	ND	ND	ND	ND
beta-Phellandrene	ND	ND	ND	ND	ND	ND	ND	1.17	ND	8.34	ND	ND
dl Limonene	14.07	ND	0.03	14.11	26.00	12.91	27.70	9.90	22.76	0.00	36.07	7.90
Cis-Ocimene	ND	ND	ND	ND	ND	ND	0.40	ND	ND	ND	ND	ND
trans-Ocimene	ND	ND	ND	ND	ND	ND	0.06	ND	ND	ND	ND	ND
γ-Terpinene	0.84	0.23	ND	ND	ND	ND	0.49	0.21	0.68	ND	ND	ND
α-Terpinolene	ND	ND	ND	ND	ND	ND	0.26	36.77	0.24	ND	ND	ND
Allo-Ocimene	ND	ND	ND	ND	ND	ND	0.12	ND	0.20	ND	ND	ND
Total	29.94	24.05	27.20	34.25	50.86	38.28	87.36	77.43	94.92	9.54	40.00	17.62
Sesqui-terpenoids												
alpha-Gurjunene	14.65	14.94	11.89	12.70	9.80	15.04	6.62	1.85	0.15	ND	18.47	17.41
alpha-Cubebene	ND	ND	ND	ND	ND	ND	ND	0.42	0.35	ND	ND	ND
trans-Caryophyllene	ND	9.99	13.90	5.92	7.37	6.45	2.40	1.10	0.21	22.90	7.01	6.49
Aromadendrene	ND	ND	ND	ND	ND	ND	0.03	ND	0.07	ND	ND	ND
β-Elemene	ND	ND	ND	ND	ND	ND	ND	ND	0.86	20.29	ND	ND
beta-Cadinene	ND	1.50	1.08	0.24	2.10	1.70	0.40	0.79	1.02	1.16	1.10	2.42
delta-Cadinene	ND	ND	ND	ND	ND	ND	0.05	0.07	0.13	ND	ND	ND
alpha-Copaene	ND	ND	ND	20.46	8.99	16.27	0.13	17.65	ND	ND	23.71	15.09
alpha-Humulene	16.01	7.65	13.98	4.52	5.56	3.39	0.57	ND	ND	12.96	4.36	4.73
beta-Selinene	39.39	41.44	32.00	21.91	15.33	19.19	1.89	ND	ND	29.39	3.68	31.37
alpha-Lonipinene	ND	ND	ND	ND	ND	ND	0.05	0.07	1.52	ND	0.67	2.57
Total	70.06	75.51	72.85	65.75	49.14	62.05	12.14	21.95	4.30	86.70	59.01	80.08

ND: not detected

Table 2 : Pearson correlation matrix of volatile profile for Bappakkai

Sample	BMP$_1$	BMP$_2$	BMP$_3$	BC$_1$	BC$_2$	BC$_3$	BM$_1$	BM$_2$	BM$_3$	BM$_4$	BM$_5$	BM$_6$
BMP$_1$	1											
BMP$_2$	0.884**	1										
BMP$_3$	0.833**	0.964**	1									
BC$_1$	0.740**	0.710**	0.688**	1								
BC$_2$	0.698**	0.538**	0.548**	0.846**	1							
BC$_3$	0.740**	0.715**	0.691**	0.977**	0.869**	1						
BM$_1$	0.317^{NS}	0.222^{NS}	0.280^{NS}	0.479**	0.769**	0.544**	1					
BM$_2$	0.039^{NS}	0.069^{NS}	0.107^{NS}	0.352*	0.305^{NS}	0.324^{NS}	0.359*	1				
BM$_3$	0.217^{NS}	0.198^{NS}	0.277^{NS}	0.393*	0.634**	0.442**	0.960**	0.396*	1			
BM$_4$	0.575**	0.651**	0.666**	0.383*	0.298^{NS}	0.361*	-0.069^{NS}	-0.125^{NS}	-0.106^{NS}	1		
BM$_5$	0.360*	0.128^{NS}	0.124^{NS}	0.703**	0.797**	0.696**	0.471**	0.285^{NS}	0.285^{NS}	0.027^{NS}	1	
BM$_6$	0.859**	0.855**	0.779**	0.908**	0.696**	0.898**	0.224^{NS}	0.148^{NS}	0.118^{NS}	0.546**	0.520**	1

Cluster analysis

Volatile composition of different samples was used to generate a dendogram for understanding the relationship between them (Fig. 1). The studied samples were divided into two main clusters with three sub-clusters in the first cluster. Cluster 1 comprised of nine samples including mother plants, control and three putative mutants. Out of these, samples BMP_1, BMP_2, BMP_3, BC_1, BC_2 and BC_3 were present in the first sub-cluster, the second sub-cluster comprised of two putative mutants *viz.*, BM_5 and BM_6, while, putative mutant BM_4 formed the solitary member of sub-cluster 3. Cluster 2 comprised of putative mutants BM_1, BM_2 and BM_3 suggesting that these three putative mutants are more distant from mother plants and control seedlings as compared to the other three putative mutants (BM_4, BM_5 and BM_6).

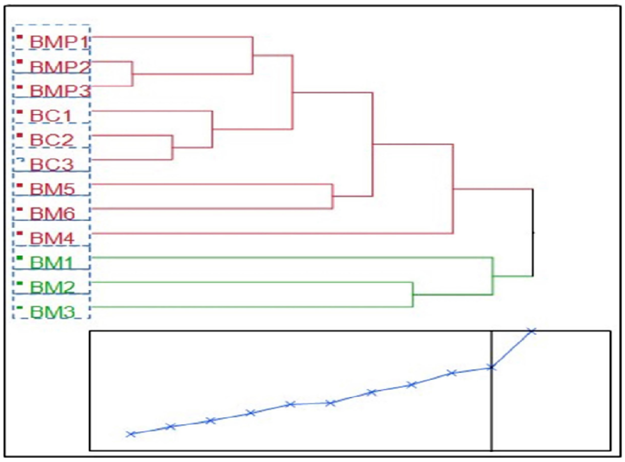

Fig. 1 : Cluster analysis of Bappakkai mother plants (BMP_1, BMP_2 and BMP_3), control sample (BC_1, BC_2 and BC_3) and selected putative mutant samples (BM_1, BM_2, BM_3, BM_4, BM_5 and BM_6)

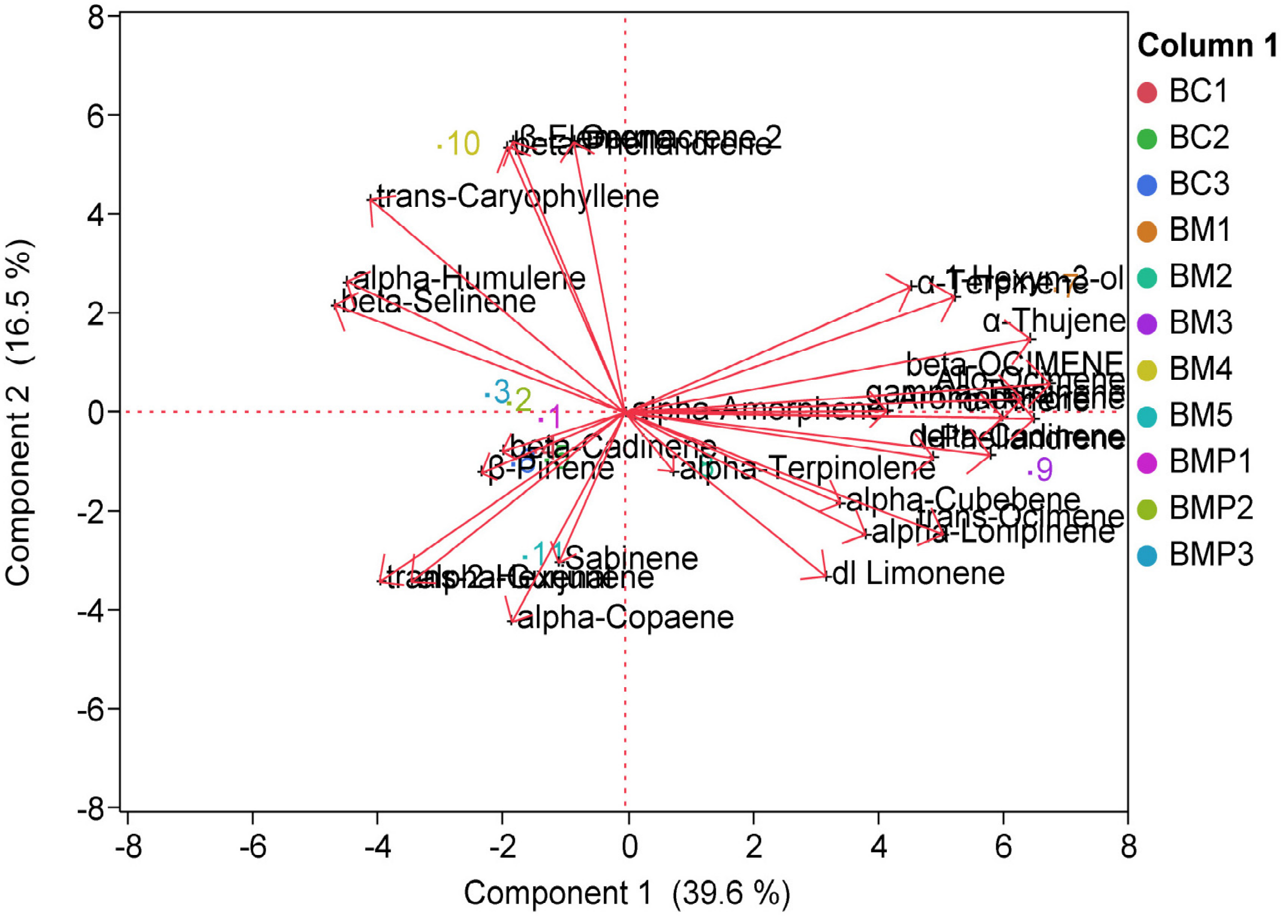

Fig. 2 : Two-dimensional bi-plot of individual VC in Bappakkai mother plants (BMP_1, BMP_2 and BMP_3), control sample (BC_1, BC_2 and BC_3) and selected putative mutant samples (BM_1, BM_2, BM_3, BM_4, BM_5 and BM_6)

Principal component analysis (PCA)

PCA results indicated that the total variability is being explained by 29 principal components out of which loading of more than 0.50 was considered significant for each factor. The first 2 principal components which collectively explained 56.14% of the total variability was depicted as two-dimensional biplot (Fig. 2). Out of these 39.63% of total variation was explained by the first principal component which had an eigenvalue of 11.89. Seventeen volatile compounds with loading (>0.5) significantly contributed to the variation in this Principal component (Table 3). Among these, 14 volatile compounds showed positive loadings wherein highest positive loading (>0.8) was exhibited by monoterpenes *viz.*, alpha-Thujene, alpha-Pinene, beta-Ocimene and Allo-Ocimene.

Table 3 : Loadings, eigen values and per cent of cumulative variance for the first two principal components

Parameter	Component	
	1	2
1-Hexyn-3-ol	0.764	0.340
trans-2-Hexenal	-0.569	-0.496
α-Thujene	0.941	0.215
α-Pinene	0.949	-
Camphene	0.662	0.368
Sabinene	-	-0.440
trans-Ocimene	0.738	-0.356
beta-Ocimene	0.986	-
β-Pinene	-0.333	-
I-Phellandrene	0.716	-
α-Terpinene	0.662	0.368
beta-Phellandrene	-	0.779
dl Limonene	0.466	-0.480
Cis-Ocimene	0.662	0.368
trans-Ocimene	0.662	0.368
γ-Terpinene	0.607	-
α-Terpinolene	-	-
Allo-Ocimene	0.921	-
alpha-Gurjunene	-0.496	-0.496
alpha-Cubebene	0.500	-
trans-Caryophyllene	-0.591	0.625
Aromadendrene	0.876	-
β-Elemene	-	0.795
alpha-Humulene	-0.645	0.384
beta-Selinene	-0.674	0.315
Eigen values	11.890	4.952
Cumulative variance (%)	39.634	56.141

CONCLUSION

Volatile profiling allowed us to differentiate between gamma irradiated and untreated plants of genotype Bappakkai. Sesquiterpenes were the major volatile compounds detected in mother plants, control plants and putative mutant seedlings, BM_4, BM_5 and BM_6 while monoterpenes dominated the volatile fractions of putative mutant seedlings, BM_1, BM_2 and BM_3. Further, the number and concentration of volatile components was found to remarkably increase with irradiation. A positive and significantly high correlation between all the mother plants, between mother plants and control seedlings as well as between the control seedlings suggests a high level of similarity or lack of variability between mother plants and control seedlings, indicating that they might be of nucellar origin. Hence, study conclude that volatile profiling can be used as a biochemical marker for characterization and validation of putative mutants in polyembryonic mango genotypes. However, further study is needed for confirmation of volatile profile as biochemical marker for mutants by testing it on a sizable progeny of genotype Bappakai and other ployembryonic mango cultivars.

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