# Comparative Analysis of Volatile Compounds of Gamma-Irradiated Mutants of Rose (*Rosa hybrida*)

**Jaihyunk Ryu, Dong-Gun Kim, Jung-Min Kim, Jae Il Lyu, Yeong Deuk Jo, Si-Yong Kang, Jin-Baek Kim, Sang Hoon Kim***

Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, Jeongeup-si, Jeollabuk-do 56212, Republic of Korea; jhryu@kaeri.re.kr (J.R); jaeil@kaeri.re.kr (J.I.L); dgkim@kaeri.re.kr (D.-G.K); jmkim0803@kaeri.re.kr (J.-M.K), jaeil@kaeri.re.kr (J.I.L), jyd@kaeri.re.kr (Y.D.J), sykang@kaeri.re.kr (S.-Y.K), jbkim74@kaeri.re.kr (J.-B.K), shkim80@kaeri.re.kr (S.H.K)

*Correspondence: shkim80@kaeri.re.kr (S.H.K); Tel.: +82-63-570-3318

Received: date; Accepted: date; Published: date

**Abstract:** Roses are one of the most important floricultural crops, and their essential oils have long been used for cosmetics and aromatherapy. We investigated the volatile compound compositions of 12 flower-color mutant variants and their original cultivars. Twelve rose mutant genotypes were developed by treatment with 70 Gy of $^{60}$Co gamma irradiation of six commercial rose cultivars. Essential oils from the flowers of the 18 genotypes were analyzed by gas chromatography–mass spectrometry. Seventy-seven volatile compounds were detected, which were categorized into five classes: hydrocarbons, terpenoids, alcohols, esters, and others. Hydrocarbons, alcohols, and esters were major components in all rose flowers. The mutant genotypes CR-S8 and CR-S9 showed higher contents of hydrocarbons than the original cultivar. In addition, CR-S1, CR-S3, and CR-S4 mutant genotypes showed higher ester contents than their original cultivar. Nonacosane, 2-methylhexacosane, and 2-methyltricosane were major volatile compounds among all genotypes. Hierarchical cluster analysis of the rose genotypes gave four groups according to grouping among the 77 volatile compounds. These findings will be useful for the selection of rose genotypes with improved volatile compounds.

Keywords: Gamma-ray, Mutant, Rose, Volatile compounds, GC-MS

## 1. Introduction

The rose belongs to the Rosaceae family, including about 200 species distributed throughout the world [1,2]. Roses are an important floricultural crop and are the most popular cut flowers because of

their various floral colors and shapes [3]. Flower color is the major horticultural characteristic of rose cultivars [3,4]. A broad range of floral colors are now available in the rose after many years of cultivar development, and many studies have investigated the contributions of rose pigments [5,6,7]. Rose flower colors are caused by the presence of pigments such as anthocyanins (cyanidin, peonidin and pelargonidin), flavonols (quercetin and kaempferol), and carotenoids (xanthophylls) [1,4,5,6,7].

Rose cultivars have developed characteristics such as long vase life, novel flower shape and color, disease tolerance, and fragrance [2,3]. Recently, many countries have made efforts to develop their own rose cultivar to produce high-quality fragrances [1,2,8]. Although wide variability in the flower has been generated through hybridization, the floriculture industry relies on a limited number of mutated traits in accordance with specific consumer expectations [3,8,9,10]. Mutation breeding involves the use of various mutagens to develop plants that exhibit a few mutated characteristics without disturbing the other characteristics of original cultivar [10,11]. Novel rose genotypes generated by radiation mutagenesis and showing improved flower traits have been developed using mutation breeding techniques [10,11,12]. In our previous work, which explored diverse variations in color and number of petals, mutations were generated by gamma-rays in three spray-type ('Lovelydia', 'Yellowbabe', and 'Haetsal') and two standard-type ('Vital' and 'Aqua') rose cultivars [12].

Rose products are in high demand throughout the world. In particular, rose essential oils have wide applications in food, cosmetics, and pharmaceuticals [1,2,13]. Although there are about 18,000 rose cultivars, only a few of them are used for fragrance applications [14,15,16]. Volatile compounds are important contributors to fragrance in roses and other flowers [16]. The chemical characterization of volatile compounds in flowers is of paramount importance for the identification of novel materials that have potential for industrial use [17]. In addition, the volatile compounds of rose are important substances in therapeutic applications because of a range of bioactivities. Rose flowers emit a strong fragrance and various volatile compounds such as hydrocarbons, alcohols, and esters have been identified in rose flower extracts [2,13,15,16,18]. These volatile compounds were found to have diverse biological activities such as antioxidant, antibacterial, antitumor, antiulcer, anti-inflammatory, and anti-parkinsonism activities; they can also decrease blood cholesterol and can be used as emollients and surfactants [16,19,20,21].

The objective of the present study was to investigate and compare the volatile compounds of rose mutant genotypes and those of original cultivars to help determine which cultivars would be suitable for the fragrance industry.

.

## 2. Results

### *2.1. Flower morphological characteristics*

The evaluation of flower morphological characteristics is presented in Table 1 and Figure 1. Mutant cultivar CR-S7, which produces white pink petals, was developed from the 'Aqua' cultivar (red-purple petal). Mutant 'CR-S2' was derived from the black red 'Blackbeauty' cultivar, and showed black pink petals with white mosaic. Mutant CR-S5 had ivory petals, while its original cultivar had pale pink petals. The mutants CR-S1, CR-S3, and CR-S4, which showed orange red or red petals, were derived from the 'Vital' cultivar (cherry red). The CR-S6 mutant with orange petals was developed from the 'Yellowbabe' cultivar. Five mutant genotypes were derived from the 'Lovelydia' cultivar: two showed changes in petal numbers and three showed changes in petal colors.


**Figure 1.** Profile of rose mutant genotypes used in this study. 1: Aqua, 2: CR-S7 (Aqua mutant), 3: Blackbeauty, 4: CR-S2 (Blackbeauty mutant), 5: Haetsal, 6: CR-S5 (Haetsal mutant), 7: Vital, 8: CR-S1 (Vital mutant), 9: CR-S3 (Vital mutant), 10: CR-S4 (Vital mutant), 11: Yellowbabe, 12: CR-S6 (Yellowbabe mutant), 13: Lovelydia, 14: CR-S8 (Lovelydia mutant), 15: CR-S9 (Lovelydia mutant), 16: CR-S10 (Lovelydia mutant), 17: CR-S11 (Lovelydia mutant), 18: CR-S12 (Lovelydia mutant).

**Table 1.** Origin and morphological characteristics of Rose genotypes used in this study.

| No. | Names | Flower color | Petal numbers | Original cultivars |
|---|---|---|---|---|
| 1 | Aqua | Red-purple (N62A*) | Normal** | |
| 2 | CR-S7 | White pink (N62D) | Low | Aqua |
| 3 | Blackbeauty | Black red (N46B) | Normal | |
| 4 | CR-S2 | Black pink (N46B) with white mosaic | Normal | Blackbeauty |
| 5 | Haetsal | Pale pink (N49C) | Normal | |
| 6 | CR-S5 | Ivory (N11D) | Normal | Haetsal |
| 7 | Vital | Cherry red (N41B) | Normal | |

| | | | | |
|---|---|---|---|---|
| 8 | CR-S1 | Orang red (N30C) | Normal | Vital |
| 9 | CR-S3 | Orang red (N32B) | Normal | Vital |
| 10 | CR-S4 | Red (N41A) | Normal | Vital |
| 11 | Yellowbabe | Yellow (N9A) | Normal | |
| 12 | CR-S6 | Orange (N25B) | Normal | Yellowbabe |
| 13 | Lovelydia | Red purple (N74B) | Normal | |
| 14 | CR-S8 | Red purple (N74D) | Normal | Lovelydia |
| 15 | CR-S9 | Red purple (N74B) | High | Lovelydia |
| 16 | CR-S10 | Light beige (N36C) | Normal | Lovelydia |
| 17 | CR-S11 | Orange purple (N68C) | Normal | Lovelydia |
| 18 | CR-S12 | Red purple with white mosaic (N74C) | High | Lovelydia |

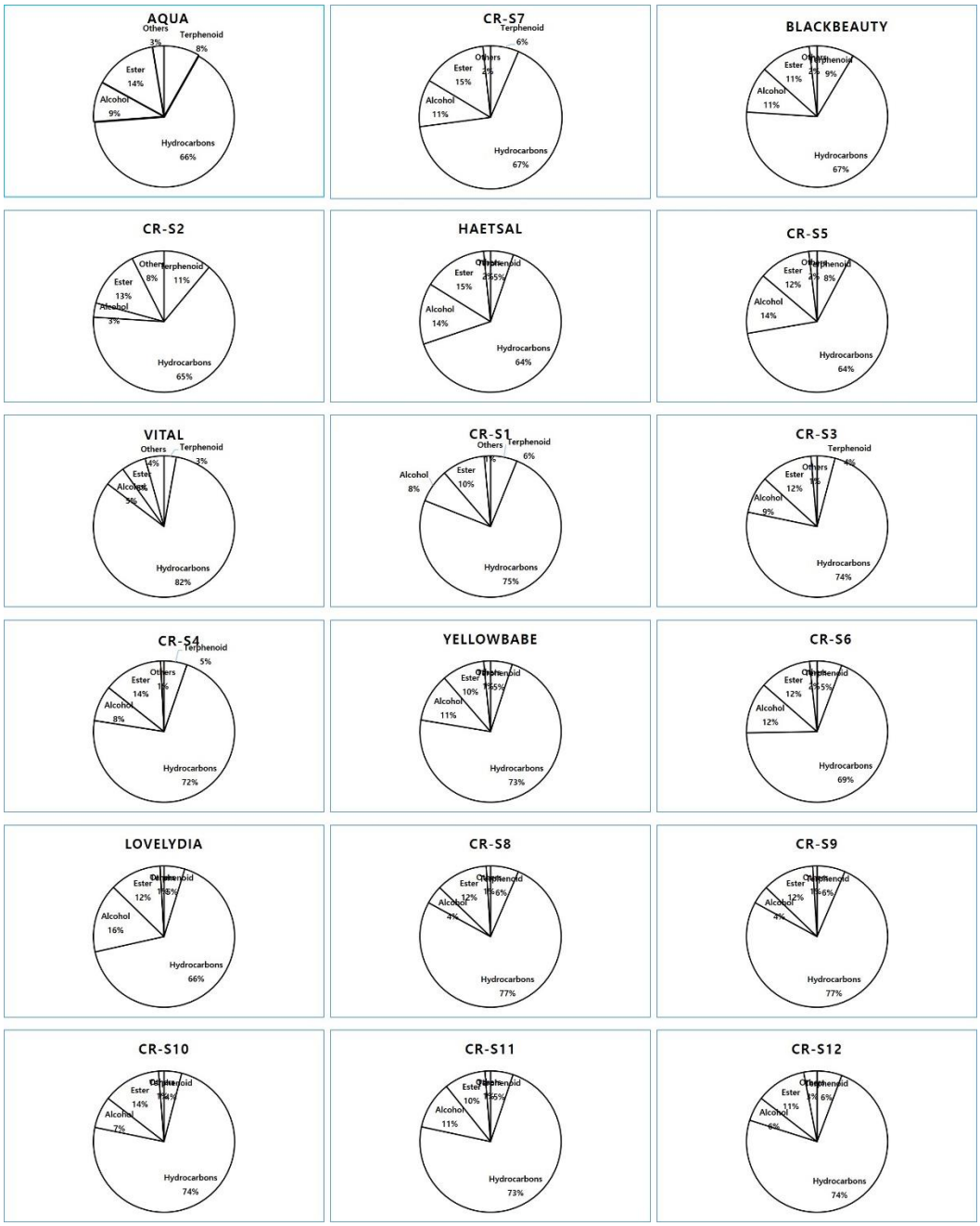
*The royal horticultural society's colour chart numbers, **Low: Under twenty, Normal: 21~40, High: over forty

*2.2 Analysis of volatile compounds*

The volatile compound constituents of the 12 rose mutants and the original cultivars were determined by gas chromatography–mass spectrometry (GC–MS). The distribution of identified volatile compounds observed from the RTX-5MS column is shown in Table S1. Seventy-seven volatile compounds were detected in the rose mutants and the original cultivars. For individual cultivars, the number of volatile compounds ranged from 40 (CR-S10) to 50 (CR-S2 and CR-S12) with an average of 47.6.

Figure 2 shows that the volatile compounds belonged to five classes: hydrocarbons, terpenoids, alcohols, esters, and others. Hydrocarbons were the dominant volatile compound category present in all rose flowers. The content of hydrocarbons in the volatiles for all rose genotypes ranged from 64.54% to 82.50% with an average value of 71.22%; the highest hydrocarbon was found in the 'Vital' cultivar. The contents of hydrocarbons in the volatiles of the CR-S8 and CR-S9 mutants were significantly higher than in the original 'Lovely Lydia' cultivar. In contrast, the hydrocarbon contents in the volatiles of CR-S1, CR-S3, and CR-S4 mutants were lower than those of the original 'Vital' cultivar. The ester contents of the all the genotypes ranged from 5.60% to 15.78% with the highest composition observed in the CR-S7 mutant. The highest alcohol content (15.78%) was observed in the 'Lovely Lydia' cultivar and the lowest alcohol content (3.26%) occurred in the CR-S2 mutant. The CR-S2 mutant had the highest terpenoid content (11.04%) while the 'Vital' cultivar had the lowest (2.80). The CR-S7 mutant showed similar constituent contents of volatile compounds when compared with the original 'Aqua' cultivar. Similar comparisons were observed for mutant CR-S5 and its original 'Haetsal' cultivar and for CR-S6 and its original 'Yellowbabe' cultivar. Mutant CR-S2 showed higher terpenoid content than the original

106 'Blackbeauty' cultivar. Mutants CR-S1, CR-S3, and CR-S4 showed higher alcohol and ester contents
107 than the original 'Vital' cultivar. Mutants CR-S8, CR-S9, CR-S10, CR-S11, and CR-S12 had higher
108 hydrocarbon contents and lower alcohol contents in the volatiles than the original 'Lovely Lydia'
109 cultivar.



110

111 **Figure 1.** Relative composition of volatile compounds released from the flower of rose mutant and those of
112 original cultivars.
113

114 Table 2 presents the ten most abundant volatile compounds detected in the GC–MS analysis of the
115 12 rose mutants and the original cultivars. The results revealed significant differences in the rose

genotypes. Nonacosane was a dominant compound in most cultivars; its highest content (18.7%) was observed in the CR-S12 mutant and the lowest (4.4%) in the 'Haetsal' cultivar. The content of 2-methylhexacosane for all rose genotypes ranged from 14.0% for 'Aqua' to 22.8% for CR-S9, with average content of 18.2%. The 2-methyltricosane contents for all rose genotypes ranged from 8.0% for CR-S6 and CR-S8 to 20.3% for the 'Vital' cultivar, with an average value of 11.4%. The tricosane contents for all genotypes ranged from 4.4% to 19.6% with the highest rate observed in the 'Vital' cultivar. Hentriacontane was found top ten volatile compounds in all cultivars except for the 'Vital' cultivar, with contents ranging from 2.8% for 'Yellowbabe' to 8.7% for CR-S8. Tetracosyl pentafluoropropionate was not observed top ten volatile compounds in mutant CR-S2 and the 'Vital' cultivar, but was found in the remaining 16 genotypes at levels between 1.8% and 6.8%. Heneicosane was observed top ten volatile compounds in 14 genotypes at levels between 2.0% and 7.6%, but was not observed in 'Aqua', CR-S7, CR-S8, and CR-S12. Octacosanol was observed top ten volatile compounds in 16 rose genotypes at levels ranging from 1.8% for CR-S12 to 6.8% for the 'Blackbeauty' cultivar, with an average content of 3.7%. Analyses for 'Vital' and CR-S2 did not show octacosanol in the top ten volatile compounds. 2-Octyl-1-decanol was listed top ten volatile compounds in 6 genotypes. Octacosane was recorded top ten volatile compounds in 9 genotypes. Hexacosane was observed top ten volatile compounds in the 'Vital' cultivar and the CR-S3 mutant. (Z)-14-Tricosenyl formate was detected in the top tent volatiles of the 'Aqua' cultivar and in the CR-S2 and CR-S12 mutants at levels of 2.7%, 4.0%, and 2.2%, respectively. The highest 1-triacontanol contents was 5.8% in CR-S2 mutant, while for the remaining cultivars, was only listed top ten volatiles for 'Aqua' and 'Blackbeauty' cultivars. Other top 10 compounds included (Z)-9-tricosene, decane, *n*-Tetracosanol, hexadecanal, tetracosane, tritriacontane, tetracosanal, octacosyl pentafluoropropionate, trifluoro-acetic acid, undecyl ester, heptacosyl heptafluorobutyrate, and dodecane.

Table 2. Top 10 volatile compounds identified from the rose mutant and those of original cultivars.

| No | Aqua | % | CR-S7 (Aqua mutant) | | Blackbeauty | | CR-S2 (Blackbeauty mutant) | |
|---|---|---|---|---|---|---|---|---|
| 1 | 2-methylhexacosane | 14.0 | 2-methylhexacosane | 19.6 | 2-methylhexacosane | 18.7 | 2-methylhexacosane | 17.6 |
| 2 | Nonacosane | 13.5 | Nonacosane | 10.4 | Nonacosane | 14.6 | Nonacosane | 16.9 |
| 3 | 2-methyltricosane | 10.2 | 2-methyltricosane | 9.9 | 2-methyltricosane | 9.3 | 2-methyltricosane | 8.2 |
| 4 | Hentriacontane | 7.7 | Hentriacontane | 8.0 | Octacosanol | 6.8 | 1-Triacontanol | 5.8 |
| 5 | Octacosanol | 6.5 | Octacosanol | 6.4 | Hentriacontane | 5.9 | Hentriacontane | 5.3 |
| 6 | Tricosane | 6.1 | Tricosane | 6.1 | Tricosane | 5.8 | Tricosane | 4.8 |
| 7 | Tetracosyl pentafluoropropionate | 4.7 | Tetracosyl pentafluoropropionate | 5.0 | Tetracosyl pentafluoropropionate | 4.9 | Octanoic acid tetradecyl ester | 4.6 |
| 8 | 1-Triacontanol | 3.6 | Heptacosyl heptafluorobutyrate | 2.0 | Heneicosane | 4.4 | (Z)-14-Tricosenyl formate | 4.0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 9 | Octacosane | 3.1 | Tritriacontane | 1.9 | 1-Triacontanol | 3.6 | Octacosane | 3.4 |
| 10 | (Z)-14-Tricosenyl formate | 2.7 | Dedecane | 1.9 | Octacosane | 1.8 | Heneicosane | 3.3 |
| No. | Haetsal | | CR-S5 (Haetsal mutant) | | Vital | | S1 (Vital mutant) | |
| 1 | 2-methylhexacosane | 16.4 | 2-methylhexacosane | 17.5 | 2-methylhexacosane | 22.0 | 2-methylhexacosane | 20.3 |
| 2 | 2-methyltricosane | 14.8 | 2-methyltricosane | 13.0 | 2-methyltricosane | 20.3 | Nonacosane | 13.7 |
| 3 | Tetracosyl pentafluoropropionate | 6.8 | 2-Octyl-1-decanol | 6.7 | Tricosane | 19.6 | 2-methyltricosane | 10.9 |
| 4 | Tricosane | 6.6 | Heneicosane | 6.6 | Heneicosane | 5.6 | Tricosane | 8.0 |
| 5 | Heneicosane | 5.7 | Tricosane | 6.4 | Hexacosane | 3.4 | Hentriacontane | 7.0 |
| 6 | 2-Octyl-1-decanol | 5.0 | Nonacosane | 5.6 | (Z)-9-Tricosene, | 3.1 | Tetracosyl pentafluoropropionate | 4.8 |
| 7 | Hentriacontane | 4.9 | Tetracosyl pentafluoropropionate | 4.9 | Tetracosanal | 2.8 | Octacosanol | 4.1 |
| 8 | Nonacosane | 4.4 | Hentriacontane | 3.8 | n-Tetracosanol-1 | 2.6 | Tritriacontane | 2.3 |
| 9 | n-Tetracosanol-1 | 3.9 | n-Tetracosanol-1 | 2.5 | Tetracosane | 2.4 | Heneicosane | 2.0 |
| 10 | Octacosanol | 2.3 | Octacosanol | 2.4 | Tetradecane | 1.5 | Octacosane | 1.7 |
| No. | S3 (Vital mutant) | | S4 (Vital mutant) | | Yellowbabe | | S6 (Yellowbabe mutant) | |
| 1 | Nonacosane | 16.5 | 2-methylhexacosane | 14.5 | 2-methylhexacosane | 19.8 | 2-methylhexacosane | 20.3 |
| 2 | 2-methylhexacosane | 14.6 | 2-methyltricosane | 13.5 | Nonacosane | 14.8 | Nonacosane | 9.9 |
| 3 | 2-methyltricosane | 12.0 | Tricosane | 12.6 | Tricosane | 9.6 | Tricosane | 8.7 |
| 4 | Tricosane | 9.6 | Nonacosane | 11.0 | 2-methyltricosane | 9.0 | 2-methyltricosane | 8.0 |
| 5 | Hentriacontane | 6.3 | Tetracosyl pentafluoropropionate | 5.9 | Heneicosane | 7.6 | Heneicosane | 6.9 |
| 6 | Octacosanol | 5.7 | Hentriacontane | 4.8 | Octacosanol | 5.4 | Octacosanol | 5.1 |
| 7 | Tetracosyl pentafluoropropionate | 5.5 | Octacosanol | 4.1 | Tetracosyl pentafluoropropionate | 5.3 | Tetracosyl pentafluoropropionate | 4.8 |
| 8 | Octacosane | 2.9 | Heneicosane | 3.7 | Hentriacontane | 2.8 | Hentriacontane | 4.7 |
| 9 | Heneicosane | 2.8 | (Z)-9-Tricosene, | 2.3 | 2-Octyl-1-decanol | 2.7 | 2-Octyl-1-decanol | 3.0 |
| 10 | Hexacosane | 1.5 | Octacosyl pentafluoropropionate | 2.2 | (Z)-9-Tricosene, | 1.7 | (Z)-9-Tricosene | 2.1 |
| No. | Lovelydia | | CR-S8 (Lovelydia mutant) | | CR-S9 (Lovelydia mutant) | | CR-S10 (Lovelydia mutant) | |
| 1 | 2-methylhexacosane | 16.8 | 2-methylhexacosane | 20.7 | 2-methylhexacosane | 22.8 | 2-methylhexacosane | 17.9 |
| 2 | 2-methyltricosane | 10.7 | Nonacosane | 17.5 | Nonacosane | 15.9 | 2-methyltricosane | 14.7 |
| 3 | 1-Decanol, 2-octyl- | 7.9 | Hentriacontane | 8.7 | 2-methyltricosane | 8.4 | Nonacosane | 9.2 |
| 4 | Nonacosane | 7.8 | 2-methyltricosane | 8.0 | Hentriacontane | 7.6 | Hentriacontane | 8.4 |
| 5 | Tricosane | 6.7 | Tricosane | 6.5 | Tricosane | 4.4 | Tricosane | 6.6 |
| 6 | Hentriacontane | 6.6 | Octacosane | 3.0 | Heneicosane | 4.1 | Tetracosyl pentafluoropropionate | 4.7 |
| 7 | Heneicosane | 6.0 | Tritriacontane | 2.9 | Octacosane | 2.7 | Heneicosane | 4.6 |
| 8 | Tetracosyl pentafluoropropionate | 4.9 | Tetracosyl pentafluoropropionate | 2.3 | Octacosanol | 2.1 | Tritriacontane | 3.3 |
| 9 | Octacosanol | 4.2 | Acetic acid, trifluoroundecyl ester | 2.2 | Dodecane | 2.0 | n-Tetracosanol-1 | 3.0 |
| 10 | Tritriacontane | 2.4 | Octacosanol | 2.0 | Tetracosyl pentafluoropropionate | 1.8 | Octacosanol | 2.0 |
| No. | CR-S11 (Lovelydia mutant) | | CR-S12 (Lovelydia mutant) | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| 1 | 2-methylhexacosane | 18.3 | Nonacosane | 18.7 | |
| 2 | 2-methyltricosane | 12.4 | 2-methylhexacosane | 16.5 | |
| 3 | Nonacosane | 12.4 | 2-methyltricosane | 10.9 | |
| 4 | Tricosane | 7.0 | Tricosane | 8.4 | |
| 5 | Heneicosane | 6.7 | Hentriacontane | 5.7 | |
| 6 | Hentriacontane | 5.0 | Octacosane | 3.9 | |
| 7 | 2-Octyl-1-decanol | 4.9 | (Z)-14-Tricosenyl formate | 2.2 | |
| 8 | Tetracosyl pentafluoropropionate | 3.6 | Tetracosyl pentafluoropropionate | 2.0 | |
| 9 | Octacosanol | 3.0 | Tetracosanal | 2.0 | |
| 10 | Octacosane | 2.4 | Octacosanol | 1.8 | |

### *2.3. Chemical hierarchical cluster analysis*

The results of the hierarchical cluster analysis are presented in Fig. 3. The 18 rose genotypes clustered into four groups, which formed two independents supergroups (Groups I and II, and Groups III, IV, 'CR-S1'). Group I contained 4 mutants ('CR-S2', 'CR-S3', 'CR-S9' and 'CR-S10') and 'Vital' cultivar. Group II contained two mutants ('CR-S4' and 'CR-S7') and two ('Haetsal' and 'Yellowbabe') original cultivars. Group III contained 4 mutants ('CR-S5', 'CR-S8', 'CR-S11' and CR-S12) and 'Lovelydia' cultivar. Group IV contained CR-S6 mutant and two ('Blackbeauty' and 'Aqua') original cultivars. The 'CR-S1' mutant was fund to belong to an independent group.

The chemical hierarchical cluster analysis divided the nine chemical compounds into four clusters. Cluster I contained 17 compounds, of which 5 compounds (tetracosanal, nonacosane, octacosane, (Z)-14-tricosenyl formate and 1-triacontanol) are listed in top ten major compounds. Cluster II contained 16 compounds, of which 5 compounds (octacosanol, tetracosyl pentafluoropropionate, tritriacontane, heneicosane and acetic acid, trifluoroundecyl ester) are listed in top ten major compounds. Cluster III contained 16 compounds, of which 3 compounds (octacosyl pentafluoropropionate, heneicosane and 2-octyl-1-decanol) are listed in top ten major compounds. Cluster IV contained 28 compounds, of which 8 compounds (dodecane, 2-methylhexacosane, decane, n-tetracosanol-1, 2-methyltricosane, (Z)-9-tricosene, hexacosane, tricosane) are listed in top ten major compounds.

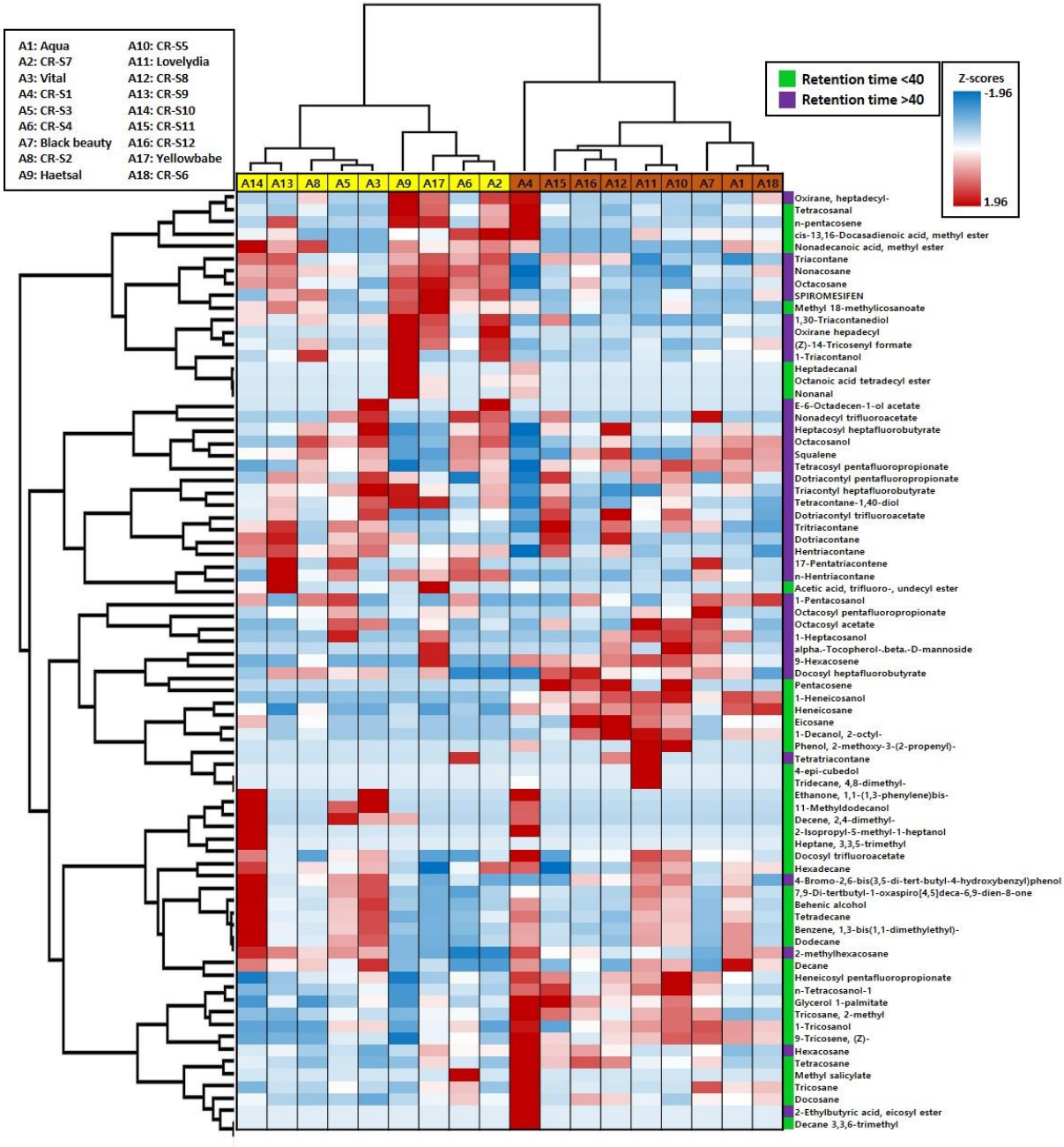



**Figure 2.** Hierarchical cluster analysis of 12 rose mutant and those of 6 original cultivars.

## 3. Discussion

Rose is a very popular ornamental crop, and there is always demand for new characteristics in horticulture and the cosmetic industry. These industries prosper on the back of new traits such as flower color and fragrance [1,8,22]. In this study, the 12 rose mutants, which were derived through gamma irradiation (70 Gy), had changed petal colors and numbers. Previous work has shown 70-Gy gamma irradiation of root cuttings to be an effective means of inducing mutations in rose plants [12,23].

Research on the pigmented components of rose has already been conducted in many studies. The dominant pigment in rose petals are carotenoids and anthocyanins, which provide yellow and pink color, respectively. Typically, orange petals result as a mixture of anthocyanins and carotenoids [4,5,6,7].

The composition of volatile compound in rose essential oils is an important determinant of oil quality [18,24]. When rose breeding is undertaken for the production of oil materials, an efficient procedure must be established for regular estimation of oil composition [1,8]. Typically, the development of new cultivars in ornamental crops is conducted through hybridization. However, hybridization can result in drastic changes in volatile compound composition [8,25]. In this study, hybridization was not used to develop aroma and oil compositions in rose cultivars. Rather, mutation breeding was performed on elite cultivars so that relatively few morphological traits were altered and oil composition could be tuned [10]. Mutagenesis using radiation has been found to be effective for introducing variability of oil composition in various crops [10,26,27].

To date, about 400 different volatile compounds have been reported in rose plants and these compounds have been categorized into to chemical groups, such as hydrocarbons, alcohols, esters, and others [15,28]. This study revealed hydrocarbons, esters, and alcohols were major volatiles in all rose genotypes. Hydrocarbons are used for industrial applications such as fragrances, paraffin, and wax [15,28]. The main rose oil paraffin contents range from 13% to 23% according to the ISO9842 rose oil standard [29]. Compared with previous reports, there were some differences in the chemical composition rates of rose oil. Oktavianawati et al. reported that they detected only hydrocarbons (100%) in three rose cultivars [13]. However, Babu et al. reported that long-chain hydrocarbons including nonadecane, heptadecane, 9-eicosene, and docosane accounted for about 21.23% of the volatiles, while alcohols made up as much as 68.13% of the total oil in the Himalayas rose [30]. Kazaz et al. reported that the main compound groups of rose oil are monoterpene alcohols and hydrocarbons [14]. The hydrocarbon contents in the CR-S8 and CR-S9 mutants were about 10% higher than that in the original cultivar. In contrast, the hydrocarbon contents in the CR-S1, CR-S3, and CR-S4 mutants were lower than that of the original cultivar. Esters and alcohols are important compounds in food, cosmetics, and medicinal products; examples include fragrances in shampoo, perfumes, soaps and creams, aromatherapy oil, and food supplements [16,31]. In addition, ester and alcohol compounds can also have bioactive properties, and can be used as emollients, surfactants, and antioxidants [14,16,17,19]. The ester contents of CR-S1, CR-S3, and CR-S4 mutants were higher than that of the original cultivar. Variation in alcohol contents was not found in the mutant genotypes. The compositions of hydrocarbons, esters, and alcohols in rose essential oil are the major compounds used to evaluate the quality of the oil [31]. It is normal that the compositions of volatile compounds are influenced by factors such as genotype, climate, and harvest times [1,2,26,30,31]. However, all of the rose genotypes were

grown under the same conditions, and any differences in volatile compounds were likely caused by genotype. This ability to control volatile compound content through genotype may have applications in rose breeding programs for oil materials [1,2,14]. Therefore, color changes of rose mutant genotypes may also be mediated by the accumulation of flower volatile compounds. This finding is similar to that of a previous study, which changed the volatile compound compositions in *Chrysanthemum* mutant cultivars generated by gamma irradiation [26].

The results of this study revealed that the flower extracts contained 77 compounds, of which nonacosane, 2-methylhexacosane, tricosane, hentriacontane, tetracosyl pentafluoropropionate, heneicosane, and octacosanol were the major volatile compounds among the rose genotypes. The major volatile compounds (nonacosane, heneicosane, and tricosane) were similar to those previously reported in eight rose accessions [Rusanov]. Moreover, many studies have reported that rose oil contains long-chain hydrocarbons, including nonadecane, heptadecane, 9-eicosene, and docosane. Nonacosane and tricosane are known to exhibit antibacterial activity [32]. 2-Methylhexacosane is reported to have antimicrobial activity and the ability to decrease blood cholesterol [33]. Hentriacontane exhibits antitumor activity and has anti-inflammatory effects through the ability to suppress NF-κB and caspase-1 activation [34]. Octacosanol is found in many plant oils and is reported to have medicinal properties. Octacosanol has important biological activities, including antioxidant, antiulcer, and anti-inflammatory activities, and is a known anti-parkinsonism agent [35]. In the CR-S3 mutant, the content of octacosanol was slightly higher than that of the original cultivar. This study showed that the flowers of the novel rose genotypes can be a rich source of various bioactive phytochemicals.

Hierarchical cluster analysis categorized the 12 rose mutant genotypes and six original cultivars according to their volatile compound similarities. The chemical hierarchical cluster analysis produced information that can be applied to select for chemotype in breeding programs and other useful information [26,36]. In this study, we found high levels of chemical diversity among the rose genotypes. Rose accessions could be divided into four major groups, where Group I mainly contained high levels of dodecane and lacked 2-methyltricosane, and Group II included four genotypes with high levels of nonacosane, octacosane, and tricontane and lacked 2-methyltricosane. The highest heneicosane contents were found in Group III. Group V, mainly contained high 1-pentacosanol, 1-tricosanol, and (*Z*)-9-tricosane and lacked heneicosane. These trends suggest that the hydrocarbon and alcohol contents of rose flowers could be used as a marker to assess chemotypes. These results could be applied to breeding programs to develop rose cultivars with improved volatile compounds.

## 4. Materials and Methods

### *4.1. Plant material*

The mutant genotypes of rose were generated by the treatment of root cuttings of each original cultivar with 70 Gy of gamma irradiation ($^{60}$Co): CR-S7 mutant was derived from 'Aqua'; CR-S2 mutant was derived from 'Blackbeauty'; CR-S5 was derived from 'Haetsal'; CR-S1, CR-S3, and CR-S4 mutants were derived from 'Vital'; CR-S6 mutant was derived from 'Yellowbabe; and CR-S8, CR-S9, CR-S10, CR-S11, and CR-S12 mutants were derived from 'Lovely Lydia'. These mutants were selected from flower-color variants and exhibited stable inheritance of the phenotype for $V_4$ generations. The radiation mutant genotypes were grown by the Radiation Breeding Research Team at the Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute. The flowers were randomly collected when fully open from the same plantation. Fresh flowers of 18 rose genotypes were subjected to hydro-distillation for 4 hours with hexane as the collecting solvent. The extracts were dried over anhydrous sodium sulfate to eliminate moisture and filtered through a polyvinylidene fluoride syringe filter (0.45 μm) for GC–MS analysis. Three replicates were used for each sample.

### *4.2. GC-MS analysis of volatile compounds*

The volatile compound compositions were analyzed using a GC–MS equipped with an Rtx-5MS column. The carrier gas was 99.99% high-purity helium with a column flow rate of 1.37 mL/min. Sample injection was performed in splitless mode. The oven temperature was initially set at 40 °C, and was gradually increased to 300 °C at 5 °C/min with a final hold for 5 min. The mass spectrometry parameters included: electron-impact ionization, 70 eV; ion source temperature, 230 °C; scan range, 40–500. The identification of each compound was performed using mass spectral libraries and Kovats retention indexes (RI). The GC–MS analysis detected volatile compounds in the rose mutants and those of the original cultivars, and compounds were tentatively identified based on a NIST library similarity index greater than 90%. The retention indices of all GC peaks were calculated with retention times of C7–C40 saturated alkane standards under the same chromatographic conditions. The RI of each compound on each column was calculated using the formula; $y$ and $z$ are carbon numbers of alkane standards, $T_{(x)}$ is the retention time of the compound, $n$ and $n$+1 represent the retention times of the alkane standards.

$$RI = 100y + 100(z - y) \times \left(\frac{T_{(x)} - T_{(n)}}{T_{n+1} - T_n}\right)$$

### *4.3. Statistical analysis*

The chemical analysis data were subjected to analysis of variance using a multiple comparisons method with the SPSS version 12 statistical software package. Differences were considered significant at the 5% level. When the treatment effect was significant, means were separated using Duncan's Multiple Range Test.

The clustering analysis of samples from the flowers of the 18 rose genotypes was performed using the complete linkage method in the SPSS software. The volatile compounds were visualized as *z*-values in the heatmap.

## 5. Conclusions

GC–MS analysis of 12 mutated rose genotypes obtained through gamma-irradiation and their six original cultivars identified 77 volatile compounds that were grouped into five functional compound categories: hydrocarbons, terpenoids, alcohols, esters, and others. Three mutant genotypes derived from the 'Vital' cultivar showed increased ester content and decreased hydrocarbon content. Chemical hierarchical cluster analysis revealed that the hydrocarbon and alcohol content of rose flowers could be used as key markers to assess chemotypes. Thus, we construe that these volatile compounds are useful for classification and identification of rose mutant genotypes. Also, our research suggests that the generation of novel rose genotypes by radiation breeding to give enhanced content of various bioactive phytochemicals may be an effective route to resources for use in the food and cosmetics industries, horticulture, and aromatherapy.

**Supplementary Materials:** The following are available online at www.mdpi.com/xxx/s1, Table S1: Volatile constituents in flower of rose genotypes by GC-MS. Figure S1: GC-MS chromatograms of the alkane standards. 1: Decane, 2: Dodecane, 3: Tetradecane, 4: Hexadecane, 5: Octadecane, 6: Eicosane, 7: Docosane, 8: Tetracosane, 9: Hexacosane, 10: Octacosane, 11: Triacontane, 12: Dotriacontane, 13: Tetratriacontane,14: Hexatriacontane. Figure S2: GC-MS chromatograms of the top 10 constituents identified in volatile compounds of the rose genotypes. Peak numbers listed in table S1.

**Author Contributions:** Conceptualization, S.H.K. and S.-Y.K.**;** methodology, J.R., D.-G.K. and J.-M.K.; validation, J.R. and D.-G.K. and J.-M.K.; formal analysis, J.R and Y.D.J.; investigation, J.R., J.I.L. and J.-B.K.; resources, S.H.K.; data curation, J.R. and J.I.L..; writing—original draft preparation, J.R.; writing—review and editing, J.B.K., S.-Y.K and S.H.K.; visualization, , J.R. and J.I.L; project administration, J.-B.K.; funding acquisition, J.-B.K. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was supported by the research program of KAERI, Republic of Korea and the Radiation Technology R&D Program through the National Research Foundation of Korea funded by the Ministry of Science and ICT.

Conflicts of interest: The authors declare no conflict of interest.

References

1. Datta, S.K. Breeding of new ornamental varieties: Rose. *Current Sci*. **2018,** 114, 1194-1206.
2. Gudin, S. Rose: genetics and breeding. *Plant Breed. Rev*., **2000**, 17, 159–189.
3. Bendahmane, M.; Dubios, A.; Raymond, O; Bris, M.L. Genetics and genomics of flower initiation and development in roses. *J. Exp. Bot*., **2013**, 64(4), 847–857.
4. Luo, H.; Deng, S.; Fu, W.; Zhang, X.; Zhang, X.; Zhang, Z. et al. Characterization of active anthocyanin degradation in the petals of rosa chinensis and brunfelsia calycina reveals the effect of gallated catechins on pigment maintenance. *Int. J. Mol. Sci.*, **2017**, 18:699.
5. Ogata, J.; Kanno, Y.; Itoh, Y.; Tsugawa, H.; Suzuki, M. Plant biochemistry: anthocyanin biosynthesis in roses. *Nature*, **2005**, 435, 757–758.
6. Eugster, C.H.; Märki-Fischer, E. The chemistry of rose pigments. *Angew. Chem. Int. Ed. Engl*., **1991**, 30, 654–672.
7. Schmitzer, V.; Veberic, R.; Osterc, G.; Stampar, F. Color and phenolic content changes during flower development in groundcover rose. *J. Am. Soc. Hortic. Sci*., **2010**, 135, 195–202.
8. Rusanov, K.; Kovacheva N.; Atanassov, I. Comparative GC/MS analysis of rose flower and distilled oil volatiles of the oil bearing rose *Rosa damascene*. *Biotechnol. Biotechnol. EG*. **2011**, 25, 2210-2216.
9. Rusanov, K.; Kovacheva, N.; Rusanova, M.; Atanassov, I. Traditional *Rosa damascena* flower harvesting practices evaluated through GC/MS metabolite profiling of flower volatiles. *Food Chem*., **2011**, 129, 1851–1859.
10. Spencer-Lopes, M.M.; Forster, B.P.; Jankuloski, L. Manual on mutation. Third edition. *Food and Agriculture Organization of the United Nations*; *FAO/IAEA*: Rome, Italy, **2018**, pp. 165~167.
11. Okamura, M.; Yasuno, N.; Ohtsuka, M.; Tanaka, A.; Shikazono, N; Hase, Y. Wide variety of flower-color and–shape mutants regenerated from leaf cultures irradiated with ion beams. Nucl. Instr. and Meth. In Phys. Res. B. **2003**, 206, 574-578.
12. Kim, S.W.; Lee, H.J.; Kim, Y.S.; Jo, Y.D.; Ryu, J.H.; Kang, S.Y.; Kim, S.H. Effect of gamma-ray on survival and mutation rates of root cuttings and unrooted cuttings in rose. **2017**, *Kor. J. Breed. Sci*. 49, 150-156.
13. Oktavianawati, I.; Letisya, N.; Citra, P.; Utari, D.P.I; Winata I.N.A.W.; Handayani W. et al. Essential oil composition of rose flower from karangpring village jember district extracted by distillation and enfleurage. *Jurnal ILMU DASAR*., **2019**, 20, 67-74.
14. Kazaz S.; Erbas, S.; Baydar, H. Breaking seed dormancy in oil rose (*Rosa damascena* Mill) by microbial inculation. *African J. Biotechnol*. **2010**, 27, 6503-6508.
15. Moein, M.; Karami, F.; Tavallali, H.; Ghasemi, Y. Composition of the Essential oil of *Rosa damascena* Mill. from South of Iran. *Iranian J. Pharmaceutical Sci.* **2010,** 6, 59-62.
16. Mohebitabar, S.; Shirazi, M.; Bioos, S.; Rahimi, R.; Malekshahi, M.; Nejatbakhsh, F. Therapeutic efficacy of rose oil: A comparehensive review of clinical evidence. *Avicenna j. Phytomed*. **2017**, 7, 206-213.
17. Bauer, K.; Garbe, D.; Surburg, H. Common fragrance and flavor materials. **2001,** *Wiley-VCH Velagsgesellschaft mbH, Weinheim, Germany*, pp.44
18. Flament I.; Debonneville C.; Furrer A. Volatile constituents of roses. 1993, *American Chemical Society, Washington, DC, USA.* pp. 269–281.
19. Umezu, T.; Ito, H.; Nagano, K.; Yamakoshi, M.; Oouchi, H.; Sakaniwa, M.; Morita, M. Anticonflict effects of rose oil and identification of its active constituents. 2002, *Life Sci*, 72, 91-102.
20. Ozkan, G., Sagdiç, O., Baydar, N.G., Baydar, H. Antioxidant and antibacterial activities of *Rosa damascena* flower extracts. *Food Sci. Technol. Int*. 2004. 10, 277–281.
21. Ulusoy, S.; Boşgelmez-Tınaz, G.; Seçilmiş-Canbay, H. Tocopherol, carotene, phenolic, contents and antibacterial properties of rose essential oil, hydrosol and absolute. *Curr. Microbiol*. 2009, 59, 554–558.
22. Narayana Gowda, J.V.; Recent advances in rose breeding. *Indian Rose Annu*., **1999**, XV, 42–43.
23. Koh, G.C.; Kim, M.Z.; Kang, S.Y. Induction of petal color mutants through gamma ray irradiation in rooted cuttings of rose. *Kor. J. Hort. Sci. Technol*. 2010. 28: 796-801.
24. Xiao, Z.; Luo, J.; Niu, Y.; Wu, M. Characterization of key aroma compounds from different rose essential oils using gas chromatography-mass spectrometry, gas chromatography–olfactometry and partial least squares regression. *Nat. Prod. Res*. 2017, 1–6.
25. Rusanov, K.; Atanassov, I. Industrial cultivation of oil bearing rose and rose oil production in Bulgaria during 21 $^{ST}$ century, directions and challenges. *Biotechnol. Biotechnol. EQ*. **2010**, 24, 1793-1798.

26. Ryu, J.; Nam, B.N.; Kim, B.R.; Kim, S.H.; Jo, Y.D.; Ahn, J.W.; Kim, J.B.; Jin, C.H.; Han, A.R. Comparative analysis of phytochemical composition of gamma-irradiated mutant cultivars of *Chrysanthemum morifolium. Molecules*, 2019, 24, 3003.
27. Kim, S.H.; Ryu, J, Kim W,J.; Kang, R.; Seo, E.; Kim, G.; Kang, S.Y.; Lee, J.D.; Ha, B.K. Identification of a new *GmSACPD-C* allele in high stearic acid mutant Hfa180 derived from gamma-ray irradiation. *Mol. Breed*. 2019. 39, 17.
28. Koksall, N.; Aslancan, H.; Sadighazadi, S.; Kafkas, E. 2015. Chemical investigation on *Rose damascena* mill. volatiles: effects of storage and drying conditions. *Acta Sci. Pol. Hortorum Cultus* 14, 105–114.
29. ISO9842, 2003. Oil of Rose (*Rosa X Damascena* Miller) 2003.
30. Babu, K.G.D.; Singh, B.; Joshi, V.P.; Singh, V. Essential oil composition of damask rose (*Rosa damascena* Mill.) distilled under different pressures and temperatures. *Flav. Frag. J.* **2002**. 17**,** 136-140.
31. Weiss, E.A. Essential oil crops. 1997. CAB International, Walingford, England, pp 393–416.
32. Mihailovi, V.; Vukovi, N.; Niforovi, N.; Soluji, S.; Mladenovi, M, MaškoviP, et al. Studies on the antimicrobial activity and chemical composition of the essential oils and alcoholic extracts of *Gentiana asclepiadea* L. J. Med. Plant Res. 2011, 5, 1164-1174.
33. Khatua, S.; Pandey, A.; Biswas, S.J. Phytochemical evaluation and antimicrobial properties of Trichosanthes dioica root extract. J. Pharma. Phytochem. 2016, 5, 410-413.
34. Kim, S.J.; Chung, W.S.; Kim, S.S.; Ko, S.G.; Um, J.Y. Antiinflammatory effect of *Oldenlandia diffusa* and its constituent, hentriacontane, through suppression of caspase-1 activation in mouse peritoneal macrophages. *Phytother. Res*. **2011**. 25, 1537-1546.
35. Wang, T.; Liu, Y.Y.; Wang, X.; Yang, N.; Zhu, H.B.; Zuo, P.P. Protective effects of octacosanol on 6-hydroxydopamine-induced Parkinsonism in rats via regulation of ProNGF and NGF signaling. *Acta Pharmacol. Sin*. **2010**, *31*, 765–774.
36. Sheela, V.L.; Sheena, A. Novel trends and achievements in breeding of tropical ornamental crops especially orchids and anthuriums: the mutation breeding approach. Wageningen Academic Publishers, Wageningen, Netherlands. 2014, pp. 141-158.