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Floral and Molecular Characterization of Gamma Rays Induced Mutants in Oil-Bearing Rose (*Rosa damascena* Mill.)

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ABSTRACT

This research was aimed to create the genetic variations for the selection of oil-bearing rose genotypes with the desirable floral features. For this reason, gamma rays at doses of 0, 100 and 200 Gy of radioactive Cobalt-60 were applied to oil-bearing rose seeds to create genetic variations. Finally, the floral and molecular characterization of oil-bearing rose plants derived from non-irradiated and irradiated seeds were carried out. A total of 48 genotypes including 17 from control (0 Gy), 18 from M100 (100 Gy) and 12 from M200 (200 Gy) together with the parental species *R. damascena* were used as genetic materials. Genetic analysis was performed by using 20 SSR primer pairs. After the electrophoresis, the bands were displayed in TIF format with the aid of the Biolab UV Tech gel imaging system. The genotypes and mutants grown from irradiated and non-irradiated seeds were significantly different with flower colours from white to dark pink and petal numbers from 5 to 100. These results also showed that the seeds in the open-pollinated flowers of oil-bearing rose had mostly heterozygous allele genes governing the floral traits. The efficacy of the SSR primers used to identify mutations was different. RA003a and RA034a primers were found to be more effective in mutation screening in oil-bearing rose genome. It was understood that the major effects of the gamma-ray irradiation were on large-scale chromosomal breaks or deficiencies. In conclusion, oil-bearing rose seeds with or without gamma-ray irradiation would be a huge selection source to breed novel varieties.

Keywords**:** *Rosa damascena* Mill., mutation breeding, gamma-ray, SSRs.

Introduction

Turkey is an important differentiation and gene center for *Rosa* genus (Yıldırım 2016). Oil-bearing rose or Damask rose (*Rosa damascena* Mill.) with about 30 pink petals is one of the most strongly scented rose species with characteristic floral scent molecules such as citronellol, geraniol, nerol and phenylethyl alcohol. Its main industrial products used in the perfume and cosmetics are rose oil, rose water, rose concrete and rose absolute, which are produced by hydrodistillation and solvent extraction processes (Anac 1984; Başer 1992; Bayrak and Akgül 1994; Aydınlı and Tutaş 2003; Aycı et al. 2005).

Turkey, Bulgaria and Iran are the leading countries which meet more than 90 percent of the world oil-bearing rose production. Oil-bearing rose as an agro-industrial product has been cultivated in the Lakes Region of Turkey (Isparta, Burdur, Denizli and Afyonkarahisar provinces) since the last quarter of the 19th century. The production area of oil-bearing rose was about 3845 hectares and annual fresh flower production was 16,560 tons in the Lakes Region of Turkey in 2019 (TUIK, 2020).

Rosa damascena Mill. is an allotetraploid species (2n=4x=28) as a hybrid of *R. gallica* L. and *R. phoenicia* Boiss. (Gudin 2000). *R. damascena*

was considered as allied to *R. alba, R. phoenicia* and *R. moschata* according to DNA analyses (Yıldırım 2016). The studies on the origin and genetics of *R. damascena* have focused especially on molecular DNA markers in recent years. Because classical genetic analyses based on phenological and morphological characteristics give very limited and inadequate information, therefore, it is absolutely necessary to be supported by molecular and biochemical techniques (Torres et al. 1993; Debener and Mattiesch 1999). For example, microsatellite genotyping demonstrated that *R. damascena* Mill. accessions from Bulgaria, Iran, India and old European Damask rose varieties possess identical microsatellite profiles, suggesting a common origin (Rusanov et al. 2005).

According to the studies on molecular analyses with RAPD, AFLP and SSR markers, while polymorphism indicating that genetic variation is not present among the individuals and populations of *Rosa damascena* plants under cultivation in Turkey and Bulgaria (Ağaoglu et al. 2000; Göktürk Baydar et al. 2004; Rusonov et al. 2005), a wide genetic diversity was determined by molecular markers among the *R. damascena* plants collected from Iran and its neighbouring areas including Syria and Pakistan (Pirseyedi et al. 2005; Babaei et al. 2007; Kiani et al. 2010; Alsemaan et al. 2011; Farooq et al. 2013).

Since oil-bearing roses have always been propagated vegetatively for hundreds of years, the current roses in the rose valleys of Turkey and Bulgaria are most probably clonal progenitors of the first planted oil-bearing roses which have been maintaining their primitive features to date. For this reason, superior or outstanding types of oil-bearing rose have not been selected through breeding in Turkey due to the lack of genetic variation among the plants under culture (Baydar et al. 2016). On the other hand, the phenotypic homogeneity caused by continuous vegetative reproduction makes it possible to produce rose oil with international standards (ISO 9842:2003) (Rusanov et al. 2009).

There is a need for different methods such as hybridization (inter- and intraspecific crosses) and induced mutation (physical or chemical mutagens) to create genetic variation in rose species including oil-bearing roses (Raev 1984; Tsvetkov 1984; Baydar et al. 2013). The other quite practical and effective methods causing genetic variation in oil-bearing roses are to derive progenies from the seeds of the plants grown only by vegetative propagation with their cuttings (Gudin 2003). Many of the seedlings derived from the seeds of open-pollinated flowers may differ genetically due to the segregation of the alleles at heterozygous loci during meiosis (Rusanov et al. 2005; Baydar et al. 2016). Microsatellites or simple sequence repeats (SSRs) are simple sequence of tandemly repeats which can presently be a short motif of di-nucleotides, or tri-nucleotides, or tetranucleotides repeated and contains in 2-6 base pairs (bp) in length (Li et al. 2004). Simple sequence repeats of few base pairs in length can find polymorphism of DNA from mutant populations. The type of DNA polymorphism could be detected only after polymerase chain reaction (PCR), amplification of DNA and separation on polyacrylamide gel electrophoresis (Wu and Steven 1993). Size polymorphism reflects variation in the number of repeats of a simple DNA sequence ranging in length from 2-6 base pairs (Chapuis and Estoup 2006). SSRs have become a popular type of co-dominant molecular marker in genetic analysis and plant breeding application and this marker system is very useful for the determination of variation among plant populations.

The primary objective of this study was to create, evaluated and molecular characterization of the genetic variations for the selection of the oil-bearing rose genotypes with the desirable floral features. Therefore, gamma rays at doses of 0, 100 and 200 Gy of radioactive Cobalt-60 were applied to the oil-bearing rose seeds to create genetic variation. The floral traits such as petal color and petal number were evaluated and molecular characterization in the oil-bearing rose plants, derived from non-irradiated and irradiated seeds with ^{60}Co gamma-rays was performed by using simple sequence repeats (SSRs) to find polymorphism of DNA from mutant populations.

Materials and Methods

Plant materials

This research was conducted at the Faculty of Agriculture, Isparta University of Applied Sciences in Isparta-a city in the south-western part of Turkey which is called “Rose Valley of Turkey” due to the presence of advanced industrial oil-bearing rose cultivation. The seeds were extracted from the mature fruits of *Rosa damascena* Mill. f. *trigintipetala* Dieck (2n=4x=28) in Isparta region in October 2007. Following the irradiation of the seeds at different doses (0, 100 and 200 Gy) of Cobalt-60 gamma-ray in Nuclear Agricultural Research Center of Ankara, Turkey, they were sown to viols in March 2008. Healthy seedlings were transferred to pots first, then grown under greenhouse conditions in 2009 and eventually planted with 1.5 m within rows and 3 m between rows with drip irrigation applied to the experimental field in March 2010 (Baydar et al. 2016).

In subsequent years (2012, 2013 and 2014), basic floral traits as petal color and petal number per flower were determined on recently opened flowers in the early morning hours during the flowering season (May and June). Five flowers from each plant were measured on the condition that the plant produced enough flowers. Petal color was assessed on recently opened flowers and measured with a portable colorimeter (Model CR-300, Minolta Camera Ltd., Osaka, Japan) as described by Sarı (2018). Color was measured in the middle of each petal (three replicates per flower) to ensure equal measurement conditions (Schmitzer et al. 2010). The fragrance density and scent molecules (not tabulated in this paper) of the fresh flowers were detected using headspace solid-phase microextraction (HS-SPME) combined with gas chromatography/mass spectrometry (GC-MS) with the help of the method explained by (Baydar et al. 2016).

The genetic analyses were also performed in total of 48 genotypes including 17 from control (0 Gy), 18 from M100 (100 Gy) and 12 from M200 (200 Gy), together with the parental species *R. damascena* Mill, by the use of SSR markers. The band patterns of each selected mutant representing each application dose of Co-60 were compared to the band pattern of control and parental plants.

DNA extraction

DNA was extracted from the fresh leaves and shoots of the plants using a Qiagen DNeasy Plant Mini Kit according to the manufacturer's instructions. The DNA quantities (ng/µL) were determined spectrophotometrically using the Thermo Scientific NanoDrop™ Spectrophotometer. The DNA quality was measured by the ratio of the absorbance values of 260 nm and 280 nm. The extracted DNA was stored at −80°C until use.

SSR analysis

Simple Sequence Repeat (SSRs) primers which were previously designed for *Rosa* species by Hibrand-Saint Oyant et al. (2008) and Kimura et al. (2006) and also used in this study were shown in Table 1. PCR amplification protocol used in this study was previously successfully applied in *R. damascena* by Göktürk Baydar et al. (2004). Each PCR reaction was prepared as follows: 25 ng template DNA, 5 pmol of each labelled reverse primer and unlabelled forward primer, 1U *Tag* DNA polymerase, 2 µL of 10×reaction buffer, 1 µL of 1×W1-detergent, 2 µL of dNTPs (1 mM), 2 µL of magnesium chloride (15 mM) in a total volume of 20 µL. The PCR reactions were carried out in a BioRad thermocycler. An initial denaturation of 94 °C/30 s was followed by 30 cycles. Denaturation and extension temperatures were 94 °C for 1 min and 72 °C for 2 min, respectively. The annealing temperature (about 58 °C for 50 s) changed according to primer pairs used. The amplified fragments were separated in the electrophoresis by 6% polyacrylamide gel using 29:1 acrylamide:bisacrylamide solution and 5% tris boric acid EDTA buffer and stained with ethidium bromide. The bands were displayed in TIF format with the aid of the Biolab UV Tech gel imaging system.

Results

Floral characteristics, DNA quantity and quality and PCR amplification of 20 SSR primers tested in the *Rosa damascena* genotypes were shown in Table 2. The genotypes which were derived by generative propagation with seeds and were gamma-irradiated with Cobalt-60 had a huge floral variation for the flower colors from white to dark pink and the petal numbers from five to a hundred as shown in Figure 1. While the petal color of the classical Isparta oil-bearing rose was pink, the petal color of the genotypes used in the study changed from white to different shades of pink. White petal color occurred in the Rd-M0 group (52 code), Rd-M100 group (18, 28/1 and 100 codes) and also Rd-M200 group (34 code) (Table 2).

It was observed that genotypes with more petals had fewer anthers (the pollen producing part of a flower**)** and genotypes with more anthers had fewer stigmas (the part of the pistil where pollen germinates). In general, those with a low number of petals (single-layered flowers with 5-7 petals) tend to bloom earlier than those with a higher number of petals (multi-layered flowers with over 25 petals). It was also observed that the flowering season of those with a low number of petals was shorter. This finding was important to show that there were close relationships between the number of petals and flowering duration according to the correlation analysis results (not tabulated). On the other hand, the flowers of single-layered genotypes were very light and had lower odor densities as well as their petal leaves were not suitable for hand collection because they were swallowed very quickly (Baydar et al. 2016). Another remarkable point by visual and sensorial inspections was that the genotypes with hairless (naked) hypanthia, pedicels and sepal leaves were less fragrant but more resistant to rose aphid (*Macrosiphum rosae* L.).

DNA quantity varied from 6.40 to 95.4 ng/µL and DNA quality (260/280 values) changed from 1.61 to 2.40 **(**Table 2). A total of 1920 (48 genotypes x 20 primers x 2 dose mutations) PCR reactions

were performed for the SSR analysis and a total of 100 polyacrylamide gels were prepared for the screening of the amplification products. Different SSR primers detected different locus losses resulted from chromosomal deletions (Table 3). All SSR markers used in the study were amplified in the parental genome of *Rosa damascena*. Among the SSR primers, RA003a (primer 8) and RA034a (primer 16) were found to be the most effective primers in oil-bearing rose genome mutation screening (Table 3). When the reaction products obtained were examined, it was determined that SSR primers being specific to locus amplified a single allele in all of the genotypes examined in the study (Figure 2a). Polymorphisms mostly occurred in the form of deletion of the entire SSR locus when compared to *R. damascena* (control) genome (Figure 2b). However single mutant also exhibited shortened specific SSR locus region due to the break on its genetic material (Figure 2c). Rd-M100-29 was identified as a mutant with the highest number of chromosomal deletions including 5 different locus losses (Table 3).

Discussion

The irradiation of gamma rays of Cobalt-60 to oil-bearing rose (*Rosa damascena* Mill.) seeds produced a broad genetic variation in the progenies in terms of floral traits like petal color and petal number (Figure 1, Table 2). These variations can be a very important genetic source for rose breeding to develop novel oil-bearing rose varieties. Even in the plants from the non-irradiated seeds (coded as M0), there is a wide variation in these traits. A wide variation in floral scent molecules such as phenyl ethyl alcohol (23.26-74.54%), citronellol (5.57-31.59%) and geraniol (3.09-26.93%) apart from the floral morphological characteristics was recorded among the seed-derived (non-irradiated) oil-bearing rose plants (Baydar et al. 2016). *R. damascena* Mill. is mainly accepted as a hybrid of *R. gallica* L. and *R. phoenicia* Boiss. (Gudin 2000). It is also thought to be a triparental origin of *R. gallica*, *R. moschata* and *R. fedtschenkoana* (Iwata et al. 2000), both of which support our observations. Our results demonstrated that oil-bearing rose may have originated from more than three parental species during the evolution for thousands of years. However, much more detailed molecular genetic studies are needed to prove this hypothesis.

The petal color was changed from white to dark pink (Table 2). However, the majority of the genotypes and the mutants had pink petal color like the parent *Rosa damascena* grown in the experimental field. This finding supports the idea that the pink color is a dominant character over other petal colors. The pink color has been shown to be inherited codominantly, with white being homozygous recessive, pink being heterozygous and darker pink being homozygous dominant, as previously confirmed by Jones (2013). The coloration of rose flowers is mainly caused by the accumulation of anthocyanins such as pelargonidin and cyanidin in the petal cells (Schmitzer et al. 2010). Roses lack blue/violet flower colors owing to the deficiency of F3′5′H and therefore lack the B-ring-trihydroxylated anthocyanins based upon delphinidin (Tanaka et al. 2008). Karami et al. (2012) found a high positive correlation (r Sq Linear=0.81) between essential oil content and anthocyanin concentration which can be used as an essential oil quantity index in *R. damascena*. It has also been reported by Nedkov et al. (2009) that the essential oil content of white rose (*Rosa alba*) is lower than that of pink rose (*Rosa damascena*).

The petal number per plant was varied from 5 to 100 (Table 2). The important and negative relationship between the number of petals and the number of anthers may be the result of the homeotic functions of MADS-box function genes such as A, B and C. As known in this model, the formation of the floral organs is controlled by three sets of functional genes from the MADS-box gene family as A (sepal and petal formation), B (petal and stamen formation) and C (stamen and carpel formation) that are expressed in certain regions of the developing flower (Causier et al. 2010). While wild roses have simple flowers typically with 5 petals per flower, modern roses have double flowers consisting of >10 petals (Bendahmane et al. 2013). The roses with double flowers might be due to a homeotic change of stamens into petals through the concept of a sliding boundary, which is also responsible for the morphological diversity of rose flowers (Dubois et al. 2010).

Because the oil-bearing rose is an allotetraploid (2n=4x=28) carrying 4 homolog chromosomes that are homologs of each other, the same gene locus is replicated at the tetrasomic level and the genome of which is highly heterozygous. As a result, the genetic variations resulting from the segregation of the alleles at heterozygous loci were also appropriate for the clonal selection of novel varieties. However, it is not easy to recognize the genetic variations caused by heterozygous alleles or gene mutations. Several different types of DNA markers may be used to determine the genetic differences in the variants obtained after mutation (Chakrabarty and Datta 2010).

In this study, twenty SSR primer pairs were used to screen the genetic structure in a population of 47 oil-bearing rose genotypes together with *Rosa*

damascena Mill.. It has been understood that SSR analysis is an effective and practical method to identify the genetic variation in oil-bearing mutant populations. There are also some researches support on the SSRs are effective primers in screening for mutations in genomes. For example, mutations caused by somaclonal variations in rice plants were successfully detected with SSR primers (Khai and Lang 2005).

However, the efficacy of the primers used to identify mutations was different in oil-bearing rose genomes (Figure 2, Table 3). RA003a and RA034a primers were found to be the most effective markers in mutation screening among other microsatellite markers. Microsatellites can be found in thousands of locations in a plant genome; in addition, it has a higher rate of mutation than other DNA regions leading to high genetic diversity. However, microsatellites cannot be amplified by PCR due to the point mutations or false base pairings where SSR primers hybridize and the expected polymorphisms may not be evident if false alleles (null alleles) cannot be distinguished. Moreover, highly polymorphic microsatellite markers are widely employed in population genetic analyses, but one potential drawback is the presence of null alleles that fail to amplify to detected levels in the PCR (Dakin and Avise 2004).

In this research, the Damask rose genotypes and mutants with distinctly different in petal color and number were distinguishable by SSR markers. However, small floral differences caused by point mutations are not always detected by SSR markers which only allow scanning a very small fraction of the rose genome (Göktürk Baydar et al. 2004). Sport mutants with clearly mutated phenotypes show identical DNA marker patterns as the single mutations leading to the altered phenotypes are derived from only a negligible small part of the DNA of the mutant genotype (Debener et al. 2000). Unlike point mutations, which affect only a single nucleotide, microsatellite mutations lead to the gain or loss of an entire repeat unit and sometimes two or more repeats simultaneously. However, since the genome analysis and the genetic map of *Rosa damascena* have not yet been prepared, SSR markers developed for other rose species such as *Rosa hybrida* were used successfully. In addition, with Single Nucleotide Polymorphism (SNP) markers developed by direct sequencing of PCR products obtained from the genomic DNA of *Rosa damascena*, more detailed information on the allele configurations of genes related to floral properties can be obtained (Rusanov et al. 2009). Furthermore, TILLING (Targeting Induced Local Lesions IN Genomes) is a well-known reverse genetics technique designed to detect unknown SNPs in genes of interest using enzymatic digestion and is widely employed in plant genomics (Wang and Shi 2015).

The results from this study showed a huge floral variability for both petal color and number among the oil-bearing rose genotypes derived by generative propagation with seeds and induced gamma-irradiation with ^{60}Co. Open-pollinating flowers of the oil-bearing rose show a high degree of outcrossing and therefore lead to a high degree of heterozygosity in the alleles or genes associated with important floral characteristics and scent molecules. So, the seed propagation of clonally-propagated *R. damascena* yields a change in the genetic segregation of the alleles especially related to the petal color and petal number. Consequently, SSR markers linked to floral characteristics of oil-bearing rose should be developed from the segregating population of oil-bearing rose and also more effective methods such as SNP and TILLING are needed for the high-throughput identification of the mutants with a range of modified functions for a particular floral gene. Instead of screening all of the base sequences of an entire *R. damascena* genome, it has been concluded that it could be better to determine the base sequences only in the related floral genes for genome comparisons.

Conclusion

In oil-bearing rose industry, since the rose flowers constitute the main parts of the production costs in the basic distillation and extraction products such as rose oil, rose water, concrete and absolute, it is important to develop novel oil-bearing rose varieties with high flower yield and high volatile oil yield for reducing the production costs. Due to the lack of sufficient genetic variations among the oil-bearing roses under culture, until now it was not possible to select superior or outstanding types by certain breeding programs in Turkey. This research reveals that seed propagation and mutation breeding can be easily utilized as a way to create effective genetic variations for floral and scent characteristics. In conclusion, oil-bearing rose seeds with or without gamma-ray irradiation could provide a huge selection source to breed novel varieties.

Acknowledgement

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Figure 1. Floral images of some *Rosa damascena* genotypes derived from non-irradiated and irradiated seeds with ^{60}Co gamma rays. In the figure, Rd refers to *Rosa damascena*; M refers to mutation; 0 (control), 100 and 200 refer to gamma ray doses. (Original)

Figure 2. a) The gel image of PCR products of SSR primer Rw5G14 in polyacrylamide gel; b) Loss of locus by Rw12J12 primer in Rd-M100-15; c) a shortened locus area by RA013a primer in Rd-M200-28.

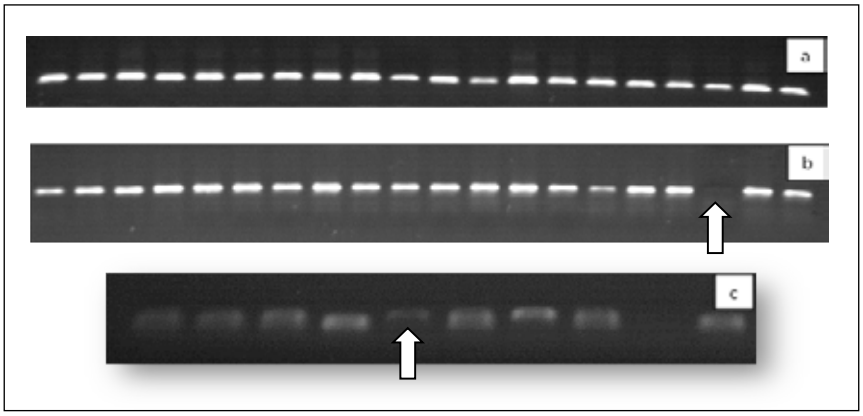

Table 1. SSR primers used in the amplification of oil-bearing roses with their respective sequences.

No	Primers	SSR Motifs	Sequence of Primers
1	Rw5G14	$(CT)_7(C)_8$	F: TGGTTTGGGGTTTTGTGTCT R: GCACAGTCTCCACCTGACAA
2	Rw12J12	$(CT)_{11}$	F: CAGTGTCCATGCTGACGAGT R: TGCTCCTGTTTTCTCTTTGCT
3	Rw55E12	$(TC)_9$	F: CGGTGGTTGGACATTAAAGC R: GGAGGCAACAGCACACTCTC
4	Rw16E19	$(TTC)_9$	F: CCAACAAACACGAGGAATGA R: CCACACTGATGTTCCAGCAC
5	Rw35C24	$(AG)_9X\ (AG)_8$	F: GGCGAATCGAGATTCAGAGA R: GGATTAGCCCAAGTCCAGGT
6	Rw34L6	$(CT)_{16}$	F: CTCCTTTAGACTCGGGACCA R: CAGGCACGCCATTTCTAACT
7	Rw52D4	$(TTC)_7$	F: GGCAGTTGCTGTGCAGTG R: TTGTGCCGACTCAAAATCAA
8	RA003a	$(GA)_{30}$	F: CAGAATTGGGTGTCCGTATG R: CAATTTTCAAAGGATAATTTGG
9	RA013a	$(AG)_{13}$	F: GAGGGGAAAGAGATACACAAA R: GTAAGACCTTGCGTGTTCATA
10	RA016a	$(AG)_{21}$	F: CAGGTGAAGAAGAGAAGGGTGT R: CCTCAGTTCATTTCAATCATCTCC
11	RA019a	$(AG)_{11}(AC)_9$	F: CGTTAGAGATCCGAGGGGGTC R: TGTCATGGTTGGGAAGTTGGCT
12	RA020a	$(AG)_{15}(AC)_{12}$	F: GTTAGAACCGAAGGCTCTAGT R: CCCGCTAAGGTGGAGACATAC
13	RA023b	$(GA)_{20}$	F: CATCCTCGGTGTTGCGTTGA R: TGTCTCCAGCAACCTTTTTTTCCC
14	RA027a	$(AG)_{25}$ $A(CAGAGA)_5$	F: ACCGTCCACAGTGTAAGAAAG R: CCCTCAAGTCTAGTAAAACCA
15	RA032b	$(GA)_{23}$	F: CGGCATCAAAGATATAGCTTCC R: AGAAATGCAAAACGCCCCTATGA
16	RA034a	$(GA)_{22}$	F: GCATAGAGAACTCGGGAATCAC R: TTCCGAAATGCCAACAACCAG
17	RA037a	$(GA)_{21}$	F: AGAGAGTATGTCGTTTGGAGGAG R: CTGCCTAAAATACCCCAAGTCAT
18	RA042a	$(GA)_{26}GT(GA)_8$	F: CAGACTTATCAATGCGATCGTGCC R: CAGCAATTCAGCAAGCCGTCTC
19	RA043a	$(AG)_{17}$	F: GCAACGTACTTCAATTTCCAC R: CAAGCTCAGAACTGAGACAC
20	RA044b	$(AG)_{14}$	F: TAGACAGATAGATATTGGCAC R: CAACTACAGATTTCTACCAACT

Table 2. Floral characteristics, DNA quantity and quality of the rose genotypes and PCR amplification of 20 SSR primers.

No	Genotypes	Petal Color	Petal Number per Flower	DNA Quantity (ng µl^{-1})	DNA Quality (260/280)	Number of Successful SSR Primers
1	Rd-M0-10*	Pink	40	19.4	2.15	19
2	Rd-M0-19	Pink	30	60.1	1.96	20
3	Rd-M0-29	Dark pink	5-10	56.7	1.90	19
4	Rd-M0-31	Pink	45-50	54.6	1.95	19
5	Rd-M0-37	Light pink	25-30	45.4	1.97	20
6	Rd-M0-43	Pink	65	42.2	1.91	20
7	Rd-M0-44	Light pink	20-25	65.6	1.92	18
8	Rd-M0-49	Pink	25-30	48.1	1.98	20
9	Rd-M0-51	Dark pink	25	29.3	1.95	20
10	Rd-M0-52	White	60	32.7	1.96	19
11	Rd-M0-57	Dark pink	5	30.2	1.96	19
12	Rd-M0-60	Red	5	35.8	1.93	19
13	Rd-M0-66	Pink	55-60	48.3	1.93	20
14	Rd-M0-67	Light pink	30	49.5	1.94	20
15	Rd-M0-70	Pink	30	31.2	1.92	16
16	Rd-M0-76	Pink	40-45	14.9	2.40	20
17	Rd-M0-77	Pink	55	37.3	1.99	20
18	Rd-M100-15	Pink	35-40	23.3	2.12	18
19	Rd-M100-18	White	35	75.3	1.92	20
20	Rd-M100-28/1	White	60-65	95.4	1.93	20
21	Rd-M100-29	Pink	25-30	74.6	1.96	15
22	Rd-M100-33	Pink	20-25	24.2	1.97	-
23	Rd-M100-37	Dark pink	35-40	54.8	1.93	19
24	Rd-M100-44	Pink	50	6.40	1.90	-
25	Rd-M100-55	Pink	35-40	54.2	1.91	20

Continuing table 2

No	Genotypes	Petal Color	Petal Number per Flower	DNA Quantity (ng µl^{-1})	DNA Quality (260/280)	Number of Successful SSR Primers
26	Rd-M100-68	Light pink	50	39.7	1.88	18
27	Rd-M100-78	Pink	85-90	57.0	1.90	18
28	Rd-M100-81	Light pink	25-30	34.5	2.04	20
29	Rd-M100-98	Light pink	95-100	55.3	1.85	20
30	Rd-M100-100	White	40-45	28.8	1.95	20
31	Rd-M100-104	Pink	5	64.9	1.92	20
32	Rd-M100-105	Pink	30-35	46.5	1.99	20
33	Rd-M100-110	Light pink	60-65	47.7	1.99	19
34	Rd-M100-111	Pink	5-6	65.2	1.94	19
35	Rd-M100-116	Pink	25	19.4	2.15	19
36	Rd-M200-4	Light pink	60	35.7	2.00	19
37	Rd-M200-9	Pink	30-35	28.4	2.06	19
38	Rd-M200-16	Dark pink	10	33.3	2.03	20
39	Rd-M200-17	Light pink	20-25	36.6	1.96	19
40	Rd-M200-18	Pink	30-35	78.1	1.92	18
41	Rd-M200-21	Light pink	10	73.7	1.61	17
42	Rd-M200-23	Dark pink	5-10	25.1	2.03	20
43	Rd-M200-26	Light pink	25	81.9	1.90	19
44	Rd-M200-28	Pink	5	47.1	1.95	19
45	Rd-M200-31	Light pink	20	40.6	2.05	18
46	Rd-M200-34	White	5	29.2	2.03	20
47	Rd-M200-35	Pink	90	43.1	1.98	20
48	*R. damascena*	Pink	30	35.6	1.90	20

*Rd refers to *Rosa damascena*; M refers to mutation; 0 (control), 100 and 200 refer to gamma ray doses

Table 3. PCR amplifications in *Rosa damascena* genotypes scanned with twenty different SSR primers.

Genotypes	SSR Primers																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
4	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+		+	+	+	+	+	+	+		+	+	+	+
8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
11	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
12	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
15	+	+		+	+	+	+		+	+	+		+	+	+		+	+	+	+
16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
17	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
18	+		+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+
19	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
21		+		+	+	+	+	+	+	+	+		+	+	+		+	+		+
23	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Continuing table 3

Genotypes	SSR Primers																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
26	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+
27	+		+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
28	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
29	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
30	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
31	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
32	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
33	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
34	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
35	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
36	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
37	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
38	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
39	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
40	+		+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
41	+	+	+	+	+	+	+		+	+		+	+	+	+		+	+	+	+
42	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
43	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
44	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+
45	+	+	+	+	+	+	+		+	+		+	+	+	+	+	+	+	+	+
46	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
47	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
48	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

*Empty (blue) areas indicate that amplification product has not occurred

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