

# Identification and Analysis of Genetic Variation among Rose Cultivars Using Random Amplified Polymorphic DNA

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Identified germplasm is an important component for efficient and effective management of plant genetic resources. Traditionally, cultivars or species identification has relied on morphological characters like growth habit or floral morphology like flower colour and other characteristics of the plant. Studies were undertaken for identification and analysis of genetic variation within 34 rose cultivars through random amplified polymorphic DNA (RAPD) markers. Analysis was made by using twenty five decamer primers. Out of twenty five, ten primers were selected and used for identification and analysis of genetic relationships among 34 rose cultivars. A total of 162 distinct DNA fragments ranging from 0.1 to 3.4 kb was amplified by using 10 selected random decamer primers. The genetic similarity was evaluated on the basis of presence or absence of bands. The cluster analysis indicated that the 34 rose cultivars form 9 clusters. The first cluster consists of eight hybrid cultivars, three clusters having five cultivars each, one cluster having four cultivars, two clusters having three cultivars each and two clusters having one cultivar each. The genetic distance was very close within the cultivars. Thus, these RAPD markers have the potential for identification of clusters and characterization of genetic variation within the cultivars. This is also helpful in rose breeding programs and provides a major input into conservation biology.

**Key words:** Polymerase Chain Reaction (PCR), Random Amplified Polymorphic DNA (RAPD)

## Introduction

The genus *Rosa* is large and complex, composed of wild and domesticated species, grouped on the basis of morphological characters. Roses are widely used as landscape and cut flower plants throughout the world. It is one of the most important groups of ornamental plants. Hybridizations and allopolyploidization have occurred frequently in this genus and make difficult the classification and the search for relationships between species and varieties (Zhang and Gandelin, 2003). Patented rose cultivars are often worth thousands of dollars and need protection from infringements. With the advent of molecular biology, several molecular marker analyses have been used in plant breeding programs. Isozyme studies were first used but their paucity limits their usefulness. The molecular approach seems to be more effective because it allows direct access to the hereditary material (the genome) and makes it possible to understand the relationships between plants (Williams *et al.*, 1990; Paterson *et al.*, 1991; Mitton, 1994). The advent of molecular markers, *e.g.* re-

striction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), mini- and microsatellites, has provided a different approach to study identification and genetic diversity. These markers have been used to classify plants that can not be identified by morphological characters. RAPD markers have proven to be a powerful tool for investigating genetic variation in various plant groups (Williams *et al.*, 1990; Welsh and McClelland, 1990; Yu *et al.*, 1993; Debener *et al.*, 1996; Rajaseger *et al.*, 1997; Lanteri *et al.*, 2001; Rout *et al.*, 2003). Randomly amplified polymorphic DNA employs gene amplification and is non-destructive as only a small quantity of DNA is needed. There are some reports on molecular characterization of rose genotypes (Hubbard *et al.*, 1992; Rajapakse *et al.*, 1992; Ben-Meir and Vainstein, 1994; Torres *et al.*, 1993; Debener *et al.*, 1996; Millian *et al.*, 1996). In the present communication findings are reported on the genetic variability among thirty-four cultivars of hybrid roses by using RAPD markers.

## Materials and Methods

### Plant material

A total of 34 hybrid rose cultivars was selected from greenhouse at the Regional Plant Resource Centre, Bhubaneswar, Orissa, India. The used cultivars for RAPD analysis are enlisted in Table I. The cultivars were of different origin. In certain cases the parents of the cultivar were also known.

### DNA extraction

DNA was extracted from fresh leaves from greenhouse raised plants of different rose cultivars by the *N*-cetyl-*N,N,N*-trimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1990). Approx. 200 mg of fresh leaves were ground to powder in liquid nitrogen using a mortar and pestle. The ground powder was transferred to a

25 ml tube with 10 ml of CTAB buffer [2% (w/v) CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris [tris(hydroxymethyl)aminomethane]-hydrochloride (pH 8.0), and 0.2% (v/v)  $\beta$ -mercaptoethanol]. The homogenate was incubated at 60 °C for 2 h, extracted with an equal volume of chloroform/isoamylalcohol (24:1) and centrifuged at  $10,000 \times g$  for 20 min (Kubata KR-2000 C, Rotor-RA-3R, Japan). DNA was precipitated from the aqueous phase by mixing with an equal volume of isopropanol. After centrifugation at  $10,000 \times g$  for 10 min, the DNA pellet was washed with 70% (v/v) ethanol, air-dried and resuspended in 10 mM Tris-HCl, pH 8.0, and 0.1 mM EDTA buffer. DNA quantifications were performed by visualizing under UV light, after electrophoresis on 0.8% agarose gel. The resuspended DNA was then diluted in TE to 5 ng/ $\mu$ l concentration for use in amplification reactions.

Table I. Hybrid rose cultivars used for the RAPD analyses.

Sl. No	Cultivar	Descendence	Country of origin, year
1	Paradise	Swarthmore $\times$ unnamed seedling	USA, 1979
2	Lovers' Meeting	Seedling $\times$ Egyptian Treasure	UK, 1980
3	Lal Bahadur	Unknown	India, 1967
4	Confidence	Peace $\times$ Michele Meilland	France, 1950
5	Sadabahar	Seedling of Frolic	India, 1969
6	Arunima	Seedling of Frolic	India, 1976
7	Montezuma	Fandango $\times$ Floradora	USA, 1955
8	Mrinalini	Pink Parfait $\times$ Christian Dior	India, 1972
9	Charisma	Gemini $\times$ Zorina	UK, 1977
10	Don Don	Seedling $\times$ Over the Rainbow	UK, 1976
11	Queen Elizabeth	Charlotte Armstrong $\times$ Floradora	USA, 1954
12	Jantar Mantar	Mirandy $\times$ Goudvlinder	India, 1982
13	Love	Unnamed seedling $\times$ Red Gold	UK, 1980
14	Indian Princess	Super Star $\times$ Granada	India, 1980
15	Shocking Blue	Unnamed seedling $\times$ Silver Star	Germany, 1974
16	Super Star	(Seedling $\times$ Peace) $\times$ (seedling $\times$ Alpine Glow)	Germany, 1960
17	Papa Meilland	Chrysler Imperial $\times$ Charles Mallerin	France, 1963
18	Taj Mahal	Manitou $\times$ Grand Slam	India, 1972
19	Raktagandha	Christian Dior $\times$ seedling of Carrousel	India, 1975
20	Christian Dior	(Independence $\times$ Happiness) $\times$ (Peace $\times$ Happiness)	France, 1959
21	Crimson Glory	Seedling of Cathrine Kordes $\times$ W. E. Chaplin	Germany, 1935
22	First Prize	Enchantment seedling $\times$ Golden Masterpiece seedling	USA, 1970
23	Anuraag	Sweet Afton $\times$ Gulzar	India, 1980
24	Italy Famous	Unknown	
25	Chandrama	White Bouquet $\times$ Virgo	India, 1980
26	Sofia Loren	Unknown	
27	Banjaran	Unknown	India, 1969
28	Tata Centenary	Mutant of bicoloured Pigalle	India, 1979
29	Gold Medal	Yellow Pages $\times$ (Granada $\times$ Garden Party)	USA, 1982
30	Dr. John Snow	Helen Traubel $\times$ seedling	UK, 1979
31	Landora	Seedling King's Ransom	UK, 1970
32	My Valentine	Little Chief $\times$ Little Curt	USA, 1975
33	Echo	Dwarf form of Rambler Tausendschön	
34	Dr. B. P. Pal	Hybrid of 2 unnamed seedlings	India, 1980

PCR amplification

Twenty five arbitrary 10-base primers (Operon Technologies Inc., Alameda, USA) were used for polymerase chain reaction (PCR). Amplification reactions were performed in 25 µl:2.0 µl of 1.25 mM each of dNTP's, 15 ng of the primer, 1 × Taq polymerase buffer, 0.5 U of Taq DNA polymerase (Genei, Bombay, India) and 20 ng of genomic DNA. DNA amplification was performed in a PTC-100 DNA Thermal Cycler (MJ Research Inc., Watertown, MA, USA) programmed for 45 cycles: 1<sup>st</sup> cycle of 3.5 min at 94 °C, 1 min at 37 °C and 2 min at 72 °C; then 44 cycles each of 1 min at 94 °C, 1 min at 37 °C, 2 min at 72 °C followed by one final extension cycle of 7 min at 72 °C. Amplified products were electrophoresed in a 1.2% (w/v) agarose (Sigma, USA) gel with 1 × TAE buffer, stained with ethidium bromide, and photographed under ultraviolet (UV) light. Gel photographs were scanned through a Gel Doc System (Gel Doc. 2000, BioRad, California, USA) and the amplification product sizes were evaluated using the software quantity one (BioRad, California, USA). The sizes of the amplification products were estimated from a 3.0 kb (100 bp to 3.0 kb) ladder (MBI Fermentas Inc., Amherst, USA). All the reactions were repeated at least two times.

Data analysis

Data were recorded as presence (1) or absence (0) of band products from the examination of photographic negatives. Each amplification fragment was named by the source of the primer (Operon), the kit letter or number, the primer number and its approx. size in base pairs. Bands with similar mobility to those detected in the negative control, if any, were not scored. Similarity indexes were

estimated using the Dice coefficient of similarity (Nei and Li, 1979). Cluster analyses were carried out on similarity estimates using the unweighted pair-group method arithmetic average (UPGMA) using NTSYS-PC version 1.80 (Rohlf, 1995).

Results and Discussion

Out of twenty-five primers, ten decamer primers (OPA-02, OPD-02, OPD-03, OPD-05, OPD-08, OPD-11, OPN-02, OPN-07, OPN-10, OPN-15) were selected, which showed good polymorphism within the 34 rose cultivars. The reproducibility of the amplification product was tested on rose DNA from three independent extractions of the 34 cultivars. Most of the amplification reactions were duplicated. Only bands that were consistently reproduced across amplifications were considered for the analysis. Bands with the same mobility were considered as identical fragments, receiving equal values, regardless of their staining intensity. When multiple bands in a region were difficult to resolve, data for that region of the gel was not included in the analysis. As a result, ten informative primers were selected and used to evaluate the degree of polymorphism within all the rose cultivars. The selected primers generated distinctive products in the range of 0.1–3.4 kb. Maximum and minimum number of bands were produced by the primers OPN-15 (21) and OPD-11 (12), respectively (Table II). A total of 162 amplified fragments was scored across 34 cultivars for the selected primers, and was used to estimate genetic relationships within the cultivars. The patterns of RAPD produced by the decamer primers OPN-07 and OPN-15 are shown in Fig. 1. The genetic variation through RAPD markers has been highlighted in number of ornamental plants including rose (Vain-

Name of primer	Sequence of the primer 5'–3'	Total number of amplification products	Number of polymorphic products	Size range [kb]
OPA-02	5'-TGCCGAGCTG-3'	16	6	0.3–2.8
OPD-02	5'-GGACCCAACC-3'	16	10	0.5–3.0
OPD-03	5'-GTCGCCGTCA-3'	14	8	0.3–2.2
OPD-05	5'-TGAGCGGACA-3'	17	8	0.4–2.4
OPD-08	5'-GTGTGCCCCA-3'	15	10	0.2–2.6
OPD-11	5'-AGCGCCATTG-3'	12	8	0.4–2.9
OPN-02	5'-ACCAGGGGCA-3'	17	6	0.1–2.7
OPN-07	5'-CAGCCCAGAG-3'	18	12	0.5–3.4
OPN-10	5'-ACAAC TG GGG-3'	16	8	0.2–3.0
OPN-15	5'-CAGCGACTGT-3'	21	10	0.1–3.0

Table II. Total number of amplified fragments and number of polymorphic fragments generated by PCR using selected random decamers.

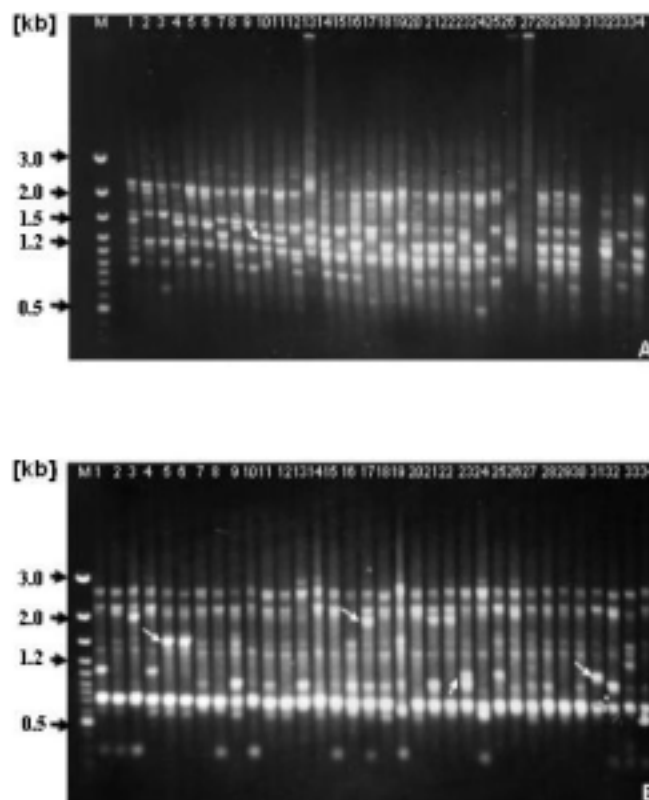


Fig. 1. RAPD patterns of 34 cultivars of *Rosa hybrida* generated by the primer OPN-07 (5'-CAGCCCAGAG-3') (A) and OPN-15 (5'-CAGC-GACTGT-3') (B). M, molecular weight ladder; Nos. 1–34 reflect the hybrid cultivars.

stein *et al.*, 1993; Debener *et al.*, 1996; Rajaseger *et al.*, 1997). The present findings showed the close variation among the 34 hybrid rose cultivars. The similarity matrix (Table III) was obtained after multivariate analysis using Nei and Li's coefficient (data not shown). The similarity matrix was then used to construct a dendrogram with the unweighted pair-group arithmetic average method (Fig. 2). The dendrogram shows nine clusters within the 34 cultivars. There was a narrow variation within the hybrid cultivars. Our results were similar to the findings of Ben-Meir and Vainstein (1994). Vainstein *et al.* (1993) reported that the genetic similarities are small within the cultivated rose groups (hybrid tea, floribunda, polyantha and miniature) by using 28 DNA fragments from microsatellite fingerprints. They also indicated a higher genetic similarity between the hybrid tea and the floribunda group than within each group. Debener *et al.* (1996) reported the higher levels of genetic variability between cultivated roses than between some wild species. The present findings

include the identification and genetic variation within 34 cultivars of rose. The dendrogram shows that the distance within the cultivars was not significantly different within the cultivars. Debener *et al.* (1996) reported that the cultivated roses do not cluster according to the classification scheme and widely used to group roses after their origin. Among the nine clusters formed, one cluster has eight hybrid cultivars (Lal Bahadur, Mrinalini, Jantar Mantar, Papa Meilland, Christian Dior, Crimson Glory, First Prize and Anuraag), three clusters have five cultivars each, one cluster has four cultivars, two clusters have three cultivars each and two clusters have one cultivar each. The genetic distances indicate that the cultivar "Tata Centenary" has 81% similarity with "Dr. John Snow". The cultivar "Sadabahar" has 72% similarity with "Arunima". The "Landora" and "Love" have 37% and 50% similarity within the 34 cultivars, respectively. The "Indian Princess" and "Super Star" have 65% similarity among themselves. The close relationships within the cul-

Table III. Similarity matrix of 34 cultivars of *Rosa hybrida*.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	
1	1																																		
2	0.7	1																																	
3	0.7	0.7	1																																
4	0.8	0.7	0.7	1																															
5	0.7	0.6	0.7	0.7	1																														
6	0.7	0.7	0.7	0.6	0.9	1																													
7	0.7	0.7	0.8	0.7	0.7	0.7	1																												
8	0.7	0.7	0.8	0.7	0.7	0.7	0.7	1																											
9	0.6	0.7	0.6	0.6	0.7	0.7	0.7	0.7	1																										
10	0.7	0.7	0.7	0.6	0.6	0.7	0.7	0.7	0.7	1																									
11	0.6	0.7	0.7	0.6	0.6	0.7	0.8	0.6	0.7	0.7	1																								
12	0.7	0.7	0.8	0.7	0.7	0.7	0.8	0.7	0.7	0.7	0.7	1																							
13	0.6	0.6	0.7	0.6	0.6	0.6	0.6	0.7	0.6	0.6	0.6	0.7	0.6	1																					
14	0.6	0.7	0.7	0.7	0.6	0.7	0.7	0.6	0.7	0.7	0.6	0.7	0.7	0.8	1																				
15	0.7	0.7	0.7	0.6	0.6	0.6	0.6	0.7	0.6	0.6	0.7	0.7	0.6	0.8	0.7	1																			
16	0.7	0.7	0.7	0.6	0.6	0.7	0.8	0.7	0.6	0.7	0.7	0.7	0.6	0.8	0.7	0.7	1																		
17	0.8	0.7	0.8	0.7	0.8	0.7	0.7	0.8	0.7	0.7	0.7	0.7	0.6	0.7	0.6	0.7	0.7	0.8	1																
18	0.7	0.7	0.7	0.8	0.7	0.7	0.8	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.6	0.7	0.7	0.7	0.6	1															
19	0.7	0.7	0.8	0.7	0.7	0.6	0.8	0.7	0.7	0.8	0.8	0.7	0.7	0.7	0.7	0.7	0.7	0.6	0.7	0.8	1														
20	0.7	0.7	0.7	0.7	0.6	0.7	0.7	0.8	0.7	0.7	0.7	0.8	0.6	0.7	0.7	0.7	0.7	0.6	0.7	0.7	0.8	1													
21	0.6	0.8	0.7	0.7	0.5	0.6	0.7	0.7	0.6	0.6	0.6	0.6	0.6	0.6	0.7	0.8	0.7	0.7	0.7	0.7	0.8	0.8	1												
22	0.7	0.7	0.7	0.7	0.7	0.7	0.5	0.6	0.7	0.7	0.6	0.7	0.8	0.6	0.7	0.7	0.8	0.7	0.7	0.7	0.7	0.8	0.8	1											
23	0.8	0.6	0.7	0.8	0.7	0.7	0.7	0.7	0.6	0.6	0.7	0.6	0.7	0.6	0.7	0.7	0.8	0.7	0.7	0.7	0.7	0.7	0.7	0.8	1										
24	0.6	0.6	0.7	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.7	0.7	0.7	0.6	0.7	0.7	0.7	0.7	0.6	0.7	0.7	0.7	0.6	1									
25	0.6	0.7	0.7	0.6	0.7	0.7	0.7	0.7	0.7	0.8	0.7	0.7	0.6	0.7	0.6	0.6	0.7	0.6	0.7	0.7	0.6	0.7	0.6	0.7	0.7	0.7	1								
26	0.7	0.7	0.7	0.6	0.7	0.7	0.6	0.7	0.6	0.6	0.7	0.8	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.8	0.7	0.7	0.7	0.7	1							
27	0.7	0.7	0.7	0.6	0.7	0.6	0.7	0.7	0.6	0.6	0.6	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	1						
28	0.7	0.8	0.8	0.7	0.7	0.7	0.7	0.7	0.6	0.6	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.8	0.7	0.7	0.7	0.7	0.7	0.7	0.7	1					
29	0.7	0.7	0.6	0.7	0.6	0.6	0.7	0.7	0.6	0.7	0.7	0.6	0.7	0.6	0.7	0.7	0.7	0.7	0.6	0.7	0.7	0.8	0.7	0.6	0.7	0.7	0.7	0.7	0.8	1					
30	0.6	0.7	0.7	0.7	0.6	0.6	0.7	0.6	0.6	0.6	0.6	0.7	0.6	0.7	0.6	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.9	0.7	1			
31	0.5	0.5	0.6	0.6	0.5	0.5	0.6	0.5	0.5	0.5	0.6	0.6	0.5	0.5	0.5	0.5	0.6	0.6	0.5	0.5	0.5	0.5	0.6	0.5	0.6	0.5	0.5	0.6	0.5	0.6	0.5	1			
32	0.7	0.7	0.7	0.7	0.6	0.6	0.7	0.6	0.6	0.7	0.7	0.6	0.6	0.6	0.7	0.6	0.7	0.7	0.7	0.7	0.6	0.6	0.6	0.6	0.6	0.5	0.7	0.6	0.7	0.6	0.7	0.5	1		
33	0.7	0.6	0.6	0.7	0.7	0.6	0.7	0.7	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.7	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.7	0.7	0.6	0.5	0.7	1		
34	0.7	0.7	0.6	0.6	0.7	0.6	0.7	0.7	0.6	0.7	0.6	0.6	0.6	0.7	0.7	0.8	0.7	0.7	0.6	0.7	0.6	0.7	0.7	0.6	0.6	0.6	0.7	0.7	0.7	0.5	0.7	0.7	0.7	1	

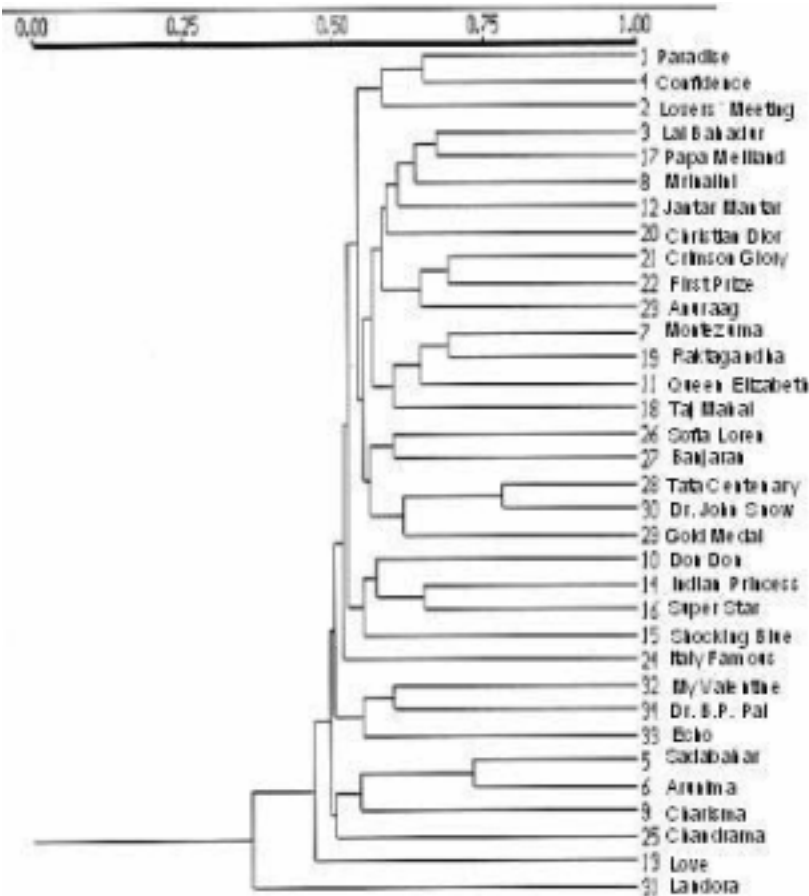


Fig. 2. Dendrogram of cluster analysis of RAPD markers. The scale indicates the fractional similarities among the cultivars.

tivars of celery were also reported by using RAPD markers (Yang and Quiros, 1993). This indicates that the RAPD markers provide a more reliable method for identification of cultivars than morphological characters. This investigation as an understanding of the level and partitioning of genetic variation within the cultivars would provide an important input into determining appropriate man-

agement strategies. This study will help in future breeding programs in roses.

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