

Morphological and molecular analyses of *Rosa damascena* × *R. bourboniana* interspecific hybrids

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ABSTRACT

Rosa damascena Mill is the most important scented rose species cultivated for rose oil production. *Rosa bourboniana* L. (Edward rose), a related species, is popular on account of its longer blooming period and ease of propagation. With an aim to combine the oil quality of *R. damascena* and recurrent flowering habit of *R. bourboniana*, two cultivars (Jwala and Himroz) of *R. damascena* were crossed with *R. bourboniana*. The F1 hybrids obtained were evaluated using morphological, random amplified polymorphic DNA (RAPD) and microsatellite (SSR) markers. Twenty-two selected RAPD and three SSR primer pairs were utilized for hybrid identification. According to presence or absence of bands RAPD and SSR markers were classified into seven types of markers. The bands specific for the pollen parent and occurring in the hybrids were good markers to confirm the hybridity. The non-parental bands expressing uniquely in hybrids were effective in distinguishing the hybrids from each other. Cluster analysis, based on Jaccard's similarity coefficient using unweighted pair group method based on arithmetic mean (UPGMA), reliably discriminated the hybrids into two main clusters. These results indicate the practical usefulness of RAPD and SSR markers in hybrid identification in scented roses. The approach is advantageous for its rapidity and simplicity, for identification of hybrids at the juvenile stage. One of the studied morphological traits – prickle density, can also complement in the identification of interspecific hybrids between *R. damascena* (♀) and *R. bourboniana* (♂).

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1. Introduction

Rosa damascena Mill is the most important scented rose species (Topalov, 1978; Staikov and Kalajiev, 1980) and is cultivated in Bulgaria, France, Italy, Turkey, Iran, Morocco USA and India for the production of rose oil (Krüssman, 1981). Besides its importance as base material for flavor and fragrance industry, rose oil is also known for anti-HIV, antibacterial and antioxidant properties (Mahmood et al., 1996; Achuthan et al., 2003; Basim and Basim, 2003; Ozkan et al., 2004). Rose oil is one of the expensive essential oils in the world (Baydar and Baydar, 2005) due to its unique combination of high valued odour constituents and low oil yields (0.045%). The total flowering period of *R. damascena* in a year is only around 25–30 days during April–May. *Rosa bourboniana* L. (Edward rose), also an essential oil (0.015%) yielding species, is popular on account of its longer blooming period and ease of propagation. The flowering in this species is sporadic and it flowers thrice a year (Sood and Nagar, 2004). Development of improved cultivars through hybridization may contribute towards increased

productivity and quality in scented rose. Hybridization of genetically different plants is followed for hybrid variety development and molecular marker techniques are often used for hybrid identification (Wolff et al., 1994). During the past several years markers like RFLP have been used in other crops. However, RFLP is costly and labor intensive. Development of PCR has allowed the introduction of RAPD approach for molecular analysis of genomes (Williams et al., 1990). The major advantage of this approach lies in exploration of large genomic portions without prior sequence information and requires small quantity of DNA. RAPD markers have been utilized by various workers for identification of hybrids in *Chrysanthemum* (Sheng et al., 2000), barley (Hoffman et al., 2003), Chilli (Mongkolporn et al., 2004), cotton (Mehetre et al., 2004) black pepper (George et al., 2005). In case of rose, RAPD markers have been successfully used for the identification of interspecific hybrids of *R. dumalis* and *R. rubiginosa* (Werlemark, 2000). Microsatellite markers are also markers of choice (Esselink et al., 2003) because they are abundant, uniformly distributed, highly polymorphic, codominant, rapidly produced by PCR and easily accessed through published primer sequences (Gupta and Varshney, 2000). Microsatellite markers have been used in identifying hybrids between red and cultivated rice (Gealy et al., 2002). The present investigation was aimed to test the hybridity of

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Table 1

The morphological characteristics of *R. damascena*, *R. bourboniana* and the putative interspecific hybrids. The data are the mean (\pm standard error) determined from 10 plants each of *R. damascena* var. Jwala (RDJ), *R. damascena* var Himroz (RDH) and *R. bourboniana* (RB) and from 11 and 57 interspecific hybrid plants – Jwala \times *R. bourboniana* (J \times B) and Himroz \times *R. bourboniana* (H \times B) respectively.

Characteristics	Genotypes				
	RDJ	RDH	RB	J \times B	H \times B
Plant height (cm)	161.1 \pm 22.1	166 \pm 17.25	187.5 \pm 29.8	131.7 \pm 40.21	136.7 \pm 44.80
Number of primary branches	4 \pm 1.75	2 \pm 0.74	4 \pm 0.48	3 \pm 1.65	2 \pm 2.16
No. of internodes/branch	55 \pm 5.8	52 \pm 3.37	48 \pm 7.84	45 \pm 9.02	48 \pm 9.03
Av. internode length	1.8 \pm 0.55	2.03 \pm 0.22	2.7 \pm 0.5	2.16 \pm 0.51	2.1 \pm 0.43
No. of prickles in top 10 nodes	6 \pm 2.7	6 \pm 1.0	2 \pm 0.4	2 \pm 0.8	2 \pm 1.1
Leaf length (cm)	13.32 \pm 1.6	12.2 \pm 1.36	11.0 \pm 1.2	9.6 \pm 2.9	9.8 \pm 2.9
No. of leaflets/leaf	6 \pm 1.0	6 \pm 0.97	5 \pm 0.6	6 \pm 0.96	5 \pm 1.1
Length of leaflets	3.6 \pm 0.39	3.5 \pm 0.42	3.9 \pm 0.38	3.04 \pm 0.88	3.3 \pm 0.9
Width of leaflets	2.6 \pm 0.2	2.3 \pm 0.26	2.6 \pm 0.27	2.1 \pm 0.57	2.2 \pm 0.52
Buds/Br	11 \pm 2.6	7 \pm 3.0	3 \pm 0.87	2 \pm 0.99	3 \pm 0.8
Width of flower	6.7 \pm 0.5	6.5 \pm 0.6	4.8 \pm 0.3	6.27 \pm 0.36	6.16 \pm 0.9
No. of petals/flower	38 \pm 5.3	32 \pm 6.0	47 (35–58) \pm 9.00	21 (5–36) \pm 12.8	33 (5–79) \pm 19.9

68 putative F1 hybrids of Jwala \times *R. bourboniana* (11), Himroz \times *R. bourboniana* (57) utilizing RAPD and microsatellite (SSR) markers and also to identify an alternative (morphological) trait to distinguish the interspecific hybrids from the parental lines.

2. Materials and methods

The experimental material comprised of three parental lines viz. *R. damascena* cv. Jwala, *R. damascena* cv. Himroz, *R. bourboniana* and 68 putative F1 hybrids Jwala \times *R. bourboniana* (11) and Himroz \times *R. bourboniana* (57), maintained in the experimental field of the Institute of Himalayan Bioresource Technology, Palampur, Himachal Pradesh, India. The hybrids were developed by adopting round cut method of emasculation followed by hand pollination (Dhyani et al., 2005) during 2002–2003. The resultant hips were harvested (Table 2) and seed collected. 15–20 seeds were sown in moist vermiculite in small Petri dishes (9 cm). Each Petri dish was sealed with cellophane and chilled for 90–120 days at 4 °C for stratification. After 30 days Petri dishes were regularly checked for seed germination and germinated seeds were transplanted in small pots. 6–10-month-old seedlings were transplanted in the field for further development and evaluation studies. The morphological data of 12 quantitative characters was recorded after 2 years of establishment of hybrid plants under field condition (Table 1).

2.1. DNA isolation

Fresh juvenile leaves from parental lines and the putative hybrids were sampled separately. The samples were frozen in liquid nitrogen and stored at –70 °C till DNA isolation. DNA was isolated from leaf tissue by a modified CTAB method (Xu et al., 2004) using increased concentration of CTAB (3%), β -mercaptoethanol (3%) and

Table 2

Details of crosses made in *Rosa damascena* and *Rosa bourboniana*.

Crosses	No. of buds pollinated	No. of hips harvested	Total no. of seeds obtained
Jwala \times <i>Rosa bourboniana</i>	1335	70	106
Himroz \times <i>Rosa bourboniana</i>	574	202	408

PVP (2%) in a high salt concentration extraction buffer. Quantification of DNA was accomplished by analyzing the DNA on 0.8% agarose gel using diluted uncut λ DNA as quantitative standard. DNA was diluted in Tris 10:EDTA 1 buffer to a concentration of approximately 20 ng/ μ l for PCR analysis.

2.2. RAPD analysis

Twenty-two selected primers (Operon Technologies Inc.) (Table 4) were used for RAPD analysis (Kaur et al., 2005). The PCR reactions were performed in 25 μ l volume containing 20 ng of DNA, 0.2 units of taq DNA polymerase (Genei, Bangalore, India), 100 mM of each dNTP, 1.5 mM MgCl₂, and 20 ng of a decamer nucleotide primer. The amplifications were carried out in a thermocycler (Gene-Amp PCR system 9700, Applied Biosystems, USA) programmed at 94 °C for 4 min, followed by 45 cycles at 94 °C for 1 min, 37 °C for 1 min and 72 °C for 2 min, then final extension at 72 °C for 7 min and stored at 4 °C. The amplified products were resolved in 1.4% agarose gel (containing 0.5 mg/ml of ethidium bromide) using 1 \times TBE buffer. RAPD gel electrophoresis profiles were visualized and imaged using Alpha Digi Doc Gel Documentation and Image Analysis System (Alpha Innotech, USA). The size of each band was estimated using 100 bp ladder plus (MBI Fermentas).

Table 3

Seven types of markers observed in hybrids of *R. damascena* (var. Jwala and Himroz) \times *R. bourboniana*.

Type of marker	Property of marker			J \times B		H \times B	
	Female	Offspring	Male	No.	%	No.	%
I	+	+	+	243	69.0	255	71.0
II	+	+	–	25	7.0	27.0	7.5
III	+	–	+	4	1.1	3.0	0.8
IV	–	+	+	48	13.6	40.0	11.2
V	+	–	–	1	0.3	0	0
VI	–	+	–	27	7.7	34.0	9.5
VII	–	–	+	4	1.1	1	0.3
Total				352		357	

2.3. Microsatellite analysis

As no microsatellite markers for *R. damascena* or *R. bourboniana* have been developed till date, 15 microsatellite primer pairs developed for *Rosa hybrida* (Yan et al., 2005) were screened for parental polymorphism and three primer pairs for the loci – Rh 48, Rh 78 and RhB510 were selected for verifying the hybridity of interspecific hybrids. DNA amplification was carried out in a 10 µl reaction volume containing 1× PCR assay buffer (50 mM KCl, 10 mM Tris–Cl, 1.5 mM MgCl₂), 25 mM of each dNTP, 20 ng of each forward and reverse primers, 0.2 units of Taq DNA polymerase (Bangalore genei) and 20 ng of DNA. The amplification reaction was carried out as above in a thermocycler programmed at 94 °C for 4 min, followed by 35 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min and final extension at 72 °C for 7 min PCR products were resolved in 3% metaphor agarose gel using 1× TBE buffer. The size of amplified fragments was determined by using 100 bp DNA ladder as size standard. The SSR profiles were visualized and captured using Alpha Digi Doc Gel Documentation and Image Analysis System (Alpha Innotech, USA).

2.4. Data analysis

Mean and standard error of morphological parameters (Table 1) was determined from 10 plants each of *R. damascena* cv. Jwala, *R. damascena* cv. Himroz and *R. bourboniana* and from 11 and 57 interspecific hybrid plants of Jwala × *R. bourboniana* and Himroz × *R. bourboniana* respectively.

2.5. Cluster analysis

All RAPD assays were repeated thrice and only the reproducible bands were scored, likewise the amplified SSR products were scored as present (1) or absent (0) for each primer genotype combination. The data entry was done into a binary data matrix and Jaccard's coefficient of similarity was generated using unweighted pair group method based on arithmetic mean (UPGMA) through the computer package NTSYS – PC 2.0 (Rohlf, 1998).

3. Results

3.1. Morphology

The morphological traits recorded of putative interspecific hybrids were unlike either parent, or identical to one parent (Table 1). Out of 12 characters examined, five characters, plant height, number of primary branches, leaf length, length of leaflets, width of leaflets, were distinctive to the interspecific hybrids. The other characters, such as number of internodes per branch and number of prickles in top 10 internodes and number of buds per bunch were similar to those of *R. bourboniana* and number of petals was closer to *R. damascena* than *R. bourboniana* (Table 1).

3.2. RAPD analysis

The twenty-two 10-mer primers selected for DNA amplification of *R. damascena* (cv. Jwala and Himroz), *R. bourboniana* and the putative interspecific hybrids (Table 4) generated a total of 263, 276, 285, 339 and 344 bands respectively showing overall 76% and 87% polymorphism. According to their presence or absence RAPD and SSR bands were classified into seven types of markers (Table 3) as per Mehetre et al. (2004) and Huang et al. (2000). The bands common in parents and hybrids were included in marker

Table 4

Selected RAPD and SSR primers, their sequences and number of hybrids identified by each primer (identified/total).

S. no.	Primer name	Sequence (5'–3')	J × B	H × B
1	OPG-14	GGATGAGACC	11/11	57/57
2	OPS-13	GTCGTTCCTG	10/11	56/57
3	OPS-14	AAAGGGGTCC	8/11	24/57
4	OPM-10	TCTGGCGCAC	9/11	56/57
5	OPV-1	TGACGCATGG	9/11	47/57
6	OPV-4	CCCCTCACGA	10/11	51/57
7	OPA-16	AGCCAGCGAA	11/11	38/57
8	OPV-9	TGTACCCGTC	10/11	40/57
9	OPS-6	GATACCTCGG	9/11	0/57
10	OPS-12	CTGGGTGAGT	0/11	0/57
11	OPS-18	CTGGCCAACT	9/11	53/57
12	OPM-4	GGCGGTGTGC	11/11	0/57
13	OPG-15	ACTGGGACTC	4/11	50/57
14	OP E-12	TTATCGCCCC	9/11	30/57
15	OPE-4	GTGACATGCC	9/11	0/57
16	OPV-12	ACCCCCCACT	10/11	56/57
17	OPV-6	ACGCCAGGT	6/11	29/57
18	OPV-7	GAAGCCAGCC	0/11	0/57
19	OPM-2	ACAACGCCTC	7/11	29/57
20	OPM-7	CCGTGACTCA	10/11	41/57
21	OPV-2	AGTCACTCCC	0/11	54/57
22	OPF-11	TTGGTACCCC	5/11	53/57
23	Rh 48	F gatagtttctgtacccccacta R ttgaccagctgcaacaaaattaga	10/11	54/57
24	Rh 78	F aaagaaacgcgaaatctatgatgc R tctgtagtggatttaaagacagg	11/11	57/57
25	Rh B510	F aaacgatagtgatctgtgggt R cactcaacctgttccactcctaat	10/11	52/57

type I, II and IV (Table 3) and were good markers to confirm hybridity in *R. damascena* cv. Jwala × *R. bourboniana* and *R. damascene* cv. Himroz × *R. bouboniana*. The bands from parents not found in hybrids were included in type III, V and VII (Table 3). The non-parental bands expressing uniquely in hybrids and were included in type VI (Table 3). Most of the primers (OPG-14, OPS-13, OPV-7, OPV-12, OPF-11, OPM-2, OPG-15, OPS-6, OPS-18, OPA-16 and OPV-4) generated type IV markers and helped in confirming the hybridity of interspecific F1 hybrids (Table 4). Three (11%) of these primers showed high resolution of the integrated pollen parental bands into the putative interspecific hybrids. In repeated trials, these operon primers (OP) (OPS-13, OPS-14 and OPG-14) consistently amplified the *R. damascena* and *R. bourboniana* bands in all 68 putative interspecific hybrids. Using these three primers a total of nine polymorphic bands were amplified in the putative interspecific hybrids (Fig. 3). For example, OPS-14 amplified a 650 bp male parent specific fragment in the hybrids (Fig. 3) and could identify 8/11 J × B hybrids and 24/57 H × B hybrids (Table 4). Whereas OPS-13 amplified 1210 and 1110 bp male parent specific fragments that together could identify 10/11 of J × B and 56/57 of H × B hybrids

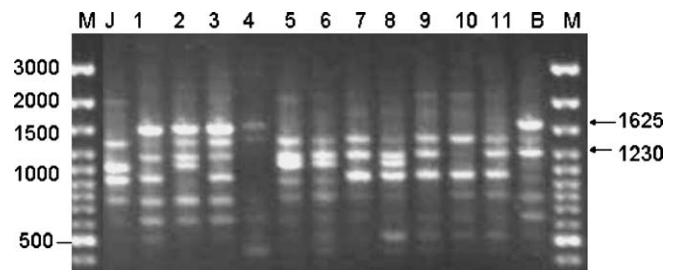


Fig. 1. RAPD profile of *R. damascena* var. Jwala (J), *R. bourboniana* (B) and putative F1 hybrids (1–11) (Jwala × *R. bourboniana*) using primer OPS-13. Male specific bands are marked with arrows. M represents 100 bp DNA ladder.

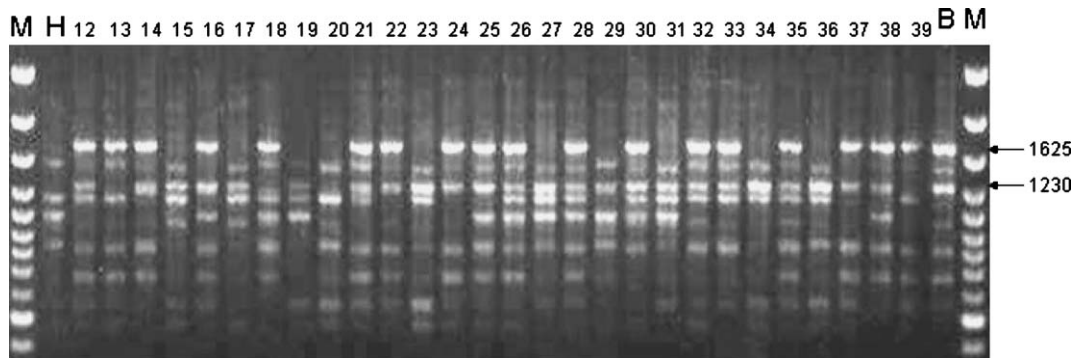


Fig. 2. RAPD profile of *R. damascena* var. Himroz (H), *R. bourboniana* (B) and putative F1 hybrids (Jwala \times *R. bourboniana*) using primer OPS-13. Male specific markers (OPS-13₁₂₁₀ and 1110 bp) are marked with arrows. M represents 100 bp DNA ladder.

(Figs. 1 and 2). All the putative hybrids tested had at least one pollen parent specific RAPD band. Certain primers (OPM-02, OPM-07 and OPG-14) generated markers of type VI and were effective in distinguishing the hybrids from each other. Hence these primers will be beneficial for the identification of specific hybrids.

3.3. Microsatellite analysis

The three SSR primers Rh 48, Rh 78 and RhB510 generated 7, 7, 11 and 7–11 alleles in *R. damascena* var. jwala, *R. damascena* var. himroz, *R. bourboniana* and interspecific hybrids respectively and could identify 9/11, 11/11 and 10/11 Jwala \times *R. bourboniana* hybrids and 55/57, 57/57 and 52/57 Himroz \times *R. bourboniana* hybrids respectively (Table 4, Fig. 3). Based on the RAPD and SSR data, we could develop fingerprints which could give details of a minimum number of primers to be used for the identification of these hybrids (Table 4). Fingerprints based on 16 fragments across the J \times *R. bourboniana* hybrids are shown in Fig. 3. Cluster analysis, based on Jaccard's similarity coefficient using UPGMA, reliably discriminated the hybrids into 2 main clusters (Fig. 4). The average similarity index across the hybrids

was 0.63. The two parental lines Jwala and Himroz were 0.90 similar, whereas similarity with the third parental line (*R. bourboniana*) was 0.75 and 0.80 respectively.

4. Discussion

Interspecific hybridization was attempted between *R. damascena* (cv. Jwala and Himroz) and its related species *R. bourboniana* to increase the spectrum of variation in its desirable traits such as oil quality and recurrent flowering. Since the parental genotypes are polyploids and highly heterozygous (being selections from an open pollinated population) recombination gives rise to variations at the gametic level and the hybrids cannot be true to type or uniform like F1's of homozygous diploid plants. Thus due to inter specific nature of the cross, hybrids offer an opportunity to combine the complimentary traits. The high level of polymorphism exhibited by the selected primers was possibly due to recombination, mutations or random segregation of heterozygous chromosomes during meiosis (Smith et al., 1996). The unique fragments helped in ascertaining the hybridity at DNA level. The non-parental bands (included in type VI) expressing uniquely in

Molecular Markers	J	B	1	2	3	4	5	6	7	8	9	10	11
OPG14 ₂₈₀₀													
OPG14 ₁₀₀₀													
OPG14 ₆₅₀													
OPG14 ₅₅₀													
OPG14 ₄₇₅													
OPS13 ₁₆₂₅													
OPS13 ₁₂₃₀													
OPS13 ₉₅₀													
OPS14 ₆₅₀													
OPV4 ₂₅₀₀													
OPA16 ₁₂₃₀													
OPM4 ₂₀₀₀													
OPM4 ₁₃₅₀													
Rh48 ₂₈₀													
Rh78 ₂₁₀													
RhB510 ₂₀₀													

Fig. 3. Diagrammatic representation of certain pollen parent specific (type IV) markers in Jwala \times *R. bourboniana* interspecific hybrids (1–11). Black colour specifies the presence of marker whereas white specifies the absence.

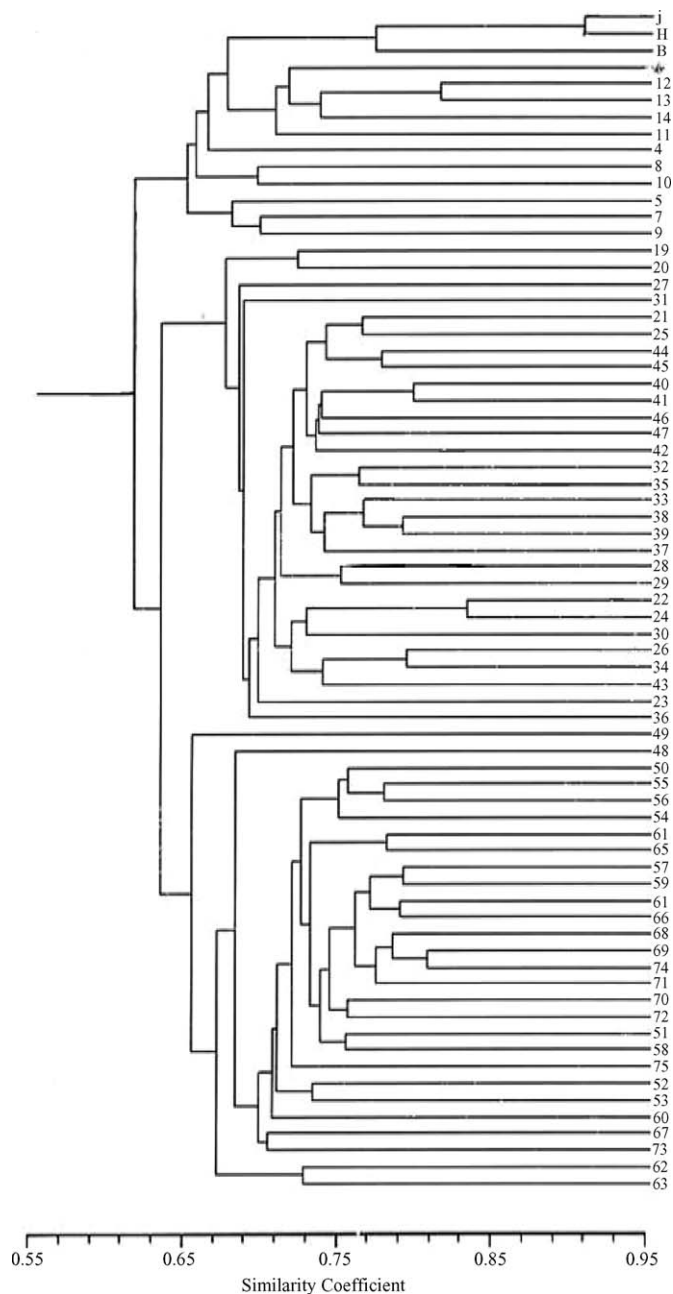


Fig. 4. Cluster diagram grouping parents [Jwala (J), Himroz (H), *R. bourboniana* (B)] and hybrids into clusters.

hybrids have direct implication in identity of specific hybrids and the assessment of genetic diversity within the hybrids. These non-parental bands may be generated from the recombination and mutation in meiosis processing during hybridization (Huchett and Botha, 1995) and may also be created by heteroduplex formation (Ayliffe et al., 1994). These results indicate the practical usefulness of RAPD and SSR markers in hybrid identification in scented rose. The approach is advantageous for its rapidity and simplicity, for identification of hybrids at the juvenile stage. Morphological characterization of the interspecific hybrids carried out depicted that prickle density was inherited as dominant and also pollen parent specific character. Quantitative character such as prickle density has also complemented in the identification of hybrids of *R. rugosa* and *R. blanda* (Mercure and Bruneau, 2008). Identification of hybrids using male parent-

specific RAPD markers has also been reported in black pepper (*Piper nigrum* L.) (George et al., 2005). Whereas, microsatellite markers have been utilized in identifying hybrids between red and cultivated rice (Gealy et al., 2002). This study shows that both dominant and co dominant markers are suitable for the identification of interspecific hybrids of *R. damascena* and *R. bourboniana*. Thus the markers can be adopted for the large scale screening of hybrids. Confirmation of hybrid nature at juvenile stage by screening with RAPD and SSR markers would be practical and feasible in perennial crops like rose. It will enable elimination of all doubtful seedlings and can save labor, space and cost. Moreover these markers can be utilized to develop SCAR markers for hybrid verification in marker assisted breeding (Lee et al., 2005).

References

- Achuthan, C.R., Babu, B.H., Padikkala, J., 2003. Antioxidant and hepatoprotective effects of *Rosa damascena*. *Pharm. Biol.* 41, 357–361.
- Ayliffe, M.A., Lawrence, G.T., Ellis, J.G., Pryor, A.J., 1994. Heteroduplex molecules formed between allelic sequences cause nonparental RAPD bands. *Nucleic Acids Res.* 22, 1632–1636.
- Basim, E., Basim, H., 2003. Antibacterial activity of *Rosa damascena* essential oil. *Fitoterapia* 74, 394–396.
- Baydar, H., Baydar, N.G., 2005. The effects of harvest date, fermentation duration and Tween 20 treatment on essential oil content and composition of industrial oil rose (*Rosa damascena* Mill.). *Ind. Crop. Prod.* 21, 251–255.
- Dhyani, D., Karthigeyan, S., Ahuja, P.S., 2005. An efficient round cut method (RCM) for emulsification of rose flowers. *Acta Horticult.* 690, 125–130.
- Esselink, G.D., Smulders, M.J.M., Vosman, B., 2003. Identification of cut rose (*Rosa hybrida*) and rootstock varieties using robust sequence tagged microsatellite markers. *Theor. Appl. Genet.* 106, 277–286.
- Gealy, D.R., Tai, T.H., Sneller, C.H., 2002. Identification of red rice, rice and hybrid populations using microsatellite markers. *Weed Sci.* 50, 333–339.
- George, J.K., Varma, R.S., Sasikumar, B., Saji, K.V., 2005. Identification of hybrids in black pepper (*Piper nigrum* L.) using male parent specific RAPD markers. *Curr. Sci.* 88, 216–218.
- Gupta, P.K., Varshney, R.K., 2000. The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica* 113, 163–185.
- Hoffman, D., Hang, A.N., Larson, S., Jones, B., 2003. Conversion of an RAPD marker to an STS marker for barley variety identification. *Plant Mol. Biol. Rep.* 21, 81–91.
- Huang, S.C., Tsai, C.C., Sheu, C.S., 2000. Genetic analysis of *Chrysanthemum* hybrids based on RAPD molecular markers. *Bot. Bull. Acad. Sin.* 41, 257–262.
- Huchett, B.I., Botha, F.C., 1995. Stability and potential use of RAPD markers in a sugarcane genealogy. *Euphytica* 86, 117–125.
- Kaur, N., Sharma, R.K., Dhayani, D., Karthigeyan, S., Ahuja, P.S., 2005. Molecular characterization of inter specific hybrids of scented roses using RAPD markers. In: Fourth International Symposium on Rose Research and Cultivation, 18–22 September, Santa Barbara, California, USA.
- Krüssman, G., 1981. *The Complete Book of Roses*. Timber Press, Portland, Oregon, 409 p.
- Lee, S.Y., Yae, B.W., Kim, K.S., 2005. Segregation pattern of several morphological characters and RAPD markers in interspecific hybrids between *Dianthus giganteus* and *D. carthusianorum*. *Sci. Hort.* 105, 53–64.
- Mahmood, N., Piacente, S., Pizza, C., Burke, A., Khan, A.I., Hay, A.J., 1996. The anti-HIV activity and mechanisms of action of pure compounds isolated from *Rosa damascene*. *Biochem. Biophys. Res. Commun.* 229, 73–79.
- Mehetre, S.S., Gomes, M., Eapen, S., 2004. RAPD analysis of hybrid nature of the offspring of *Gossypium hirsutum* × *G. raimondii*. *Curr. Sci.* 87, 25–28.
- Mercure, M., Bruneau, A., 2008. Hybridization between the escaped *Rosa rugosa* (rosaceae) and native *R. blanda* in eastern North America. *Am. J. Bot.* 95, 597–607.
- Mongkolporn, O., Dokmaihom, Y., Kanchana Udomkan, C., Pakdeevanaporn, P., 2004. Genetic purity test of F1 hybrid capsicum using molecular analysis. *J. Horticult. Sci. Biotechnol.* 79, 449–451.
- Ozkan, G., Sagdic, O., Baydar, N.G., Baydar, H., 2004. Antioxidant and antibacterial activities of *Rosa damascena* flower extracts. *Food Sci. Technol. Int.* 10, 277–281.
- Rohlf, F.J., 1998. NTSYS-PC Numerical Taxonomy and Multivariate Analysis System, Version 2.0. Exeter Software, Setauket, New York.
- Sheng, C.H., Chu, C.T., Chian, S.S., 2000. Genetic analysis of *Chrysanthemum* hybrids based on RAPD molecular markers. *Bot. Bull. Acad. Sin.* 41, 257–262.
- Smith, J.F., Burke, C.C., Wagner, W.L., 1996. Interspecific hybridization in natural population of *Cryptandra* (Gesneriaceae) on the Hawaiian Islands: evidence from RAPD markers. *Plant Syst. Evol.* 200, 61–77.
- Sood, S., Nagar, P.K., 2004. Changes in endogenous polyamines during flower development in two diverse species of rose. *Plant Growth Regul.* 44, 117–123.
- Staikov, V., Kalajiev, I., 1980. Study of oil roses (*Rosa damascena* Mill.) originated from India, Morocco, Iran and Bulgaria. *Plant Sci. XVII*, 58–68 (in Bulgarian).
- Topalov, V., 1978. The Kazanlak rose and the rose production in Bulgaria. *Christo Danov, Plovdiv* (in Bulgarian).

- Werlemark, G., 2000. Evidence of apomixes in hemisexual dogroses, *Rosa* section Caninae. *Sex Plant Reprod.* 12, 353–359.
- Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A., Tingey, S.V., 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* 18, 6531–6535.
- Wolff, K., Peters, V.R., Hofstra, H., 1994. RFLP analysis in chrysanthemum. I. Probe and primer development. *Theor. Appl. Genet.* 88, 472–478.
- Xu, Q., Wen, X., Deng, X., 2004. A simple protocol for isolating genomic DNA. From chestnut rose (*Rosa roxburghii* Tratt) for RFLP and PCR analyses. *Plant Mol. Biol. Rep.* 22, 301a–301g.
- Yan, Z., Denneboom, C., Hattendorf, A., Dolstra, O., Debener, T., Stam, P., Visser, P.B., 2005. Construction of an integrated map of rose with AFLP, SSR, PK, RGA, RFLP, SCAR and morphological markers. *TAG* 110, 766–777.