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G. Scovel · H. Ben-Meir · M. Ovadis
H. Itzhaki · A. Vainstein

RAPD and RFLP markers tightly linked to the locus controlling carnation (*Dianthus caryophyllus*) flower type

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Abstract Flower doubleness as a breeding characteristic is of major importance in carnation (*Dianthus caryophyllus*), one of the major cut-flowers sold worldwide, since flower architecture is of the utmost value in ornamentals. Based on the number of petals per flower, carnations are grouped into "single", "semi-double" and "double" flower types. The first have five petals and are easily distinguishable, but of no economic value to the carnation industry. Flowers of standard and spray varieties, which constitute the largest market share, are usually of the double and semi-double type, respectively. These flower types are not easily distinguishable due to phenotypic overlaps caused by environmental conditions. To study the inheritance of this trait, several progeny segregating for flower type were prepared. Based on the number of single-flower type fullsibs among the offspring, we found that this phenotype is expressed only in plants homozygous for the recessive allele and that a dominant mutation in this allele causes an increase in petal number. Using random decamer primers, we identified a random amplified polymorphic DNA (RAPD) marker which is tightly linked to this recessive allele. The RAPD marker was cloned and used to generate a restriction fragment length polymorphic (RFLP) marker. This RFLP marker could discriminate with 100% accuracy between the semi-double and double-flower phenotypes in carnations of both Mediterranean and American groups. The advantages of RFLP over RAPD markers and their applicability to marker-assisted selection in carnation are discussed.

Key words Flower phenotype · *Dianthus caryophyllus* · RAPD (random amplified polymorphic DNA) · SCAR (sequence characterized amplified region) · RFLP (restriction fragment length polymorphism)

G. Scovel and H. Ben-Meir contributed equally to the experimental work presented in this study

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G. Scovel · H. Ben-Meir · M. Ovadis · H. Itzhaki
A. Vainstein (✉)
The Kennedy-Leigh Centre for Horticultural Research and
The Otto Warburg Center for Biotechnology in Agriculture,
The Hebrew University of Jerusalem, Rehovot 76-100, Israel
Fax: 972-8-946-8263
E-mail: vain@agri.huji.ac.il

Introduction

Flower development is controlled by a highly intricate mechanism(s). In recent years, significant progress has been made in delineating the processes of floral meristem initiation and the differentiation into flowers with unique organs at defined positions. A large number of homeotic genes have been isolated and analyzed with respect to expression patterns. Models have been proposed for their function and the interaction between them (Schwarz-Sommer et al. 1990; Meyerowitz 1997). These models have been mainly derived from the characterization of homeotic mutants in *Arabidopsis* and *Antirrhinum*. To some extent, the analysis of floral development in these plants has enabled an evaluation of the applicability of these models to other plant species (Pnueli et al. 1991; Van der Krol and Chua 1993; Zuker et al. 1997). In ornamentals, flower architecture is of the utmost importance. Yet very little, if anything, is known of the genetics governing horticulturally important traits in general, and flower shape and form in particular, in the major ornamentals (rose, carnation, chrysanthemum, etc). Nevertheless, in recent years studies have begun to emerge on flower development in these economically important plant units (Zuker et al. 1997).

Carnation (*Dianthus caryophyllus)* is one of the leading commodities in the ornamental industry (Jensen and Malter 1995). Based on its flower's phenotype, carnations can be grouped into three categories:

"single", "semi-double" and "double" (Conners 1913; Imai 1938). Single flowers have five petals and are easily distinguishable. However, they are of no value to the carnation industry, where standard and spray varieties from American and Mediterranean groups control most of the market. Flowers of most standard varieties are double, and those of the spray varieties are usually semi-double (Holley and Baker 1991). Hence, doubleness as a breeding characteristic is highly important in carnations. The double flowers have up to 120 petals and the number of petals in the semi-doubles is usually in the range of 20–80 per flower. In addition, whereas in double flowers there are usually no anthers, semi-doubles may have 15–20 anthers (Holley and Baker 1991). Nevertheless, it is not always easy to differentiate between these two flower types. Mainly environmental conditions strongly affect the numbers of petals and anthers per flower and hence cause phenotypic overlap (Holley and Baker 1991). Although very little is known of the genetics of doubleness in carnation (Conners 1913; Saunders 1917; Brooks 1960), 80 years ago Saunders (1917) had already suggested that carnation flower phenotype is a monogenic trait and the locus involved was designated "D".

Numerous studies on an array of organisms have shown the benefits of DNA markers. These include genotype identification, the construction of genetic maps, molecular tagging of various agronomic traits, and gene isolation. A variety of single and multilocus DNA markers have been developed (Tanksley et al. 1989; Williams et al. 1990; Mohan et al. 1997). Among these, random amplified polymorphic DNA (RAPD), and their derivative sequence characterized amplified region (SCAR), and restriction fragment length polymorphism (RFLP) markers are the simplest to produce. Although these are not the most informative ones, as compared, for example, to amplified fragment length polymorphism (AFLP) or simple sequence repeat (SSR) markers, much of the information accumulated to-date has been generated using RAPD/RFLP markers (Paran et al. 1991; Jacobs et al. 1996; Mohan et al. 1997). The purpose of the study reported here was to identify DNA markers linked to the genetic locus controlling carnation flower type. We describe single-locus inheritance of the trait and the very tight tagging of the locus by RAPD and RFLP markers.

Materials and methods

Plant material

Carnation genotypes were obtained from R. Shemi Ltd., Israel, and grown under standard greenhouse conditions. An F_2 population (total 74 fullsibs) segregating for flower phenotype and analyzed by RAPD was prepared by selfing breeding line 2217. The scoring of flower phenotype (single, semi-double and double) was based on the number of petals/anthers. However, due to strong phenotypic overlap between the semi-double and double flower-phenotypic classes, based on this scoring method, not all offspring were included in the RAPD analyses. In several cases, in order to distinguish between genotypes heterozygous and homozygous for flower type, individual F_2 plants were selfed and the flower phenotype of the progeny analyzed. To assess the inheritance of flower phenotype, several different crosses were made, as described in the table legends. Six halfsibs (two lines from each cross) analyzed by RAPD were derived from crosses between line 2217 and cvs Eveline, Splendid and Ashley. For RFLP analyses, the following randomly chosen and unrelated genotypes of American and Mediterranean groups were used: cvs White Sim and Nora of the American type; cvs Saturn, Montelisa, Francesco and Reiko and breeding lines 2507, 1342, 1684, 431–1, 432–1, 730–2 of the Mediterranean type; cvs Scarlette, Carmit, Natilla, Duett, Ashley and Shelly and breeding lines 293, 1446, 578–20 and 1278–10 resulting from crosses between American and Mediterranean cultivars.

All crosses were carried out in the fall/winter and seeds were collected and sown in the summer. Seedlings (2-months old) were transplanted to the trial greenhouse in September and data were recorded during the following winter and spring.

RAPD analysis

DNA was extracted from young leaves by the CTAB (cetyltrimethylammonium bromide) procedure as described previously (Tzuri et al. 1991). RAPD analyses were performed with random decamer primers from Operon Technologies (Alameda, Calif.) using a programmable thermal controller (PTC-100, M.J. Research Inc., Mass.). PCR reactions were carried out in a 25-µl volume containing 25 ng of genomic DNA, 0.1 mM of each dNTP, 25 ng of primer, 10 mM Tris HCl (pH 8.2), 50 mM KCl, 1.5 mM $MgCl_2$ and 1 unit of *Taq* DNA polymerase (Advanced Biotechnologies Ltd., UK). The reaction mixture was overlaid with 30 µl of mineral oil and subjected to PCR. The PCR conditions were: 94°C for 5 min followed by 40 cycles of 1 min at 94°C, 1 min at 35°C and 2 min at 72°C, followed by 5 min at 72°C. The RAPD products were resolved on a 1.5% (w/v) agarose gel in TBE buffer (0.13 M Tris, 0.07 M boric acid and 2.45 mM EDTA, pH 8.4). Gels were stained with ethidium bromide, photographed under ultraviolet light and analyzed by Southern blotting as described below.

SCAR development

The polymorphic RAPD fragment of interest generated by the OPR02 primer ($OPR02_{782}$) was resolved on an agarose gel and the DNA was purified with a 'Geneclean' kit (Bio 101 Inc., La Jolla, Calif.). DNA was blunt-end cloned into the *Sma*I site of pBluescript KS and sequenced using a *Taq* DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems). Specific SCAR primers were synthesized based on the $OPR02_{782}$ fragment sequence, and were designated BH1, BH2 – corresponding to the internal sequence of the $OPR02_{782}$ fragment, and BH3, BH4 – containing the ten original bases of the OPR02 RAPD primer and an additional ten bases for BH3 and nine bases for BH4 according to the sequence of the cloned $OPR02_{782}$ fragment:

BH1: 5′-GGCAGCGACGACAACACCAAA-3′;
BH2: 5′-CAGATGAGCGTGAGGAAAACA-3′;
BH3: 5′-CACAGCTGCCCAGCACGACA-3′;
BH4: 5′-CACAGCTGCCGATATTATT-3′.

The PCR conditions for the SCAR primers were as follows: 94°C for 3 min, 50°C for 2 min and 72°C for 2 min, followed by 40 cycles of 94°C for 30 sec, 54°C for 1 min, and 72°C for 1 min, and finishing with 72°C for 10 min. The SCAR reaction mixtures were the same as those used for the RAPD reaction.

Southern-blot analysis

DNA (10 μg) was digested with *Eco*RI, *Kpn*I and *Hae*III restriction enzymes (*Eco*RI has a unique restriction site within the $OPR02_{782}$ fragment and *Kpn*I and *Hae*III do not cut the fragment) and was electrophoresed through a 20-cm long, 0.8% (w/v) TBE agarose gel at 50 V for 24 h. DNA was transferred to a nylon membrane (Hybond N +, Amersham) by capillary blotting, according to the manufacturer's recommendations. Membranes were hybridized with the $OPR02_{782}$ fragment which was P^{32}-labelled by random priming (Feinberg and Vogelstein 1984). Pre-hybridization and hybridization were carried out as described previously (Ben-Meir and Vainstein 1994). The membranes were washed at 65°C for 20 min twice in 2 × SSC (0.15 M NaCl; 0.015 M sodium citrate), 0.1% SDS, and once in 1 × SSC, 0.1% SDS. When Southern blots were performed with PCR products, two washes of 2 × SSC were performed at 65°C for 20 min. Membranes were exposed to an imaging plate (Fujix Bas 1000) for 2–7 h. The plate was then read in an imaging plate reader (Fujix Bio Imaging Analyzer Bas 1000).

Results

Genetic analyses of flower type

Based on the flower phenotype, carnations were grouped into single, semi-double and double categories (Fig. 1). To characterize the inheritance of the "flower phenotype" trait, a series of crosses were performed and the percentage of offspring with the single-flower phenotype (unequivocally identifiable) in the progeny was evaluated. As can be seen from Table 1, only single-flower phenotype siblings were generated following selfing of six different single-flower breeding lines. Selfing of semi-double flower breeding lines yielded offspring of all three phenotypic classes. The number of single-flower siblings in the progeny (for all six progeny) was not significantly different from the expected value for a 1 : 3 segregation ratio by the χ^2 test (Table 1).

To further characterize the single-locus Mendelian inheritance of the flower-phenotype trait, additional crosses were performed (Table 2). The number of offspring with the single-flower phenotype in two progenies derived from a cross between varieties of the semi-double flower phenotype was not significantly different from the expected value for the 1 : 3 segregation ratio by the χ^2 test. Crosses between varieties of semi-double and single-flower phenotypes yielded offspring about half of which were of the single-flower phenotype. No plants with single-flower phenotype were generated from crosses between varieties of single- and double- flower phenotypes. Hence the single-flower phenotype is expressed only in carnation plants homozygous for the recessive allele, hereafter termed "d".

Fig. 1 Three carnation flower types. From left to right – single, semi-double and double flower phenotypes

Identification of RAPD markers linked to the d allele

The F_2 population (derived by selfing semi-double flower b.l. 2217) segregating for flower phenotype was used to detect the DNA marker linked to the locus controlling the flower-phenotype trait. RAPD screening was initially carried out with DNA from the Dd semi-double parental line (b.l. 2217) and five dd single-flower and two DD double-flower fullsibs. One of the analyzed primers, OPR02, yielded a 782-bp amplification product ($OPR02_{782}$) only in the parental line and in offspring with the single-flower phenotype. To further substantiate linkage, additional fullsibs were screened with the OPR02 primer. In all cases, the 782-bp product was observed as expected based on flower phenotype, i.e. the 782-bp DNA fragment was generated from DNA of all single and semi-double flower lines analyzed and absent in all double-flower lines (Fig. 2 A). The 782-bp DNA fragment generated by OPR02 from DNA of the parental 2217 line was cloned and used as a probe in a Southern-blot analysis

Table 1 Segregation of the single-flower phenotype in self-pollinated progeny. From left to right, selfed genotypes of the single-flower phenotype were b.l. 658-1, 669-21, 293-1, 270-1, 276-12, 2217-60 and of the semi-double flower phenotype were b.l. 2217, 2217-57, 431-1, 730-2, 450-1, 971-1. *P*, the right-tail probability, is shown in parentheses

Parental phenotype	Number of fullsibs with single-flower phenotype over the total number of fullsibs for different progeny					
Single	112/112	87/87	78/78	71/71	59/59	176/176
Semi-double	16/74	85/359	16/69	29/120	10/54	17/52
	$(0.7 > P > 0.5)$	$(0.7 > P > 0.5)$	$(0.8 > P > 0.7)$	$(0.95 > P > 0.8)$	$(0.3 > P > 0.2)$	$(P = 0.2)$

Table 2 Segregation of the single-flower phenotype in directed open-pollinated progeny. Progeny were obtained from crosses between genotypes with a semi-double flower phenotype (b.l. 2217 × cv Duett, b.l. 432-1 × cv Nora, from left to right); between genotypes with semi-double and single-flower phenotypes (cv White Sim × b.l. 1202-1, cv White Sim × b.l. 1278-10, cv 971-2 × b.l. 1326-1, cv White Sim × b.l. 1278-2, from left to right), and between genotypes with single- and double-flower phenotypes (b.l. 578-20 × cv Francesco, b.l. 1278-10 × cv Saturn, b.l. 578-20 × cv Montelisa, b.l. 578-20 × cv Reiko, from left to right). *P*, the right-tail probability, is shown in parentheses

Parental phenotype	Number of fullsibs with a single-flower phenotype over the total number of fullsibs for different progeny			
Semi-double × semi-double	124/528 ($0.5 > P > 0.3$)	19/97 ($0.3 > P > 0.2$)		
Semi-double × single	11/20 ($0.7 > P > 0.5$)	9/22 ($0.5 > P > 0.3$)	8/20 ($0.5 > P > 0.3$)	11/21 ($0.95 > P > 0.8$)
Single × double	0/107	0/120	0/59	0/76

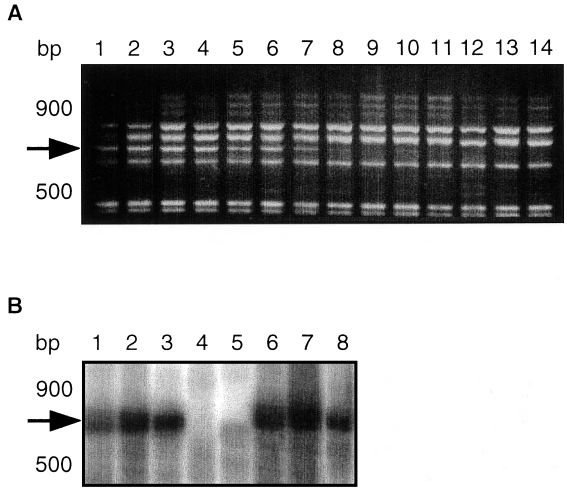

Fig. 2A, B RAPD amplification of genomic DNA using primer OPR02. **A** PCR was performed with F_2 progeny of self-pollinated b.l. 2217 (semi-double flower phenotype, shown in *lane 7*). *Lanes 1 through 4* – offspring 2217-24, 2217-30, 2217-33, 2217-45, with a single-flower phenotype; *lanes 5, 6* – offspring 2217-3, 2217-57, with a semi-double phenotype, and *lanes 8 through 14* – offspring 2217-1, 2217-12, 2217-13, 2217-21, 2217-37, 2217-40, 2217-43, with a double-flower phenotype. The *arrow* indicates the phenotype-specific 782-bp ($OPR02_{782}$) RAPD fragment. **B** Southern-blot analysis of PCR-amplified products using this $OPR02_{782}$ RAPD fragment as a probe. *Lanes 1 through 8* – 2217-24, 2217-30, 2217-33 (single-flower phenotype); 2217-1, 2217-37 (double-flower phenotype); 2217-3, 2217-57, 2217 (semi-double flower phenotype)

of PCR-amplified products from several fullsibs. As expected, the probe hybridized only with $OPR02_{782}$ generated from DNA of fullsibs with single and semi-double flower phenotypes (Fig. 2 B).

To evaluate the tightness of the linkage, RAPD analysis using OPR02 was performed with the fullsibs (50 lines) which could be definitely identified with respect to flower phenotype. In all cases, the 782-bp fragment was generated only from DNA of dd (single) or Dd (semi-double) flower lines. Moreover, six halfsibs (four double and two semi-double flower lines) analyzed were also clearly identified by the OPR02 RAPD marker as expected, based on the flower phenotype. It is worth noting that when the RAPD fragment was scored among randomly chosen fullsibs from b.l. 2217 progeny, the segregation of the marker did not significantly differ ($0.5 < P < 0.7$) from the expected 3:1 (band present:absent) segregation for a single gene in an F_2 population. In contrast, the RAPD marker was not applicable to genotypes which were not related to the progeny derived from the selfing of b.l. 2217.

SCAR and RFLP marker development

To overcome some of the limitations of RAPD markers, we set out to develop a SCAR marker (Paran and Michelmore 1993). Specific primers (BH1 and BH2) were designed according to the internal sequence of the $OPR02_{782}$ RAPD fragment. PCR amplification using these primers did not generate SCAR markers able to differentiate between single and double-flower phenotypes, yielding a fragment of the predicted size (650 bp) in both cases. When we used primers synthesized according to the sequence of OPR02 and the 5′ and 3′ end sequences of the $OPR02_{782}$ RAPD fragment (BH3 and BH4), a PCR fragment of the expected size was generated but no polymorphism between fullsibs was revealed.

To develop an alternative marker which would reveal polymorphism under conditions more stringent than those used in RAPD analysis, the cloned $OPR02_{782}$ RAPD fragment was used as an RFLP probe. Initially, the RFLP analysis was performed with DNA of six F_2 fullsibs from 2217 progeny (four double-flower and two single-flower lines) and two unrelated lines, 293 (single-flower phenotype) and 2507 (double-flower phenotype). Digestion of DNA with *Hae*III yielded a 1.8-kb polymorphic band that co-segregated with the single-flower phenotype (Fig. 3), whereas the *Kpn* I and *Eco*RI restriction enzymes failed to generate a polymorphism. Ten additional fullsibs from the 2217 progeny were analyzed by RFLP and in all cases the

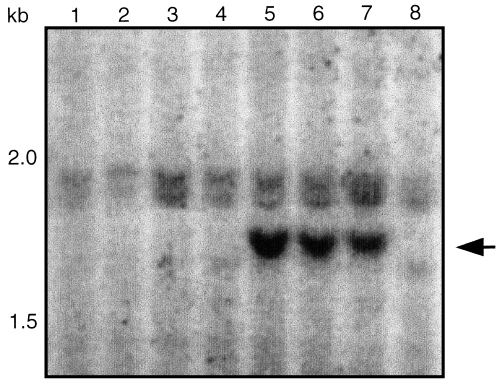

Fig. 3 A flower phenotype-specific RFLP marker. DNA from six F_2 fullsibs (2217-37, 2217-43, 2217-44, 2217-21, 2217-30, 2217-45, derived by selfing of b.l. 2217, *lanes 1 through 6,* respectively) and two unrelated genotypes (b.l. 293 and b.l. 2507, *lanes 7 and 8*, respectively) was digested with the restriction enzyme *Hae*III. The $OPR02_{782}$ RAPD fragment was used as a probe in a Southern-blot analysis. 2217-37, 2217-43, 2217-44, 2217-21, b.l. 2507 – double-flower phenotype; 2217-30, 2217-45, b.l. 293 – single-flower phenotype. The *arrow* indicates the phenotype-specific 1.8-kb RFLP marker

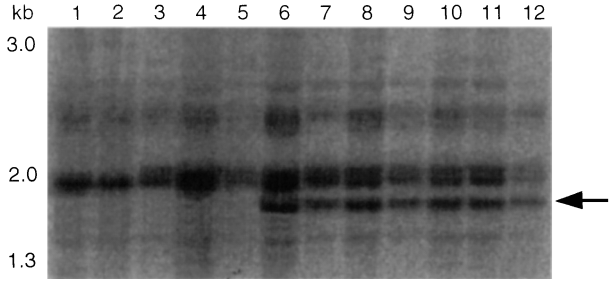

Fig. 4 RFLP analysis of 12 unrelated genotypes. DNA was digested with the restriction enzyme *Hae*III and the Southern blot was probed with the $OPR02_{782}$ RAPD fragment as described in Materials and methods. The arrow indicates the phenotype-specific 1.8-kb RFLP marker. *Lanes 1 through 12*, respectively: cvs Montelisa, Francesco, Reiko, Saturn, b.l. 1342 of the double-flower phenotype; cvs Scarlette, Carmit, Natilla, White Sim, Nora of the semi-double flower phenotype, and b.l. 578-20, 1278-10 of the single-flower phenotype

marker co-segregated with the expected phenotype. To further evaluate the possibility of using this RFLP marker in genotypes genetically unrelated to 2217 progeny, 20 genotypes belonging to American- and Mediterranean-type carnations were analyzed. In all cases the RFLP marker correctly discriminated between flower phenotypes. The 1.8-kb band was observed in all lines of dd (single) flower and Dd (semi-double) flower phenotypes and not in DD (double) flower phenotypes. The RFLP patterns for 12 of these unrelated genotypes are shown in Fig. 4.

Discussion

In ornamentals, and carnation in particular, saturated genetic maps have never been constructed. Moreover, to the best of our knowledge, no marker for any trait of horticultural importance has been reported. In ornamentals, DNA markers are currently used to identify varieties and to analyze inter- and intra-specific genetic relatedness (Torres et al. 1993; Ben-Meir and Vainstein 1994; Yamagishi 1995; Rajapakse and Ballard 1997). To effectively employ DNA markers in the breeding of ornamentals, a number of traits, at least those of horticultural importance, need to be tagged. This would enable "pyramiding" the genes and generating elite cultivars. With this as our long-term goal, we have generated carnation families segregating for a number of traits. In the present study, we report on RAPD/RFLP markers which can discriminate between double and semi-double flower phenotypes. Based on the number of single-type flowers in progeny of a number of crosses (Tables 1 and 2), we show that in carnation, as in petunia (Van der Krol and Chua 1993), the recessive d allele is responsible for the single-flower phenotype and that a dominant mutation causes increased petal numbers. PCR-based analyses of over 50 fullsibs and several halfsibs revealed that the $OPR02_{782}$ RAPD marker is very tightly linked to the d allele. To-date, over 100 genotypes have been analyzed and in all cases 100% co-segregation has been observed (data not shown).

RAPD markers have numerous advantages. The analysis is rapid, simple, and does not involve radioactive material. On the other hand, the RAPD technique is highly sensitive to reaction conditions, dominant in nature, and does not usually enable detection of a single locus (Williams et al. 1991; Williamson et al. 1994; Yang and Korban 1996; Mohan et al. 1997). Hence this marker is not useful, for example, in marker-assisted breeding programs. However, based on the sequence of the DNA fragment of interest identified by the RAPD approach, SCAR markers can be generated. These are not sensitive to reaction conditions, enable the detection of a single locus, and can potentially be converted into co-dominant markers (Paran and Michelmore 1993). In the present study, however, as in some other reports (Jacobs et al. 1996), conversion of the RAPD to a SCAR marker led to loss of polymorphism. Moreover, SCAR primers containing the sequence of the OPR02 RAPD primer and the 5′ and 3′ end sequences of the $OPR02_{782}$ RAPD fragment of interest were not useful in screening for the trait under study. Nevertheless, by comparing the sequence of the DNA fragment amplified by SCAR primers in double-flower lines with that of single-flower lines, it may be possible to generate (as in other studies, e.g. Nair et al. 1996) polymorphic, co-dominant SCAR markers.

In the present study, the $OPR02_{782}$ RAPD marker co-segregating with the d allele was used as an RFLP probe. Using the *Hae*III restriction enzyme, which does not cut within the $OPR02_{782}$ fragment, an RFLP marker tightly linked to the d allele was obtained. This marker, similar to the RAPD one, could also be scored

only as a dominant locus (presence or absence of the band). It is worth noting that several additional, non-polymorphic bands were revealed by the RFLP marker, although in contrast to other studies (Nair et al. 1996), no repetitive sequences were found in the $OPR02_{782}$ RAPD fragment. Using this RFLP marker, rather than the RAPD one, we could identify with 100% accuracy the flower phenotype in genotypes not genetically related to the original line 2217-derived segregating family. Discrimination of flower phenotype was successful with carnations of both the Mediterranean and American groups. Hence, although Southern blotting is still required, this RFLP marker may be used immediately in breeding for spray or standard carnation varieties by screening, respectively, for or against semi-double flower genotypes. Moreover, the RFLP marker opens the way to cloning and characterizing the gene(s) involved in the determination of flower phenotype in carnations.

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References

Ben-Meir H, Vainstein A (1994) Assessment of genetic relatedness in roses by DNA fingerprint analysis. Sci Hortic 58:115–121

Brooks HJ (1960) Cytological and genetical study of the carnation, *Dianthus caryophyllus*, with special reference to the production of triploids. PhD thesis, University of Connecticut, USA

Conners CH (1913) Multiplication of floral parts in the carnation. Am Soc Hort Sci 10:93–99

Feinberg AP, Vogelstein B (1984) A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 137:266–267

Holley WD, Baker R (1991) Carnation production II. Kendall/Hunt Publishing, Colorado State University

Imai Y (1938) The genes for double flowers in the commercial varieties of the perpetual carnation. Jap J Genet 14:63–65

Jacobs JME, van Eck HJ, Horsman K, Arens PFP, Verkerk-Bakker B, Jacobsen E, Pereira A, Stiekema WJ (1996) Mapping of resistance to the potato cyst nematode *Globodera rostochiensis* from the wild potato species *Solanum vernei*. Mol Breed 2:51–60

Jensen MH, Malter AJ (1995) Protected agriculture, a global review. World Bank Technical Paper 253:144–146

Meyerowitz EM (1997) Genetic control of cell division in developing plants. Cell 88:299–308

Mohan M, Nair S, Bhagwat A, Krishna TG, Yano M, Bhatia CR, Takuji S (1997) Genome mapping, molecular markers and marker-assisted selection in crop plants. Mol Breed 3:87–103

Nair S, Kumar A, Srivastava MN, Mohan M (1996) PCR-based DNA marker linked to a gall midge resistance gene, *Gm4t*, has potential for marker-aided selection in rice. Theor Appl Genet 92:660–665

Paran I, Michelmore RW (1993) Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theor Appl Genet 85:985–993

Paran I, Kesseli RV, Michelmore RW (1991) Identification of RFLP and RAPD markers linked to downy mildew resistance genes in lettuce by using near-isogenic lines. Genome 34:1021–1027

Pnueli L, Abu-Abeid M, Zamir D, Nacken W, Schwarz-Sommer Z, Lifschitz E (1991) The MADS-box gene family in tomato: temporal expression during floral development, conserved secondary structures and homology with homeotic genes from *Antirrhinum* and *Arabidopsis*. Plant J 1:255–266

Rajapakse S Ballard RE (1997) Cultivar identification using molecular methods. In: Geneve RL, Preece JE, Merkle SA (eds) Biotechnology of ornamental plants. CAB International, Wallingford, UK (Biotechnology in agriculture series, no 16, pp 153–164)

Saunders ER (1917) Studies in the inheritance of doubleness in flowers, II. *Meconoposis*, *Althaea* and *Dianthus*. J Genet 6:165–184

Schwarz-Sommer Z, Huijser P, Nacken W, Saedler H, Sommer H (1990) Genetic control of flower development: homeotic genes in *Antirrhinum majus*. Science 250:931–936

Tanksley SD, Young ND, Paterson AH, Bonierbale MW (1989) RFLP mapping in plant breeding – new tools for old science. Bio Technology 7:257–264

Torres AM, Millan T, Cubero JI (1993) Identifying rose cultivars using random amplified polymorphic DNA markers. HortScience 28:333–334

Tzuri G, Hillel J, Lavi U, Haberfeld A, Vainstein A (1991) DNA fingerprints of ornamental plants. Plant Sci 76:91–97

Van der Krol AR, Chua N-H (1993) Flower development in petunia. Plant Cell 5:1195–1203

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535

Williams MNV, Pande N, Nair S, Mohan M, Bennett J (1991) Restriction fragment length polymorphism analysis of polymerase chain reaction products amplified from mapped loci of rice (*Oryza sativa* L.) genomic DNA. Theor Appl Genet 82:489–498

Williamson VM, Ho JY, Wu FF, Miler N, Kaloshian I (1994) A PCR-based marker tightly linked to the nematode resistance gene, *Mi*, in tomato. Theor Appl Genet 87:757–763

Yamagishi M (1995) Detection of section-specific random amplified polymorphic DNA (RAPD) markers in *Lilium*. Theor Appl Genet 91:830–835

Yang H, Korban SS (1996) Screening apples for OPD20/600 using sequence-specific primers. Theor Appl Genet 92:263–266

Zuker A, Tzfira T, Vainstein A (1997) Cut-flower improvement using genetic engineering. Biotech Adv 16(1):33–79