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Construction of a genetic linkage map for roses using RAPD and AFLP markers

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Abstract A segregating population of diploid rose hybrids (2n = 2x = 14) was used to construct the first linkage maps of the rose genome. A total of 305 RAPD and AFLP markers were analysed in a population of 60 F_1 plants based on a so-called "double-pseudotestcross" design. Of these markers 278 could be located on the 14 linkage groups of the two maps, covering total map lengths of 326 and 370 cM, respectively. The average distances between markers in the maps for 93/1–117 and 93/1–119 is 2.4 and 2.6 cM, respectively. In addition to the molecular markers, genes controlling two phenotypic characters, petal number (double versus single flowers) and flower colour (pink versus white), were mapped on linkage groups 3 and 2, respectively. The markers closest to the gene for double flowers, *Blfo,* and to the gene for pink flower colour, *Blfa,* cosegregated without recombinants. The maps provide a tool for further genetic analyses of horticulturally important genes as, for example, resistance genes and a starting point for marker-assisted breeding in roses.

Key words *Rosa* · Genetic mapping · Molecular markers · Flower colour · Petal number

Introduction

Roses are among the economically most important ornamental crops world-wide. Despite their commercial importance and intensive efforts to breed new varieties, little information is available about the inheritance of the important characters of cultivated roses (De Vries and DuBois 1978; 1984, Debener 1999; Gudin 1998). Detailed genetic analyses have been hampered by the fact that most cultivated rose varieties are highly heterozygous tetraploids which suffer from severe inbreeding depression after selfing. Furthermore, most diploid species are self-incompatible and are not recurrent flowering, i.e. they do not flower in the first year after sowing. Consequently, breeding strategies have mostly been based on the production of F_1 progeny and subsequent selection of new varieties from these populations. This strategy is possible because roses can be easily propagated vegetatively.

Due to increasing demands regarding the quality of new rose varieties, especially resistances to pests and pathogens, new and more sophisticated breeding strategies are needed (DeVries and DuBois 1996; Gudin 1998). The introgression of genes from wild rose species into the genetic background of modern roses could be carried out in a more effective way if the genetic determinants of the relevant traits and their chromosomal locations were known.

Genetic linkage maps would facilitate genetic investigations (Staub et al. 1996; Gebhardt and Salamini 1992; Paterson et al. 1991) and the implementation of more sophisticated breeding strategies. High-density linkage maps would also help locate or tag genes of interest for marker-assisted breeding and map-based cloning. The advent of molecular marker techniques greatly facilitated the construction of linkage maps, especially after the development of polymerase chain reaction (PCR)-based markers including random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) markers (Williams et al. 1990; Vos et al. 1996). The first molecular marker maps were published mainly for annual crops (Staub et al. 1996). More recently, maps have also been developed for *Malus*, *Prunus*, *Eucalyptus* and other tree species (Grattapaglia and Sederoff 1994; Hemmat et al. 1994; Kubisiak et al. 1995). In these species and in roses it is difficult to produce segregating populations based on inbred genotypes. However, the relatively high heterozygosity allows the application of the "double-pseudotestcross" strategy (Grattapaglia and Sederoff 1994). Two highly heterozygous individuals are

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Table 1 Selective AFLP primers used to generate markers in the segregating population 94/1

AFLP primer combination	*Hin*dIII primers	*Mse*I primers
AFLP1	GACTGCGTACCAGCTTACC	GATGAGTCCTGAGTAAAAG
AFLP2	GACTGCGTACCAGCTTACC	GATGAGTCCTGAGTAAATA
AFLP3	GACTGCGTACCAGCTTACA	GATGAGTCCTGAGTAAACT
AFLP4	GACTGCGTACCAGCTTACG	GATGAGTCCTGAGTAAACT
AFLP5	GACTGCGTACCAGCTTACT	GATGAGTCCTGAGTAAACG
AFLP6	GACTGCGTACCAGCTTACT	GATGAGTCCTGAGTAAAAG
AFLP7	GACTGCGTACCAGCTTACC	GATGAGTCCTGAGTAAAAG
AFLP8	GACTGCGTACCAGCTTACG	GATGAGTCCTGAGTAAATC
AFLP9	GACTGCGTACCAGCTTACA	GATGAGTCCTGAGTAAAAG
AFLP10	GACTGCGTACCAGCTTACC	GATGAGTCCTGAGTAAACG
AFLP11	GACTGCGTACCAGCTTATT	GATGAGTCCTGAGTAAACG
AFLP12	GACTGCGTACCAGCTTAGA	GATGAGTCCTGAGTAAACG

crossed for which no prior genetic information is available. As a result a maximum of four alleles may segregate in such a population. This type of cross is particularly useful if dominant PCR-based markers like RAPD and AFLP markers are utilised. Molecular markers in roses have already been applied in a number of cases to investigate polymorphisms in natural populations (Reynders-Aloisi and Bollereau 1996) and between rose varieties (Debener et al. 1996; Ben Meir and Vainstain 1994; Hubbard et al. 1992, Torres et al. 1993), to infer phylogenetic relationships (Millan et al. 1996) and to perform the first genetic analyses (Debener and Mattiesch 1996).

Here we describe the construction of the first linkage maps constructed for diploid roses, based on RAPD and AFLP markers and the localisation of two genes controlling important morphological traits, petal number and flower colour.

Materials and methods

Plant material, crosses

A population of 60 F_1 hybrids from a cross between the diploid rose genotypes 93/1–117 and 93/1–119 was used as the mapping population. This F_1 population segregates for double versus simple flowers and pink versus white flower colour and is perpetual flowering. The parental genotypes are half-sibs resulting from open pollination of the diploid genotype 81/42–15. The genotype 81/42–15 was derived from a breeding programme in which diploid hybrids between *Rosa multiflora* and garden roses were produced (Reimann-Philipp 1981). Plants were maintained in the greenhouse at the Institute for Ornamental Plant Breeding in Ahrensburg. The cultivation of plants, crosses and a first characterisation of morphological characters in this population is described in Debener (1999). The parental genotypes or DNA samples of the parental genotypes are available upon request from the authors.

DNA isolation

Young leaves were harvested in the greenhouse, washed and placed in the dark at room temperature for 2 days prior to DNA extraction. The DNA was isolated according to Suhl and Korban (1996) with the following modifications: after incubation of the ground leaf material in extraction buffer (2% CTAB, 3% PVP, 1.4 *M* NaCl, 20 m*M* EDTA, 100 m*M* TRIS/HCl pH 8.0) and 0.5 vol 8 *M* LiCl for 15 min at 65°C, the samples were extracted three times with chloroform. After precipitation with ethanol and resuspension in TE, the carbohydrates present in the samples were digested for 3 h at room temperature with Driselase (5 mg/ml in 10 m*M* MES, Sigma Aldrich, Deisenhofen, Germany), and RNA was digested for 30 min with RNAse A (10 µg/ml, Boehringer, Germany) followed by a phenol/chloroform extraction. The DNA was then precipitated again with ethanol, resuspended in TE and quantified as described in Debener and Mattiesch (1998).

RAPD analysis

The RAPD analyses were performed with primers of different lengths as described in Debener and Mattiesch (1998).

In addition to using primers of 10 bases (10 mers, Primer names starting with OP) purchased from Operon technologies (Alameda, Calif., USA), we used primers of 15 and 20 bases (15 mers and 20 mers, Primer names starting with P) as single primers and in pairwise combinations. The primer sequences are available from the internet homepage of the Federal Centre for Breeding Research on Cultivated Plants (www.bafz.de/internet_e/index.htm) under the subtopic collections or upon request from the authors.

AFLP analysis

AFLP analyses were performed as described in Vos et al. (1995) with the following modifications: 250 ng of genomic DNA were digested overnight with the restriction enzymes *Hin*dIII and *Mse*I. After ligation with *Hin*dIII and *Mse*I adaptors preamplifications were performed with primers carrying one selective nucleotide at the 3´end followed by a second amplification with three selective nucleotides at the 3´end. For the AFLP primer combinations 1–5, selective *Hin*dIII primers were end-labelled with γ–[^{32}P]-dATP, and the reaction products were separated on 6% denaturing polyacrylamide sequencing gels and autoradiographed. For the primer combinations 6–12, selective *Hin*dIII primers were end-labelled with the IRD 800 dye (MWG Biotech, Ebersberg, Germany) and analysed on a LICOR 400 automatic sequencer (MWG Biotech, Ebersberg, Germany). The sequences of the selective AFLP primers are listed in Table 1.

Scoring of data

Scoring of the morphological traits double flowers and pink flower colour is described in Debener (1999). PCR fragments generated in the RAPD and AFLP analyses were scored as dominant markers. Markers were recorded as present or absent, coded according to the JOINMAP programme manual (Stam and Van Ooijen 1995), and stored in ASCII text files. The marker designation for the markers generated by the 10-mer primers start with the capital

Table 2 Analyses of RAPD markers in three subsequent steps with the parental genotypes and segregating F_1 genotypes

Type of primer	Number of primers analysed with the parents	Number of primers analysed with the parents+ 12 F_1 plants	Number of primers analysed with the parents + 48 F_1 plants
10 mers	167	38	14
15 mers	25	13	8
15-mer combinations	300	117	33
20 mers	25	13	6
20-mer combinations	300	111	53
Total	817	292	114

Table 3 Analysis of marker segregation for three different marker types in the mapping population

	From93/1–117	From93/1–119	From both parents	Total
Markers analysed in the segregating population	119	127	59	305
Expected segregation ratios	1:1	1:1	3:1	
Markers with deviation from the expected ratio at $P = 0.005$	27(23%)	14 (11%)	9 (15%)	50 (16%)

letters OP followed by a letter and number code as given by the supplier and the number of the marker fragment separated from the primer code by a hyphen (e.g. OPA18_1). The designations of markers from 15- and 20-mer primers start with a P followed by a three-letter code for the primer and finally the fragment number separated by a hyphen. For primer combinations, primer numbers are separated by a slash (e.g. P469/474_1). The primer combinations used for the AFLP markers were numbered from 1–12 according to Table 1. Different marker fragments are separated from the primer combinations by a hyphen.

Map construction

Map construction was performed with the JOINMAP programme package (Stam and Van Ooijen 1995). The marker data were collected separately for markers present only in parent 93/1–117, present only in parent 93/1–119, and for markers present in both parents. All markers that did not fit the expected ratios (1:1 for markers present in only one parent and 3:1 for the markers present in both parents) were excluded from the computation of the core maps. Separate maps for each parent were computed with a LOD of 4.0 for grouping the markers into linkage groups and an LOD of 0.01 and a recombination threshold of 0.49 for mapping (ripple value = 1, jump threshold = 3, triplet threshold = 6, Kosambi mapping function). In a second step markers with skewed segregation ratios as well as segregating markers heterozygous in both parents were assigned to the linkage groups of the core map. The final map was constructed with the parameters mentioned before. To avoid the disturbance of the marker-order of the core map, we used fixed order files.

Results

RAPD and AFLP analysis

To minimise experimental artefacts in the RAPD analyses, we applied a four-step procedure. First, individual primers for the 10-, 15- and 20-mer primers and pairwise primer combinations for the 15- and 20-mer primers were used in RAPD-PCR reactions to amplify fragments for the two parents. The complexity of the fragment patterns obtained with 15- and 20-mer primers was comparable to that of the 10-mer primers in that an average of 8 fragments were amplified per primer or primer combination. Those primers and primer combinations that resulted in clear amplification patterns and strong polymorphic bands between the parents were used in a second step to analyse both parents and 12 F_1 individuals. The fraction of primers that produced fragment patterns compatible with the first analyses, and in which at least 1 band segregated to fit a 1:1 ratio, were further tested in a third step with the remaining 48 F_1 plants. Reactions with primers that produced clear banding patterns were repeated in a fourth step with a random set of 20 F_1 plants. In the third and fourth step, markers segregating in ratios of 1:1 and 3:1 were recorded.

From 817 single primers and primer combinations 114 (14%) were finally analysed in the whole mapping population, resulting in 179 segregating marker fragments. The remaining primers and primer combinations either produced no polymorphic marker fragments, or the fragment patterns were not sufficiently clear and reproducible for unambiguous scoring. The results of the RAPD analyses are listed in Table 2.

As the AFLP reactions turned out to yield several clearly segregating polymorphic markers for every primer combination (data not shown), 12 combinations were chosen at random for analysis with the parental lines and 12 F_1 plants. In a second experiment the remaining 48 F_1 genotypes were analysed, followed by a third independent experiment with a subset of 20 randomly chosen genotypes. A total of 126 segregating marker bands with an average of 10.5 marker bands per primer combination resulted from the AFLP analyses.

From a total of 305 segregating RAPD and AFLP markers 119 were inherited from parent 93/1–117, 128 from parent 93/1–119 and 60 markers were inherited from both parents. The segregation ratios were tested with a standard chi^2-test. The markers with significant deviations from the expected segregation ratios at $P= 0.005$ varied from 11% to 23% (Table 3). The proportion of markers

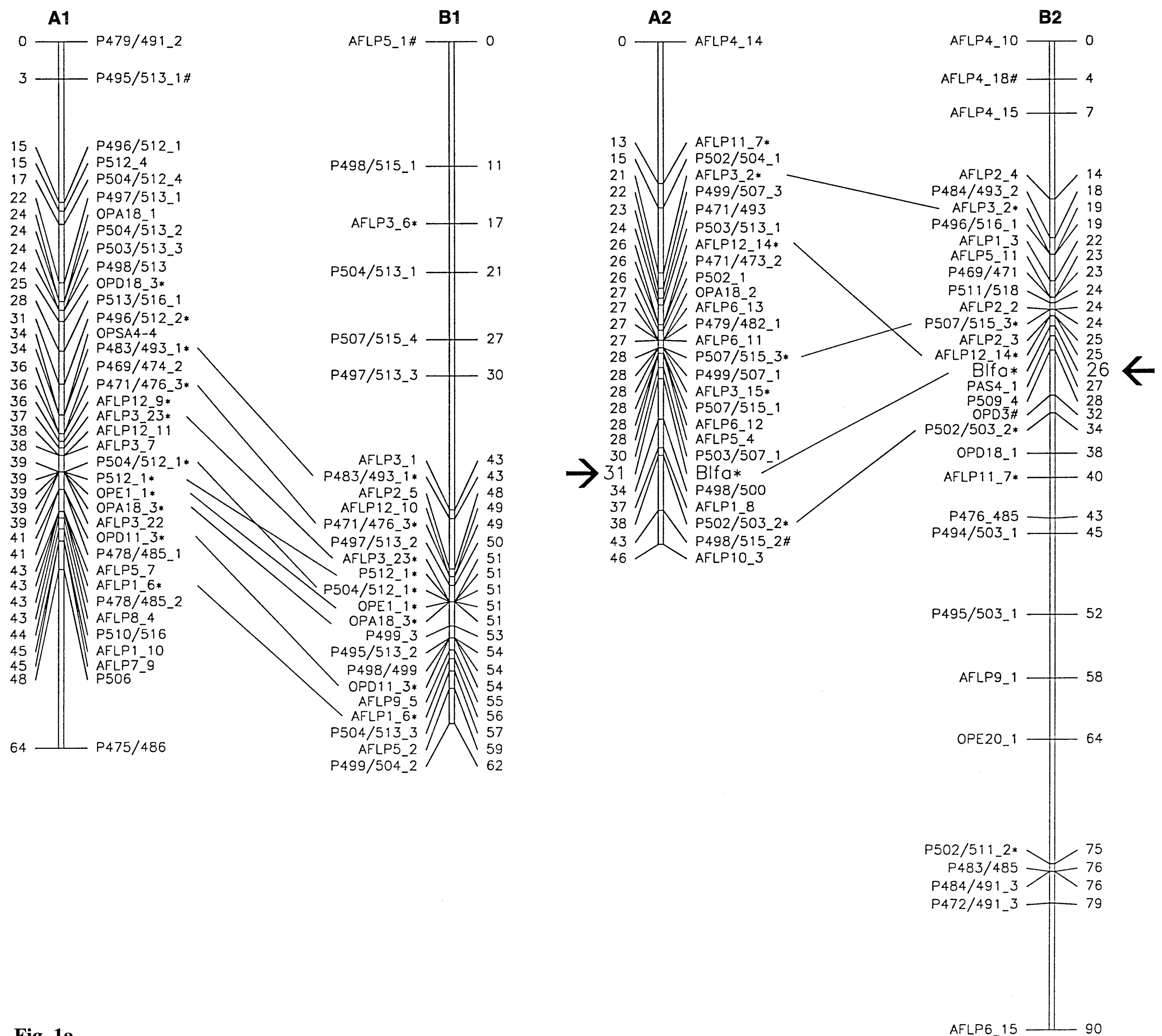

Fig. 1a

Fig. 1a-c Linkage maps of the diploid rose genotypes 93/1–117 and 93/1–119. *A1-A7* Linkage groups based on markers of the core map inherited from 93/1—117, B1-B7 linkage groups based on markers of the core map inherited from 93/1–119. Markers with skewed segregation ratios added at the second round of map construction are marked with a #. Markers inherited from both parents are marked with a *. Distances between markers are given in centi Morgans. Markers inherited by both parents and common to linkage groups are connected by *solid lines*. The two genes controlling the phenotypic characters double flowers, *Blfo,* and pink flower colour, *Blfa,* are denoted by *arrows*

with skewed segregation ratios was higher for markers inherited from 93/1–117 than for those from 93/1–119.

Map construction

One hundred and two markers from parent 93/1–117 and 121 markers from 93/1–119 which displayed no distorted segregation were grouped with a LOD score of 4.0. As a result 7 linkage groups containing at least 3 markers could be assembled for each parent (Fig. 1).

In addition, two and three pairs of linked markers were detected for the parents 93/1–117 and 93/1–119 respectively (data not shown); 20 markers were unlinked. After core maps had been established, 32 markers with distorted segregation ratios and 60 segregating markers heterozygous in both parents were tested for linkage to the core map. Among the 60 markers segregating from both parents no distinction was made on the basis of their segregation ratios. Only those markers with at least three links to markers within a particular group were considered. Of these 68 could be placed on linkage groups of the core map. Of the 7 linkage groups for each parent 6 could be joined through 32 of these markers (Fig. 1). For 1 linkage group of each parent no

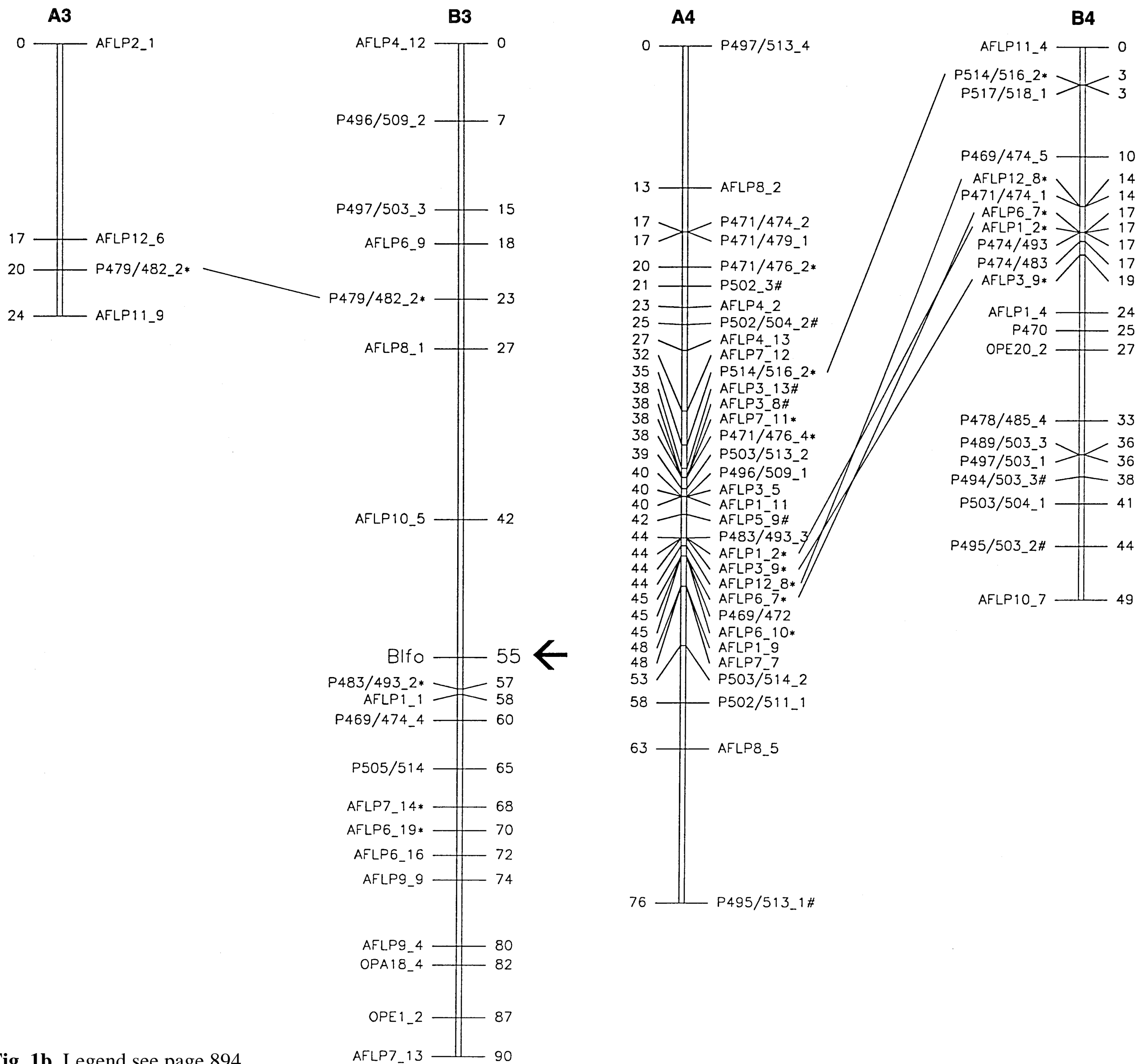

Fig. 1b Legend see page 894

linkage to any common marker could be detected, and therefore these groups could not be joined.

Based on the common markers the orientation of the linkage groups A1, B1, A2, B2 A4 and B4 could be inferred. For the other groups, the orientation was not possible due to missing common markers (A6, B6, A3 and B3) or contradictions in the map positions of these markers (A5, B5, A7 and B7). In total 278 markers (157 RAPD and 119 AFLP markers) could be assigned to the 14 linkage groups.

The map of parent 93/1–117 spans a total length of 326 cM with an average distance between markers of 2.4 cM. The map of 93/1–119 covers a total of 370 cM with an average marker density of 2.6 cM. Several markers on linkage groups A1, B1, A2 and, to a lesser extend B2 and A4, are strongly clustered (Fig. 1). The largest gap in the map is located on linkage group A5 between the markers P503/504_3 and P498/503_1 and spans 16 cM.

Mapping of phenotypic characters

Two genes controlling phenotypic characters segregate in the mapping population. Parent 93/1–117 has simple (5 petals) pink flowers, whereas parent 93/1–119 has double (>15 petals) pink flowers. Double flowers segregate versus simple flowers in agreement with a ratio of 1:1, indicating a monogenic or oligogenic inheritance of this trait (Debener 1999). Linkage analysis along with the molecular markers place the corresponding gene in the centre of linkage group B3 (designated *Blfo*) between the markers P483/493_2 and AFLP 10_5. The direct map distance in a two-point analysis to the closest

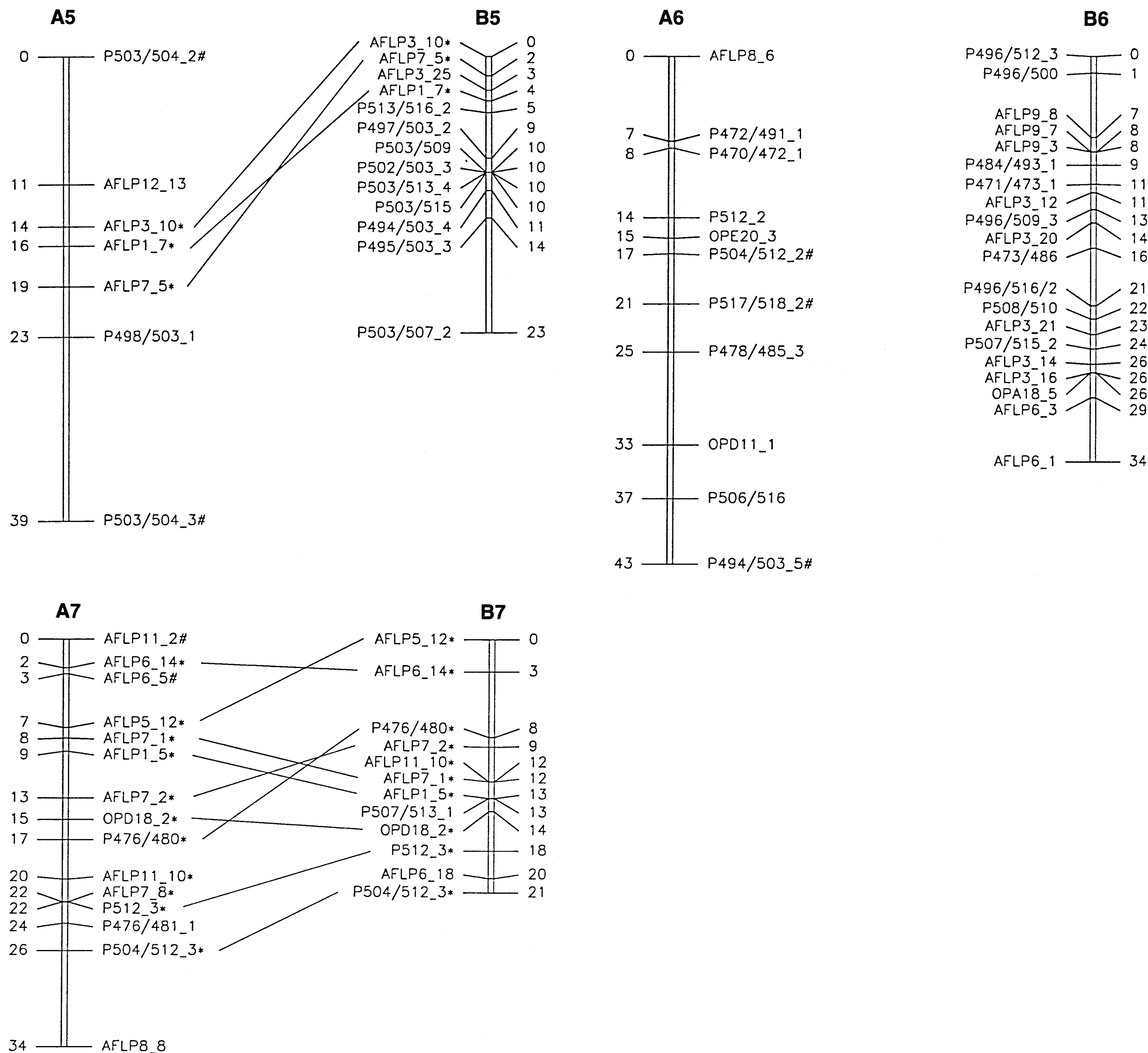

Fig. 1c Legends see page 894

markers is 0 cM to P483/493_2 (LOD = 8.4), 3.5 cM to AFLP 1_1 (LOD = 13.1) and 15.3 cM to AFLP 10_5 in coupling. Marker P469/474_4 is linked with 10.2 cM (LOD = 9.7) in repulsion.

Pink segregates versus white flower colour in accordance with a 3:1 ratio, indicating a monogenic or oligogenic inheritance as well (Debener 1999). Tight linkage of the corresponding gene (designated *Blfa*) could be detected on linkage group B2 to the markers OPD3 (0 cM, LOD = 6.9), AFLP1_3 (0 cM, LOD = 5.1), AFLP2_2 (0 cM, LOD = 5.6), AFLP2_3 (0 cM, LOD = 5.6), AFLP5_11 (0 cM LOD = 5.6), OPD18_1 (0 cM, LOD = 4.4), P469/471 (0 cM, LOD = 6.0), P507/513_3 (1.9 cM, LOD = 10.8), AFLP 12_14 (5.8 cM, LOD = 7.3). On linkage group A2 *Blfa* was tightly linked to the markers P511/513 (o cM, LOD 3.0), P507/513_3 (1.9 cM, LOD = 10.8) and AFLP 12_14 (5.8 cM, LOD = 7.3).

Discussion

In the current paper we describe the construction of the first molecular marker maps for roses. To date, no linkage data have been published for the rose genome. A total of 305 AFLP and RAPD markers were analysed in a segregating population of 60 F_1 individuals; 278 of these markers could be assigned to 14 linkage groups on maps for each parent.

Marker segregation

Apart from 119 markers inherited from the parent 93/1–117 and 128 markers inherited from 93/1–119, 60 segregating markers were present in both parents (Table 2). This relatively high proportion of 19.2% may be explained by the fact that both parents are half-sibs and re-

sulted from open pollination of genotype 81/42–15. Therefore, it is expected that both genotypes share a minimum of 25% of all alleles and that the fraction of markers of this type is higher than in other mapping projects based on pseudotestcross strategies (Hemmat et al. 1994; Grattapaglia and Sederoff 1994).

The majority of RAPD markers was generated with combinations of 15- and 20-mer primers (Table 2). This strategy has been shown previously to be effective for the generation of large numbers of RAPD markers with a limited number of primers (Debener and Mattiesch 1998). The fraction of markers which could be reliably scored in the segregating population was higher for the 15- and 20-mer primers than for the 10-mer primers. However, even the efficiency of the longer RAPD primers was low compared to the AFLP technique where every primer combination yielded reliable marker data in the segregating population.

Map construction

The distribution of markers over 14 linkage groups corresponds to the chromosome number of 2x = 14 in the genome of diploid roses. However, some of the linkage groups, for example groups A3, A5 and B7, contain only small numbers of markers. Because the mapping population of 60 F_1 individuals is relatively small, linkage to marker pairs and single markers so far unlinked to the main linkage groups might have gone undetected. These putative gaps in the map might be closed by analysing more markers in a larger mapping population. The present maps span a total of 326 and 370 cM for 93/1–117 and 93/1—119, respectively. Although these values are much smaller than map lengths from other plant species, such as apple with 950 cM (Hemmat et al. 1994), tomato with 1276 cM (Tanksley et al. 1992) and rice with 1670 cM (Nagamura et al. 1993), they are comparable to values obtained for Arabidopsis with 520 cM (Hauge et al. 1993), sugar beet with 508 cM (Nilsson et al. 1997) and diploid strawberry with 445 cM (Davis and Yu 1997). The average distance between the markers of 2.4 cM for the map of 93/1–117 and 2.6 cM for the map of 93/1–119 provides a medium-density marker coverage compared to the high-density maps of, for example, tomato (Tanksley et al. 1992) and Arabidopsis (Hauge et al. 1993) and low-density maps as for strawberry (Davis and Yu 1997) and Citrus (Cai et al. 1994).

Markers on most linkage groups are clustered in one or two areas. The clustering of markers on specific areas of the linkage groups is a commonly observed phenomenon. One explanation is the reduced recombination rate around centromeres (Tanksley et al. 1992; AlonsoBlanco et al. 1998) as well as the tendency of some marker types like AFLP and RAPD markers to map in clusters (AlonsoBlanco et al. 1998; Nilsson et al. 1997). Part of the clustering could also be explained by the observation that the joint mapping of the markers heterozygous only in one parent (building the core map) and the markers heterozygous in both parents caused a reduction in the average distance between the markers (data not shown). The extreme clustering of markers on linkage groups A1, B1 and A2 exceeds the degree of clustering observed on the other linkage groups. Possible explanations for this phenomenon are the presence of a self-incompatibility factor on that linkage group or a general reduction of recombination due to foreign chromosomal material introgressed in earlier breeding programmes (Reimann-Philipp 1981). A clear distinction between these possibilities would require the analysis of a large numbers of intercrosses among genotypes of the population 94/1 for self-incompatibility alleles and the mapping of clustered markers in other populations.

Localisation of genes controlling phenotypic characters

The localisation of two genes controlling phenotypic characters, double flowers and pink flower colour, on the current map close to molecular markers is a first step in utilising the map for marker-assisted selection. Both traits are important ornamental characters for both cut and garden roses (DeVries and DuBois 1996, Krüssmann 1986). A knowledge of the genetic complexity of these traits as well as of their chromosomal location provides valuable information for further analyses of the genetics of these traits as well as for their manipulation in breeding schemes. As other populations segregating for both traits are available (Debener 1999), it can now be tested whether genes with the same chromosomal locations are responsible for the observed segregation.

Application of the map

The current maps can be applied in several ways. The medium-density marker coverage obtained with the current maps are a good starting point for marker-assisted selection projects. As, for example, modern rose varieties lack efficient resistance genes against the major fungal pathogens, these genes can only be introgressed from wild rose species into the genome of modern varieties by intensive backcross breeding. The tetraploid, highly heterozygous nature of most modern varieties makes this very complicated and time-consuming. Any information on the distribution of markers distributed over the map as well as on markers linked to the ornamental traits may facilitate the progress of gene introgression while minimising linkage drag of unwanted characters. The problem of transferring data from the dominant PCR-based AFLP and RAPD markers to other segregating populations can be overcome by turning a marker framework covering the entire map into SCAR (sequence characterised amplified regions, Paran and Mitchelmore 1993), CAPS (cleaved amplified polymorphic sequences, Konieczyn and Ausubel 1993) and restriction fragment length polymorphism (RFLP) markers. Marker data generated in other populations

can be mapped onto the current map with the same strategy. Populations segregating for other horticulturally important traits as, for example, the presence of prickles, the occurrence of recurrent flowering and resistance to black spot, are already available (Debener 1999; von Malek and Debener 1998), and the first tightly linked markers generated in tetraploid segregating populations are now mapped (von Malek and Debener, in preparation). An interesting approach could also be the utilisation of marker data from maps generated in the apple and *Prunus* mapping projects (Hemmat et al. 1994, Baird et al 1996). The degree of synteny between rosaceous genomes can now be investigated based on commonly mapped markers. In these projects, genes for resistance against the major pathogens as well as genes controlling several morphological characters have been located on the maps. Mapping of RFLP markers from these maps onto the rose map could help to locate the appropriate rose genes more efficiently.

Another application of the rose maps is the map-based cloning of interesting genes. Genome size estimations for diploid rose species vary between 0.2 and 1.6 pg per haploid genome for *Rosa wichuraiana* and *R. multiflora,* respectively (Bennet et al. 1991). More recent data report genome sizes of 0.49 pg per haploid genome for *R. rugosa* var 'alba' (Yokoya and Roberts, personal communication), which is about 1.5 –2× the size of the *Arabidopsis* genome. These conditions together with high-density marker systems as AFLP markers would make the map-based cloning strategy a realistic approach for the cloning of rose genes. Markers from the current map might therefore be useful in "chromosome walking" or "chromosome landing" projects.

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References

AlonsoBlanco C, Peters AJM, Korneef M, Lister C, Dean C, van den Bosch N, Pot J, Kuiper MTR (1998) Development of an AFLP based linkage map of Ler, Col and Cvi *Arabidopsis thaliana* ecotypes and construction of a Ler/Cvi recombinant inbred line population. Plant J 14: 259–271

Baird WV, Ballard RE, RajapakseS, Abbott AG (1996) Progress in *Prunus* mapping and application of molecular markers to germplasm improvement. HortScience 31: 1099–1106

Bennett MD, Smith JB (1991) Nuclear DNA amount in angiosperms. Philos Trans R Soc London Ser B 334: 309–345

Ben-Meir H, Vainstein A (1994) Assessment of genetic relatedness in roses by DNA fingerprint analysis. Sci Hortic 58: 158–164

Cai Q, Guy CL, Moore GA (1994) Extension of the linkage map in *Citrus* using random amplified polymorphic DNA (RAPD) markers and RFLP mapping of cold-acclimation-responsive loci. Theor Appl Genet 89: 606–614

Davis TM, Yu H (1997) A linkage map of the diploid strawberry *Fragaria vesca*. J Hered 88:215–221

Debener T (1999) Genetic analysis of horticulturally important characters in diploid roses. Gartenbauwissenschaft 64:14–20

Debener, T, Mattiesch L (1996) Genetic analysis of molecular markers in crosses between diploid roses. Acta Hortic 424: 249–253

Debener T, Mattiesch L (1998) Effective pairwise combination of long primers for RAPD analyses in roses. Plant Breed 117: 147—151

Debener T, Bartels C, Mattiesch L (1996) RAPD analysis of genetic variation between a group of rose cultivars and selected wild rose species. Mol Breed 2: 321–327

De Vries DP, Dubois LAM (1978) On the transmission of the yellow flower colour from *Rosa foetida* to recurrent flowering hybrid tea-roses. Euphytica 27: 205–210

De Vries DP, Dubois LAM (1984) Inheritance of the recurrent flowering and moss characters in F_1 and F_2 Hybrid Tea x *Rosa centifolia muscosa* (Aiton) Seringe populations. Gartenbauwissenschaft 49: 97–100

De Vries DP, Dubois LAM (1996) Rose breeding: past, present, prospects. Acta Horticulturae 424: 241–248

Gebhardt C, Salamini F (1992) Restriction fragment length polymorphism analysis of plant genomes and its application to plant breeding. Int Rev Cytol 135: 201–237

Grattapaglia D, Sederoff R (1994) Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudotestcross mapping strategy and RAPD markers. Genetics 137: 1121–1137

Gudin S (1998) Rose: genetics and breeding. Appl Bot (in press)

Hauge BM, Hanley SM, Cartinhour S, Cherry JM, Goodmann HM, Korneef M, Stam P, Chang C, Kempin S, Medrano L, Meyerowitz EM (1993) An integrated genetic/RFLP map of the *Arabidopsis thaliana* genome. Plant J 3: 745–754

Hemmat M, Weeden NF, Manganaris AG, Lawson DM (1994) Molecular marker linkage map for apple. J of Hered 85: 4–11

Hubbard M, Kell J, Rajapakse S, Abbott A, Ballard R (1992) Restriction fragment length polymorphism in rose and their use for cultivar identification. HortScience 27: 172–173

Konieczyn A, Ausubel FM (1993) A procedure for mapping *Arabidopsis* mutations using co-dominant ecotype-specific PCR-based markers. Plant J 4: 403–410

Krüssmann G (1986) Rosen, rosen, rosen. Verlag Paul Parey, Berlin Hamburg

Kubisiak TL, Nelson CD, Nance WL, Stine M (1995) RAPD linkage mapping in a longleaf pine x slash pine F_1-family. Theor Appl Genet 90: 1119–1127

Millan F, Osuma F, Cobos S, Torres A, Cubero JI (1996) Using RAPDs to study phylogenetic relationships in *Rosa*. Theor Appl Genet 92: 273–277

Nagamura Y, Yamamoto K, Harushma Y, Wu J, Antonio BA, Sue N, Shomura A, Lin S-Y, Miyamoto Y, Toyama T, Kirihara T, Shimizu T, Wang Z -X, Tamura Y, Ashikawa I, Yano M, Kurata N (1993) A high density STS and EST linkage map of rice. Rice Genome 2: 11–13

Nilsson NO, Hallden C, Hansen M, Hjerdin A, Säll T (1997) Comparing the distribution of RAPD and RFLP markers in a high density linkage map of sugar beet. Genome 40: 644–651

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

Paterson AH, Tanksley SD, Sorrels ME (1991) DNA markers in plant improvement. Adv Agron 46: 39–90

Reimann-Philipp R (1981) Cytogenetics and breeding in diploid roses from the triploid hybrid *R.multiflora* x garden cultivars. In: Proceedings Eucarpia Ornamentals Meet Rose Breed pp 27–29

Reynders-Aloisi S, Bollereau P (1996) Characterization of genetic diversity in genus *Rosa* by random amplified polymorphic DNA. Acta Hortic 424: 253–259

Stam P, Van Ooijen JW (1995) JoinMap version 2.0: Software for the calculation of genetic linkage maps, CPRO-DLO, Wageningen, The Netherlands

Staub JE, Serquen FC, Gupta M (1996) Genetic markers, map construction, and their application in plant breeding. Hort Sci 31: 729–741

Suhl IW, Korban SS (1996) A highly efficient method for isolating genomic DNA from plant tissues. Plant Tissue Cult Biotechnol 2: 113–116

Tanksley SD, Ganal MW, Prince JW, de Vincente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Paterson AH, Pineda O, Röder MS, Wing RA, Wu W, Young ND (1992) High density molecular linkage maps of the tomato and potato genomes. Genetics 132: 1141–1160

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

von Malek B, Debener T (1998) Genetic analysis of resistance to blackspot (*Diplocarpon rosae*) in tetraploid roses. Theor Appl Genet 96:228–231

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Fritjers A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new concept for DNA fingerprinting. Nucleic Acids Res 23: 4407–4414

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