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Two genetic linkage maps of tetraploid roses

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Abstract A tetraploid F_2 progeny segregating for resistance to black spot, growth habit, and absence of prickles on the stem and petioles was used to construct genetic linkage maps of rose. The F_1 of the progeny, 90–69, was created by crossing a black spot-resistant amphidiploid, 86–7, with a susceptible tetraploid, 82–1134. The F_1 was open-pollinated to obtain 115 seedlings. AFLP and SSR markers were used to eliminate seedlings produced through cross-fertilization. The remaining progeny set of 52 F_2 plants was used to study the inheritance of 675 AFLPs, one isozyme, three morphological and six SSR markers. AFLP markers were developed with three combinations of restriction enzymes, *Eco*RI/*Mse*I, *Kpn*I/*Mse*I and *Pst*I/*Mse*I. Most of the markers appear to be in simplex or single-dose and segregated 3:1 in the progeny. One linkage map was constructed for each parent using only the single-dose markers. The map of 86–7 consists of 171 markers assigned to 15 linkage groups and covering more than 902 cM of the genome. The map of 82–1134 consists of 167 markers assigned to 14 linkage groups and covering more than 682 cM of the genome. In the AFLP analysis, *Eco*RI/*Mse*I generated nearly twice as many markers per run than *Pst*I/*Mse*I. Markers developed with three restriction enzyme combinations showed a mixed distribution throughout the maps. A gene controlling the prickles on the petiole was located at the end of linkage group 7 on the map of 86–7. A gene for malate dehydrogenase locus 2 was located in the middle of linkage group 4 on the map of 86–7. These first-generation maps provide initial tools for marker-assisted selection and gene introgression for the improvement of modern tetraploid roses.

Keywords *Rosa* · Linkage maps · Single-dose markers · Inheritance of markers · Genome size

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Introduction

Roses are among the most-important ornamental plants worldwide. There are more than 120 rose species and 20000 commercial rose cultivars. Most of the modern rose cultivars are tetraploid (x=7) or triploid hybrids derived from eight to ten wild diploid rose species and a few tetraploid species. Rose breeding has exploited specific combining abilities to produce highly heterozygous, vegetatively produced cultivars with the desired combinations of color, form, fragrance, and hardiness. Modern hybrid tea cultivars are popular for their pointed buds, large blooms with multiple rows of petals, vivid colors, and recurrent flowering throughout the growing season. Despite these attractive features, hybrid tea roses and many other modern roses are susceptible to a host of diseases such as black spot (*Diplocarpon rosae* Wolf) and powdery mildew (*Sphaerotheca pannosa* var. *rosae* Lév). Genetic resistances to these diseases exist in some of the rose species. Furthermore, some rose species are free of prickles on their stems and petioles. The rose breeders' challenge is to transfer these desirable traits from wild-species roses (2n=14) to the cultivated tetraploid rose (4n=28) while maintaining the superior floral traits of the modern roses. Understanding how important rose traits are inherited in tetraploid roses, and developing a marker data base for use in introgression of beneficial genes from diploid roses, can greatly accelerate the production of superior rose germplasm.

The inheritance of only a few rose traits is understood, e.g., recurrent flowering, controlled by a single recessive gene (De Vries and Dubios 1984), resistance to black spot fungus *Diplocarpon rosae* race 5, controlled

by a single dominant gene (Von Malek and Debener 1998), petal number, controlled by quantitative trait loci (Debener et al. 2000), and flower color, controlled by one or a few genes (Debener and Mattiesch 1999). Until recently there was no gene map developed for any rose, nor were there markers linked to any of the important traits which could be used by rose breeders. Debener and Mattiesch (1999) published a genetic map for the diploid *Rosa multiflora* Thunb. that located the genes controlling petal number and flower color. Maps like this provide initial tools for the breeders. However, no genetic maps are available for tetraploid roses that can be used by rose breeders attempting to introduce desirable genes into the background of modern roses.

The size of the rose genome has been the subject of controversy for many years. Bennet and Smith (1991) reported that the nuclear DNA content of diploid *Rosa wichuraiana* Crép. was 0.2 pg/2C, while Dickson et al. (1992) estimated the size of another diploid species *R. multiflora* Thunb. Ex J. Murr to be 1.65 pg/2C. The earlier measurement is likely an underestimation due to the technique employed. Recently, Yokoya et al. (2000) published the genome size of many rose species. Their genome size estimates are more comparable to that of Dickson et al. (1992).

This paper reports the genomic size of three diploid roses and describes the first linkage maps in tetraploid roses locating a gene controlling prickles on petiole. We also report the nature of inheritance of molecular markers, which will eventually lead to understanding how the genes controlling commercially valuable traits are inherited in tetraploids.

Materials and methods

Plant material

The rose progeny was produced by crossing 86–7, an amphidiploid resistant to black spot, with 'Basye's Blueberry' (82–1134), a tetraploid moderately susceptible to black spot. Amphidiploid 86–7 contains genomes of two highly black-spot-resistant species, *R. wichuraiana* 'Basye's Thornless' and *Rosa rugosa* Thunb. var. *rubra* (Byrne et al. 1996). The 86–7 bush is a sprawling ground cover with white flowers of five petals. Its stems and petioles have prickles, traits inherited from the *rugosa* parent. The maternal parent of the cross, 82–1134, is a spreading bush with pink flowers consisting of ten or more petals. This tetraploid is free of prickles on both stems and petioles. One F_1 hybrid, 90–69, has a high level of field resistance to black spot, pink flowers and prickles on stems and petioles. This F_1 was open-pollinated to obtain 115 seedlings. Since roses self-fertilize as well as cross-fertilize, we anticipated that some of these progeny would be a result of outcrossing. AFLP, SSR, and isozyme markers were used to identify and remove those seedlings produced through cross-fertilization, and to obtain an F_2 mapping progeny set.

Nuclear genome size

The nuclear DNA content of diploid parents of amphidiploid 86–7, *R. wichuraiana* 'Basye's Thornless', *R. rugosa* var. *rubra*, and another diploid rose species *Rosa chinensis* 'Roulettii' were measured at the flow cytometry core research facility at the Center for Biotechnology, University of Nebraska, Lincoln. Sprigs of the three rose species were collected, wrapped in moist paper towels and shipped overnight to Lincoln. The procedure used to analyze nuclear DNA content in rose cells was modified from that of Arumuganathan and Earle (1991). Chicken red blood cell nuclei were used as the internal standard. For flow cytometric analysis, 50 mg of fresh leaf tissue was placed on ice in a sterile 35×10 mm plastric Petri dish. The tissue was sliced into 0.25-mm to 1-mm segments in a solution containing 10 mM of $MgSO_4.7H_2O$, 50 mM of KCl, 5 mM of Hepes, pH 8.0, 3% PVP-10 (polyvinyl pryrrolidine), 3 mM of dithiothreitol, 0.1 mg/ml of propidium iodide (PI), 1.5 mg/ml of DNAse-free RNAse (Boehinger Mannheim) and 0.25% Triton X-100. The suspended nuclei were withdrawn using a pipettor, filtered through a 30-µm nylon mesh, and incubated at 37°C for 30 min prior to flow cytometric analysis. The suspension of sample nuclei was spiked with chicken erythrocyte nuclei (prepared in above solution) and analyzed with a FACscan flow cytometer (Becton-Dickinson). For each measurement, the propidium iodide fluorescence area signals (FL2-A) from 1000 nuclei were collected and analyzed by CellQuest software (Becton-Dickinson, San Jose, Calif.) on a Macintosh computer. The mean position of the G0/G1 (nuclei) peak for the sample and the internal standard was determined by CellQuest software. The mean nuclear DNA content of each plant sample, measured in picograms, was based on 1000 scanned nuclei.

AFLP analysis

Young leaves from seedlings of the rose progeny grown at Texas A & M University were collected on ice and shipped overnight to Clemson University. Total genomic DNA was extracted from 1 g of fresh leaf tissue (Jarret and Austin 1994) and quantified using a minifluorometer (Hoefer TK 100). DNA working samples of 100 ng/µl were prepared for each of the progeny. AFLP analysis (Vos et al. 1995) was carried out using the following three different restriction enzyme combinations; *Eco*RI/*Mse*I, *Kpn*I/*Mse*I and *Pst*I/*Mse*I. For *Eco*RI/*Mse*I, AFLP Analysis System I (Life Technologies) was used. For the remaining two restriction enzyme combinations, adapters and primers were synthesized (Integrated DNA Technologies). Pre-amplification primers consisted of the following single selective nucleotide for each enzyme, except for *Pst*I; *Eco*RI-A, *Mse*I-C and *Kpn*I-A. No selective nucleotide was used in the pre-amplification primer for *Pst*I. In the selective amplification step, the number of additional primer bases (two or three for each primer) required to obtain the optimum number of clearly scorable bands was first determined for each restriction enzyme combination before applying it to the entire progeny set. Additional bases for the two primers of restriction enzymes were selected carefully in order to avoid duplication and the clustering of genomic fragments generated from AFLP analysis.

The following primer combinations were employed; E-AAC+M-CAT, E-AGG+M-CTG, E-ACC+M-CAC, E-AAG+M-CTT, E-ACA+M-CAA, E-ACT+M-CAG, E-ACG+M-CTC, E-AGC+M-CCA; P-GC+M-CCA, P-GC+M-CAC, P-CA+M-CTG, P-AG+M-CAG, P-CC+M-CTC, P-AC+M-CTT, P-CG+M-CAC; K-AA+M-CAC, K-AT+M-CTC, K-AG+M-CAT, K-AT+M-CCC, K-AA+M-CTG, and K-AG+M-CCA. *Eco*RI, *Kpn*I or PstI primers were end-labeled with [ϒ ^{33}P] (New England Nuclear) and T4 polynucleotide kinase (Promega). PCR products were separated on 6% denaturing polyacrylamide gels, which were pre-run to reach 45°C. Four microliters of samples were loaded on to gels and electrophoresed at 70 V for 2 h 30 min. Kodak Biomax MR films were exposed to vacuum-dried gels for 3 days and developed.

SSR analysis

We screened 13 peach SSR primers (received from A. Abbott, Clemson University), five apple SSR primers (Guilford et al. 1997), and three sour cherry SSR primers (received from A.

Iezzoni, Michigan State University) in the mapping progeny. The primers and their sequences are given in Table 1. Reaction mixtures (10 µl total volume) contained 1.5 pmol of primers, 200 µM of dNTPs, 0.25 U of *Taq* polymerase (Qiagen), 10 mM of Tris-HCl (pH 8.7), 50 mM of KCl, 3.0 mM of $MgCl_2$ and 14 ng of genomic DNA. PCR reactions were performed in a MJ Research PTC-100 thermal cycler programmed for one step of denaturation at 94°C for 5 min, followed by 31 cycles of denaturation at 94°C for 45 s, primer annealing for 30 s and primer extension at 72°C for 30 s. The starting annealing temperature for each primer is 5°C greater than the temperature given in Table 1. In the first ten cycles the annealing temperature was lowered by 0.5°C in each cycle. After the 10th cycle, the annealing temperature given in Table 1 was maintained for the remaining 21 cycles. A final extension step was carried out at 72°C for 10 min. Prior to PCR with all samples, a range of annealing temperatures, lowered from that used with the homologous species, was tested and optimized for use in rose. PCR products were first run on agarose gels to verify amplification and, upon observation of clear bands, PCR was performed again with one of the primer ends labeled with [ϒ ^{33}P] and run on 6% denaturing polyacrylamide gels.

Isozyme analysis

One isozyme, malate dehydrogenase (E.C. 1.1.1.37), was assayed in the progeny. Sample preparation and electrophoretic procedures were modified from Byrne and Littleton (1988) as follows. Samples were prepared with 150 mg of diced leaf tissue, 0.1 g of polyvinylpolypyrrolidone (PVPP), and 2 ml of extraction buffer [100 ml of Na-phosphate buffer, pH 7.3, 2.5 g of polyvinylpyrrolidone (PVP40), 1 ml of mercaptoethanol, and 0.25 ml of Tween 80]. The extracts were soaked in filter paper wicks and run on a morpholine citrate starch-gel system (pH 6.1). All samples were run at least twice to confirm isozyme phenotypes.

Morphological characters

Morphological traits segregating in the progeny include prickles on the stem, prickles on the petiole, and the bush growth habit. Rose bushes were evaluated for prickles on stem and petiole and scored as present or absent. The variation in the size, number or the density of prickles was not taken into account. Prickle evaluation on the petiole included the rachis of the compound leaf as well as the petioles of each leaflet. The growth habit of 86–7 and 82–1134 is a sprawling ground cover and a spreading bush, respectively. The F_1 has an intermediate phenotype, a sprawling bush. Therefore, bush growth habit in the F_2 was scored as a co-dominant marker. Though the field black-spot resistance data were not available from the young seedlings at this time, we expect the F_2 progeny to segregate for the trait.

Inheritance of markers

Markers originating from each parent were scored according to the standard coding system using A, B, C, D and H of JoinMap (Stam and Van Ooijen 1995) and Mapmaker/EXP 3.0 (Lincoln et al. 1992), and maintained as two separate data sets. Chi-square (χ^2) tests of goodness-of-fit were performed on segregation data for all markers and three morphological traits evaluated in the 90–69 rose progeny. Each marker was tested for segregation ratios expected for simplex (single-dose) and duplex (double-dose) under both the disomic and tetrasomic inheritance patterns possible in this progeny. Markers deviating significantly (P>0.05 level) from the expected ratios were rejected for assuming segregation at that ratio. Segregation tests for 3:1 or 1:2:1 ratios were carried out using the JoinMap single-locus analysis module 'SLA.' Segregation tests for other ratios were done manually.

Linkage analysis and map construction

All markers, except growth habit and Mdh-2, were scored as dominant markers. Only the single-dose markers (Wu et al. 1992), i.e., the dominant markers that segregated 3:1 or the codominant markers that segregated 1:2:1, regardless of disomic or tetrasomic modes of transmission, were included in the map construction. Linkage analysis was performed (LOD >3.5) with JoinMap 2.0 (Stam 1993; Stam and Van Ooijen 1995). Linkage groups with less than four markers were not ordered and omitted from the map. Map construction was carried out with the Haldane mapping function.

Results and discussion

Estimation of nuclear genome size

Nuclear DNA content estimated by flow cytometry indicates that *R. wichuraiana* 'Basye's Thornless', *R. rugosa* var. *rubra* and *R. chinensis* 'Roulettii' have 1.30 (±0.01), 1.10 (±0.02) and 1.36 (±0.01) pg/2C, respectively. Recently, Yokoya et al. (2000) published the genome size of many rose species estimated using propidium iodide staining. Their study included different varieties of the above three species. Their genome size estimates are highly comparable to our measurements, though slightly smaller (an average of 15%). Their size estimates for *R. wichuraiana* Crép., *R. rugosa* var. *alba* W. Robins and *R. chinensis* Jacq. are 1.13, 0.98 and 1.16 pg/2C, respectively. Despite this small difference between the two sets of measurements, these results indicate that diploid roses have a comparatively small nuclear genome among flowering plants, (approximately 1200 Mbp/2C), only about 4× the size of *Arabidopsis thaliana* (Arumuganathan and Earle 1991).

Isozyme analysis

The rose progeny did not segregate for band 3 representing the Mdh-1 locus. However, the bands representing Mdh-2 were polymorphic among these rose genotypes. The amphidiploid parent of 90–69 exhibited the three-banded phenotype (bands 7, 8 and 9) which is derived from its parents each of which were homozygous for either band 7 (*R. wichuraiana*) or band 9 (*R. rugosa* var. *rubra*). The other parent of 90–69, 82–1134, also had a three-banded pattern (bands 9, 10 and 11). In the case of each of these roses, the middle band is a heterodimer created by the combination of the protein subunits of the outside bands. Thus the allelic composition of the tetraploid parents of 90–69 are 7, 7, 9, 9 for 86–7 and 9, 9, 11, 11 for 82–1134 (Kim and Byrne 1996). 90–69 exhibits a five-banded pattern 7, 8, 9, 10, 11, which combines all the alleles of its parents. Given the higher density of the middle band, its genotype is 7, 9, 9, 11. The F_2 progeny segregated for bands 7, 9 and 11, and their genotypes were deciphered according to the presence and the intensity of the bands. The isozyme was scored and ana-

Table 1 SSR primers tested in rose

Source species, primer (other names)	Sequence 5′–3′	Annealing temperature	Amplification of rose DNA[a]	Polymorphism in mapping progeny
Peach				
Pchgms1 (B3D5)	ggatcattgaactacgtcaatcctc gggtaaatatgcccattgtgcaatc	40.5°C	Complex band pattern	
Pchgms2 (B10H3)	gtcaatgagttcagtgtctacactc aatcataacatcattcagccactgc	55°C	Distinct PCR products	Polymorphic
Pchgms3 (B6B1)	acggtatgtccgtacactctccatg caacctgtgattgctcctattaaac	45.5°C	Distinct PCR products	Polymorphic
Pchgms4 (ppEG)	atcttcacaaccctaatgtc gttgaggcaaaagacttcaat	46°C	Complex band pattern	
Pchgms5 (B10B9)	cccgtagatttcaacgtcatctaca ggttcactctcacatacactcggag	42°C	No PCR products	
Pchgms6 (B4G3)	cattgttcatgggaggaatt agaacattcctaaaggagca	46°C	Distinct PCR Products	Not polymorphic
Pchgms7 (B1H1)	ttggctgcaggtcgacggatcc tcttccgaggcattgccacaaa	40°C	No PCR products	
Pchgms8 (B1G6)	catctgggcacacctaaagtcttgg agtgccaagcttggctgcaggtcga	45.5°C	Complex band pattern	
Pchcms1	gttacacctctgtcaca cttggctggcattccta	42°C	No PCR products	
Pchcms2	agggtcgtctctttgac cttcgtttcaaggcctg	45°C	Weak bands	
Pchcms3	ctgcagaacactactga gctttgcaaccaccagc	49°C	Single PCR Product	Not polymorphic
Pchcms4	ctcacgctatttctcgg cctcgacgaagagctcg	54°C	Complex band pattern	
Pchcms5	cgcccatgacaaactta gtcaagaggtacaccag	45°C	Distinct PCR Products	Not polymorphic
Apple				
23f1	ggaagagtgcgaaggcaa tgtcccaaccaccgcata	46°C	Complex band pattern	
26c6	gacgaagaactcgccggagc cgaggaccaacccacacacaa	55°C	Single PCR product	Not polymorphic
23g4	tttctctctctttcccaactc agccgccttgcattaaatac	46°C	Weak bands	
02b1	ccgtgatgacaaagtgcatga atgagtttgatgcccttgga	47°C	Distinct PCR products	Not polymorphic
01a6	aggattgctggaaaaggagg ttagacgacgctacttgtcct	40°C	No PCR product	
Sour Cherry				
GA77		41°C	Distinct PCR products	Not polymorphic
GA34	gaacatgtggtgtgctggtt tccactaggaggtgcaaatg	41°C	No PCR products	
GA25	gcaattcgagctgtatttcagatg cagttggcggctatcatgtcttac	41°C	Complex band pattern	

[a] Results oberved in polyacrylamide gels

lyzed in the progeny in two ways, as a dominant marker for the presence or absence of each allele, and as a co-dominant marker.

AFLP analysis

In order to avoid clustering of markers on certain regions of the chromosome maps, we have taken two precautions. First, AFLP markers were developed with three different restriction enzyme combinations. These are *Eco*RI/*Mse*I, *Kpn*I/*Mse*I and *Pst*I/*Mse*I. The optimum number of additional primer bases required to obtain the maximum number of clearly scorable bands was first investigated for each restriction enzyme combination before applying that combination to the entire progeny set. Second, stringent measures were taken in selecting additional bases for the two primers of restriction enzymes. A change in only one base (e.g., using AAG vs AAC) can produce the same genomic fragments since a single base change may or may not alter PCR amplification. We selected very different base combinations (e.g., AAG vs CGT) to avoid the duplication of genomic fragments generated from AFLP analysis.

AFLP analysis with all three restriction enzyme combinations was effective in generating markers in rose. In the selective amplification step, primers with three additional selective bases for each restriction enzyme in *Eco*RI/*Mse*I (i.e., +3/+3) yielded an optimum number of bands per gel (60–70). Primers with less selective bases (+2/+3 or +3/+2) yielded too many bands and concealed polymorphisms. In contrast, for the *Kpn*I/*Mse*I and *Pst*I/*Mse*I enzyme combinations, primers with +2/+3 additional selective bases produced well-separated bands equivalent in number and dispersion to those produced by +3/+3 selective bases of *Eco*RI/*Mse*I. Therefore, by optimizing the number of additional selective bases used in selective amplification, we were able to develop the largest number of markers that could be scored confidently. These optimum primer combinations were employed in generating markers for the purpose of identification of seedlings produced by outcrossing of the F_1 and subsequent map construction.

SSR analysis

Microsatellite or simple sequence repeat (SSR) markers are highly polymorphic and informative. Some of the SSR primers developed in one species may be effective in detecting polymorphism in other related species. We tested 21 SSR primer pairs developed in peach, apple or sour cherry, in our rose mapping population (Table 1). After tests with varying the annealing temperatures, 16 primer pairs were successful in amplifying rose DNA. Out of these, six gave rise to complex banding patterns with over ten bands and two other primer sets produced only weak bands. The remaining eight primer sets produced distinct PCR products, comparable in size with the corresponding PCR products of the homologous species. PCR products of two of these primers, B10H3 and B6B1, developed from the peach mapping project, showed polymorphisms and segregated in F_2 progeny of the current rose mapping population and have been mapped. In addition to providing markers on the map, these two SSR markers were useful in identifying those seedlings produced from the cross-pollination of 90–69. Six SSR primers out of eight did not produce any useful polymorphisms. This is a high proportion considering that SSR markers are usually highly polymorphic. The regions represented by these primers are conserved across the species, genera and even subfamilies. Though all these species belong in the family Rosaceae, they are in different subfamilies, rose in the Rosoideae, peach and sour cherry in the Prunoideae, and apple in the Maloideae. It appears that regions amplified by these primers are well-conserved across subfamilies, since they were designed and optimized for peach, apple and sour cherry, yet amplified rose DNA. However, these regions may be too well conserved to allow the observation of significant polymorphism in rose.

Table 2 Inheritance of markers segregating in 86–7 and 82–1134

Type	Markers arising from 86–7	Markers arising from 82–1134
Simplex	242 (72%)	243 (70%)
Duplex	62 (18%)	79 (22%)
Other[a]	33 (10%)	26 (8%)
Total	337	348

[a] Other markers include those with distorted segregation ratios

Identification of seedlings resulting from outcrossing

The presence of strong bands in the open-pollinated progeny that are absent in the F_1 90–69 was used to identify the outcrossers. Since AFLP markers are PCR-based and are known to produce occasional nonspecific bands, the following selection criteria were adopted: (1) the presence of non-F_1 bands in more than one primer combination, and (2) the presence of more than one non-F_1 band in each primer combination. Using these selection criteria, and after screening with more than 20 primer combinations, 63 of the 115 plants tested were identified as resulting from outcrossing. The remaining 52 plants formed the progeny for linkage analysis.

Inheritance of markers and rose phenotypic traits

Cytogenetic studies indicate multivalent formation involving up to 8 of the 28 chromosomes in amphidiploid 86–7 and its progeny (Ma and Byrne 1998). The formation of multivalents indicates that these tetraploids may be segmental allopolyploids and that traits can be inherited either disomically or tetrasomically. Regardless of the nature of inheritance (disomic or tetrasomic), dominant markers in simplex (single-dose) segregate 3:1 in the F_2 progeny. However, for the dominant markers in duplex (double-dose), segregation ratios vary from 15:1 for disomic and 35:1 for tetrasomic inheritance patterns. We observed that about 70% of markers originating from either parent segregate 3:1 in the F_2 progeny (χ^2 not significant at $P>0.05$) and are in simplex (Table 2). Single-dose markers originating from 82–1134 indicate that it also consists of two different genomes. Indeed the origin of the tetraploid 82–1134 indicates this to be true. 82–1134 is a backcross progeny derived from crossing the F_1 hybrid 74–193 with one of its parents, 65–626. 74–193 is the cross between 65–626 (*Rosa carolina* L.×hybrid tea 'Hugh Dickson') and 62–322 (*Rosa virginiana* Mill.×hybrid tea 'Betty Morse').

About 20% of the markers originating from either parent appear to segregate either 15:1 or 35:1, as expected from markers in duplex under the disomic and tetrasomic inheritance patterns possible in tetraploids. Due to the dominant nature of most markers, the population size was not large enough to discriminate markers in duplex between disomic and tetrasomic inheritance patterns (15:1 vs 35:1). Even if only one plant showed the ab-

Table 3 Comparison of AFLP markers generated with three restriction enzyme pairs

Enzyme/ primer combination	Number of primers tested	Total # of markers generated	Single dose markers	Number of markers mapped[a]	Coverage/ marker cM
E-AXX M-CXX	8	315	200 (63%)	140 (44%)	8.1
K-AX M-CXX	6	216	163 (75%)	113 (52%)	7.6
P-XX M-CXX	7	144	114 (80%)	81 (56%)	9.3

[a] Excluding markers in small linkage groups not drawn on maps and unlinked markers

sence of a marker (51:1), which would most favor tetrasomic inheritance, the population size was not large enough to reject disomic inheritance at P=0.05. Furthermore, a small percentage of markers appear to segregate 1:1, and these are assumed to have a distorted or another segregation pattern. (χ^2 significant for 3:1,15:1 or 35:1). We conclude that most of the markers analyzed follow simple Mendelian inheritance and are controlled by genes specific to one of four sets of genomes. These results are compatible with chromosome-pairing research conducted by Ma and Byrne (1998). It would be interesting to identify which chromosomes form multivalents and thus give rise to tetrasomic inheritance.

Several rose characteristics were evaluated for their genetic basis of inheritance. For prickles on the stem, the segregation ratio for the 52 F_2 progeny was 32 present: 20 absent (χ^2 for testing this ratio for 3:1=5.03, df=1, significant at 0.05), indicating the nonsimplex origin for this trait. For prickles on the petiole, the segregation ratio was 36 present:14 absent, two F_2 plants were not scored for this trait (χ^2 for testing 3:1=0.24, df=1, not significant at 0.05 level). Therefore, the absence of prickles on the petiole appears to segregate 3:1 and probably is controlled by a single recessive gene. It appears that absence of prickles on the stem and petiole segregate independently. Segregation of the growth habit scored as a codominant marker in the progeny (17:20:14) was also not significantly different from 1:2:1 (χ^2=2.73, df=2) demonstrating its single-gene inheritance. While the two parents of 90–69 show variation in growth habit, i.e., 86–7 is a sprawling ground cover and 82–11134 is a spreading bush, the F_1 has an intermediate phenotype, sprawling bush. This allows us to infer that the trait is under incomplete dominance. The F_2 progeny segregated 12:28:5 for isozyme Mdh-2 scored as a codominant marker. This ratio is not different from 1:2:1 (χ^2=4.87, df=2, not significant at the 0.05 level) demonstrating its single-gene inheritance.

Comparison of three restriction enzymes in developing markers for mapping

An average of 39, 36 and 21 markers were scored from a single primer pair with *Eco*RI/*Mse*I, *Kpn*I/*Mse*I and *Pst*I/*Mse*I, respectively (Table 3). These numbers also reflect the level of polymorphism obtained with each restriction enzyme combination, as the total number of bands developed was kept approximately the same. *Eco*RI/*Mse*I generated nearly twice as many markers per run than *Pst*I/*Mse*I. Most of the markers (63–80%) developed with all three restriction enzyme pairs appear to be in simplex or single-dose and segregated 3:1 in the progeny (χ^2 not significant at P>0.05). *Pst*I/*Mse*I markers had a somewhat higher percentage of single-dose markers than *Eco*RI/*Mse*I markers. Markers produced with methylation-sensitive *Pst*I are expected to be found more in gene-rich areas of the genome than markers developed with methyl-insensitive *Eco*RI. The latter group of markers may arise more often from the repetitive sequences of the genome.

Linkage analysis and map construction

All AFLP markers were scored as dominant markers. SSR markers generated from the two primer pairs (B10H3 and B6B1) were also scored as dominant markers because they yielded three and five bands, respectively, in denaturing gels. Allelic relationships of these bands and their origin from a single locus were not clear, hence the individual bands were scored as dominant markers (e.g., B6B1A, B6B1B, etc.). Isozyme Mdh-2 was scored in two ways, as a codominant marker and as a dominant marker for each allele to test the accuracy of codominant genotyping.

One linkage map was constructed for each parent using only the single-dose markers. The map of 86–7 consists of 171 markers assigned to 15 linkage groups and covering more than 902 cM of the genome (Fig. 1). The map of 82–1134 consists of 167 markers assigned to 14 linkage groups and covering more than 682 cM of the genome (Fig. 2). Additionally, there are a number of small linkage groups with less than four markers in both maps (data not drawn). These include, one group with two markers and 12 unlinked markers for 86–7, and two linkage groups with three markers, seven groups with two markers, and 15 unlinked markers for 82–1134. These small linkage groups and unlinked markers are a result of small population size coupled with the low map saturation. A gene controlling the prickles on the petiole was located at the end of linkage group 7 on the map of 86–7. No markers are closely linked to this trait. The closest marker (EACAMCAA16) is 17.6 cM away from the gene. A gene for Mdh-2 was located in the middle of linkage group 4 on the map of 86–7. Mdh-2 mapped to

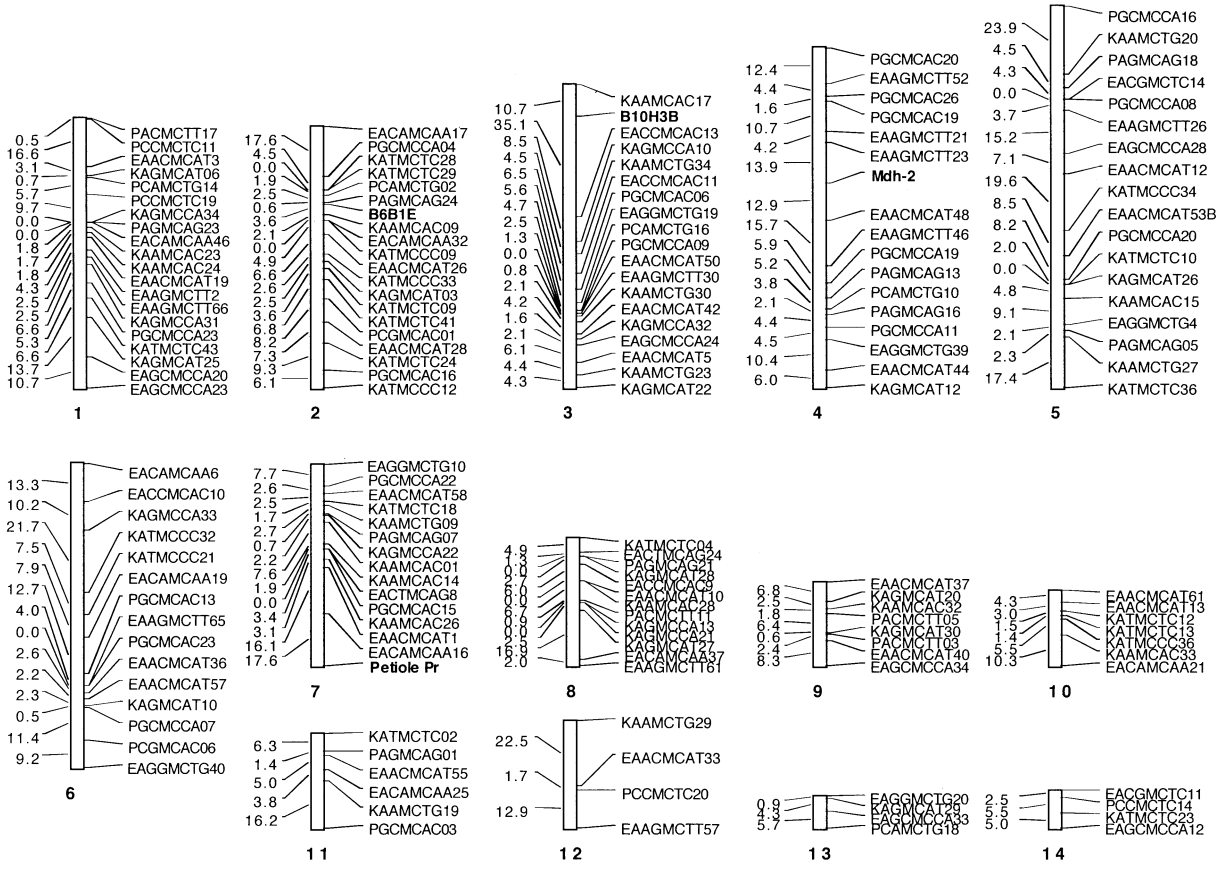

Fig. 1 Linkage map of amphidiploid rose 86–7 constructed with single-dose markers. Linkage groups are numbered arbitrarily. Markers are indicated to the right of the linkage groups and Haldane distances are given to the left of the group. The restriction enzyme used in the AFLP analysis is indicated by the first letter of the marker followed by the selective bases of both primers (MseI was used as the frequent cutter in all AFLP markers). SSR markers are *highlighted*. Linkage groups with less than four markers were not drawn

the same location regardless of the manner in which it was scored (dominant or codominant). The markers flanking Mdh-2 are 13.9 and 12.9 cM away from the gene. Mdh-2, scored as a codominant marker, was not linked to any other marker of 82–1134. Growth habit was not mapped in both maps since no other marker was linked to this trait.

The two maps could not be anchored to each other since no marker is common to both maps. Two SSR markers (B6B1E and B10H3B) were mapped to linkage groups 2 and 3, respectively, in the 86–7 map, and one SSR marker (B6B1C) was placed in linkage group 15 in the 82–1134 map. SSR markers B6B1C and B6B1E are two bands arising from a single SSR primer pair. However, their allelic relationship is not clear at this time. If these two bands are indeed allelic, they would provide anchoring points between the two maps. The SSR markers have the added advantage of being useful in providing anchoring loci between the maps of these related species in the Rosaceae for genome comparisons among the species. However, the two SSR markers developed in peach are not placed on the peach map due to the lack of linkage with other markers (A. G. Abbott, personal communication). Therefore, these two SSRs could not be used to anchor rose and peach maps at this time.

In the AFLP analysis, *Eco*RI/*Mse*I generated nearly twice as many markers per run than *Pst*I/*Mse*I. Markers developed with all three restriction enzyme combinations showed a mixed distribution throughout the maps. In a tomato integrated AFLP-RFLP map, markers developed with *Eco*RI/*Mse*I are reported to be clustered in the centromeric regions of chromosomes while markers generated with *Pst*I/*Mse*I showed a more-even distribution throughout the chromosomes (Haanstra et al. 1999). A fairly mixed distribution of *Eco*RI/*Mse*I and *Pst*I/*Mse*I markers in the rose maps perhaps reflect the low level of saturation. However, compared to *Eco*RI/*Mse*I and *Kpn*I/*Mse*I AFLP markers, *Pst*I/*Mse*I markers had a slightly greater distribution in both rose maps. Overall, *Pst*I/*Mse*I AFLP markers provided the highest percentage of mapped markers.

Based on the published saturated maps (Lister and Dean 1993; Cho et al. 1998; Haanstra et al. 1999) an average of 100–150 cM map distance can be expected per chromosome regardless of the physical length. Applying this to the 14 linkage groups in tetraploid roses, we estimate the map length of the rose genome to be 1400–2100 cM. Therefore, the present genetic maps

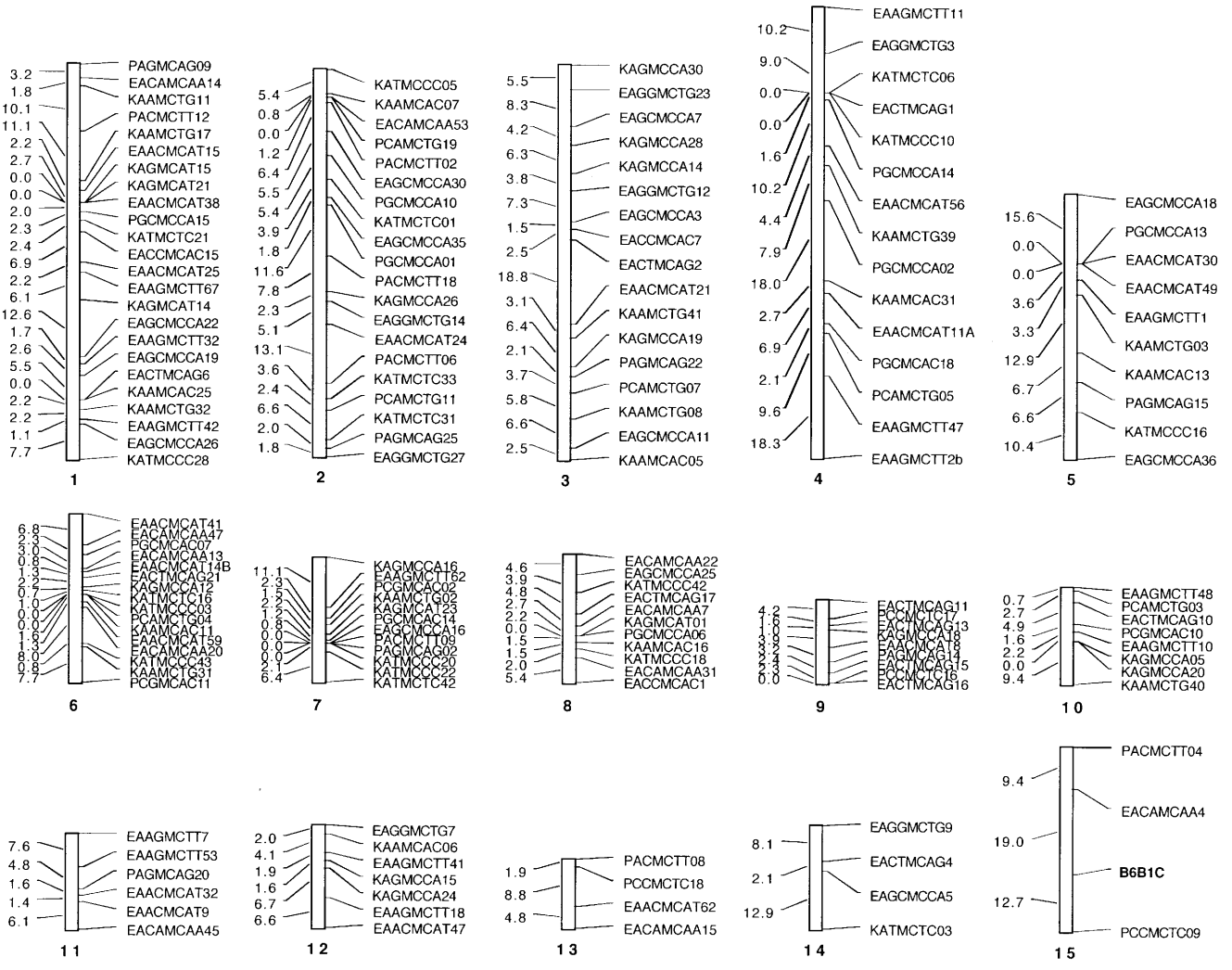

Fig. 2 Linkage map of tetraploid rose 'Basye's Blueberry' (82–1134) constructed with single-dose markers. Linkage groups are numbered arbitrarily. Markers are indicated to the right of the linkage groups and Haldane distances are given to the left of the group. The restriction enzyme used in the AFLP analysis is indicated by the first letter of the marker followed by the selective bases of both primers (MseI was used as the frequent cutter in all AFLP markers). The SSR marker is *highlighted*. Linkage groups with less than four markers were not drawn

may cover less than half of the genomes in each parent. About 200 additional F_2 plants from the same F_1 (90–69) have since been developed and will be included in future segregation analyses to increase the resolution of the maps. The tetraploid nature of the modern roses complicates the genetic analysis of inheritance and map construction. Segregating populations in polyploids provide more genotypes than the diploids. Restricting mapping to the single-dose markers is clearly not very efficient and there is a need for more-efficient statistical algorithms to utilize the large amount of genetic information generated by DNA markers in polyploids. The AFLP technique offers a very efficient and rapid method for map construction. However, in polyploid species it has limited value, especially when used in conjunction with F_2 mapping populations. Some of these disadvantages include the large progeny size required to detect linkages of markers in repulsion. As a result, homolog-specific maps have to be constructed in small progeny sets. The development of codominant SSR, STS or RFLP markers will help identify the corresponding homologs in each map. These first-generation maps provide initial tools for future map saturation and the localization of valuable rose traits. These maps will also serve as the starting points for map-based cloning of important rose traits.

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