J. Amer. Soc. Hort. Sci. 131(3):380–387. 2006.

Microsatellite Marker Development in Rose and its Application in Tetraploid Mapping

L.H. Zhang[1]
Department of Genetics, Biochemistry & Life Science Studies, Clemson University, Clemson, SC 29634

D.H. Byrne[2]
Department of Horticultural Sciences, Texas A&M University, College Station, TX 77843

R.E. Ballard
Department of Biological Sciences, Clemson University, Clemson, SC 29634

S. Rajapakse
Department of Genetics, Biochemistry & Life Science Studies, Clemson University, Clemson, SC 29634

Additional index words. *Rosa wichurana, rugosa,* genetic maps, polyploid, SSR

Abstract. **Microsatellite or simple sequence repeat (SSR) markers were developed from *Rosa wichurana* Crépin to combine two previously constructed tetraploid rose (*Rosa hybrida* L.) genetic maps. To isolate SSR-containing sequences from rose a small-insert genomic library was constructed from diploid *Rosa wichurana* and screened with several SSR probes. Specific primers were designed for 43 unique SSR regions, of which 30 primer pairs gave rise to clear PCR products. Seventeen SSR primer pairs (57%) produced polymorphism in the tetraploid rose 90-69 mapping family. These markers were incorporated into existing maps of the parents 86-7 and 82-1134, which were constructed primarily with AFLP markers. The current map of the male parent, amphidiploid 86-7, consists of 286 markers assigned to 14 linkage groups and covering 770 cM. The map of the female tetraploid parent, 82-1134, consists of 256 markers assigned to 20 linkage groups and covering 920 cM. Nineteen rose SSR loci were mapped on the 86-7 map and 11 on the 82-1134 map. Several homeologous linkage groups within maps were identified based on SSR markers. In addition, some of the SSR markers provided anchoring points between the two parental maps. SSR markers were also useful for joining small linkage groups. Based on shared SSR markers, consensus orders for four rose linkage groups between parental maps were generated. Microsatellite markers developed in this study will provide valuable tools for many aspects of rose research including future consolidation of diploid and tetraploid rose genetic linkage maps, genetic, phylogenetic and population analyses, cultivar identification, and marker-assisted selection.**

Roses are one of the most important ornamental plants in the world today. All roses belong to the genus *Rosa* L. of the Rosaceae. There are about 200 rose species and more than 18,000 commercial rose cultivars (Gudin, 2000). Most of the commercial cultivars are complex tetraploid (x=7) or triploid hybrids derived from 8 to 10 wild diploid and a few tetraploid rose species. Roses have a small genome within the angiosperms, about 600 Mb, (Rajapakse et al., 2001; Yokoya et al., 2000) which is only four times the size of *Arabidopsis thaliana* (L.) Heynh. We have previously constructed two genetic maps for the tetraploid rose family 90-69 based primarily on AFLP markers (Rajapakse et al., 2001). In addition, three diploid families have been mapped in rose (Crespel et al., 2002; Debener and Mattiesch, 1999; Dugo et al., 2005; Yan et al., 2005). There are no molecular markers common to anchor or combine the homolog specific maps of tetraploid 90-69. The development of highly polymorphic, codominant markers would allow us to anchor and combine the two tetraploid maps, as well as to further consolidate tetraploid maps with diploid maps in order to produce a consensus gene map for rose.

Microsatellites or simple sequence repeats are tandem repeats of short DNA motifs of 1–6 bp, scattered in clusters of ≈50–100 bp throughout plant and animal genomes. SSR markers, generated by PCR amplification of the repeat regions using specific sequences flanking the SSRs as primers, are highly polymorphic and robust PCR-based markers that are easily transferable between research groups. SSRs can produce informative codominant markers, especially useful in tracking multiple alleles in polyploid mapping. SSRs provide valuable tools in many facets of genetic analyses including consolidation of genetic linkage maps and germplasm characterization.

From a previous rose genetic mapping study (Rajapakse et al., 2001), only a limited proportion of heterologous SSR markers generated from related genera such as peach [*Prunus persica* (L.) Batsch], apple (*Malus domestica* Borkh.), and sour cherry (*Prunus cerasus* L.) were found to be useful for rose. Of the 21 primer pairs developed in peach, apple and sour cherry, and tested in rose, only 38% of peach, 40% of apple and 33% of sour cherry primers produced distinct PCR products in rose. There were no rose SSRs available for screening at the time. Esselink et al. (2003) reported the development of 24 microsatellite markers using enriched small-insert libraries from rose (*R. hybrida*) These markers have been used to identify hybrid tea cut rose and

Received for publication 23 Aug. 2005. Accepted for publication 12 Feb. 2006. This study was made possible through grants from Fred C. Gloeckner Foundation, the American Rose Society, and the U.S. Dept. of Agriculture/National Research Initiative (1999-3551). The authors would like to thank Clemson Univ. Genomic Institute for allowing access to the facilities for this research and Steve Weitz for helping with SSR analysis.
[1]Current address: IAREC, Washington State Univ., Prosser, WA 99350.
[2]Corresponding author. E-mail: d-byrne@tamu.edu; Tel.:979-862-3072; Fax: 979-845-0627.

rootstock cultivars and recently incorporated into a diploid rose map (Yan et al., 2005).

In the present research, a small-insert genomic library was constructed in rose and screened with several SSR probes. Based on the sequence information from the SSR-positive clones, specific primers were designed to amplify SSR regions. SSR markers were incorporated into the existing tetraploid rose maps. In addition, a few SSRs developed in gene regions of peach and apple were also placed in these maps. SSR markers were used to identify homeologous groups within maps as well as to anchor and combine the two homolog specific parental maps.

Materials and Methods

Construction of a genomic library. A diploid rose, *R. wichurana* 'Basye's Thornless', was used to construct a small-insert rose genomic library. Total genomic DNA was extracted from fresh young leaves (Jarret and Austin, 1994) and purified further by a $CsCl_2$ gradient. DNA samples were quantified using a minifluorometer (Hoefer TK 100; Hoefer Scientific Instruments, San Francisco, Calif.). Rose DNA (1 μg) was digested completely with 5 U *Sau*3A1 (Promega Corp., Madison, Wis.) for 3 h at 37 °C, and then heated at 65 °C for 20 min to inactivate the restriction enzyme. A sample of restriction digestion was electrophoresed on a 1.2% agarose gel in 1X TBE buffer. Most of the digested fragments were observed to be in the range of 200–1000 bp. The remaining digested DNA was cloned into dephosphorylated, *Bam*H1 digested, pUC118 vectors using a 1:1 (vector to insert) molar ratio. Ligation was performed in a 25-μL volume with 3U T4 ligase and 1X ligation buffer (Promega). Ligated plasmids were electro-transformed into *E. coli* JM109 cells, which were then grown overnight at 37 °C on plates with ampicillin, X-gal (5-bromo-4-chloro-3-indolyl-beta-D-galactoside), and IPTG (isopropylthio-beta-D-galactoside). The positive transformants were robot picked (Genetix Q-bot; Genetix Ltd., New Milton, U.K.) and stored in 384-well plates in freezing LB media containing 36 mM K_2HPO_4, 13.2 mM KH_2PO_4, 1.7 mM sodium citrate, 6.8 mM $(NH_4)_2SO_4$, 4 mM $MgSO_4$, 4.4% glycerol, and 50 $\mu L \cdot mL^{-1}$ ampicillin.

Isolation of SSR-containing clones from the library. A total of 24,576 clones in the library were stamped in duplicate onto high-density Hybond nylon filters (Amersham Pharmacia Biotech, Piscataway, N.J.) by the procedure outlined at the Clemson University Genomics Institute (2005) using the Genetix Q-bot robot (Genetix, Boston, Mass). The nylon filters were hybridized separately with 17 different γ^{33}P-ATP end-labeled oligo nucleotide probes containing several repeat motifs (Fig. 1). Southern hybridizations were performed overnight at the optimum annealing temperature for each probe.

SSR primer development. About 250 positive clones identified from screening the library with SSR probes were sequenced to ensure the presence of repeats and to design specific primers to amplify SSR regions. In order to minimize the amount of unsuccessful sequencing, SSR-positive clones were pre-screened for insert length using PCR amplification. Positive clones larger than 100 bp were selected for sequencing either on ABI PRISM 377 sequencer (for clones <300 bp) (Applied Biosystems, Foster City, Calif.) or LI-COR Long Reader 4200 sequencer (for clones >300 bp) (LI-COR, Lincoln, Nebr.). Forward and reverse sequences obtained separately were joined using Sequencher 4.1 (Gene Codes Corp., Ann Arbor, Mich.). To eliminate redundancy, all SSR-containing sequences were checked for duplication and

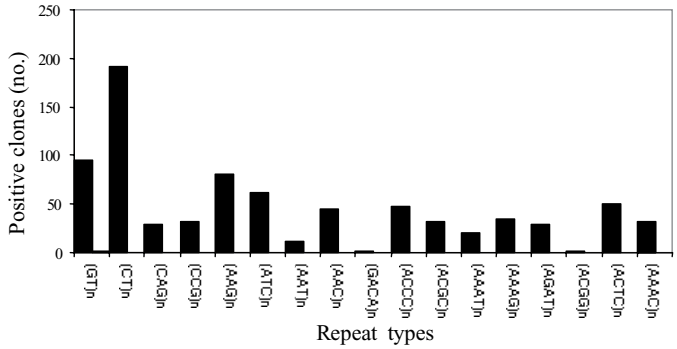

Fig. 1. Number of genomic clones in a small-insert genomic library constructed from diploid *R. wichurana* that were identified as containing simple sequence repeats.

subjected to BLAST search against online Genbank database (Altschul et al., 1997). Primer3 (Rozen and Skaletsky, 2000) was used to design SSR primers. The candidate primer sequences were checked for their GC base pair content, formation of hairpins, and other secondary structures. Forty-three different pairs of primers were designed and ordered from IDT (Integrated DNA Technologies, Coralville, Iowa).

PCR amplification of SSR loci. Primers designed for specific SSR regions were first tested in PCR amplifications using *R. wichurana* DNA, which was used to build the genomic library. If needed, gradient PCR was performed to optimize the annealing temperature to get clear PCR products. PCR was carried out with one primer end-labeled using γ^{33}P-ATP and T4 polynucleotide kinase (Promega) and one unlabeled primer in a total volume of 10 μL using a MasterCycler (Eppendorf, Hamburg, Germany). The basic PCR profile was: 30 cycles of 30 s at 94 °C, 30 s at the optimized annealing temperature, 1 min at 72 °C, and 10 min at 72 °C for final extension. This basic PCR profile was imposed with lowering the annealing temperature in a touchdown process starting at 5 °C higher than the optimized annealing temperature for each primer, then decreasing 0.5 °C for every cycle with total 10 cycles (Don et al., 1991). PCR products were separated on 6% polyacrylamide denaturing gels in 1X TBE at 70 W, and marker bands were detected by autoradiography.

SSR marker analysis in the tetraploid 90-69 mapping progeny. The rose mapping family consisted of 52 selfed seedlings from the F_1 plant 90-69, a hybrid between amphidiploid 86-7 and tetraploid 'Basye's Blueberry' (82-1134). The amphidiploid 86-7 combines the diploid genomes of *R. wichurana* and *R. rugosa* Thunberg. The female parent 82-1134 has *R. carolina* L. and *R. virginiana* Miller in its background (Rajapakse et al., 2001). The same 52 seedlings were screened with SSR primers developed in this study.

In addition to the new rose SSRs developed from this study, eight SSR primers developed from the *evergrowing* (*evg)* gene region in *P. persica* (pchgms10, pchgms11, pchgms12, pchgms13, pchgms14, pchgms36, pchgms40, and pchgms41; Wang et al., 2002), and two SSR primers from the scab [*Venturia inaequalis* (Cooke) Wint.] resistance gene *Vb* in *M. domestica*. (CH02C11 and CH02D11; Liebhard et al., 2002; 2003), were also tested in the 90-69 family. The PCR amplification with these primers followed a touchdown program similar to that used with rose SSR primers. Annealing temperatures given for the original species were used.

Linkage analysis. Markers arising from those SSR primers that produced four distinct bands and showed clear codominant

segregation patterns were scored as codominant markers. Markers of other SSR primers that gave rise to a null allele or less than four alleles were scored as dominant markers. SSR marker data were combined with previously generated AFLP, heterologous SSR, isozyme and phenotypic marker data (Rajapakse et al., 2001). Chi-square (χ^2) tests of goodness-of-fit were performed on segregation data for all markers. Linkage analysis was performed (LOD >3.5) with JoinMap 2.0 (Stam, 1993; Stam and Van Ooijen, 1995) (LOD is the logarithm base 10 of the ratio of the likelihood assuming linkage and the likelihood assuming nonlinkage). First, only the single-dose markers (i.e., the dominant markers that segregated 3:1 or codominant markers that segregated 1:2:1) regardless of disomic or tetrasomic modes of transmission, were grouped and mapped. Then, the order of these single-dose markers served as the fixed order for adding double-dose and distorted markers to the linkage groups including double-dose and distorted AFLP markers that were not used in earlier map construction (Rajapakse et al., 2001).

Results

Abundance of SSRs in the rose genome. The genomic library constructed in this study consisted of 24,576 clones. The average insert size estimated by PCR amplification of a sample of ≈100 clones was 300 bp. This library is, therefore, estimated to cover about 1.2% of the R. wichurana genome (628 Mbp). It was screened with 17 di-, tri-, and tetra-repeat probes to determine the relative abundance of these repeat sequences in the rose genome (Fig. 1). A total of 794 SSR-positive clones were identified with all 17 probes. This is equivalent to 3% of clones in this small-insert genomic library. Assuming even distribution, the average frequency of 17 SSR types combined was one in every ≈18.6 kb in the rose genome.

Among the 794 SSR-positive clones, 286 clones were identified from two types of di-nucleotide repeats, 258 clones from six different tri-nucleotide repeats, and 250 clones from nine different tetra-nucleotide repeats (Fig. 1). Among these 17 repeat base pair motifs tested, the most common was $(CT)_n$. Sequencing of about 250 of SSR-positive clones revealed that many had multiple repeat types. Clones with large inserts >500 bp (base pair) were more suitable for development of sequence tagged sites (STS) and were not used in SSR primer design. A few clones had repeat regions close to the end of the sequence that prohibited primer design. A few SSR-positive clones contained single nucleotide repeats, which were not screened for. Forty-three SSR-positive clones that represent all mono-, di-, tri-, and tetra-nucleotide repeat motifs were selected for primer design. BLAST search of the sequences indicated that one clone (Rw29B1) was a part of a MADS-box gene (MADS box is a highly conserved sequence motif found in a family of transcription factors). It was among the clones selected for primer design and later mapped.

SSR primer development and optimization of annealing temperature. Of the 43 specific SSR primer pairs tested in rose, 30 gave clear, reliable amplification products (Table 1). The remaining primer pairs produced too many products or none at all. Twelve of the 30 rose SSR primers amplified di-nucleotide repeats regions, 10 tri-nucleotide regions, and 1 mono-nucleotide region. Seven SSRs contained multiple simple repeats. Primers were screened with a gradient of annealing temperatures if the original annealing temperature tested did not result in clear PCR products. The annealing temperatures for the 30 SSR primers varied from 45.4–58.1°C (Table 1). A few primer pairs had close annealing temperatures and electrophoretically resolvable products. Therefore, it was possible to multiplex PCR amplification of these primer pairs. All rose SSR primers are identified by the prefix Rw, which stands for *R. wichurana*, followed by the clone number from the genomic library, eg. Rw5D11.

Incorporation of SSR markers into maps of 86-7 and 82-1134. Initially, SSR primers that amplified up to four bands in the tetraploid F_1 90-69 were considered to represent a single locus. Primers that amplified 5–8 bands were assumed to represent two loci, and primers that gave rise to more than eight bands were considered to represent more than two loci. Subsequently, upon mapping marker bands separately, allelic relationships between the bands and the number of loci represented by the SSR primers were revised. Many SSR primers were found to amplify more than one locus per diploid genome set in the 90-69 family (Table 1). Twenty primers amplified a single locus, 5 two loci and 5 more than two loci. Of the 30 SSR primer pairs tested, 17 (57%) primer pairs showed polymorphism between the two parents and segregated in the F_2 mapping progeny. Compared to these, in the previous study, only two out of 21 (10%) heterologous SSRs developed from peach, apple and sour cherry showed polymorphism and segregated in F_2 progeny of 90-69 family (Rajapakse et al., 2001). When SSR primers amplified a single locus, and the allelic composition of F_2 progeny at that locus could be clearly deduced, those markers were scored as codominant markers. When the primers amplified a single locus but the allelic composition of the F_2 progeny at the locus was not clear, or when primers amplified more than one locus, each band was treated separately as a dominant marker. Seventeen SSR primers that gave rise to polymorphism in the 90-69 family generated 31 markers of which 25 were scored dominant and 6 codominant. Of the 31 markers generated from the rose SSR primers, 27 (87%) were single dose markers that segregated 3:1 or 1:2:1 in the F_2 progeny. The other four were distorted; they neither segregated 3:1 (single dose) nor 15:1 (double dose). The percentage of single dose markers in SSRs (87%) was much higher than in AFLP markers (70%; Rajapakse et al., 2001). Eleven rose microsatellite loci derived from 9 primer pairs were located on 8 linkage groups in the 82-1134 map (Fig. 2), and 19 microsatellite loci derived from 16 primer pairs were located on 11 linkage groups in the 86-7 map (Fig. 3). A single SSR marker was not linked to any other marker and hence not mapped.

Of the eight peach microsatellites screened in the 90-69 family in the present study, seven either produced complex band patterns or did not amplify rose DNA. Peach SSR pchgms41, which was closely linked to the *evg* locus (Bielenberg et al., 2003; Wang et al., 2002), amplified three polymorphic bands (identified as A, B and C) representing two loci in the 90-69 rose progeny. Two of these markers, pchgms41C and A, were mapped on the seventh and eighteenth linkage groups of the 82-1134 map, respectively, and are likely alleles of a single locus identified as pchgms41a (Fig. 2). A third marker from this SSR, pchgms41B, was located on the eighth linkage group of the 86-7 map and represents a second locus pchgms41b (Fig. 3). Of the two apple SSR primers, CH02C11 amplified a single locus in rose while CH02D11 gave rise to complex multiple bands. CH02C11 was mapped on the fourth and ninth groups of the 82-1134 map, the seventh and fourteenth groups of the 86-7 map.

The new maps of this tetraploid progeny incorporate the newly developed rose SSR markers, three peach SSR markers from pchgms41, and the apple SSR CH02C11 (Figs. 2 and 3). Double dose and distorted AFLP markers not included in earlier

Table 1. Microsatellite markers developed in rose.

SSR name	Primer base pair sequence (5′-3′)	Repeat type	Product size[z] (bp)	Annealing temp (°C)	Loci amplified in F_1 90-69 (no.)
Rw8B8	GGTAACCAACTTAGCGTTGA ATGGCTGCTTCTCTCCTT	$(TG)_{12}(AG)_{12}$	123	54.0	2
Rw3K19	GCCATCACTAACGCCACTAAA GCGTCGTTCGCTTTGTTT	$(CAA)_6$	418	56.0	1
Rw10J19	GCGAGTTGACGACGAGTT GGGTGGGCTTCCTTAGTTA	$(GAA)_9$	373	55.0	>2
Rw4E22	ATGGGAGACAGAGGTGTAAG TCCTAACTCTCGGTGGAGAT	$(GA)_2(GAA)_5$	183	54.3	>2
Rw10M24	TTAATCCAAGGTCAAAGCTG TCTCTTTCCCTCCTCACTCT	$(CT)_7(TA)_4$	252	53.5	1
Rw12D5	CCCCTATGCTACACCACAA AAGGCTCCAAAGCTTCAC	$(CTTT)_2(CTT)_4$	406	54.9	2
Rw14A5	CCCTCAAAACCCCTCTTA CGTAATAACTGTCCGGTCTC	$(GAA)_4$	124	52.4	>2
Rw1F9	GTTGAAGGTAATAAATAACTGAAG CAAGGGACGGTAATAAAATC	$(ATT)_6$	295	45.4	1
Rw3N19	CTGGCTGGTTCTCTTTCTG ATGGGTCGTCGTCGATATG	$(CT)_{20}$	125	54.3	1
Rw5D11	CAGATTCGCCGTAGCCCTTAC ATCCGAACCCCGACCTGAC	$(CT)_{14}$	254	54.3	1
Rw11E5	GATACCGCGAAGGTGTAGT GAGTGAAAACTCTGCAATCA	$(CT)_{10}$	173	49.3	3
Rw14H21	ATCATGTGCAGTCTCCTGGT AATTGTGGGCTGGAAATATG	$(GT)_{16}(GA)_{15}$	145	54.7	1
Rw17I7	CAGGTAATTTGCGGATGAAG GATCCGCCGTTTCCAGT	$(GCC)_8(ACC)_3$	216	58.1	1
Rw18N19	CCCGAGAAAGAGACAGTAAA ATCGAGAGAGACACCGACTC	$(CTT)_6$	250	54.4	1
Rw22A3	AGAGAATTGAAAAGGGCAAG GAGCAAGCAAGACACTGTAA	$(TTC)_6$	150	52.9	2
Rw22B6	ACAGTGAGTTGTTCGCTTCT TTCATTGCTAGGAAGCAGTA	$(CAT)_7$	158	53.9	2
Rw23H5	AAGCTCTGCCATTGTCCACT GCCCCTCCAAACTTAACCTC	$(CA)_9$	125	57.3	2
Rw27A11B	TGTTCCCTTTTAATGAATTAGC GTTCATCCCTTCAAACCAC	$(AG)_{12}$	313	51.3	1
Rw29B1	GTGGCAAGCTCTATGAGTTC CCCACCTTAAATTAGCTTCA	$(AATT)_4(AT)_4$	352	52.6	1
Rw32D19	GAAGTCCAGAGCCAATTCCA AGGGTCCTCATCCACCACTT	$(GAA)_7$	540	54.0	1
Rw45E24	CAGTTTCATTGCTCGTCTTC TATACATGATTCGGGCCTTC	$(CT)_{45}$	319	50.9	1
Rw46O8	ACATGTGGTGCTGTGTTT GTACAGGCCACTGCTGTC	$(CT)_{10}$	307	50.0	>2
Rw48N6	GAGGGCGATCTTCGTATTCTC GGGGCAATTGAAGGGTTTAG	$(ATA)_9$	272	50.0	1
Rw49N14	AAGCACACAGCCCTATCATC GGCCTTTCTAGGGTTTTCTG	$(GAT)_7$	247	50.2	1
Rw50N23	AATTGGTATATTCTGGTAAGTAG ATAGGAGGGTGATGACTAAC	$(CT)_{10}$	567	50.9	1
Rw55C6	GTGGATTTTCAGAGATACGC TCACAGACAGGACCACCTAT	$(CT)_{11}$	265	50.0	1
Rw55D22	GATCCGTTTAAGTAACCTTT CCACAAGGATTCTGATTTAT	$(G)_{12}$	201	50.9	1
Rw60A16	GCAAATCTCTCAGTAAACCT AAGGTCTTCCCTAATACTCA	$(CT)_{53}$	368	50.9	2
Rw61F2	GTTGGAATTGCAGAGGTGAT AACTAAAGGCAGGCCACTAA	$(GA)_{14}$	250	50.0	1
Rw62C4	GGCAGAGTGCTGTGTTAAGT AGGTGGAGTGTGCATCTAAC	$(CT)_{24}$	345	50.0	1

[z]Product size of *Rosa wichurana*.

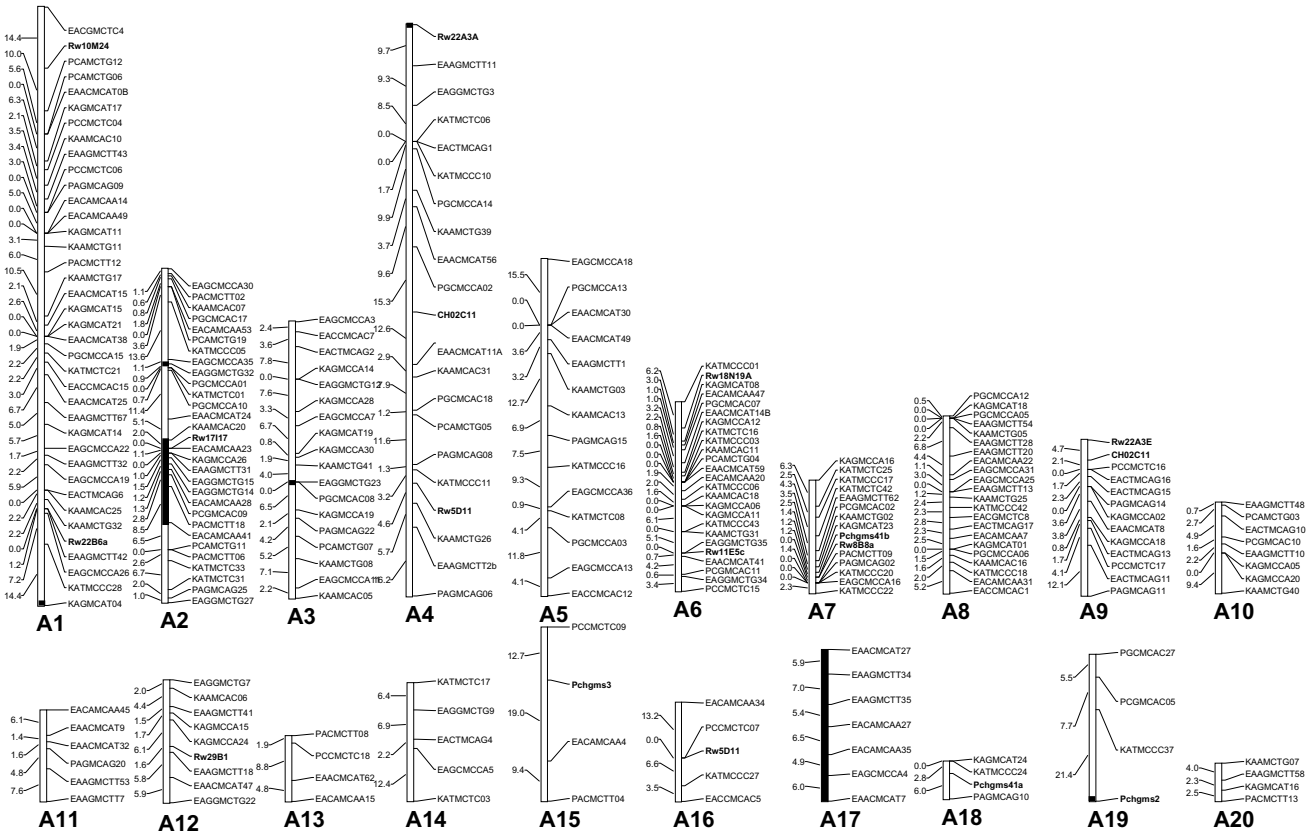

Fig. 2. Linkage map of the tetraploid rose 'Basye's Blueberry' 82-1134 (A-map) after new SSR markers and double-dose AFLP markers not included in the earlier map (Rajapakse et al., 2001) were added. SSR markers are highlighted in bold. Linkage groups with less than four markers are not drawn. Locations of distorted markers along the linkage groups are indicated in black.

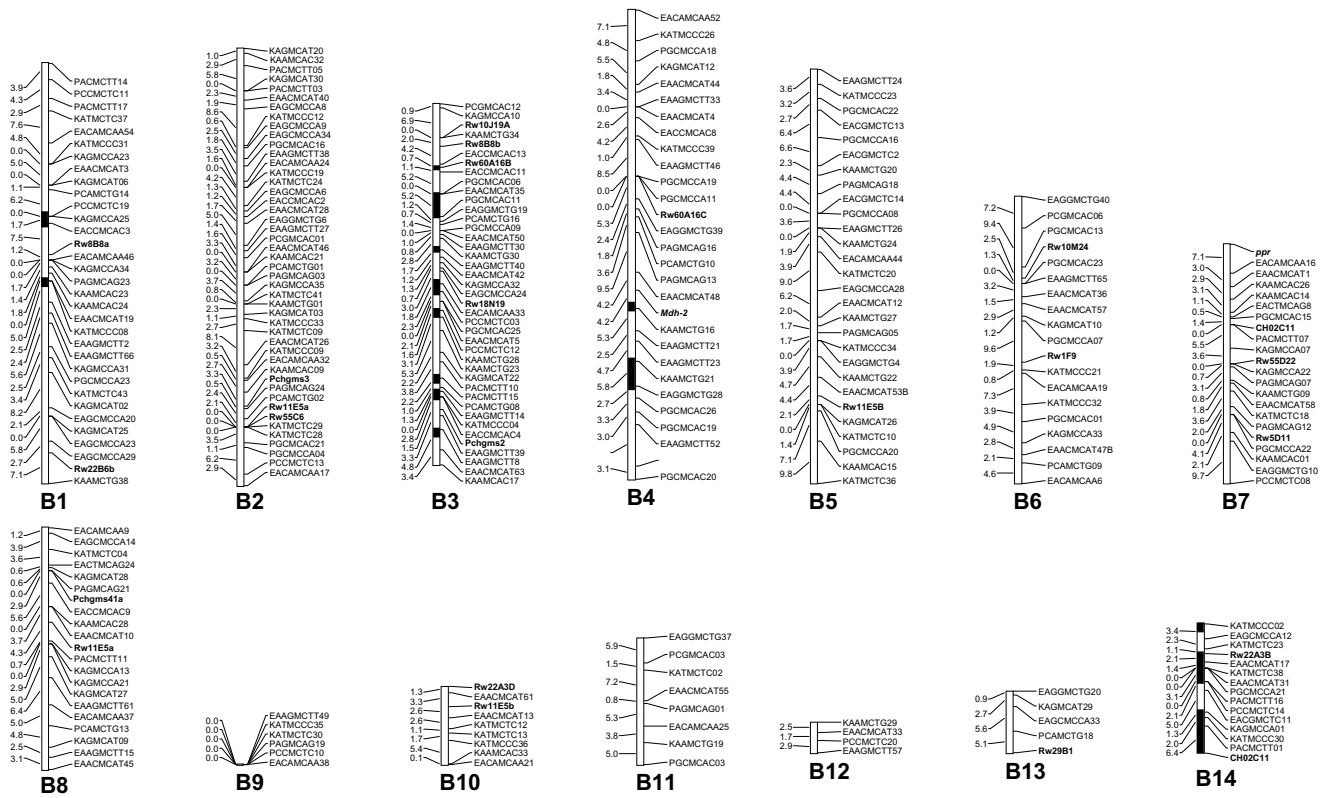

Fig 3. Linkage map of the amphidiploid rose 86-7 (B-map) after new SSR markers and double-dose AFLP markers not included in the earlier map (Rajapakse et al., 2001) were added. SSR, morphological and isozyme markers are highlighted in bold. Linkage groups with less than four markers are not drawn. Locations of distorted markers along the linkage groups are indicated in black.

maps were also used in the linkage analysis this time. Two peach microsatellites (pchgms2 and pchgms3), isozyme *Mdh-2*, and absence of prickles on the petiole (*ppr*) located in previous maps (Rajapakse et al., 2001) were also included in the new maps. The new map of 82-1134 (A-map) consists of 256 markers assigned to 20 linkage groups and covering more than 920 cM. It locates a total of 17 SSR loci (11 from rose) on 11 linkage groups. The new map of 86-7 (B-map) consists of 286 markers assigned to 14 linkage groups and covering more than 770 cM. This map locates a total of 24 SSR loci (19 from rose) on 11 groups, isozyme *Mdh-2* on linkage group 4, and the only morphological marker in the maps, absence of prickles on the petiole (*ppr*) on linkage group 7.

In the two 90-69 maps, 15% of the markers showed segregation distortion. Distorted markers, indicated in black on both tetraploid maps (Figs. 2 and 3), showed clustering on some linkage groups. In the 82-1134 (A) map, distorted markers were mapped to six linkage groups. Among these, A2 showed a cluster of distorted markers in the center whereas for A17 all markers were distorted. For the other four groups, there was only one distorted marker located possibly either on the telomeric or centromeric region on each chromosome. For the 86-7 (B) map, distorted markers were mapped to four groups and showed several clusters or single markers.

Anchoring and combining the two parental maps. One of the main purposes of developing SSR markers was to anchor and combine the two homolog specific tetraploid rose maps. Aiming for this goal, first, several homeologous linkage groups (of diploid genome sets) within each parental map were identified based on common markers or linkages to common markers (Fig. 4A–D). For the A-map of 82-1134, four groups were identified as being homeologous. They are A1 and A7, A4 and A9+A16, A15 and A18, and A6 and A19. A4 has two SSR markers in common with A9 (CH0211 and Rw22A3), and one (Rw5D11) in common with A16 (Fig. 4B). This indicated that A9 and A16 are likely parts of one linkage group. For the B-map, B1 and B6, B10+B7 and B14, B2 and B8, and B3 and B4 are identified as homeologous groups. B14 has CH02C11 in common with B7, and Rw22A3 with B10. Based on these markers, B7 and B10 are combined into one linkage group, which is homeologous with B14. Although some groups did not share markers directly, they could also be identified as being homeologous groups using markers shared with other maps, for example, A6 and A19 (Fig. 4D).

In addition, homologous relationships for linkage groups between A and B maps were established using SSR markers (Fig. 4A–D). Four sets of linkage groups from the two parental sides were recognized. These groups are as follows: A1and A7 vs. B1 and B6, A4 and (A9+A16) vs. (B7+B10) and B14, A15 and A18 vs. B2 and B8, and A6 and A19 vs. B3 and B4. In addition, two small groups A12 and B13 were recognized as homologous groups using Rw29B1, a rose SSR primer set that amplified a MADS box gene region (not drawn). Although some other groups shared the markers generated from the same SSR primer pairs, their relatedness is not clear at this time due to the uncertainty of allelic relationships among the markers.

Based on shared SSR markers, common linkage groups were consolidated and consensus orders for four out of seven rose chromosomes were generated (Fig. 4A–D). The first consensus

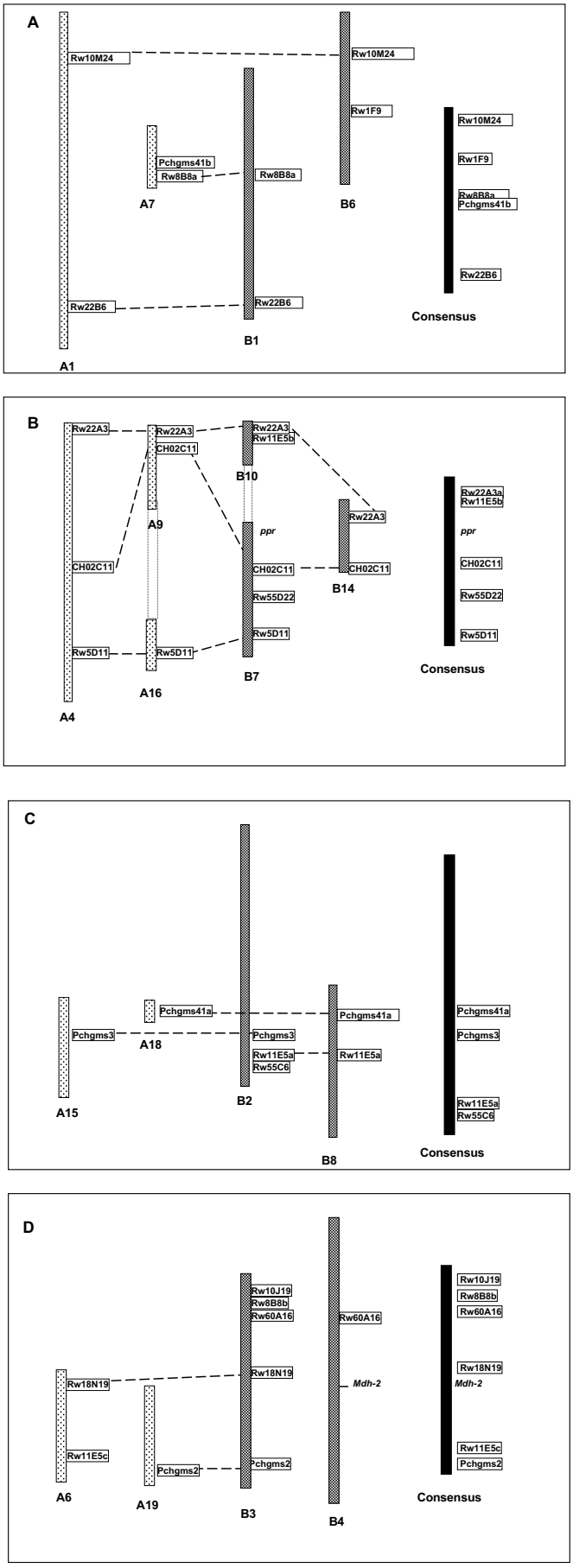

Fig. 4. (*Right*) (**A–D**) Linkage groups of the two parental tetraploid rose (*Rosa hybrida*) maps, 82-1134 (A-map) and 86-7 (B-map) anchored by common SSR markers. AFLP markers were omitted from the groups. Most likely consensus order was constructed for each group. Map distances are not calculated and the consensus map is not drawn to scale.

group (Fig. 4A) consists of rose SSR markers Rw10M24, RwIF9, Rw8B8a and Rw22B6, and peach SSR pchgms41b. Rw10M24 in this group is a typical codominant SSR marker, which has the ability to track all four tetraploid alleles. One locus amplified from peach SSR pchgms41 derived from the *evg* gene region (pchgms41b) also mapped to this group and possibly located very close to Rw8B8a (Fig. 4A). (Note that the other locus pchgms41a amplified by the same peach primer set is located in the third consensus group). The second consensus group (Fig. 4B) consists of rose SSR markers Rw22A3a, Rw11E5b, Rw55D22 and Rw5D11, and apple SSR CH02C11. Rw5D11 is an ideal codominant marker, which could trace all four alleles in tetraploids. The morphological trait prickles in the petiole (*ppr*) is located in this group. The third consensus group of tetraploid maps (Fig. 4C) consists of two rose (Rw11E5a and Rw55C6), and two peach (pchgms41a and pchgms3) markers. The fourth consensus group (Fig. 4D) locates the most SSR markers. They are Rw10J19, Rw8B8b, Rw60A16, Rw 18N19, Rw11E5c and pchgms2. In addition, isoenzyme *Mdh*-2 is also located in this group.

Discussion

The small coverage of the genomic library constructed in this study (1.2%) and possible clustering of microsatellites in some regions of the genome may not permit meaningful conclusions to be drawn on SSR distribution in the entire rose genome. However, assuming the library provides an unbiased representation of the genome and that SSRs are evenly dispersed, this study revealed that SSRs are abundant in the rose genome. In this study, twice as many CT base pair repeats as GT base pair repeats were observed in the rose genome, which is consistent with results from SSR characterization in peach (Georgi et al., 2002; Sosinski et al., 2000). In another rose SSR study, in which the libraries were enriched for SSR development, more SSR clones containing GT base pairs than CT base pairs were obtained (Esselink et al., 2003). Differences in SSR types and frequencies have been found between genomic regions relating to differing evolutionary constraints (Li et al., 2004).

In this study, 30 out of 43 new microsatellite primer pairs developed from *R. wichurana* successfully amplified DNA of the tetraploid mapping population, which has a diverse genetic background. The female parent of the cross, 82-1134, has *R. carolina* and *R. virginiana* in its background while the male parent, amphidiploid 86-7, has the background of *R. wichurana* and *R. rugosa* (Rajapakse et al., 2001). This demonstrates conservation of many of the microsatellite regions among rose species. Conserved microsatellite regions found between the parental roses in this study were used to consolidate homologous linkage groups and develop consensus maps for four out of seven rose chromosomes. Furthermore, conservation of linkages of some of the markers observed in these tetraploid maps have also been observed in a diploid *R. multiflora* Thunb. 97/7 rose map produced in our lab (manuscript in preparation). For example, three out of four rose SSR markers in the first consensus group of the present study are linked in the diploid *R. multiflora* 97/7 map as well. They are Rw10M24, RwIF9, and Rw 8B8a. Linkage between two markers in the second consensus group (i.e., Rw22A3 and CH02C11, has also been observed in the diploid *R. multiflora* 97/7 map). Pchgms41a and Rw11E5, mapped to the third consensus group of the present study are linked to each other in our diploid *R. multiflora* 97/7 map as well. A gene controlling double corolla (*Dc*) and another controlling repeat blooming ability (*rb*) are linked to the latter two markers in the diploid maps. Anchoring with maps locating such different morphological traits will enable to cross utilize marker information between maps.

Map distances for the consensus groups of the present map are not calculated with this initial group of common SSR markers since the homeologous linkage groups originating from different species may not be of the same size. As more SSRs are developed from rose and mapped in different species, eventually it will be possible to determine the distances in the consensus maps and identify chromosomal regions shared by the species as well regions of chromosomal rearrangements among the rose species. A second set of SSR markers is now being developed from the additional SSR-positive clones selected from the genomic library for this purpose. Some SSRs that represented multiple loci mapped to different linkage groups showing regions of possible internal duplications within the rose genome. For example loci from Rw11E5 mapped to two consensus groups.

Peach microsatellite primer pchgms41 amplifies a single locus in peach and two loci in diploid rose. Markers such as this can be used to identify regions of gene duplication between peach and rose and thus aid in the comparative mapping of the Rosaceae. Two peach SSR markers, pchgms2 and pchgms3, mapped in tetraploid rose maps, allowed anchoring with the *Prunus* L. reference map (Aranzana et al., 2003). The *Prunus* reference map was developed using an almond × peach F_2 progeny. Pchgms2, located in G4 of the *Prunus* reference map, mapped to the fourth consensus set in rose (Fig. 4D). Pchgms3, located in G1 of the *Prunus* map, mapped to third consensus set in rose (Fig. 4C). From our experience, pchgms2 appears to be a quite useful marker for comparative mapping in the Rosaceae, as it has been found to amplify DNA of a wide range of *Prunus* species, strawberry (*Fragaria* ×*ananassa* Duchesne ex Rozier) and rose.

A long-term goal of this rose research is to understand the genes controlling important traits in rose, and use this knowledge to assist selection of breeding progeny in rose. Five SSR markers (Rw22A3, Rw11E5b, CH02C11, Rw55D22, and Rw5D11) are located in the same linkage group in the map of 86-7 with the morphological trait, prickle on the petiole. These SSR markers can serve as reference points towards developing markers tightly linked to the gene for marker-assisted selection in the future.

In a preliminary study, the usefulness of the developed microsatellite markers in germplasm characterization was tested with a group of rose species and cultivars. Results of this germplasm study are not included here and will be presented separately. In that study, 28 out of 30 SSR primer pairs (>90%) displayed polymorphism among rose species and cultivars tested. As expected, most SSRs exhibited a high level of variation among the roses, and allowed an easy differentiation of all rose cultivars tested. Thus, microsatellite markers developed in this study will be useful for a wide range of applications in rose genetics including map comparison and consolidation, germplasm characterization, population analyses, and creating a marker database for rose cultivar identification. In fact, some of these SSR markers have already been used to test the phylogenetic relationships between oil bearing *Rosa damascena* Mill. accessions (Rusanov et al, 2005).

Literature Cited

Altschul, S.F., T.L. Madden, A.A. Schaffer, J. Zhang, Z. Zhang, W. Miller, and D.J. Lipman. 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Res. 25:3389–3402.

Aranzana, M., A. Pineda, P. Cosson, E. Dirlewanger, J. Ascasibar, G.

Cipriani, C. Ryder, R. Testolin, A. Abbott, G. King, A. Iezzoni, and P. Arús. 2003. A set of simple-sequence repeat (SSR) markers covering the *Prunus* genome. Theor. Appl. Genet. 106:819–825.

Bielenberg, D., Y. Wang, G. Reighard, R. Scorza, and A. Abbott. 2003. Sequencing and the analysis of the peach Evg locus. HortScience 38:738 (Abstr.).

Clemson University Genomics Institute. 2005. CUGI protocols. 19 Aug. 2005 <http://www.genome.clemson.edu/protocols>.

Crespel, L., M. Chirollet, C.E. Durel, D. Zhang, J. Meynet, and S. Gudin. 2002. Mapping of qualitative and quantitative phenotypic traits in *Rosa* using AFLP markers. Theor. Appl. Genet. 105:1207–1214.

Debener, T. and L. Mattiesch. 1999. Construction of a genetic linkage map of roses using RAPD and AFLP markers. Theor. Appl. Genet. 99:891–899.

Don, R.H., P.T. Cox, B.J. Wainwright, K. Baker, and J.S. Mattick. 1991. Touchdown PCR to circumvent spurious priming during gene amplification. Nucleic Acids Res. 19:4008.

Dugo, M.L., Z. Satovic, T. Milan, J.I. Cubero, D. Rubiales, A. Cabrera, and A. M. Torres. 2005. Genetic mapping of QTLs controlling horticultural traits in diploid roses. Theor. Appl. Genet. 111:511–520**.**

Esselink, G.D., M.J.M. Smulders, and B. Vosman. 2003. Identification of cut rose (*Rosa hybrida*) and rootstock varieties using robust sequence tagged microsatellite site markers. Theor. Appl. Genet. 106:277–286.

Georgi, L.L., Y. Wang, D. Yvergniaux, T. Ormsbee, M. Iñigo, G. Reighard, and A.G. Abbott. 2002. Construction of a BAC library and its application to the identification of simple sequence repeats in peach [*Prunus persica* (L.) Batsch]. Theor. Appl. Genet. 105:1151–1158.

Gudin, S. 2000. Rose: Genetics and breeding. Plant Breeding Rev. 17:159–189.

Jarret, R.L. and D. Austin. 1994. Genetic diversity and systemic relationships in sweetpotato [*Ipomoea batatas* (L.) Lam.] and related species as revealed by RAPD analysis. Genetic Resources Crop Evol. 41:165–173.

Li, Y-C., A.B. Korol, T. Fahima, and E. Nevo. 2004. Microsatellites within genes: Structure, function and evolution. Mol. Biol. Evol. 21:991–1007.

Liebhard, R., L. Gianfranceschi, B. Koller, C. D. Ryder, R. Tarchini, E. Van de Weg, and C. Gessler. 2002. Development and characterisation of 140 new microsatellites in apple (*Malus domestica* Borkh.). Mol. Breeding 10:217–241.

Liebhard, R., B. Koller, L. Gianfranceschi, and C. Gessler. 2003. Creating a saturated reference map for the apple (*Malus domestica* Borkh.) genome. Theor. Appl. Genet. 106:1497–1508.

Rajapakse, S., D.H. Byrne, L. Zhang, N. Anderson, K. Arumuganathan, and R.E. Ballard. 2001. Two genetic linkage maps of tetraploid roses. Theor. Appl. Genet. 103:575–583.

Rozen, S. and H.J. Skaletsky. 2000. Primer3 on the WWW for general users and for biologist programmers, p. 365–386. In: S. Krawetz and S. Misener (eds.). Bioinformatics methods and protocols: Methods in molecular biology. Humana Press, Totowa, NJ.

Rusanov, K., N. Kovacheva, B. Vosman, L. Zhang, S. Rajapakse, A. Atanassov, and I. Atanassov. 2005. Microsatellite analysis of Rosa damascena Mill. accessions reveals genetic similarity between genotypes used for rose oil production and old damask rose varieties. Theor Appl Genet. 111:804–809.

Sosinski, B., M. Gannavarapu, L.D. Hager, L.E. Beck, G.J. King, C.D. Ryder, S. Rajapakse, W.V. Baird, R.E. Ballard, and A.G. Abbott. 2000. Characterization of microsatellite markers in peach (*Prunus persica* (L.) Batsch). Theor. Appl. Genet. 101:421–428.

Stam, P. 1993. Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. Plant J. 3:739–744.

Stam, P. and J.W. Van Ooijen. 1995. JoinMap version 2.0: Software for calculation of genetic linkage maps. CPRO-DLO, Centre for Plant Breeding and Reproduction Research, Wageningen, The Netherlands.

Wang, Y., L.L. Georgi, T.N. Zhebentyayeva, G.L. Reighard, R. Scorza, and A.G. Abbott. 2002. High-throughput targeted SSR marker development in peach [*Prunus persica* (L.) Batsch]. Genome 45:319–328.

Yan, Z., C. Denneboom, A. Hattendorf, O. Dolstra, T. Debener, P. Stam, and P.B. Visser. 2005. Construction of an integrated map of rose with AFLP, SSR, PK, RGA, RFLP, SCAR and morphological markers. Theor. Appl. Genet. 110:766–777.

Yokoya, K., A.V. Roberts, J. Mottley, R. Lewis, and P. Brandham. 2000. Nuclear DNA amounts in roses. Ann. Bot. 85:557–561.