Theor Appl Genet (2000) 101:421–428

B. Sosinski · M. Gannavarapu · L.D. Hager
L.E. Beck · G.J. King · C.D. Ryder · S. Rajapakse
W.V. Baird · R.E. Ballard · A.G. Abbott

Characterization of microsatellite markers in peach [*Prunus persica* (L.) Batsch]

Received: 18 June 1999 / Accepted: 6 December 1999

Abstract Microsatellites have emerged as an important system of molecular markers. We evaluated the potential of microsatellites for use in genetic studies of peach [*Prunus persica* (L.) Batsch]. Microsatellite loci in peach were identified by screening a pUC8 genomic library, a λZAPII leaf cDNA library, as well as through database searches. Primer sequences for the microsatellite loci were tested from the related Rosaceae species apple (*Malus×domestica*) and sour cherry (*Prunus cerasus* L.). The genomic library was screened for CT, CA and AGG repeats, while the cDNA library was screened for $(CT)_n$- and $(CA)_n$-containing clones. Estimates of microsatellite frequencies were determined from the genomic library screening, and indicate that CT repeats occur every 100 kb, CA repeats every 420 kb, and AGG repeats every 700 kb in the peach genome. Microsatellite-containing clones were sequenced, and specific PCR primers were designed to amplify the microsatellite-containing regions from genomic DNA. The level of microsatellite polymorphism was evaluated among 28 scion peach cultivars which displayed one to four alleles per primer pair. Five microsatellites were found to segregate in intraspecific peach-mapping crosses. In addition, these microsatellite markers were tested for their utility in cross-species amplification for use in comparative mapping both within the Rosaceae, and with the unrelated species *Arabidopsis thaliana* L.

Key words Rosaceae · Peach · *Prunus* · Microsatellite · Simple sequence repeats

Communicated by M.A.Saghai Maroof

B. Sosinski · M. Gannavarapu · L.E. Beck · S. Rajapakse
R.E. Ballard · A.G. Abbott (✉)
Department of Biological Sciences, Clemson University,
Clemson SC 29634, USA
e-mail: aalbert@clemson.edu
Fax: 864-656-0435

W.V. Baird
Department of Horticulture, Clemson University,
Clemson SC 29634, USA

L.D. Hager
Department of Genetics, University of Georgia,
Athens GA 30602, USA

C.D. Ryder
Department of Plant Genetics and Biotechnology,
Horticulture Research International, Wellesbourne, Warwick,
CV35 9EF, UK

Present address:
B. Sosinski, Department of Plant Pathology,
North Carolina State University, Raleigh NC 27695, USA

Introduction

Peach is a member of the Rosaceae, which ranks as the third most agronomically important plant family in temperate regions and includes such economically important species as apple, apricot, plum, sweet and sour cherry, almond, strawberry, and rose. Unlike many of the other family members, peach is a self-pollinated diploid (2n=16), and has a small genome size of approximately 5.9×10^8 base pairs/diploid nucleus (Baird et al. 1994), about twice the size of the genome of *Arabidopsis thaliana* L. (Arumuganathan and Earle 1991). These factors, combined with the fact that peach has a relatively short juvenile stage (3–4years) for a perennial tree species, make peach the ideal genetic system for the identification and characterization of important genes in Rosaceae species.

Thorough genetic studies, including cultivar identification and linkage mapping, require the use of cost-effective DNA markers that tag distinct, evenly spaced genomic intervals. Restriction fragment length polymorphisms (RFLPs) were utilized successfully towards this end within the Rosaceae (Nybom and Schall 1990; Eldredge et al. 1992; Foolad et al. 1995), but they are costly and time-consuming to produce. The emergence of polymerase chain reaction (PCR)-based random amplified polymorphic DNA (RAPD) markers allows the rapid and cost-effective identification of polymorphic markers (Hemmat et al. 1994; Lu et al. 1996). Due to their reduced transportability between peach populations

(Rajapakse et al. 1995), and because of their usually dominant mode of inheritance, RAPD markers are generally unsuitable for comparative mapping studies.

Two other PCR-based marker systems are used for the identification of polymorphic DNA markers. Amplified fragment length polymorphism (AFLP) analysis (Vos et al. 1995) produces as many as 30 polymorphisms per primer pair in peach, and has been used to construct linkage maps (Lu et al. 1998, Dirlewanger et al. 1999). Although AFLP analysis is a powerful, cost-effective method for identifying DNA polymorphism, AFLP markers are generally dominant, requiring conversion to sequence tagged sites (STSs) for application in comparative mapping studies and for practical use in marker-assisted selection (Lu et al. 1999). On the other hand, microsatellite (simple sequence repeat) markers are PCR-based and exhibit codominant inheritance. In plants, these markers have been used widely for cultivar identification and genetic mapping (Bell and Becker 1994; Guilford et al. 1997; Cipriani et al. 1999), and are a powerful genetic resource for phylogenetic studies (Provan et al. 1996). Therefore, microsatellite markers are suitable for comparative genetic studies, and can facilitate the integration of genetic maps both within the Rosaceae and across wider taxonomic boundaries.

In this study, we show that microsatellite markers are highly polymorphic, abundant, and transportable between peach cultivars and crosses. Furthermore, we demonstrate that the microsatellites developed in Rosaceae species are useful for cross-species amplification and may have utility in both intra- and inter-family comparative mapping analyses.

Materials and methods

Plant material

Genomic DNA was isolated from fresh young peach leaves using the CTAB protocol described by Eldredge et al. (1992). DNA from the following 28 peach scion cultivars was used for this study: 'Anita', 'Babygold 5', 'Babygold 7', 'Babygold 8', 'Babygold 9', 'Belle', 'Bicentennial', 'Biscoe', 'Boone County', 'Cresthaven', 'Empress', 'Flordadawn', 'Flordaking', 'Goldcrest', 'Jayhaven', 'Junegold', 'Majestic', 'Maycrest', 'Maygold', 'Queencrest', 'Rio Oso Gem', 'Springbrite', 'Springcrest' (CA, GA, and SC sources), 'Springold', 'Sunbrite', and 'Sweethaven'. In addition, 48 F_2 individuals from the cross 'New Jersey Pillar'×KV77119 (USDA), 48 F_2 individuals from the cross 'Bailey'×'Suncrest', and 55 F_2 individuals from the cross Lovell×Nemared (rootstocks) were screened for linkage analysis whenever polymorphism was detected between the parents. For analysis of cross-species amplification, genomic DNA was obtained from the following: *A. thaliana* 'Columbia'; apricot cultivar 'Goldrich', from Dr. Maria L. Badenes, Instituto Valenciano de Investigaciones Agrarias, Valencia, Spain; sour cherry cultivars 'Erdi Botermo' and 'Rheinische Schattenmorelle', from Dr. Amy Iezzoni, Michigan State University, USA; and rose breeding lines (TAMU) 86–7 (amphidiploid), 82–1134 (tetraploid), and 90–69 (F_1 from the cross 86–7×82–1134).

Library construction

A peach genomic library was made from *Eco*RI-digested peach DNA from the cultivar 'Bicentennial' cloned into the plasmid pUC8 (Eldredge et al. 1992). Duplicate filters of 1056 unique clones were produced by stamping the colonies onto a Hybond N+ (Amersham International) nylon membrane, and processed according to the manufacturer's instructions. The filters were then hybridized with microsatellite probes (see Library Screening section, below). Hybridization-positive plasmids were isolated by the alkaline-lysis method (Sambrook et al. 1989) from 5-ml LB-ampicillin cultures grown overnight with shaking at 37°C. When the cloned insert size was greater than 1 kb, the insert was further digested with an additional 4- or 6-base cutter. The restriction fragments were then separated by agarose-gel electrophoresis, and blotted onto Hybond N+ nylon membranes by the alkaline-transfer method described by the manufacturer. This membrane was screened with the original microsatellite probe, and the microsatellite-containing fragment was then electroeluted from a subsequent gel and subcloned by ligation into either pUC118 or pBluescript.

The cDNA library was constructed by isolating mRNA from young leaves of the cultivar 'Suncrest' using a lithium-chloride method (Sambrook et al. 1989) and subsequent purification of the polyA+ fraction with streptavidin beads (Promega). The *Eco*RI-adapted cDNA was made using the cDNA Synthesis System (Pharmacia), and was then cloned into λZAPII (Stratagene) as specified by the manufacturer. Approximately 1000 phages were plated, and duplicate filters of the library were lifted, processed, and alkaline-fixed using standard methods. The filters were then hybridized with microsatellite probes (see Library Screening section, below). Hybridization-positive clones were subjected to three rounds of phage purification, and the phagemids were excised using ExAssist (Stratagene). The resulting clones were grown and purified in the same fashion as the genomic clones.

Library screening

All prepared library filters were separately screened with a $(CA)_{10}$ and a $(CT)_{10}$ oligonucleotide probe, and the genomic filters were also screened with an $(AGG)_{10}$ probe. The probes were end-labeled with either γ^{32}P-ATP or γ^{33}P-ATP, using T4 polynucleotide kinase (Promega). Hybridizations of cDNA library filters were carried out at 52°C with 5 ml of hybridization buffer [7% sodium dodecyl sulfate (SDS), 0.25 M sodium phosphate buffer pH 7.4], after a 2-h pre-hybridization. The filters were rinsed three times with 2×SSC (0.3 M NaCl, 0.03 M sodium citrate) and once with 0.5×SSC, for 20 min each time, at 52°C. Filter replicas of the genomic clones in pUC8 were hybridized at 50°C [$(CT)_{10}$ probe] or 55°C [$(AGG)_{10}$ and $(CA)_{10}$ probes] and washed four times with 2×SSC and once in 0.5×SSC for 15 min each time, at the same temperature as the hybridization. The filters were then exposed to Kodak X-Omat AR X-ray film for 2–5 days at –80°C, and the microsatellite-containing clones were identified by observation of a hybridization signal for the same clone on both of the duplicate filters.

Microsatellite clone sequencing and primer design

Microsatellite-containing clones were sequenced using the Dye Terminator Sequencing kit (PE Biosystems) and an ABI 373 Stretch sequencer. The sequencing reactions were primed with either M13 universal primers (pUC clones) or T3 and T7 promoter primers (pBluescript/phagemid). An additional microsatellite was identified by searching GenBank and EMBL for *Prunus* sequences that contain microsatellite repeats. The peach endo-beta-1,4-glucanase gene (GenBank accession #X96856) was found to contain a $(CT)_{21}$ repeat, hereafter referred to as pchgms4. For the detection of microsatellite loci, primers (Integrated DNA Technologies) were designed for regions upstream of and downstream from

the repeat region that had an approximately 60% GC content, to yield an expected amplicon of about 200 bp. Microsatellite markers isolated from the genomic library are designated "pchgmsX", while those isolated from the cDNA library are designated "pchcmsX."

PCR amplification and visualization of microsatellites

Peach microsatellites were PCR-amplified as follows: 1×PCR buffer (10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$), 200 µM of each dNTP, 0.12 µM of each primer, 0.5 U of DNA polymerase [either Ampli*Taq* Gold (PE Biosystems) for "hot-start", or FisherBiotech*Taq*], and 4 ng of peach genomic DNA in a 10-µl final volume. Prior to amplification, 0.8 pg per reaction of one arbitrarily chosen primer was end-labeled with γ^{33}P-ATP using T4 polynucleotide kinase. PCR reactions were performed in an MJR PTC-200 DNA Engine by an initial denaturation for 4 min at 94°C followed by 32 cycles of: 45 s at 94°C, 30 s at 50–60°C, 30 s at 72°C; and a final extension of 5 min at 72°C. The PCR products were denatured by the addition of 1 vol of 80% formamide/dye solution, and heating for 3 min at 94°C. They were then visualized by electrophoresis for 2.5 h at 70 W on a 6% polyacrylamide, 42% urea sequencing gel in 1×TBE (90 mM Tris, 90 mM boric acid, 2 mM EDTA). After electrophoresis, the gel was dried and exposed to X-ray film for 1–4 days.

Linkage and statistical analysis for polymorphic microsatellites

Whenever a microsatellite was found to be polymorphic between any of the parents of the crosses under study, an attempt was made to assign linkage. Markers were first subjected to a chi-square test to determine goodness of fit for an F_2 model. Linkage analysis was performed using MapMaker v2.0 for Macintosh (Lander et al. 1987) with a critical LOD of 3.0 and a theta of 0.4. Marker distances were calculated using Kosambi's mapping function. The heterozygosity of the microsatellite loci was determined by H=1 – (total frequency for each of the homozygotes detected in the peach cultivars).

Results

Frequency and polymorphism level of microsatellites in peach

The frequency of microsatellites in the peach genome was initially estimated by repeat-probe hybridization of 1056 unique plasmid genomic clones having an average insert size of 2 kb. While the $(AGG)_{10}$ probe hybridized with only 0.28% of the clones, the $(CA)_{10}$ probe hybridized with 0.47% of the clones, and the $(CT)_{10}$ probe hybridized with 1.98% of the clones, corresponding to three, five and 21 hybridization-positive clones respectively. Similar results were obtained when the cDNA library was screened with the dinucleotide probes. In this case the $(CA)_{10}$ probe hybridized with ten clones (or 1.0% of the clones screened), while the $(CT)_{10}$ probe hybridized to 22 clones (or 2.2% of the clones screened). From the genomic-library screening, using an average insert size of 2 kb, it was estimated that CT repeats occur every 100 kb, CA repeats occur every 420 kb, and AGG repeats occur every 700 kb in the peach genome.

Screening of 1000 peach-leaf cDNAs yielded 10 and 22 positive clones for $(CA)_{10}$ and $(CT)_{10}$, respectively. This indicates that CT repeats are approximately twice as abundant as CA repeats in peach-leaf transcripts.

From these plasmid and cDNA library screenings, clones were chosen for microsatellite development. In the case of plasmid genomic clones, hybridization-positive clones were characterized for their insert size. Those less than 1 kb were sequenced directly, while a subset of those with greater than 1 kb inserts were digested with either *Sau*-3A1 or one of several six-base-cutting restriction enzymes. The resultant fragments were subcloned to obtain a microsatellite containing insert smaller than 1 kb for sequencing. In the case of the cDNA library screenings, the resultant positive clones were sequenced directly without further subcloning. All microsatellites from peach were developed in this manner, except for pchgms4 which was derived from a sequence database search.

All microsatellite loci amplified from the 28 peach cultivars displayed between one and four putative alleles (one or two alleles/cultivar) at the expected size for each locus (Fig. 1). Heterozygosity values ranged from of 0.21 to 0.56, with an average value of 0.45 (Table 1). Of the five genomic clones studied, only pchgms5, which contains a $(CA)_9$ repeat, failed to reveal any poly-

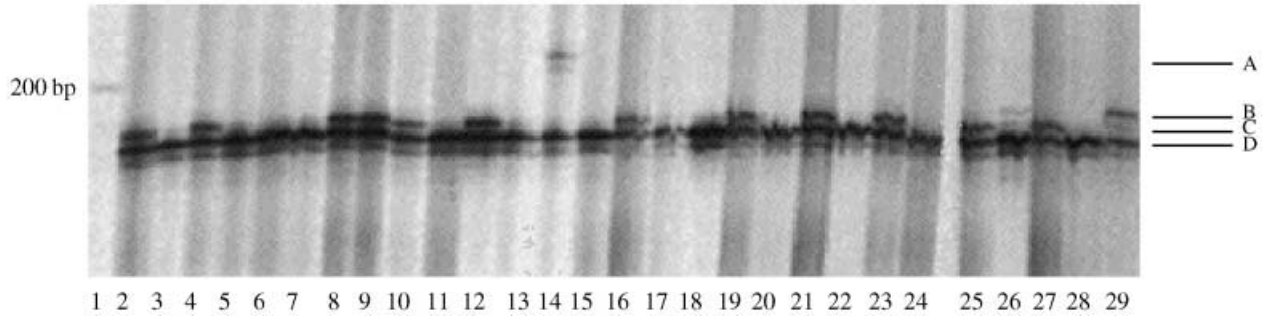

Fig. 1 Microsatellite polymorphism in peach cultivars and rootstocks, amplified by the primer set pchgms1. Allele designations are provided on the right side of the figure (**A**–**D**). *Lane 1* 100 bp marker with the 200 bp band seen. *Lanes 2–29* refer to the cultivars in the following order: 'Spring Crest (GA)', 'Sunbrite', 'Springold', 'May Gold', 'Spring Brite', 'Anita', 'Spring Crest (CA)', 'Queen Crest', 'May Crest', 'Flordadawn', 'Empress', 'June Gold', 'Flordaking', 'Baby Gold 5', 'Spring Crest (CA)', 'Biscoe', 'Cresthaven', 'Bicentennial', 'Gold Crest', 'Jay Haven', 'Rio Oso Gem', 'Belle', 'Sweet Haven', 'Majestic', 'Baby Gold 9', 'Baby Gold 8', 'Baby Gold 7' and 'Boone County'

Table 1 Polymorphic microsatellite markers identified in this study

Marker	Primer sequences	Repeat type	Predicted length[a]	No. of alleles	Heterozygosity
pchgms1	GGG TAA ATA TGC CCA TTG TGC AAT C GGA TCA TTG AAC TAC GTC AAT CCT C	$(AC)_{12}(AT)_6$	194	4	0.54
pchgms2	GTC AAT GAG TTC AGT GTC TAC ACT C AAT CAT AAC ATC ATT CAG CCA CTG C	$(CT)_{24}$	163	2	0.49
pchgms3	ACG GTA TGT CCG TAC ACT CTC CAT G CAA CCT GTG ATT GCT CCT ATT AAA C	$(CT)_{14}$	179	3	0.33
pchgms4	ATC TTC ACA ACC CTA ATG TC GTT GAG GCA AAA GAC TTC AAT	$(CT)_{21}$	174	3	0.49
pchgms5	CCA GTA GAT TTC AAC GTC ATC TAC A GGT TCA CTC TCA CAT ACA CTC GGA G	$(CA)_9$	160	1	Not polymorphic
pchcms1	GTT ACA CCT CTG TCA CA CTT GGC TGG CAT TCC TA	$(CA)_9(TA)_{14}$ $(GA)_{13}$	166	4	0.52
pchcms2	AGG GTC GTC TCT TTG AC CTT CGT TTC AAG GCC TG	$(CA)_8$	180	3	0.49
pchcms3	CTG CAG AAC ACT ACT GA GCT TTG CAA CCA CCA GC	$(CT)_{19}$	220	2	0.21
pchcms4	CTC ACG CTA TTT CTC GG CCT CGA CGA AGA GCT CG	$(CA)_9$	225	1	Not polymorphic
pchcms5	CGC CCA TGA CAA ACT TA GTC AAG AGG TAC ACC AG	$(CA)_9(TA)_8$	246	3	0.56

[a] The predicted length was determined from the sequencing results for the isolated clones. Therefore the lengths for pchgms1, pchgms2, pchgms3, and pchgms4 are based upon the allele found in 'Bicentennial', while the remaining lengths were determined from the 'Suncrest' alleles

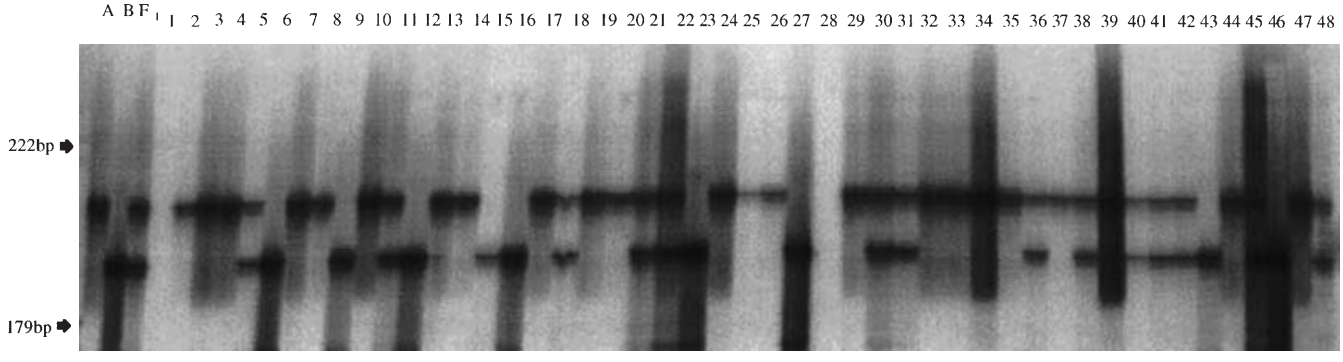

Fig. 2 Segregation of microsatellite pchgms1 in 'New Jersey Pillar'×KV7119. From left to right: *A* 'New Jersey Pillar'; *B* KV7119, F_1; *1–48* F_2 progeny of selfed F_1

morphism in the material tested. Likewise, only one cDNA-derived microsatellite, pchcms4, which contains a $(CA)_{10}$ repeat, was monomorphic in all of the tested material. These results indicate that microsatellites identified in this study have an overall polymorphism level of 80% in peach germplasm, in contrast to a level of approximately 25% for RFLP probes (Eldredge et al. 1992). When the polymorphic microsatellite markers were used for DNA fingerprinting, they successfully discriminated between the 28 scion cultivars (data not shown); however, this required the use of all eight polymorphic markers thus far identified.

Microsatellite polymorphism in peach mapping populations

All of the polymorphic microsatellites of genomic origin were also polymorphic between the parents of the intraspecific peach crosses under study. In contrast, in these mapping crosses, microsatellite loci derived from cDNA sequences (pchcms designation) were not polymorphic. Markers pchgms3 and pchgms2 were polymorphic in 'New Jersey Pillar'×KV7119, but failed to map to any existing linkage groups. Similarly, marker pchgms5 segregated in 'Bailey'×'Suncrest', but could not be assigned to any previously determined linkage group. Marker pchgms1, on the other hand, was found to be informative in both Lovell×Nemared and 'New Jersey Pillar'×KV7119 (Fig. 2). In the Lovell×Nemared family this marker (formerly denoted B3D5) was mapped to linkage group 1, which contains important rootstock characters for resistance to the root-knot nematode, *Meloidogyne* sp (Lu et al. 1998). In the 'New

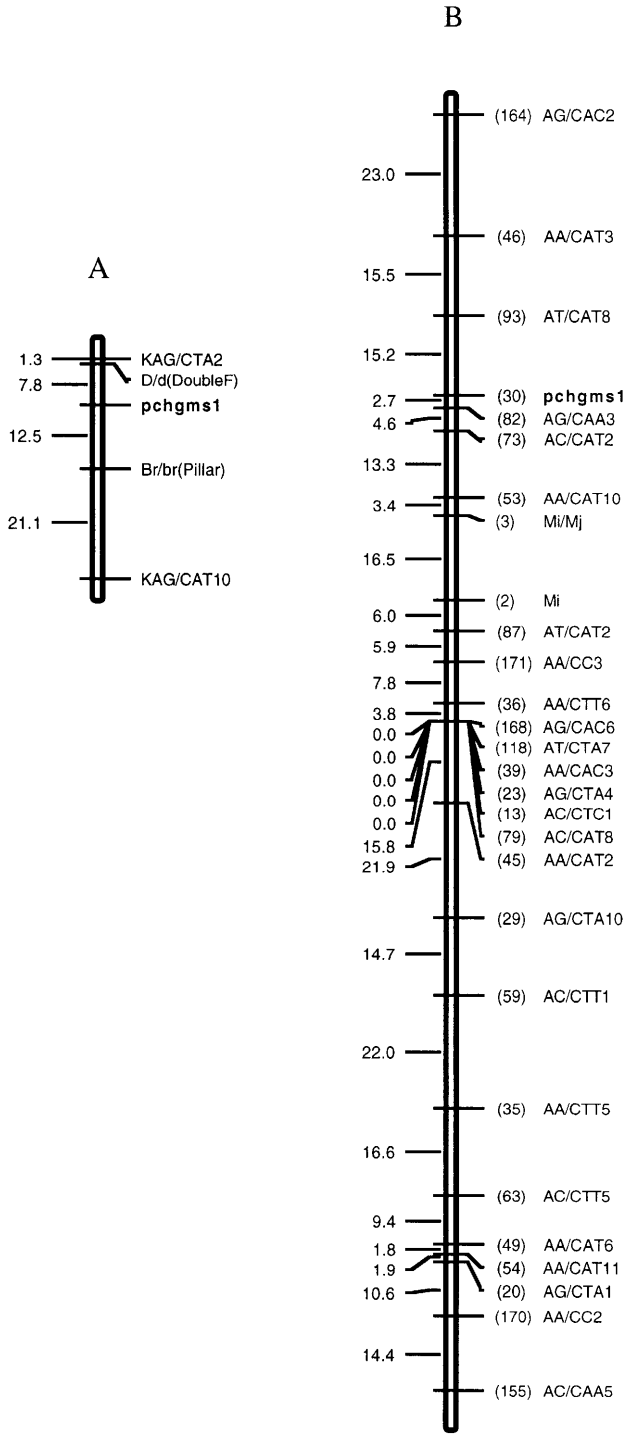

Fig. 3A, B Linkage groups showing position of microsatellite pchgms1 on: **A** Linkage group 1 from 'New Jersey Pillar'× KV7119. **B** Linkage group 1 from Lovell×Nemared replacing the former designation of locus B3D5 (Lu et al. 1998) with pchgms1. Other markers depicted are phenotypic markers: D/d double flower; Br/br pillar; Mi/Mj resistance to the nematodes, *M. incognita* and *M. javanica*; Mi resistance to *M. incognita;* or AFLP markers

Table 2 Cross-species amplification using peach microsatellite primers

Marker	Apricot	Sour Cherry	Rose	*A. thaliana*
pchgms1	+[a]	+	–[c]	+
pchgms2	+	+	+	+
pchcms1	+	+	+	–
pchcms2	+	+	+	+
pchcms3	+	*[b]	*	+
pchcms4	+	*	*	+
pchcms5	+	*	*	+

[a] + indicates amplification of a single product of the appropriate length
[b] * indicates amplification displaying a complex banding pattern
[c] – no amplification

Jersey Pillar'×KV7119 family, pchgms1 was assigned to linkage group 1. This group contains two important morphological characters: (1) *Double Flower* (*Dl*), which appears to control organ number in all whorls (Abbott, unpublished data), and (2) *Pillar* (*Pi*), which gives the tree an upright morphology due to acute branch angle (Scorza et al. 1989). Marker pchgms1 was assigned a position between these two characters at a distance of 7.8 cM from *Double Flower* and 12.5 cM from *Pillar*. To-date, this is the only locus identified between these two morphological characters in our linkage analysis (Fig. 3). This marker has also been identified as an anchor locus for merging the two linkage groups in a combined map of three individual maps (Sosinski et al. 1998).

Microsatellites in rosaceae and cross-species amplification

In addition to the microsatellites identified in peach, microsatellite primers were obtained from apple and sour cherry (see Table 3). These primers were tested in peach, apricot (*Prunus armeniaca* L.), and the unrelated species *A. thaliana.* Seven peach microsatellite primers (pchgms1, pchgms2, and pchcms1–5) were tested to evaluate their effectiveness for cross-species amplification. All of these primer sets successfully produced an amplified product of the expected length in apricot, all sets except pchcms1 produced a product in *A. thaliana*, and four sets produced a product in rose and/or sour cherry (Table 2; see example in Fig. 4). For the five apple primer sets: 01a6, 02b1 and 26c6 amplified an appropriate-sized product in peach (60%); 01a6, 02b1, 23f1 and 26c6 produced an amplicon in apricot (80%), but showed either a complex banding pattern or a product of unexpected size; while in *A. thaliana*, 02b1 and 26c6 were the only sets capable of amplification (40%) (Table 3). For the five cherry microsatellites, all of the primer sets tested produced an amplified product in peach and apricot. However, PS05C03 and PS12A02 did not work for *A. thaliana*, giving a success rate of 80% (Table 3).

Table 3 Primers used for cross-species microsatellite amplification, and their success for amplification

Marker	Sequences 5′-3′	Source	Peach	Apricot	*A. thaliana*
01a6	Guilford et al. 1997	Apple	+[a]	++	–[b]
02b1	Guilford et al. 1997	Apple	+	++	+
23f1	Guilford et al. 1997	Apple	–	++	–
23g4	Guilford et al. 1997	Apple	–	–	–
26c6	Guilford et al. 1997	Apple	+	++	+
PS12A02	GCC ACC AAT GGT TCT TCC AGC ACC AGA TGC ACC TGA	Cherry	+	+	-
PS01H03	TGA GGA GCA TAA TGA CAG T TCA CCA TGT GTC ATA CT	Cherry	+	+	+
PS05C03	AGA TCT CAA AGA AGC TGA AGC TTA TGC ATA TAC CTG	Cherry	+	+	–
PS07A02	CAG GGA AAT AGA TAA GAT G TCT AAT GGT GGT GTT CAT T	Cherry	+	+	+
PS08E08	CCC AAT GAA CAA CTG CAT CAT ATC AAT CAC TGG GAT G	Cherry	+	+	+

[a] + indicates amplification and intensity of amplified product
[b] – indicates no detectable amplification

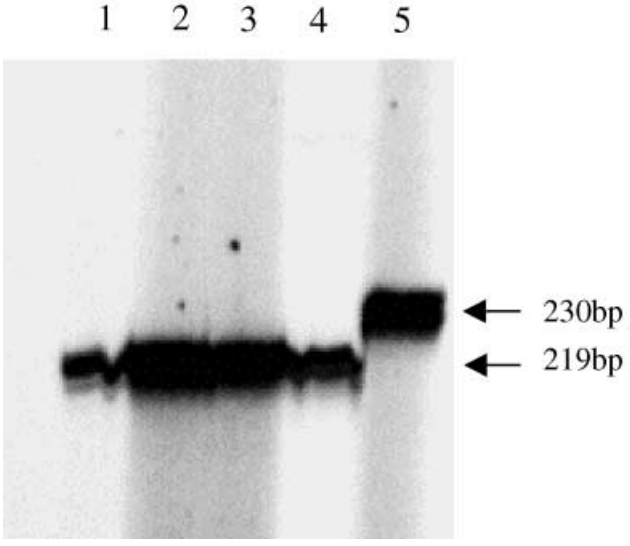

Fig. 4 Cross-species amplification using peach microsatellite primer set pchcms4. *Lane 1* 'Suncrest', lane 2 'Bailey', lane 3 F_1 of 'Bailey×'Suncrest', lane 4 *A. thaliana* ecotype 'Columbia', lane 5 apricot cultivar 'Goldrich'. Sizes of products are indicated at the right

Discussion

From this study, we see that microsatellites occur in the peach genome at frequencies comparable to those seen in other plant species. CT repeats occur at least once in every 100 kb in peach, compared to once in every 120 kb in apple (Guilford et al. 1997) and every 225 kb in rice (Wu and Tanksley 1993). In concurrence with previous observations, CA repeats were less frequent, occurring once in every 420 kb in peach. In apple and rice, CA repeats occur every 190 kb and 480 kb respectively. Of the microsatellite motifs we have evaluated in peach, the AGG repeat motif was found to be the least common, occurring once in every 700 kb. Low frequencies of occurrence of trinucleotide repeats have also been reported by other researchers; in apple trinucleotide repeats occur every 3 Mb (Guilford et al. 1997), while in wheat trinucleotides are as much as ten-times less frequent than dinucleotide repeats (Ma et al. 1996). The number of alleles observed for microsatellites in peach appeared to be relatively low (one to four) when compared to other species such as apple (one to nine) (Guilford et al. 1997). However, the polymorphism level in peach germplasm is still quite satisfactory (average heterozygosity=0.45) for most genetic studies. Our estimates of peach microsatellite heterozygosity and general utility agree well with those of Cipriani et al. (1999).

Since the markers generated from microsatellite sequences identify significant levels of polymorphism, are highly transportable, and occur in reasonable abundance, it is evident that microsatellites have significant potential for genetic mapping, map merging, and cultivar identification in peach. In the case of mapping, with this small set of microsatellites examined, we were able to map one locus in two different maps. Other loci, although polymorphic, did not locate on known linkage groups. Both maps are not completely saturated (manuscripts in preparation) and increased placement of markers in these maps would be expected to incorporate these microsatellites. In regard to cultivar identification, it is interesting to note that analysis of three different sources of the cultivar 'Springcrest' revealed polymorphisms among them. It is more likely that this represents misidentifications of trees in germplasm orchards than actual clonal variation; however, this result emphasizes the power of microsatellite analysis for cultivar identification.

In recent years, comparative chromosomal and subchromosomal genome studies of key crop species in several important families have been reported. These include: the Poaceae (Whitkus et al. 1992; Ahn and Tanksley 1993; Devos et al. 1993; Grivet et al. 1994; Huang and Kochert 1994; Kurata et al. 1994; Lin et al. 1995; van Deynze et al. 1995; Dufour et al. 1996; Saghai Maroof et al. 1996); the Brassicaceae (Kowalski et al. 1994; Bohuon et al. 1996; Lagercrantz and Lydiate 1996; Lagercrantz et al. 1996); and the Solanaceae (Tanksley et al. 1992). Researchers working on Rosaceae species (including peach, apple, sour cherry, apricot, almond,

strawberry and rose) have initiated a comparative study within the family with the goal of creating a unified map for the Rosaceae (Joobeur et al. 1998). Due to its diploid nature, economic importance, and small genome size, peach is being explored as a model species within the Rosaceae for comparative mapping and for the positional cloning of important genes.

We have shown that the microsatellite primers developed in peach have high utility for cross-species amplification both within the Rosaceae and in the model dicot plant *A. thaliana*. This latter point is particularly significant, since the *A. thaliana* genome is currently being sequenced, and the ability to identify syntenic genomic regions between it and the genomes of species within the Rosaceae could potentially aid in the identification and characterization of important genes in the Rosaceae. Furthermore, we have observed that the microsatellites developed in other species of Rosaceae, in this case apple and cherry, may be useful for synteny and cloning studies with peach. In the light of these results, it is likely that microsatellites will prove to be the marker of choice for comparative studies within the Rosaceae, including the study of the collinearity of genomes, and in some cases for similar studies with unrelated species such as *A. thaliana*.

Acknowledgements We thank Dr. Ralph Scorza for generously supplying peach leaf material for this study, and Dr. Anne Westman for her editorial contributions. This research was partially supported by a grant from the USDA, National Research Initiatives Competitive Grants Program (NRICGP) 93–37300–8818. The reported experiments comply with the current laws of the United States of America (USA).

References

Ahn S, Tanksley SD (1993) Comparative linkage maps of the rice and maize genomes. Proc Natl Acad Sci USA 90:7980–7984

Arumuganathan K, Earle E (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9:208–218

Baird WV, Estager AS, Wells JK (1994) Estimating nuclear DNA content in peach and related diploid species using laser flow cytometry and DNA hybridization. J Am Soc Hort Sci 119: 1312–1316

Bell CL, Becker JR (1994) Assignment of 30 microsatellite loci to the linkage map of *Arabidopsis*. Genomics 19:137–144

Bohuon EJ, Keith DJ, Parkin IAP, Sharpe AG, Lydiate DJ (1996) Alignment of the conserved C genomes of *Brassica oleracea* and *Brassica napus*. Theor Appl Genet 93:833–839

Cipriani G, Lot G, Huang WG, Marrazzo MT, Peterlunger E, Testolin R (1999) AC/GT and AG/CT microsatellite repeats in peach [*Prunus persica* (L) Batsch]: isolation, characterisation and cross-species amplification in *Prunus*. Theor Appl Genet 99:65–72

Devos KM, Milan T, Gale MD (1993) Comparative RFLP maps of the homeologous group-2 chromosomes of wheat, rye and barley. Theor Appl Genet 85:784–792

Deynze AE van, Nelson JC, O'Donoughue LS, Ahn SN, Siripoonwiwat W, Harrington SE, Yglesias ES, Braga DP, McCouch SR, Sorrells ME (1995) Comparative mapping in grasses. Oat relationships. Mol Gen Genet 249:349–356

Dufour P, Grivet L, D'Hont A, Deu M, Trouche G, Glaszmann JC, Hamon P (1996) Comparative genetic mapping between duplicated segments on maize chromosomes 3 and 8 and homeologous regions in sorghum and sugarcane. Theor Appl Genet 92:1024–1030

Dirlewanger E, Moing A, Rothan C, Svanella L, Pronier V, Guye A, Plomion C, Monet R (1999) Mapping QTLs controlling fruit quality in peach [*Prunus persica* (L) Batsch]. Theor Appl Genet 98:18–31

Eldredge L, Ballard R, Baird WV, Abbott A, Morgens P, Callahan A, Scorza R, Monet R (1992) Application of RFLP analysis to genetic mapping in peaches. HortScience 27:160–163

Foolad MR, Arulsekar S, Becerra V, Bliss FA (1995) A genetic linkage map of *Prunus* based on an interspecific cross between peach and almond. Theor Appl Genetics 91:262–269

Grivet L, D'Hont A, Dufour P, Hamon P, Roques D, Glaszmann JC (1994) Comparative genome mapping of sugar cane with other species within the Andropogoneae tribe. Heredity 73:500–508

Guilford P, Prakash S, Zhu JM, Rikkerink E, Gardiner S, Bassett H, Forster R (1997) Microsatellites in Malus×Domestica (apple): abundance, polymorphism and cultivar identification. Theor Appl Genet 94:249–254

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

Huang H, Kochert G (1994) Comparative RFLP mapping of an allotetraploid wild rice species (*Oryza latifolia*) and cultivated rice (*O. sativa*). Plant Mol Biol 25:633–648

Joobeur T, Viruel MA, de Vicente MC, Jauregui B, Ballester J, Dettori MT, Verde I, Truco MJ, Messeguer R, Batelle I, Quarta R, Dirlwanger E, Arus P (1998) Construction of a saturated linkage map for *Prunus* using an almond×peach F_2 progeny. Theor Appl Genet 97:1034–1041

Kowalski SP, Lan TH, Feldmann KA, Paterson AH (1994) Comparative mapping of *Arabidopsis thaliana* and *Brassica oleracea* chromosomes reveals islands of conserved organization. Genetics 138:499–510

Kurata N, Moore G, Nagamura Y, Foote T, Yano M, Minobe Y, Gale M (1994) Conservation of genome structure between rice and wheat. Bio/Technology 12:276–278

Lagercrantz U, Lydiate DJ (1996) Comparative genome mapping in *Brassica*. Genetics 144:1903–1910

Lagercrantz U, Putterill J, Coupland G, Lydiate D (1996) Comparative mapping in *Arabidopsis* and *Brassica*, fine scale genome collinearity and congruence of genes controlling flowering time. Plant J 9:13–20

Lander E, Green P, Abrahamson J, Barlow A, Daly M, Lincoln S, Newberg L (1987) MAPMAKER: an interactive computer program for constructing primary linkage maps of experimental and natural populations. Genomics 1:174–181

Lin YR, Schertz KF, Paterson AH (1995) Comparative analysis of QTLs affecting plant height and maturity across the Poaceae, with reference to an interspecific sorghum population. Genetics 141:391–411

Lu ZX, Reighard GL, Baird WV, Rajapakse S, Abbott AG (1996) Identification of peach rootstock cultivars by RAPD markers. HortScience 31:127–129

Lu ZX, Sosinski B, Reighard GL, Baird WV, Abbott AG (1998) Construction of a genetic linkage map and identification of AFLP markers for resistance to root-knot nematodes in peach rootstocks. Genome 41:199–207

Lu ZX, Sossey-Alaoui K, Reighard GL, Baird WV, Abbott AG (1999) Development and characterization of a codominant marker linked to root-knot nematode resistance, and its application to peach rootstock breeding. Theor Appl Genet 99:115–122

Ma ZQ, Roder M, Sorrells ME (1996) Frequencies and sequence characteristics of di-, tri-, and tetra-nucleotide microsatellites in wheat. Genome 39:123–130

Nybom H, Schall BA (1990) DNA 'fingerprints' applied to paternity analysis in apples (Malus×domestica). Theor Appl Genet 79:763–768

Provan J, Powell W, Waugh R (1996) Microsatellite analysis of relationships within cultivated potato (*Solanum tuberosum*). Theor Appl Genet 92:1078–1084

Rajapakse, S, Belthoff LE, He G, Estager AE, Scorza R, Verde I, Ballard RE, Baird WV, Callahan A, Monet R, Abbott AG (1995) Genetic linkage mapping in peach using morphological, RFLP and RAPD markers. Theor Appl Genet 90:503–510

Saghai Maroof MA, Yang GP, Biyashev RM, Maughan PJ, Zhang Q (1996) Analysis of the barley and rice genomes by comparative RFLP linkage mapping. Theor Appl Genet 92:541–551

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Scorza R, Lightner GW, Liverani A (1989) The pillar tree and growth habit analysis of compact×pillar progeny. J Am Soc Hort Sci 114:991–995

Sosinski B, Lu ZX, Tabb A, Sossey-Alaoui K, Rajapakse S, Glassmoyer K, Scorza R, Reighard G, Ballard RE, Baird WV, Abbott AG (1998) Use of AFLP and RFLP markers to create a combined linkage map in peach [*Prunus persica* (L.) Batsch] for use in marker assisted selection. Acta Hort 465:61–68

Tanksley SD, Ganal MW, Prince JP, de Vicente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB (1992) High density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141–1160

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

Whitkus, R, Doebley J, Lee M (1992) Comparative genome mapping of sorghum and maize. Genetics 132:1119–1130

Wu KS, Tanksley SD (1993) Abundance, polymorphism and genetic mapping of microsatellites in rice. Mol Gen Genet 241: 225–235