

Genetic linkage maps of rose constructed with new microsatellite markers and locating QTL controlling flowering traits

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Abstract New microsatellites markers [simple sequence repeat (SSR)] have been isolated from rose and integrated into an existing amplified fragment-length polymorphism genetic map. This new map was used to identify quantitative trait locus (QTL) controlling date of flowering and number of petals. From a rose bud expressed sequence tag (EST) database of 2,556 unigenes and a rose genomic library, 44 EST-SSRs and 20 genomic-SSR markers were developed, respectively. These new rose SSRs were used to expand genetic maps of the rose interspecific F_1 progeny. In addition, SSRs from other *Rosaceae* genera were also tested in the mapping progeny. Genetic maps for the two parents of the progeny were constructed using pseudo-testcross mapping strategy. The maps consist of seven linkage

groups of 105 markers covering 432 cM for the maternal map and 136 markers covering 438 cM for the paternal map. Homologous relationships among linkage groups between the maternal and paternal maps were established using SSR markers. Loci controlling flowering traits were localised on genetic maps as a major gene and QTL for the number of petals and a QTL for the blooming date. New SSR markers developed in this study will provide tools for the establishment of a consensus linkage map for roses that combine traits and markers in various rose genetic maps.

Keywords Genetic map · Flowering traits · Rosa

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Introduction

Rose is one of the most commonly cultivated ornamental plants in the world. Rose production, encompassing cut flower, garden and potted plants, is increasing worldwide. Most rapidly expanding among these is the cut rose production, which has grown from 5,370 ha in 1994 (Gudin 2000) to about 8,500 ha in 2003 (Blom and Tsujita 2003). Despite its economical value, little is known about the inheritance of phenotypic traits in the genus *Rosa*, to which all roses belong. Nevertheless, this genus presents a wide range of phenotypic variability and a high level of genetic heterozygosity. The genetic basis of cultivated roses is narrow in comparison with the vast spectrum of genetic resources existing in the wild (Wylie 1954). Although there are more than 140 *Rosa* species (Wisseman 2003), it is believed that only 11 of them have contributed to the origin of modern cultivars (Gudin 2000).

Genus *Rosa*, along with *Malus*, *Prunus*, *Fragaria* and *Rubus*, is a member of the family *Rosaceae*. The basic chromosome number (x) of *Rosa* is 7, and 2C DNA

amount of diploid roses varies from 0.78 to 1.29 pg (Yokoya et al. 2000).

Rose breeding is a long process, and a large number of phenotypic traits are observed in full-grown plants. The ability of rose breeders to select interesting parents for promising crosses and to identify progenies with favourable combinations of characters is still empirical and hampered by the limited knowledge of the genetic basis of important traits such as flowering, pathogen resistance, fragrance and plant architecture. Thus, the development of genetic maps locating genes controlling phenotypic characters can improve the speed and precision of rose-breeding programs by marker-assisted progeny selection.

Genetic maps have been previously constructed in diploid (Debener 1999; Debener and Mattiesch 1999; Crespel et al. 2002; Dugo et al. 2005) and tetraploid (Rajapakse et al. 2001) roses. These maps were constructed with dominant amplified fragment-length polymorphism (AFLP) and randomly amplified polymorphic DNA markers. Yan et al. (2005) constructed a genetic map in a diploid rose family with simple sequence repeat (SSR) markers developed by Esselink et al. (2003). Recently, the tetraploid rose maps have been expanded with a new set of SSR markers (Zhang et al. 2006).

Several major rose traits have been located on rose genetic maps. These include flower color and double corolla (Debener and Mattiesch 1999), prickles on the petiole (Rajapakse et al. 2001), single seasonal blooming and double corolla (Crespel et al. 2002), black spot resistance (Linde et al. 2004; Malek et al. 2000), and quantitative traits prickles on the stem (Crespel et al. 2002), leaf size, days to flowering and resistance to powdery mildew (Dugo et al. 2005).

In an attempt to make marker technology widely available for rose, easily transferable, highly polymorphic, codominant markers such as SSRs must be developed. SSRs have been detected in higher plants and are widely distributed throughout the plant genomes (Morgante and Olivieri 1993), in contrast to AFLP markers that are preferentially localised in centromeric regions and in clusters (Vuylsteke et al. 1999). SSR frequency in plants has been assessed by oligonucleotide hybridization, and the presence of SSR was detected every 8–60 kb of the genome (Panaud et al. 1996; Echt and May-Marquardt 1997). Genomic SSR markers have been previously developed in rose (Esselink et al. 2003; Yan et al. 2005; Zhang et al. 2006). SSRs appear to be abundant in rose, with an estimated density of one microsatellite present every ~19 kb (Zhang et al. 2006).

SSR markers have been shown to be highly polymorphic, a property that would permit their application as molecular markers in rose population genetics, *Rosaceae* systematics and genome mapping. SSR markers have been used to estimate genetic variation between hybrid tea and rootstock rose varieties (Esselink et al. 2003), to understand reproduction in dog roses (Nybom et al. 2004) and to test

the phylogenetic relationships between oil-bearing *Rosa damascena* Mill. accessions (Rusanov et al. 2005).

In the present study, we developed two sets of new rose microsatellites, one from a *Rosa wichuraiana* Crépin genomic library (Zhang et al. 2006) and the other from expressed sequence tags (ESTs) generated from cDNA libraries of rose vegetative and floral buds (Foucher et al., submitted). Latter SSRs represent the first report of SSR isolated from EST in rose. Both kinds of SSRs were used to expand the AFLP genetic map previously reported (Crespel et al. 2002) and to obtain a comprehensive genetic linkage map of the diploid rose genome. Using codominant SSR markers, we were able to identify homologous linkage groups (LGs) between the two parental maps. Furthermore, using the new maps, we investigated the genetic control of number of petals (NP) and blooming date (BD) in the diploid progeny.

Materials and methods

Mapping population and DNA isolation

The F₁ mapping population used in this study has been previously used to develop an AFLP genetic map (Crespel et al. 2002). This interspecific population of 91 diploids was generated from a cross between dihaploid rose, H190, obtained from haploidisation of tetraploid *Rosa hybrida* cv Zambra (Meynet et al. 1994) and a hybrid of diploid *R. wichuraiana*, *Rw*, originating from Jardin de Bagatelle (Paris, France). H190 and *Rw* were chosen as parents because they differ in important horticultural traits; for instance, H190 is a recurrent blooming, double flowered, prickle-free bush susceptible to black spot and powdery mildew, whereas *Rw* is single seasonal blooming, single flowered, pricked, and ground cover partially resistant to black spot and powdery mildew. Moreover, H190 has 20–25 petals (a mean of 23 petals for five flowers counted) and flowers later than *Rw* in the field. The latter two traits, NP and BD, were scored in the 91 hybrids grown on their own roots and cultivated outdoors in pots, at INRA in Angers, France.

‘Félicité et Perpétue’ and ‘New Dawn’ cultivars used in the measurement of the nuclear genome size were obtained from the Nursery Loubert (<http://www.rosesloubert.com>). Young unfolded leaves (100 mg) were collected from all roses, and DNA was extracted from fresh leaves (Dneasy[®]96 Plant Kit, QIAGEN).

Nuclear genome size

The nuclear DNA content of diploid parents of the mapping population, diploid ‘Félicité et Perpétue’ and triploid ‘New Dawn’ cultivars, were measured using a cytometer (ISV,

CNRS, Gif-sur-Yvette, France). Fresh leaves (1–2 cm²) were finely chopped with a sharp razor blade in 600 µL of Galbraith buffer (MgCl₂ 45 mM, NaNO₃ 30 mM, MOPS acid 20 mM pH 7.0) supplemented with 0.1% (w/v) Triton X-100 and 1% (w/v) Polyvinyl-pyrrolidone 10.000, in the presence of same amount of *Petunia hybrida* cv PxPc6 [2C=2.85 pg DNA/nucleus, GC ratio=41.0% (Marie and Brown 1993)]. The extract was then filtered through a 48-µm mesh and treated with 40 µg/ml of RNase A (Roche) for 10 min on ice and stained with 50 µg/ml propidium iodide. The samples were analysed on an EPICS Elite ESP cytometer (Coulter, Hialeah, FL). Excitation of fluorochrome was performed by an air-cooled Argon laser (Uniphase) at 488 nm, 20 mW.

Development of microsatellite markers

The new rose SSR markers developed in this study are originated from two different sources: rose genomic DNA and rose EST. A genomic library from *R. wichuraiana* had been previously constructed, and about 800 SSR-positive clones had been identified by screening the library with 17 different SSR probes (Zhang et al. 2006). In the present study, 384 SSR-positive genomic clones from this *R. wichuraiana* library were sequenced by Ouest-Genopole[®]. The two cDNA libraries have been constructed from vegetative and floral buds of *R. wichuraiana* and *R. hybrida* Black Baccara[®], respectively. Five thousand ESTs from these libraries have been sequenced and found to be clustered in 2,556 unigenes (Foucher et al., submitted). The EST sequences are available at the URGI public web server (<http://www.urg.versailles.inra.fr/GnpSeq/>).

In the two sets of sequences (384 genomic clones and 2,556 unigenes from the EST bank), SSR detection and primer design were carried out using Ouest-Genopole[®] web server (http://genoweb.univ-rennes1.fr/Serveur-GPO/outils_access.php3?id_syndic=1/). This pipeline for SSR detection and primer design was developed by combining two softwares: Sputnik (<http://www.cbi.labri.fr/outils/Pise/sputnik.html>) for SSR detection and Primer3 (http://www.frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) for primer design (Rozen and Skaletsky 2000). The search for SSR was carried out with selection criteria of repetition length greater than 20 bp (base pairs) for di-, 15 bp for tri- and 16 bp for tetra-nucleotides. Primers were synthesised by Sigma Genosys. For those EST sequences found to contain microsatellites, search for open reading frame (ORF) was performed using ORF Finder (<http://www.ncbi.nlm.nih.gov/gorf/gorf.html>) and Blastx (<http://www.ncbi.nlm.nih.gov/BLAST>) tools.

In addition to the new rose SSRs identified from genomic and EST clones in this study, SSRs already developed from other *Rosaceae* genera such as *Prunus* (Dirlewanger et al. 2002), *Malus* (Liebhard et al. 2003; Sosinski et al. 2000; Stam 1993) and *Fragaria* (B.

Denoyes-Rothan, unpublished results) were also used to screen the mapping population.

Polymerase chain reaction amplification and visualisation of microsatellites

Some of the SSRs were analysed by gel electrophoresis coupled with silver staining, whereas others were analysed through a capillary sequencer. For those SSRs analysed by gel electrophoresis, polymerase chain reaction (PCR) was performed in a 10-µl volume containing 1X AmpliTaq buffer, 0.15 mM of each deoxyribonucleotide triphosphate (Promega), 2 mM MgCl₂, 0.15 µM of each primer, 0.5 units of RedTaq DNA polymerase (Sigma) and 10 ng of genomic DNA. General PCR profiles used were: classical profile [94°C 2 min, (94°C 30 s, annealing temperature 15 s, 72°C 30 s) for 35 cycles, 72°C 7 min] and touchdown profile [94°C 2 min, (94°C 30 s, annealing temperature+5°C 15 s, 72°C 30 s) for ten cycles with decrease of 0.5°C by cycle, (94°C 30 s, annealing temperature 15 s, 72°C 30 s) for 25 cycles, 72°C 7 min]. PCR products were mixed with an equal volume of 2× gel loading buffer (80% deionized formamide, 10 mM ethylenediamine tetraacetic acid pH 8.0, 1 mg/ml xylene cyanol, 1 mg/ml bromophenol blue) and denatured at 95°C for 5 min. Products were separated on 5% denaturing polyacrylamide sequencing gels by electrophoresis at 60 W for 2 h. An improved procedure described by Creste et al. (2001) was used for silver nitrate staining of gels.

For those SSRs analysed through a capillary sequencer, an economic fluorescent labelling method of PCR fragments was used (Schulke 2000). SSR forward primers were modified by 5' concatenation with the 18-mer 5'-CACGACGTTG TAAAACGAC-3' primer, which permitted concurrent fluorescent labelling of PCR products by a third primer (M13) incorporated with a fluorophore (ROX). The PCR mix was the same as the previously described mix except for the primers, extended forward and reverse primers (0.04 µM) and M13 primers (0.15 µM). The PCR profile used was 94°C 2 min, (94°C 30 s, 61°C 15 s) for two cycles, (94°C 30 s, 59°C 15 s) for two cycles, (94°C 30 s, 57°C 15 s) for two cycles, (94°C 30 s, 55°C 15 s, 72°C 30 s) for 25 cycles, 72°C 7 min.

PCR products were resolved on an ABI 3100 capillary electrophoresis array (Applied Biosystems) using GeneScan-500 [ROX] as the size standard (Applied Biosystems) on Ouest-Genopole[®] platform. The Genotyper software (Applied Biosystems) was used to analyse electrophoregrams.

Linkage map construction

Map construction in each parent was carried out using JoinMap version 3.0 (Stam 1993). Parental linkage maps were constructed using AFLP markers described in Crespel et al. (2002) and SSR markers developed in this study. Markers

segregating from one or both parents were scored separately. For both maps, LGs were determined using a minimum of logarithm of odds ratio (LOD) threshold of 5.0, and map construction was performed using Kosambi mapping function with the following JOINMAP parameters: Rec=0.45, LOD=1.0, Jump=5, first and second runs. The resulting linkage maps were drawn using MapChart software.

Evaluation of phenotypic traits and statistical analysis

In the present study, number of petals, designated NP, was counted from three flowers on each of the 91 F₁ hybrids and analysed as both a major gene and a quantitative trait. To analyse NP as a major gene, flowers having less than ten petals were considered single, and flowers with ten or more petals, corresponding to two complete whorls, were considered double.

BD, the date of first appearance of a floral bud with sepals taken off corresponding to the stage 1 described by Put and Clercx (2003), was recorded in the mapping population during one growing season.

Statistical analysis of the phenotypic data was carried out using Statistical Analysis System (SAS) software (SAS Institute, 1989). Analysis of variance (ANOVA) was performed using the general linear model (GLM) procedure to test the significance of the genotype effect. Broad-sense of heritability of genotypic means within the progeny was calculated using the following formula: $h^2 = \sigma^2g / (\sigma^2g + \sigma^2e/n)$, where n is the mean number of replicates per genotype, σ^2g is the genetic variance (i.e. inter-genotype variance) and σ^2e is the residual error variance.

Additive quantitative trait locus (QTL) effects were detected using the simple interval mapping procedure of MapQTL software (Van Ooijen and Maliapaard 1997). QTLs with a maximum LOD score greater or equal to 3.0 were declared significant. For each significant QTL, confidence intervals corresponding to a LOD score drop of 1 or 2 on either side of the likelihood peak were calculated.

Results

Identification of new microsatellite markers in rose

In the present study, 156 new rose microsatellites were developed. Sixty-three were from rose genomic clones (designated genomic-SSR) and 93 from rose EST clones (designated EST-SSR). Through sequencing 384 SSR-positive clones from the *R. wichuraiana* genomic library of Zhang et al. (2006), we have identified 63 new genomic-SSRs. Fifty (79%) are di-nucleotide repeats, 12 tri-nucleotide repeats and one was a mono-nucleotide repeat. Among the di-nucleotide repeats, 39 are of the AG/GA/TC/CT repeat motif, 10 are of

AC/CA/TG/GT and one is a TA/AT repeat. Twelve tri-nucleotide repeats are distributed as AAG (5), CCG (4), AAC (2) and GCA (1). The single mono-nucleotide repeat was (A).

From the 2,556 EST unigene sequences developed in our laboratory (Foucher et al., submitted), 93 SSRs have been selected. Among these EST-SSRs, 52.2% were di-nucleotide repeats, 44.6% were tri-nucleotide repeats and 3.2% were tetra-nucleotide repeats. The only di-nucleotide motif found was AG/GA/CT/TC, whereas a variety of tri-nucleotide repeats were found, of which the most common is AAG/AGA/GAA/CTT/TTC/TCT (22%). The localisation of microsatellites in the transcribed regions was studied. No ORF was detected in 5 of the 93 sequences. Di-nucleotide repeats were predominantly located in the 5' untranslated regions (UTR; 89%) and less frequently in ORF (6.5%) or in the 3' UTR (4.5%). Tri-nucleotide repeats were distributed across transcribed regions, principally in ORF (55%) and also in 5' UTR (37%). Of the three tetra-nucleotide microsatellites, two are located in the coding sequence and one in 5' UTR.

Characterization of SSR loci

In addition to these 156 new rose SSRs, previously described SSR from other *Rosaceae* genera (Sosinski et al. 2000; Dirlewanger et al. 2002; Liebhard et al. 2003; B. Denoyes-Rothan unpublished results) and from rose genomic DNA (Zhang et al. 2006) have been characterized. Therefore, we have analysed a total of 231 *Rosaceae* microsatellites (Table 1) in the mapping population.

Among the 63 genomic-SSR tested, 43 (~68%) failed to give any product or gave complex profiles of amplified products and were not further studied. The 20 (~32%) remaining primer pairs amplified fragments in the range of the expected size for each microsatellite. Fourteen out of 20 genomic-SSRs (Table 2) showed polymorphism between parents H190 and *Rw* (Table 1). The six markers monomorphic in the mapping population are not presented in Table 2. Primer sequences of these markers are available upon request, as they could be polymorphic in other populations. From the SSRs previously characterized from the same genomic library (Zhang et al. 2006), 16 SSRs (*Rw*1F9, *Rw*8B8, *Rw*10J9, *Rw*10M24, *Rw*11E5, *Rw*14A5, *Rw*14H21, *Rw*17I17, *Rw*22A3, *Rw*22B6, *Rw*23H5, *Rw*27A11b, *Rw*29B1, *Rw*55C6, *Rw*55D22 and *Rw*61F2) were also tested and only one (*Rw*23H5) was monomorphic between the parents of our population (Table 1).

Of the 93 EST-SSR, 26 (~28%) failed to give any product, and 23 (~25%) gave rise to a PCR product that had a larger size than the expected, suggesting the presence of introns between the primer sites in the genomic sequence (Table 1). Eighteen (~19%) are monomorphic in the mapping population; their sequences are not included in Table 1 but available upon request. Twenty-six (28%)

Table 1 SSR markers used in this study, grouped by source and characterisation in the mapping population

SSR source	Number tested	Able to amplify correct size	Polymorphic	Mapped		Common H190 and Rw
				H190	Rw	
<i>Prunus</i>	17	3 (17.5%)	3 (100%)	1	2	1
<i>Malus</i>	25	2 (8%)	1 (50%)	1	0	0
<i>Fragaria</i>	17	13 (76%)	7 (54%)	1	1	1
Rose genomic, this study	63	20 (32%)	14 (70%)	12	11	9
Rose genomic (Zhang et al. 2006)	16	16 (100%)	15 (94%)	11	11	4
Rose EST	93	44 (47%)	26 (59%)	17	18	9
TOTAL	231	98 (42.5%)	66 (67%)	43	43	24

Percentage of SSR able to amplify is related to the total number of tested SSR. Percentage of polymorphic SSR is related to SSR able to amplify gDNA of Rose.

displayed polymorphism between parents of the population (Tables 1 and 3). Two EST-SSR primer pairs amplified multiple loci, two in C139 and three in H9B01 (Table 3).

Among the 59 heterologous *Rosaceae* microsatellites tested, only five have been used to construct the genetic map. Indeed, as shown in Table 1, of the 17 *Prunus* SSRs tested, only 3 [pchgms3 (Sosinski et al. 2000), BPPCT28 (Dirlewanger et al. 2002) and pchgms40 (Wang et al. 2002)] are able to amplify rose gDNA and are used to

screen the mapping population. From 25 *Malus* SSR primers, two gave expected-size amplified fragment, but only one [CH02C11 (Liebhard et al. 2003)] is polymorphic. Transferability of *Fragaria* SSRs was high, as half of the *Fragaria* SSRs tested displayed polymorphism between parents of the mapping population (Table 1). Nevertheless in this work, only one (F3-7C, B. Denoyes-Rothan) has been mapped. Analysis of the six remaining *Fragaria* SSRs is in progress in a synteny study between *Fragaria* and *Rosa*.

Table 2 Properties of rose polymorphic SSR primer pairs (F, forward; R, reverse) developed from genomic DNA

Name	SSR motif	Annealing temperature (°C)	Expected size (bp)	N° loci amplified in F1 HW	Primer (5'-3')
Rw5G14	(CT) ₇ (C) ₈	55	231	1	F: TGGTTTGGGGTTTTGTGTCT R: GCACAGTCTCCACCTGACAA
Rw12J12	(CT) ₁₁	55	162	1	F: CAGTGTCCATGCTGACGAGT R: TGCTCCTGTTTTCTCTTTGCT
Rw15D15	(CT) ₁₁	55	203	1	F: CGGCTAGCAATCAGTGACAA R: GGTCTCCCTAATGCCCAAT
Rw16E19	(TTC) ₉	55	160	1	F: CCAACAAACACGAGGAATGA R: CCACACTGATGTTCCAGCAC
Rw20I17	(TG) ₉	50	210	1	F: TTCCTCTTCTCCTCCCTCGT R: AGCAGTTTCTGGCGAGTTA
Rw23F13	(CT) ₁₄	59,3	172	1	F: TGCATTATCCCTCTCACTG R: TCAAATGCATGCTGAAAGGA
Rw25J16	(TC) ₈	TD50	177	1	F: TGGACCTTCCCTTTGTTTCC R: GCTTGCCACATATTGTTGA
Rw35C24	(AG) ₉ X (AG) ₈	55	247	1	F: GGC GAATCGAGATTCAGAGA R: GGATTAGCCCAAGTCCAGGT
Rw34L6	(CT) ₁₆	55	216	1	F: CTCCTTTAGACTCGGGACCA R: CAGGCACGCCATTTCTAACT
Rw52D4	(TTC) ₇	55	174	1	F: GGCAGTTGCTGTGCAGTG R: TTGTGCCGACTCAAAATCAA
Rw54N22	(TC) ₉ X (TC) ₈	55	169	1	F: CTC AACTTCCCCGCCTTATC R: CTCGGCAGCTCCACTATCTC
Rw55E12	(TC) ₉	55	225	1	F: CGGTGGTTGGACATTAAGC R: GGAGGCAACAGCACACTCTC
Rw59A12	(TC) ₁₉	61	220	1	F: GGAAGGTGCGTATGCAAAAT R: GAGAGGCTCATGCGCTTTAT
Rw62D8	CT rich	56,1	123	2	F: CCTGACTCTAACCTCGTGCT R: TCCATCATCAACTTCGTCTG

TD Touchdown

Table 3 Properties of rose polymorphic SSR primer pairs (F, forward; R, reverse) from EST library

Name	SSR motif	Annealing temperature (°C)	Expected size (bp)	N° loci amplified in F1 HW	Primer (5'–3')	Homology
C139	(CT) ₂₂	55	244	2	F: AAAACCGCACACACACTC R: GTCGTCCGAGCAGAAGGAT	AF227979 ribosomal protein S16
C172	(AAG) ₈	55	151	1	F: ACAACACCAACTAGAACTTGAGC R: GCTCAACAGCAACAACCTCA	No homology
C187	(AAG) ₇	55	225	1	F: TGTGCCTCGAGAGGTTTCTT R: GTCAGCTGAAGCACTGGTGA	AY739290 disulfite isomerase
CL2002	(TCAT) ₄	55	195	1	F: GAAGCAGGGAAGATCCATGA R: GGCCCAATGCTCACACTAAT	No homology
CL2845	(AAG) ₁₇	55	300	1	F: ACAACCCGTAACACGACCTG R: ATATGGTGCCTTTGGTGGAA	No homology
CL2980	(AG) ₁₆	55	234	1	F: CCCTATTCGATTCGAGTGC R: ACTTGCTCGACGGATACAC	NM_123761 ADHIII
CL2996	(CCG) ₁₇	55	183	1	F: GCCACCATAGCCAGAGACAT R: GGGCAGAGAAGAAGTTGACG	No homology
CL3881	(TTTG) ₄	55	241	1	F: GACAACGACCACCACTTG R: CCAAAGCAACATTGTCAAAGA	AC145165 unknown protein
CTG21	(TTC) ₂₀	55	123	1	F: CACAGTTTCCATTAACACAGCA R: CAAGAGGAGGCAAGAGGATG	AF220491 O-methyltransferase (OMT1)
CTG329	(GAA) ₁₀	55	190	1	F: ATTTTGAAATCGGTGGGTGA R: CGGAGAATCCATGAAGCAAT	NM_101485 TMS membrane family protein
CTG356	(GGA) ₁₅	55	172	1	F: GTCATCAAGGAGGACCAGGA R: GATCAGCGACCACCATGTC	No homology
CTG623	(CT) ₁₆	55	280	1	F: CCCCTGACCCAACAAGAGTA R: GGCTTCTGAAAACCCAGTCA	No homology
H1F03	(AGG) ₅	55	247	1	F: CCCTAGCTCTCTCCCTCTCC R: AGCACATTCTTGAGGGTTGG	Cnsoa431 unknown protein
H2F12	(TC) ₁₉	55	244	1	F: TGGCCAACCTCTCTCTGTCT R: TCCCAGCTTCGCTTTGTTAT	No homology
H4F06	(AAG) ₅	55	214	1	F: TTTAAAGCCTCCCCTCTTCC R: CCTACCGGACAATCAGAGGA	LO7291 nucleic acid binding protein BT005004 putative PHD-type zinc finger protein
H5F12	(AAG) ₅ AGG (AAG) ₃	55	156	1	F: CACAGAAACGAAGCGCAGTA R: GCTCGAAGAAGTCTGGATG	No homology
H9B01	(CT) ₁₁	55	232	>3	F: TCTGGTTGGTGATGATGAGC R: CGTAAACCATTCCTGTCTCC	NM_123565 ORMDL family protein
H9B07	(AAG) ₆	55	226	1	F: TGTGGTTTTGCCTCACAAAG R: GACAATGACCCTTCAAACATCA	Ay639647 GMPase
H10D03	(CT) ₁₁	55	222	1	F: CAATTCAAACACCCTCTCT R: CGCAGAGTCAACGAACCATA	No homology
H20D08	(CT) ₁₀	55	240	1	F: TTCGGCTCTCTCTCTGCTC R: GACATTACAGCGACGAAGCA	AF339025 pectate lyase
H20G01	(CT) ₉	55	225	1	F: CCCCTCCCTCTCTCTACAA R: TGATGAGGTTGTTGCGATG	No homology
H22C01	(TC) ₉	55	228	1	F: TCATAACCAACCATCTCCATCA R: AGGATTTCAACCAGAACACG	Ay96516 putative GH3 protein
H22E04	(AAG) ₇	55	241	1	F: GACATCACCACCACAAG R: AACCAAGTTTCCAGTTCCA	AC120988 unknown protein
H22F01	(CT) ₁₀	55	227	1	F: ACCATTTCCGAGCGACTCTA R: GAGGAGGAGGTGTGAATGGA	No homology
H23O17	(CT) ₁₁	55	218	1	F: ACACCAAGCAAACCAAAACC R: AGCACGAAAACCGAGAGAGA	No homology
H24D11	(CT) ₁₀	55	168	1	F: CCTCCTCAGCTTTCCTCCTT R: CAGCAACCATCTCTTCGTGA	CNS0A26C unknown protein

Map construction

Segregation data obtained from 91 F_1 progeny for AFLP (Crespel et al. 2002), genomic-SSRs, EST-SSRs and heterologous *Rosaceae* SSRs were used to generate the parental maps. A total of 126 loci derived from AFLP (73), genomic-SSR (24), EST-SSR (24) and heterologous *Rosaceae* SSR (5) were analysed to construct the map of maternal parent H190 (Table 4, Fig. 1). The genetic linkage map of the H190 has seven LGs (identified as A1–A7) and covers 432 cM of its genome (Table 4, Fig. 1). Of the 126 loci analysed, 106 were placed on the maternal parent map at a LOD score of 5.0. Of the 106 mapped markers, 27 (~26%) departed from the expected Mendelian segregation ($p < 0.05$); these are distributed primarily along three LGs A2, A3 and A6. These distorted markers are identified on the map with asterisks (Fig. 1).

A total of 158 loci derived from AFLP (106), genomic-SSR (26) EST-SSR (22) and heterologous *Rosaceae* SSR (4) were used to construct the paternal map of *R. wichuraiana* hybrid *Rw*. Of the 158 loci, 137 were assigned on seven LGs (identified as B1–B7) at a LOD score of 5.0 and covering 438 cM of its genome (Table 4, Fig. 1). Fourteen markers (~10%) have segregation ratios significantly different from the expected Mendelian segregation ($p \leq 0.05$); these were placed on two LGs B2 and B3 and identified on the map with asterisks (Fig. 1).

Nuclear genome size

To evaluate the physical genome size of the parents of the mapping population, nuclear 2C DNA amounts have been measured for both parental roses as well as that of two cultivars, ‘Félicité et Perpétue’ and ‘New Dawn’. The DNA amounts of the two cultivars were 1.27 pg (SD±0.02 pg) and 1.75 pg (SD±0.01 pg) for ‘Félicité et Perpétue’ and ‘New Dawn’, respectively. These values were comparable with the 2C DNA amounts previously reported, i.e. 1.29 pg (SD±0.02 pg) and 1.76 pg (SD±0.08 pg) for ‘Félicité et Perpétue’ and ‘New Dawn’, respectively (Yokoya et al. 2000). The 2C DNA amounts of the parental roses measured in this study were similar, 1.18 pg (SD±0.02 pg) and 1.16 pg (SD±0.01 pg), respectively, for H190 and *Rw*. The genomic size of *Rw* measured in this study is comparable with the size of *R. wichuraiana* (1.13±0.08 pg) obtained by Yokoya et al. (2000).

Anchoring of the maternal and paternal parental maps

SSR markers that showed heterozygosity in both parents (types *abxac* and *abxcd*) were used as anchor points between maternal and paternal genetic maps (Table 4, Fig. 1). The homology of LGs A1/B1, A4/B4 and A7/B7 was established by two common SSR markers for each

group: *Rw*25J16 and *Rw*34L6 for A1/B1, F3-7C and *Rw*16E19 for A4/B4 and CTG623 and *Rw*55D22 for A7/B7. Three SSRs, CTG329, *Rw*27A11b and *Rw*59A12, allowed us to define the homology between A2 and B2 groups. The greatest number of anchoring SSR markers, 5, was observed between A3/B3, A5/B5 and A6/B6. SSR markers C139, *Rw*55E12, CL3881, *Rw*20I17 and H2F12 anchor A3 and B3 groups, whereas SSR markers BPPCT28, *Rw*10M24, *Rw*15D15, H4F06 and *Rw*5G14 link A5 and B5 groups. Maternal LG A6 is homologous to paternal LG B6 based on SSR markers *Rw*52D4, CL2845, H24D11, H20G01 and H22C01.

Phenotypic data analysis

We analysed two phenotypic traits, NP and the BD, in the F_1 population (Fig. 2). This study expands the results for NP previously named double flower and designated *d6* and performed only on recurrent blooming hybrids (Crespel et al. 2002). A strong genotype effect (p value<0.001) was observed for NP by ANOVA. The ratio 1:1 for double versus single flowers (40:39) in the mapping population suggests the effect of a major gene for doubleness as described by Debener et al. (2001). Thus, the maternal parent H190, characterized by double flowers (>10 petals) would be heterozygous *NP/np*, whereas the paternal parent *Rw*, derived from *R. wichuraiana*, characterized by five petals in the corolla, would be double recessive *np/np*. For single flowers corresponding to a number of whorls lower than two, the distribution ranged from mean values of 5 to 8.33. The double flowers displayed a distribution of NPs ranging from mean values of 10 to 180. The major gene for NP is located in the LG A4. Tight linkage between the major gene NP and AFLP markers E2M2.07 (LOD=13.7) and E2M5.06 (LOD=16) could be detected on LG A4. Difference in map positions observed between major genes NP and *d6* identified by Crespel et al. (2002) is due to three hybrids that have a mean of petal number between 7.33 and 8.33, previously considered as double flowers but considered as single flowers in this study, as flowers with less than ten petals corresponding to less than two complete whorls were considered single in this study.

According to Debener et al. (2001), the distribution of NP in double-flowered group (Fig. 2a) could suggest a polygenic inheritance of this trait. Broad-sense heritability of the NP was 0.54 for single-flowered sub-group and 0.97 for double-flowered sub-group. Effects of additive QTL was studied with MapQTL software for double-flowered sub-group. A QTL was detected with R^2 of 29% and a LOD score of 3 on B4 in *Rw* map (Fig. 1). As A4 and B4 are anchored by two SSRs, both the major gene (NP) and additive QTL controlling NP in the double flowers were localised on this A4/B4 consensus LG.

Table 4 Distribution of SSRs and AFLP markers in the linkage groups of the maternal (A1–A7) and paternal (B1–B7) maps

	AFLP	Rose genomic SSR	Rose SSR-EST	SSR <i>Rosaceae</i>	Total	Length	SSR anchor points
Maternal linkage groups							
A1	23	2	4	0	29	66	2
A2	8	8	4	0	20	94	3
A3	12	4	3	1	20	63	5
A4	9	2	0	1	12	64	2
A5	5	4	1	1	11	78	5
A6	2	2	5	0	9	34	5
A7	2	1	1	1	5	33	2
Total	61	23	18	4	106	432	24
Paternal linkage groups							
B1	13	3	1	0	17	32	2
B2	4	2	3	0	9	106	3
B3	0	2	4	0	6	23	5
B4	17	2	0	1	20	60	2
B5	13	6	3	1	23	65	5
B6	23	3	5	0	31	75	5
B7	24	4	4	0	32	77	2
Total	94	22	20	2	138	438	24

For BD in the F_1 progeny (distribution shown in Fig. 2b), a strong QTL (LOD=6.38), explaining 34% of the phenotypic variance, was detected on the B4 LG (Fig. 1). As the locus controlling recurrent blooming located by Crespel et al. (2002) and QTL affecting BD were located on the same LG, we analysed their interaction by the GLM procedure. The recurrent blooming group flowered significantly earlier than the non-recurrent single seasonal blooming group ($R^2=0.16$, as shown in Fig. 2b).

Discussion

One of the major goals of this study was to develop new easily transferable SSR markers in rose. A total of 64 new rose SSR markers were generated from this study, 44 from EST sequences and 20 from a genomic DNA bank. Out of these, 40 SSRs have displayed polymorphism between the parents of the mapping population and have been mapped. The remaining 24 SSRs, although not polymorphic in the mapping population, gave rise to clear PCR products, and they might be useful in other rose populations and studies. In addition to the new rose SSRs generated in this study, 15 SSRs developed from rose genomic DNA (Zhang et al. 2006) and 4 SSRs developed from other *Rosaceae* species have also been mapped in the diploid population.

EST as a good source of SSR markers

EST databases are increasingly becoming available for a wide range of plants and plant tissues. Bioinformatic tools

allow easy searching for SSRs in these EST databases. In this study, a set of new rose SSRs was developed using such strategy. We searched a rose bud EST database of 2,556 unigenes obtained after analysis of 5,000 EST sequences (Foucher et al., submitted). In the rose bud EST database, 3.6% of the sequences contained repeats of 20 nucleotides for di-, 15 for tri- and 16 for tetra-nucleotide repeats. This frequency is comparable to those obtained for *Vitis* (2.5%, Scott et al. 2000), maize and rice (1.5 and 4.7%, respectively, Kantety et al. 2002) and recently for the *Rosaceae* species (4%, Jung et al. 2005) but lower than those reported in *Capsicum* (10.5%, Huang et al. 2001) and wheat (7.5%, Nicot et al. 2004). These differences may be explained by SSR length criteria used in these different studies.

Jung et al. (2005) showed that frequency of SSRs in rose is 59% for di-, 35% for tri- and less than 5% for tetra-nucleotides. This analysis for SSR sequences was performed on rose petal EST databases of Channeliere et al. (2002) and Guterman et al. (2002). In our study, this order of repeats remains the same with slightly different frequencies (52.2, 44.6 and 3.2%, for di-, tri- and tetra nucleotides, respectively). In both studies, the most common type of repeat motif is $(CT)_n$ for di-nucleotides and $(GAA)_n$ for tri-nucleotides.

The ESTs used in this study were obtained through 5'-end sequencing of directionally cloned full-length cDNA (Foucher et al., submitted). This could explain the over-representation of SSRs in the 5' UTR. Although the di-nucleotide SSRs are over-represented in the 5' UTR, an equal frequency of tri-nucleotide SSRs was found between 5' UTR and ORF. Moreover, tri-nucleotide microsatellites

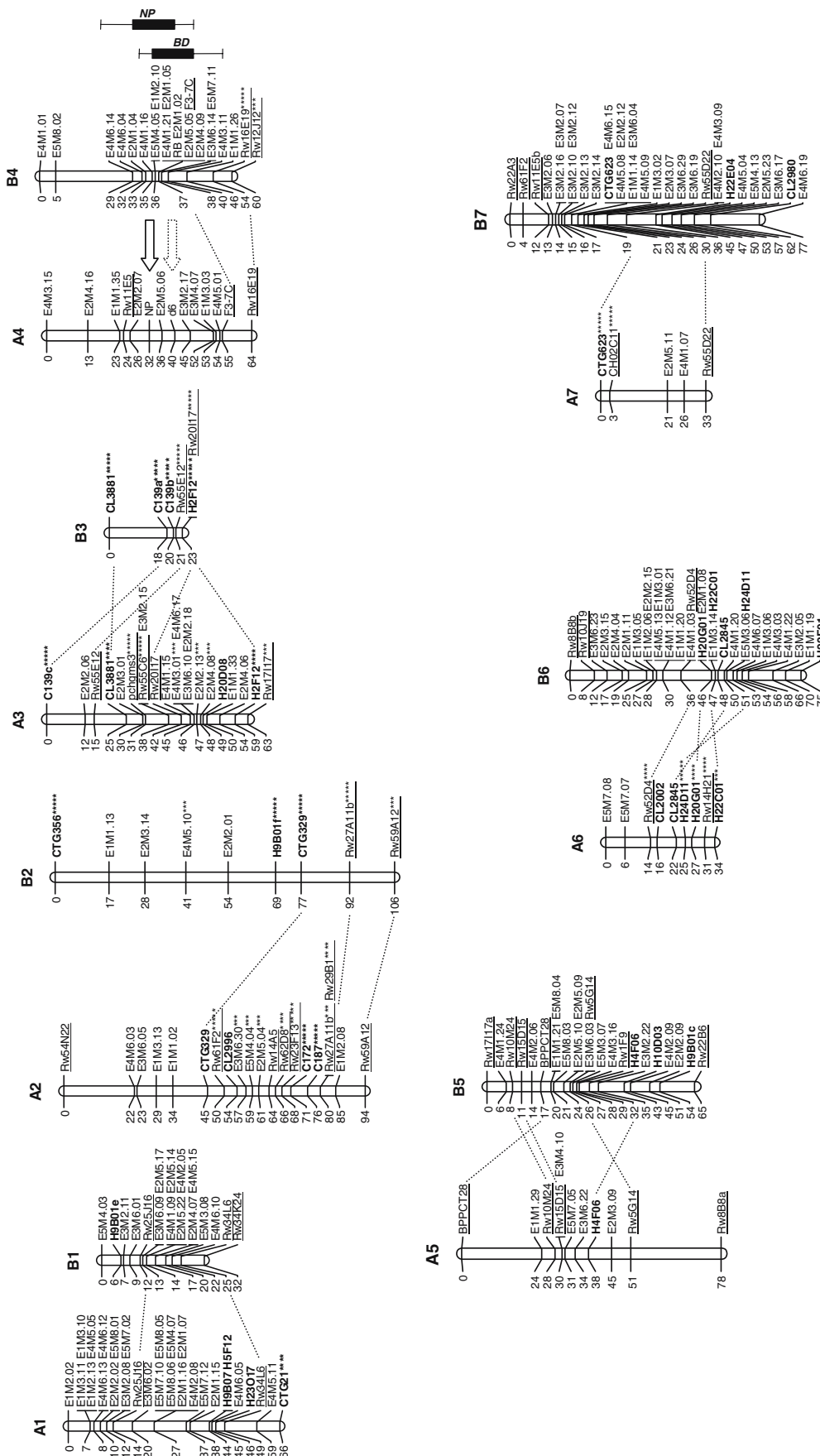
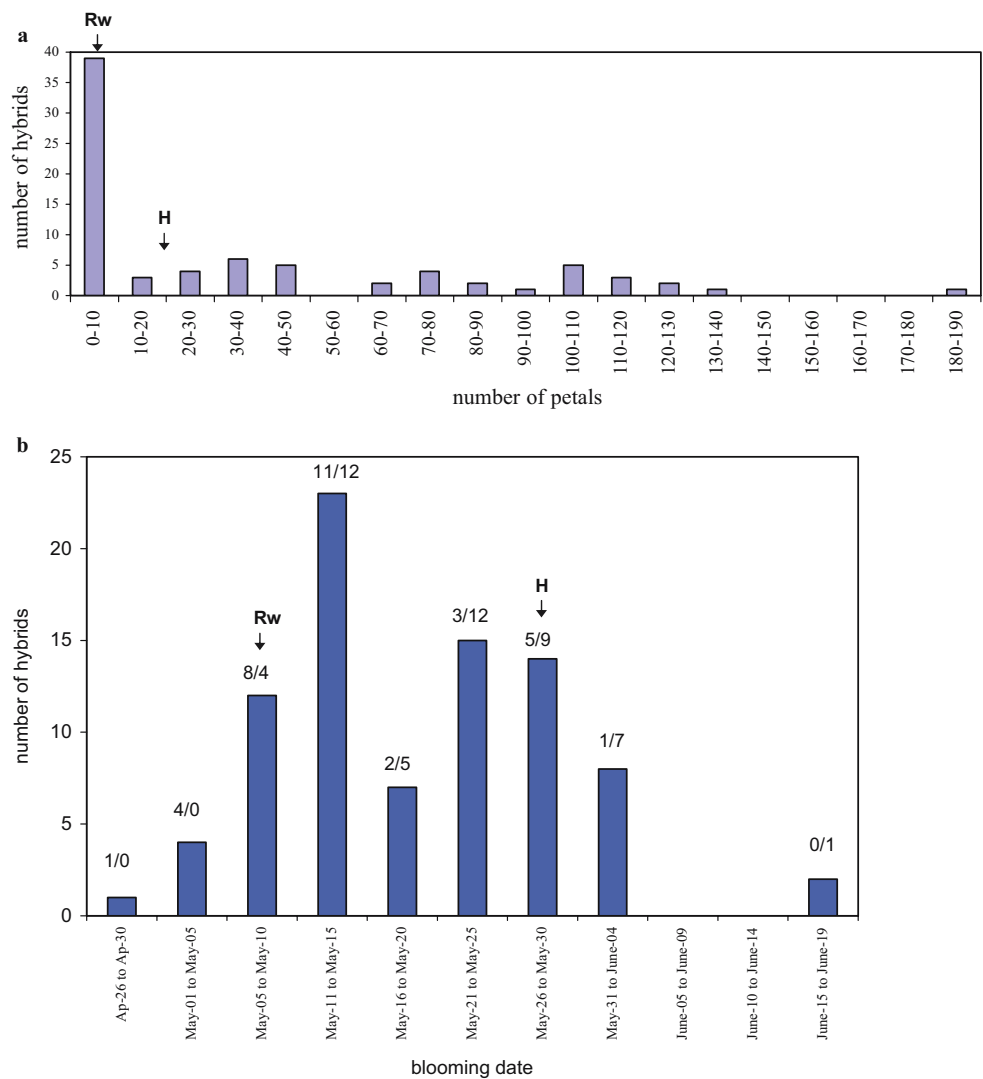


Fig. 1 Linkage maps of the dihaploid H190 (A1–A7) and the hybrid of diploid species *R. wichuraitana* (B1–B7) developed from 91 hybrids of an F_1 population. EST-SSRs are indicated in **bold**, and genomic-SSRs are *underlined*. AFLP markers have been previously located by Crespel et al. (2002). Map distances in cM are listed on the *left* and loci on the *right* of each linkage group. SSRs common between maternal and paternal maps are linked by *dotted lines*. Level of distorted markers are indicated with an *asterisk* (***) $p < 0.01$, **** $p < 0.001$, ***** $p < 0.0001$). Localization of the major gene controlling number of petals (*NP*) is indicated by an *arrow*, and the corresponding QTLs for blooming date (*BD*) and NP are represented by a *box prolonged with lines*, which respectively stretch over the “LOD 1” and “LOD 2” confidence intervals

Fig. 2 Distribution in the 91 F₁ progeny of **a** the number of petals per flower recorded as the average of 3 flowers per plant. **b** The blooming date, recorded as the date of first appearance of a floral bud from April 26, 2005. Parental values are indicated by arrows (*Rw* for *R. wichuraiana* hybrid and *H* for H190). The number of recurrent/non-recurrent genotype for each bloom period *i* indicated above the graph



were more abundant in ESTs (44.6%) than in genomic sequences (19%). Tri-nucleotide repeats do not lead to frame shift mutation and, as proposed by Morgante et al. (2002), tri-nucleotide microsatellites in the coding region could be a result of mutation pressure and positive selection for specific amino acid stretches.

Fewer SSRs derived from ESTs displayed polymorphism in our inter-specific mapping population (59%) compared to the SSRs derived from genomic sequences (70%; Table 1). Similar results have been found in other plants (Blair et al. 2003; Cho et al. 2000; Schloss et al. 2002), as EST sequences are generally more conserved than genomic sequences. Although SSRs derived from EST are less polymorphic than genomic SSR, the EST-SSRs could be more transferable inter-*genus* or intra-*genus* than anonymous genomic sequences. As a large number of SSRs can be very easily developed from the available EST databases, they can be effectively

used in the construction of a consensus genetic map for rose as well as synteny studies with other *Rosaceae* species.

The use of SSRs to construct genetic maps

Microsatellite markers generated were used to enrich a genetic map previously constructed with AFLP markers (Crespel et al. 2002). Genome coverage and LGs in the original maps were considerably improved by the addition of SSRs. As a result, seven LGs covering a total length of 438 cM for the paternal parental map and 432 cM for the female parental map were obtained, showing an increase in the genome coverage of 81 and 52.5%, respectively, for maternal and paternal maps, compared to the previous maps (Crespel et al. 2002).

Recently, genetic maps for roses have been constructed with different types of molecular markers such as AFLP, SSR, PK, RGA, RFLP, SCAR and morphological markers

(Yan et al. 2005). The genome coverage for *Rosa multiflora* was statistically estimated to be about 500 cM (Yan et al. 2005), whereas the two maps in the present study cover an average of 435 cM of parental genomes. The 2C amount of *R. multiflora*, estimated to be 1.66 pg (Dickson et al. 1992), is larger than the genomes of both maternal and paternal roses mapped in the present study (average of 1.16 pg). This difference in physical genome size might explain the difference in the length of genetic maps obtained by Yan et al. (2005) and in the current study, although genetic and physical map distances are not always correlated.

The density of markers in maps of the present study is lower (one marker every 4 and 3 cM for maternal and paternal maps, respectively) than the density (0.4–0.7 markers per cM) of the maps developed in Yan et al. (2005). Moreover, several large gaps are present in the maps of the present study. The largest gap (27 cM) is located on LG A5 (Fig. 1). Adding new SSR markers to the maps, such as those from other studies on *Rosa* (Yan et al. 2005), from *Rosa* EST (Channeliere et al. 2002; Guterman et al. 2002) and/or other *Rosaceae* species, will help fill these gaps and further saturate the maps.

Some markers, 26 and 10% of maternal and paternal parental markers, respectively, exhibited segregation distortion. These markers are located on several LGs. Markers with distorted segregation have already been reported in *Rosaceae* species (Aranzana et al. 2003; Liebhard et al. 2003; Sargent et al. 2004) and in rose (Crespel et al. 2002; Debener and Mattiesch 1999; Yan et al. 2005). Gametophytic selection by sub-lethal genes conferring “low viability” of zygote, embryo or seedling, could be located near the distorted markers in maps. In rose, the average rate of seed germination is around 40% (Meilland-Richardier Int., personal communication). Moreover, the origin of female dihaploid H190 (Meynet et al. 1994) could be explained by the large proportion of distorted markers in this genotype. This hypothesis is discussed in detail by Crespel et al. (2002).

The maternal and parental maps developed in this study were integrated using the codominant SSR markers. For this integration, 24 SSRs shared by the two maps were used.

Localization of genes controlling important rose traits

This map was constructed to locate major genes as well as QTL controlling interesting rose characters. We tested the integrated map for the localization of genomic regions involved in NP and BD. We have shown that petal number is controlled by a major gene and a QTL as previously proposed by Debener (1999) and located on A4 and B4 integrated LG. These results have been obtained from small number of flowers into 91 hybrids. An extended study could be realised to obtain additional QTL with smaller effects on a more significant number of flowers and/or on a higher population. Petal number has been previously located on

other genetic maps on LG D10-2 (Dugo et al. 2005) and LG B3 (Debener and Mattiesch 1999). Based on *NP* gene, we can suggest that A4/B4 LGs (this study) would be homologous to LG D10-2 (Dugo et al. 2005) and LG B3 (Debener and Mattiesch 1999). This hypothesis can be tested by developing common markers (as SSR) on these different LGs.

A strong QTL controlling BD was located on the same integrated LG A4/B4. Dugo et al. (2005) detected two QTLs, *Df1* and *Df2* controlling flowering time on two LGs different from D10-2. Thus, the process or the variability of the BD could be different in our population compared to this previous study; indeed, date of flowering is known to be a complex process involving numerous genes. Moreover, in Dugo et al. (2005), flowering time was recorded when 50% of flowers were fully opened, whereas in our study, flowering time was recorded when the first flower reached a specific stage.

The integrated A4/B4 group locates important major genes and QTLs controlling several flowering-related genes. These include recurrent blooming, BD, NP and presence of prickles (Crespel et al. 2002). Thus, this integrated LG could contain genes with pleiotropic effect or cluster of genes implied in important ornamental traits, which have been selected by breeders.

Anchoring with other rose maps

Some of the SSR markers provide anchor points between maps constructed in the present study and the tetraploid rose maps (Zhang et al. 2006). The apple SSRs CH02C11 and Rw55D22, mapped to A7 in the present map, are placed on the second integrated LG of the tetraploid map that also carry SSRs Rw22A3, Rw11E5 and Rw5D11 (Zhang et al. 2006). Indeed, Rw22A3 and Rw11E5 are located on the B7 of the present map. Thus, SSRs Rw55D22 and CH02C11 on LG A7 and Rw22A3 and Rw11E5 on B7 are homologous to the second integrated LG of the tetraploid map (Zhang et al. 2006). Furthermore, Rw10M24, one of the four SSRs that established homology between A5 and B5 groups (this study), is mapped to the first consensus group of the tetraploid consensus map (Zhang et al. 2006). Three SSRs, present in the consensus tetraploid map (Rw1F9, Rw8B8a and Rw22B6), are located on A5 (Rw8B8a) and B5 (Rw1F9 and Rw22B6), suggesting that A5/B5 is homologous to the first consensus group of the tetraploid maps.

Several important qualitative and quantitative rose traits have been located in different rose maps (Debener 1999; Debener and Mattiesch 1999; Crespel et al. 2002; Dugo et al. 2005; Yan et al. 2005; Zhang et al. 2006). SSR markers developed in this and other studies will provide tools for the establishment of a consensus linkage map for roses that combine traits and markers in the various maps. This consensus map locating important qualitative and quantita-

tive traits will allow new marker-assisted approaches to breeding complex traits in rose.

The use of SSR markers to study synteny in *Rosaceae* species

A large number of SSR markers have now been characterized in *Rosaceae*; notably in *Malus* (Liebhard et al. 2003), *Prunus* (Dirlewanger et al. 2002; Sosinski et al. 2000), *Fragaria* (Sargent et al. 2004; B Denoyes-Rothan, unpublished results) and recently in rose (Yan et al. 2005; Zhang et al. 2006; this work). The development of molecular markers shared by the members in this family is an important tool for comparative genetics and to study synteny in *Rosaceae* family. Although SSR markers are attractive for this purpose, development of markers that would be shared by at least some genera is difficult and time consuming. In this study, the transportability of microsatellites developed from *Fragaria* to rose was rather good (50%); however, the transportability of SSR markers from *Malus* and *Prunus* to rose was poor (less than 10%). To date, only four markers developed from other *Rosaceae* species have been located on the rose maps of the present study. Only one marker among them, BPPCT028, is known to be transportable to *Prunus*, *Malus* and *Fragaria* species (Dirlewanger et al. 2002). Thus, SSRs appear to be good transportable markers for synteny study within the Rosoideae tribe of the *Rosaceae* that include strawberry, raspberry and rose but not suitable for synteny between different tribes as shown with the low transportability between *Malus/Prunus* and *Rosa*. Markers more suitable for synteny studies between distantly related genera could be conserved ortholog set markers (Fulton et al. 2002).

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References

- Aranzana MJ, Pineda A, Cosson P, Dirlewanger E, Ascasibar J, Cipriani G, Ryder CD, Testolin R, Abbott A, King GJ, Iezzoni AF, Arus P (2003) A set of simple-sequence repeat (SSR) markers covering the *Prunus* genome. *Theor Appl Genet* 106:819–825
- Blair MW, Pedraza F, Buendia HF, Gaitan-Solis E, Beebe SE, Gepts P, Tohme J (2003) Development of a genome-wide anchored microsatellite map for common bean (*Phaseolus vulgaris* L.). *Theor Appl Genet* 107:1362–1374
- Blom TJ, Tsujita MJ (2003) Cut rose production. In: Roberts AV, Debener T, Gudin S (eds) *Encyclopedia of rose science*, vol 2. Elsevier Academic Press, Oxford, pp 594–600
- Channeliere S, Riviere S, Scalliet G, Szecsi J, Jullien F, Dolle C, Vergne P, Dumas C, Mohammed B, Huguency P, Cock JM (2002) Analysis of gene expression in rose petals using expressed sequence tags. *FEBS Lett* 515:35–38
- Cho YG, Ishii T, Temnykh S, Chen X, Lipovich L, McCouch SR, Park WD, Ayres N, Cartinhour S (2000) Diversity of microsatellites derived from genomic libraries and GenBank sequences in rice (*Oryza sativa* L.). *Theor Appl Genet* 100:713–722
- Crespel L, Chirollet M, Durel CE, Zhang D, Meynet J, Gudin S (2002) Mapping of qualitative and quantitative phenotypic traits in *Rosa* using AFLP markers. *Theor Appl Genet* 105:1207–1214
- Creste S, Tulman Neto A, Figueira A (2001) Detection of single sequence repeat polymorphisms in denaturing polyacrylamide sequencing gels by silver staining. *Plant Mol Biol Report* 19:299–306
- Debener T (1999) Genetic analysis of horticulturally important morphological and physiological characters in diploid roses. *Gartenbauwissenschaft* 64:14–20
- Debener T, Mattiesch L (1999) Construction of a genetic linkage map for roses using RAPD and AFLP markers. *Theor Appl Genet* 99:891–899
- Debener T, Von Malek B, Mattiesch L, Kaufmann H (2001) Genetic and molecular analysis of important characters in roses. *Acta Hort* 547:45–49
- Dickson EE, Arumuganathan K, Kresovich S, Doyle J (1992) Nuclear DNA content variation within the *Rosaceae*. *Am J Bot* 79:1081–1086
- Dirlewanger E, Cosson P, Tavaud M, Poizat C, Moing A, Zanetto A, Aranzana MJ, Arus P (2002) Development of peach SSRs and their use in fingerprinting peach and sweet cherry cultivars. *Acta Hort* 592:245–252
- Dugo ML, Satovic Z, Millan T, Cubero JI, Rubiales D, Cabrera A, Torres AM (2005) Genetic mapping of QTLs controlling horticultural traits in diploid roses. *Theor Appl Genet* 111:511–520
- Echt CS, May-Marquardt P (1997) Survey of microsatellite DNA in pine. *Genome* 40:9–17
- Esselink GD, Smulders MJM, Vosman B (2003) Identification of cut rose (*Rosa hybrida*) and rootstock varieties using robust sequence tagged microsatellite site markers. *Theor Appl Genet* 106:277–286
- Fulton TM, van der Hoeven R, Eannetta NT, Tanksley SD (2002) Identification, analysis, and utilization of conserved ortholog set markers for comparative genomics in higher plants. *Plant Cell* 14:1457–1467
- Gudin S (2000) Rose: genetics and breeding. *Plant Breed Rev* 17:159–189
- Guterman I, Shalit M, Menda N, Piestun D, Dafny-Yelin M, Shalev G, Bar E, Davydov O, Ovadis M, Emanuel M, Wang JH, Adam Z, Pichersky E, Lewinsohn E, Zamir D, Vainstein A, Weiss D (2002) Rose scent: genomics approach to discovering novel floral fragrance-related genes. *Plant Cell* 14:2325–2328
- Huang S, Zhang B, Milbourne D, Cardle L, Yang G, Guo J (2001) Development of pepper SSR markers from sequence databases. *Euphytica* 117:163–167
- Jung S, Abbott A, Jesudurai C, Tomkins J, Main D (2005) Frequency, type, distribution and annotation of simple sequence repeats in *Rosaceae* ESTs. *Funct Integr Genomics* 5:139–143
- Kantety RV, Rota MI, Matthews DE, Sorrells ME (2002) Data mining for simple sequence repeats in expressed sequence tags from barley, maize, rice, sorghum and wheat. *Plant Mol Biol* 48:501–510
- Liebhard R, Koller B, Gianfranceschi L, Gessler C (2003) Creating a saturated reference map for the apple (*Malus × domestica* Borkh.) genome. *Theor Appl Genet* 106:1497–1508

- Linde M, Mattiesch L, Debener T (2004) Rpp1, a dominant gene providing race-specific resistance to rose powdery mildew (*Podosphaera pannosa*): molecular mapping, SCAR development and confirmation of disease resistance data. *Theor Appl Genet* 109:1261–1266
- Malek BV, Weber WE, Debener T (2000) Identification of molecular markers linked to Rdr1, a gene conferring resistance to blackspot in roses. *Theor Appl Genet* 101:977–983
- Marie D, Brown SC (1993) A cytometric exercise in plant DNA histograms, with 2C values for 70 species. *Biol Cell* 78:41–51
- Meynet J, Barrade R, Duclos A, Siadous R (1994) Dihaploid plants of roses (*Rosa* × *hybrida*, cv ‘Sonia’) obtained by parthenogenesis induced using irradiated pollen and in vitro culture of immature seeds. *Agronomie* 14:169–175
- Morgante M, Olivieri AM (1993) PCR-amplified microsatellites as markers in plant genetics. *Plant J* 3:175–182
- Morgante M, Hanafey M, Powell W (2002) Microsatellites are preferentially associated with nonrepetitive DNA in plant genomes. *Nat Genet* 30:194–200
- Nicot N, Chiquet V, Gandon B, Amilhat L, Legeai F, Leroy P, Bernard M, Sourdille P (2004) Study of simple sequence repeat (SSR) markers from wheat expressed sequence tags (ESTs). *Theor Appl Genet* 109:800–805
- Nybohm H, Esselink GD, Werlemark G, Vosman B (2004) Microsatellite DNA marker inheritance indicates preferential pairing between two highly homologous genomes in polyploid and hemisexual dog-roses, *Rosa* L. sect. *Caninae* DC. *Heredity* 92:139–150
- Panaud O, Chen X, McCouch SR (1996) Development of microsatellite markers and characterization of simple sequence length polymorphism (SSLP) in rice (*Oryza sativa* L.). *Mol Gen Genet* 252:597–607
- Put HMC, Clerckx ACM (2003) Xylem structure and function of cut roses. In: Roberts AV, Debener T, Gudín S (eds) *Encyclopedia of rose science*, vol 2. Elsevier Academic Press, Oxford, pp 529–549
- Rajapakse S, Byrne DH, Zhang L, Anderson N, Arumuganathan K, Ballard RE (2001) Two genetic linkage maps of tetraploid roses. *Theor Appl Genet* 103:575–583
- Rozen S, Skaletsky HJ (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) *Bioinformatics methods and protocols: methods in molecular biology*. Humana Press, Totowa, NJ, pp 365–386
- Rusanov K, Kovacheva N, Vosman B, Zhang L, Rajapakse S, Atanassov A, Atanassov I (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
- Sargent DJ, Davis TM, Tobutt KR, Wilkinson MJ, Battey NH, Simpson DW (2004) A genetic linkage map of microsatellite, gene-specific and morphological markers in diploid *Fragaria*. *Theor Appl Genet* 109:1385–1391
- Schloss SJ, Mitchell SE, White GM, Kukatla R, Bowers JE, Paterson AH, Kresovich S (2002) Characterization of RFLP probe sequences for gene discovery and SSR development in *Sorghum bicolor* (L.) Moench. *Theor Appl Genet* 105:912–920
- Schulke M (2000) An economic method for the fluorescent labeling of PCR fragments. *Nat Amat* 18:233–234
- Scott KD, Eggler P, Seaton G, Rossetto M, Ablett EM, Lee LS, Henry RJ (2000) Analysis of SSRs derived from grape ESTs. *Theor Appl Genet* 100:723–726
- Sosinski B, Gannavarapu M, Hager LD, Beck LE, King GJ, Ryder CD, Rajapakse S, Baird WV, Ballard RE, Abbott AG (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
- Van Ooijen JW, Maliepaard C (1997) MapQTL version 3.0: software for the calculation of QTL positions on genetic maps. In: *Advances in biometrical genetics. Proceedings of the tenth meeting of the EUCARPIA Section Biometrics in Plant Breeding*, Poznan, Poland, 14–16 May 1997
- Vuylsteke M, Mank R, Antonise R, Bastiaans E, Senior ML, Stuber CW, Melchinger AE, Lubberstedt T, Xia XC, Stam P, Zabeau M, Kuiper M (1999) Two high-density AFLP(R) linkage maps of *Zea mays* L.: analysis of distribution of AFLP markers. *Theor Appl Genet* 99:921–935
- Wang Y, Georgi LL, Zhebentyayeva TN, Reighard GL, Scorza R, Abbott AG (2002) High-throughput targeted SSR marker development in peach (*Prunus persica*). *Genome* 45:319–328
- Wissemann V (2003) Classification. In: Roberts AV, Debener T, Gudín S (eds) *Encyclopedia of rose science*, vol 1. Elsevier Academic Press, Oxford, pp 111–117
- Wylie AP (1954) The history of garden roses. *Masters memorial lecture 1954*. *J R Hort Soc* 79:555–571
- Yan Z, Denneboom C, Hattendorf A, Dolstra O, Debener T, Stam P, Visser PB (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, Roberts AV, Mottley J, Lewis R, Brandham PE (2000) Nuclear DNA amounts in roses. *Ann Bot* 85:557–561
- Zhang LH, Byrne DH, Ballard RE, Rajapakse S (2006) Microsatellite marker development in rose and its application in tetraploid mapping. *J Am Soc Hortic Sci* 131(3):380–387