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ORIGINAL PAPER

Z. Yan · C. Denneboom · A. Hattendorf · O. Dolstra
T. Debener · P. Stam · P. B. Visser

Construction of an integrated map of rose with AFLP, SSR, PK, RGA, RFLP, SCAR and morphological markers

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Abstract A high-density genetic map with a number of anchor markers has been created to be used as a tool to dissect genetic variation in rose. Linkage maps for the diploid 94/1 population consisting of 88 individuals were constructed using a total of 520 molecular markers including AFLP, SSR, PK, RGA, RFLP, SCAR and morphological markers. Seven linkage groups, putatively corresponding to the seven haploid rose chromosomes, were identified for each parent, spanning 487 cM and 490 cM, respectively. The average length of 70 cM may cover more than 90% of the rose genome. An integrated map was constructed by incorporating the homologous parental linkage groups, resulting in seven linkage groups with a total length of 545 cM. The present linkage map is currently the most advanced map in rose with regard to marker density, genome coverage and with robust markers, giving good perspectives for QTL mapping and marker-assisted breeding in rose. The SSR markers, together with RFLP markers, provide good anchor points for future map alignment studies in rose and related species. Codominantly scored AFLP markers were helpful in the integration of the parental maps.

Keywords Rose · Parental maps · Integrated map · Molecular marker

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Z. Yan · C. Denneboom · O. Dolstra (✉) · P. B. Visser
Plant Research International,
Wageningen University and Research Centre,
P.O. Box 16, 6700 AA Wageningen, The Netherlands
E-mail: Oene.Dolstra@wur.nl
Tel.: +31-317-477006
Fax: +31-317-418094

A. Hattendorf · T. Debener
Institute for Ornamental Plant Breeding,
Federal Centre for Breeding Research on Cultivated Plants,
Bornkampsweg 31, 22926 Ahrensburg, Germany

Present address: T. Debener
Department of Horticulture, Hannover University,
Herrenhäuser Str. 2, 30419 Hannover, Germany

P. Stam
Laboratory of Plant Breeding,
Wageningen University and Research Centre,
P.O. Box 386, 6700 AJ Wageningen,
The Netherlands

Present address: P. B. Visser
Instituto Agroforestal Mediterráneo,
Universidad Politécnica de Valencia,
Camino de Vera s/n, 46022 Valencia, Spain

Introduction

Rose (*Rosa*) is the most important ornamental crop in the floriculture industry. The genus *Rosa*, belonging to the Rosaceae family, includes more than 150 species and thousands of cultivars (Gudin 2000). Most modern roses do not belong to a simple rose species but are complex hybrids derived from 7–10 species (Gudin 2000; Zhang 2003). Wild species are often diploids ($2n = 2x = 14$) while almost all cultivated roses are tetraploids ($2n = 4x = 28$). Rose chromosomes are fairly small with an average DNA content of 1.1 pg/2C for diploid roses (Yokoya et al. 2000). The genome size is estimated to be about four times larger than that of *Arabidopsis thaliana* (Debener and Mattiesch 1999; Rajapakse et al. 2001). Despite the low chromosome number and small genome size, little is known on the genetics of rose (De Vries and Dubois 1996; Gudin 2000). This is largely due to characteristics like a high degree of heterozygosity, varying ploidy levels between species, difficulties in sexual reproduction, low seed set and poor seed germination. However, current advances in molecular genetic mapping have enhanced the understanding of rose genetics and the genes controlling important traits, including resistance to fungal diseases (Debener 2003; Rajapakse et al. 2001; Crespel et al. 2002; Von Malek et al. 2000; Kaufmann et al. 2003). Furthermore, the future availability of dense genetic maps will facilitate the identification of quantitative trait loci (QTLs), and provide

markers for marker-assisted breeding, map-based cloning of genes and the introgression of beneficial genes from wild species into modern cultivars (Liebhard et al. 2003; Rajapakse 2003).

The first molecular genetic linkage map for rose covering over 300 markers was published by Debener and Mattiesch (1999) using a diploid population derived from *Rosa multiflora* hybrids. Seven pairs of homologous linkage groups were identified with RAPD (randomly amplified polymorphic DNA) and AFLP (amplified fragment length polymorphism) markers. Genes controlling pink flower colour (*Blfa*) and double flower (*Blfo*) were localized. Debener et al. (2001) extended their map with additional AFLP, SSR (simple sequence repeat or microsatellite), RFLP (restriction fragment length polymorphism) and SCAR (sequence specific amplified region) markers, and were able to map a resistance gene (*Rdr*1) to blackspot (*Diplocarpon rosae*). Rajapakse et al. (2001) developed two genetic maps based on a tetraploid population and identified genes for prickles and the enzyme malate dehydrogenase. Crespel et al. (2002) published an AFLP map based on a dihaploid population and localized genes controlling the number of prickles, double corolla and recurrent blooming. These maps so far have a medium marker density and have provided initial tools for genetic research and marker-assisted breeding of roses (Rajapakse 2003). For better comparison of the different maps an advanced map for roses is needed with full genome coverage and also including a wide set of polymorphic PCR-based anchor markers. Codominant markers, such as SSRs and RFLPs, would allow alignment of homologous linkage groups between maps, and facilitate marker transfer across populations as well as across related species.

AFLP markers have widely been used for map construction and map saturation in rose-related species such as peach (Sosinski et al. 1998) and apple (Maliepaard et al. 1998; Liebhard et al. 2003) as well as other crops (Haanstra et al. 1999; Chagné et al. 2002). The utility of AFLP markers was improved by the possibility to score them codominantly (Castiglioni et al. 1999; Piepho and Koch 2000). Sequence-based markers, such as resistance gene analogues (RGA) that are based on the conserved sequences of leucine-rich repeats (LRRs) and nucleotide binding sites (NBSs), most likely lead to target genes for disease resistance. Sequences based on protein kinase (PK) motifs lead specifically to this class of genes which are involved in signal transduction processes in plants (Bent 1996; Van der Linden et al. 2004). The mapping of RGA and PK markers on linkage maps has been used as a candidate gene approach to identify genes and pseudogenes with a possible role in the resistance mechanisms to various pathogens (Foolad et al. 2002; Quint et al. 2002; Mohler et al. 2002; Donald et al. 2002; Di Gaspero and Cipriani 2003).

The linkage maps for diploid rose presented in this paper are composed of AFLP, SSR, PK, RGA, RFLP, SCAR and morphological markers. A number of markers, especially the developed SSRs, provide good anchor points on the maps for the alignment of diploid and tetraploid rose maps. The maps serve as an essential step towards a reference map of rose. This paper gives a detailed description and discussion on the approach we took for construction of the map and the alignment of the parental and integrated maps.

Materials and methods

Mapping population

A diploid rose population, 94/1, derived from a cross between 93/1-119 (P119) and 93/1-117 (P117) (Debener and Mattiesch 1999) and consisting of 88 individual genotypes, was used to generate the present genetic map. The parents are half sibs resulting from open pollination of a diploid genotype 81/42-15, originating from a cross between *Rosa multiflora* and unidentified garden roses. Genomic DNA of each genotype of the population was extracted from young leaves according to Esselink et al. (2003).

Marker analysis

AFLP marker analysis was performed as described by Vos et al. (1995) with some minor modifications, using two restriction enzyme combinations, *Eco*RI/*Mse*I (E-M) and *Pst*I/*Mse*I (P-M). A total of 500 ng genomic DNA was used for each sample. Pre-amplification was performed with the E01/M02 and P01/M02 primers each containing one additional base (E01 +A, P01 +A, and M02 +C). Selective amplification was carried out with primers that contained two (only in the *Pst*I primers) or three (in the *Pst*I, *Eco*RI and *Mse*I primers) additional selective nucleotides. Forward *Pst*I and *Eco*RI primers were labelled with fluorophores (6FAM, HEX, NED) at the 5' ends. All PCRs were performed on a Perkin Elmer 9600 thermocycler (Perkin Elmer/Applied Biosystems) under standard conditions. Electrophoresis was performed on an ABI Prism 377 DNA Sequencer (Perkin Elmer) with 5% denaturing polyacrylamide gels.

Semi-automated scoring of the amplified fragments was performed with the programs GeneScan 3.1.2 and Genotyper 2.5 (Perkin Elmer/Applied Biosystems). Polymorphic AFLP fragments with a clear segregation pattern, i.e. discriminative at ± 0.5 bp within a size range of 50–500 bp and a peak height (intensity of fragment) of more than 100 were selected, labelled and either dominantly scored as 0 (fragment absent) or 1 (fragment present), or codominantly scored as 0 (fragment absent) or 1 (homozygous fragment present) or 2 (heterozygous fragment present). In the case of codominant scoring, the peak heights of the segregating markers were scored, taking into account the peak heights of the flanking non-segregating markers.

Rose genomic DNA libraries enriched for dinucleotide and trinucleotide SSRs were constructed (Esselink et al. 2003). The clones were sequenced and primers were generated according to Esselink et al. (2003). The ''Rh'' SSR primers used in this study are available upon request from Plant Research International, The Netherlands. The ''MicD'' and ''RMS'' SSRs are available from the Federal Centre for Breeding Research on Cultivated Plants, Institute for Ornamental Plant Breeding, Germany.

Protein kinase profiling was performed according to the protocol described in Van der Linden et al. (2004) with some modifications. In brief, 400 ng genomic DNA of each sample was digested with *Mse*I then adapter ligated, followed by amplification of PK-specific fragments using a two-step PCR procedure. PCR products were radioactively labelled by primer extension using the γ-[^{33}P]ATP-labelled protein kinase primer and an adapter primer and separated on a 6% polyacrylamide sequencing gel. Marker patterns were visualized by autoradiography.

A rose RGA library was established containing expressed and genomic rose RGAs according to Pan et al. (2000) with different degenerate primers based on conserved motives of the NBS-LRR resistance genes. The clones were sequenced and specific primers for the RGAs were designed and used for genotyping of the population. PCR amplifications were performed with 25 ng genomic DNA in 25 µl assays containing 0.1 mM dNTPs, 0.5 μM of each primer (MWG Biotech AG, Ebersberg, Germany) and 0.5 U *Taq* DNA polymerase, in a buffer consisting of 10 mM Tris pH 8.3, 50 mM KCl, 2 mM $MgCl_2$ and 0.01% gelatine. The following PCR program was used: 5 min at 95°C, then 35 cycles of 1 min at 94°C, 1 min at 58°C and 1 min at 72°C, and 30 min at 72°C for the final extension. Polymorphism of the PCR products was visualized by SSCP analysis on 0.5× MDE gels (Slabaugh et al. 1997).

A total of 51 previously generated markers (Debener and Mattiesch 1999; Debener et al. 2001) were chosen as bridge markers: 26 AFLP markers (coded as ''AFLP'', 2 markers on each parental linkage group except on A6), 4 SSR markers (coded as ''MicD''), 11 RFLP markers (coded as ''RGF'' or ''BMA''), 6 SCAR markers (coded as ''PAS'') and 4 morphological markers including pink flower colour (*Blfa*), double flower (*Blfo*), resistance to black spot (*Rdr*1) and powdery mildew (*Mildew*).

Marker segregation type

Markers were divided into uni-parental markers, being markers heterozygous in either the female or the male parent, and bi-parental markers that showed heterozygosity in both parents. The coding of the marker segregation types is indicated in Table 1. Segregation types 1, 2 and 3 were scored dominantly, while marker types 4, 5 and 6 were scored codominantly.

Map construction

JoinMap version 3.0 (Stam 1993; Van Ooijen and Voorrips 2001) was used for linkage analysis and map calculations. After all the marker data had been imported, different sets of marker data were set up with a selection of marker loci by using the ''excluding'' function.

Parental maps were separately constructed by using different sets of marker data. Each parental linkage map was constructed using its uni-parental and common bi-parental markers. As for the bi-parental markers, separate data sets with and without type 3 markers were employed in order to enable the comparison of the marker orders before the less informative type 3 markers were added. JoinMap used the defined marker data to perform a stepwise building of the map by adding one marker at a time, and to estimate the recombination frequencies between a given pair of markers that were then used to determine the linear arrangement of markers by minimizing the number of recombination events in the data set (Stam 1993). The marker order in a linkage group was determined by calculation of the goodness-of-fit criterion to find the best fitting order and

Table 1 Markers grouped by marker type and segregation type. Segregation types: *ab* × *aa* (type 1) for markers segregating only in paternal P117 with two alleles; *aa* × *ab* (type 2) for markers segregating only in maternal P119 with two alleles; *ab* × *ab* (type 3) for markers segregating in both parents with two alleles; *ab* × *cd* (type 4) for markers segregating in both parents with four alleles; *ab* × *ac* (type 5) for markers segregating in both parents with three alleles and *cd* × *cd* (type 6) for markers segregating in both parents with two alleles. Segregation types 1, 2 and 3 are dominantly scored markers, the rest are codominantly scored markers. *PRI*: Plant Research International, Wageningen University and Research Centre, The Netherlands; *BAFZ*: Institute for Ornamental Plant Breeding, Ahrensburg, Germany

Marker type	Uni-parental markers		Bi-parental markers				Total	Source
	ab × *aa* 1	*aa* × *ab* 2	*ab* × *ab* 3	*ab* × *cd* 4	*ab* × *ac* 5	*cd* × *cd* 6		
AFLP	107	113	89	0	0	11	320	PRI
PK	8	7	9	0	0	0	24	PRI
SSR	18	24	6	4	18	4	74	PRI/BAFZ
RGA	27	24	0	0	0	0	51	BAFZ
Total	160	168	104	4	18	15	469	

simultaneously calculating the map positions corresponding to that order (Stam 1993; Van Ooijen and Voorrips 2001). Linkage groups were determined using a LOD threshold of 5.0 and map construction was performed using the Kosambi mapping function with JoinMap parameter settings as follows: Rec = 0.45, LOD = 1.0, Jump = 5, "first run" and "second run". The "first run" option resulted in a stepwise build-up of a map by adding markers one by one with best goodness-of-fit for all markers. Subsequently, the "second run" option was applied in an attempt to add previously omitted markers to the map (Van Ooijen and Voorrips 2001). A "third round" option that enforces the mapping of problematic markers was not employed. Mapped markers were then inspected and some of the markers were removed when they showed insufficient linkage and conflict with other markers, e.g. those having low pair count, a high chi-square contribution ($\chi^2 \geq 3.0$) and inconsistent order. The "fixed order" option was used. To this end, the marker order of a set of markers evenly distributed per linkage group was employed prior to adding type 3 markers.

Integrated linkage groups were built up by "merging" the pair-wise marker data from homologous parental linkage groups having common anchor markers. The same parameters as mentioned above were employed to test the robustness of the linkages. The resulting marker order was compared to that of the homologous parental linkage groups. In cases where the resulting order in the integrated map was clearly conflicting with that in either of the parental maps, the order in one parental linkage group was taken as a fixed (reference) order.

The resulting linkage maps were drawn and the comparison of the integrated and the parental maps was performed by using MapChart software (Voorrips 2001).

Nomenclature of markers and linkage groups

Newly developed AFLP markers were coded according to Keygene's nomination system comprising a letter code for restriction enzyme, followed by a figure for the combination of selective nucleotides (Haanstra et al. 1999) and the size of the fragment in base pairs. SSR markers start with "Rh", or "RMS"; PK markers with "PK" and RGA markers with "RGA". The last digit of the marker represents the code of the segregation type (Table 1). The coding of the linkage groups and their orientation corresponds to that defined by Debener and Mattiesch (1999).

Estimation of genome coverage

The proportion of the diploid rose genome covered by each of the parental maps was calculated (Stam, unpublished program) by repeated sampling of markers from the maps without replacement. The average map length covered by a single marker sample of a given size was first calculated. The average coverage of the maps was based on 20,000 samples. The asymptotic upper limit was estimated by increasing the sample size up to the actual number of markers in the maps and by fitting an exponential curve to the relation between sample size and average map length covered. The validity of this procedure has been verified extensively using simulated mapping data (Stam, unpublished results).

Results

Segregating markers

The mapping study comprised a total of 469 newly generated AFLP, SSR, PK and RGA markers, which are grouped in Table 1. AFLP analysis, based on 56 AFLP primer pairs, including 33 E-M and 23 P-M, resulted in 320 polymorphic markers (Table 1). Out of these markers 220 were uni-parental and 100 bi-parental. From the bi-parental markers 11 were codominantly scored.

From the enriched SSR libraries, 149 new clones were sequenced and primers were designed based on their flanking sequences. Of these primers 58 showed correct amplification and were added to the Rh SSR primer database at Plant Research International, The Netherlands. A set of 42 primer pairs from the database and 16 RMS SSR primer pairs showed polymorphisms in the present population and resulted in 74 polymorphic markers (Table 1), 26 of which could be scored codominantly. The size of the alleles found for a subset of the mapped SSR markers in the present population as well as the primers needed to generate the markers are shown in Table 2. The numbers of alleles detected in the present population ranged from two to four.

Protein kinase profiling resulted in 24 PK markers, using a gene-specific primer based on a protein kinase specific variant. A total of 51 RGA markers were generated with 32 RGA primer pairs. Both PK and RGA markers were dominantly scored.

Different segregation types were assigned for the markers (Table 1). Among the 469 markers, 328 markers were uni-parental, of which 160 (34%) were derived from parent P117 (type 1) and 168 (36%) from parent P119 (type 2). The remaining 141 markers showed a bi-parental inheritance, of which 104 (22%) were dominantly scored (type 3) and 37 (8%) were codominantly scored (types 4, 5 and 6).

Before the map was constructed all the markers were subjected to a chi-square test using the expected segregation ratios given by the JoinMap program. Of all markers, 101 (22%) showed distorted segregation ($P \leq 0.05$, chi-square test). Of these markers, 64 were derived from P117, 14 from P119 and 23 from both parents, of which 15 were type 3 markers, indicating that the distorted markers mainly originated from parent P117. These distorted markers were included in the

Table 2 A selection of the SSR markers used for mapping. The sizes of DNA fragments (in base pairs) as well as the chromosome location (LG) of the SSR markers are indicated. The annealing temperature was 50°C for all the primers. Additional SSR information is available upon request from the authors

SSR	Forward (5′ → 3′)	Reverse (5′ → 3′)	Size (bp)	LG
Rh79	ttcttcttgctcgccattttgatt	gaacgtccaccaccacccactctg	135, 147, 149	1
RhAB9-2	gtcaatttgtgcataagctc	gtgagaacagatgagaaatg	101, 108, 124	1
Rh48	gatagtttctctgtaccccaccta	ttgaccagctgcaacaaaattaga	99, 107	2
Rh80	catgccaaacgaaatgagtta	ttatctaaagggctgctgtaagtt	134, 148	2
Rh96	gccgatggatgccctgctc	agattccctgcgacattcacattc	267, 276, 294	2
RhB510	aaacgataggtgaatctgtgggt	cactcaaccttgtccactcctaat	159, 161	2
Rh50	tgatgaaatcatccgagtgtcag	tcactttcattggaatgccagaat	336, 339, 340, 343	3
Rh58	acaatttagtgcggatagaacaac	ggaaagcccgaaagcgtaagc	248, 252, 254, 269	3
Rh59	cgcggatgaagctagtgaatcagt	ctagcccatctcagtatccctcacc	197, 200, 216	3
RhABT12	caagtttgtctccttggacc	catagatgattatcctagagcc	166, 172, 180	4
Rh65	agtacgccgacgcagatccagtga	acggcgttgtaggtcgtcattctc	128, 130, 132	4
Rh78	aaagaaacgcgaaatctatgatgc	tctggatgggatttaaaagacagg	216, 250	4
Rh77	caactgaaaggaacaaatggatgt	ggaatggcttgtaaatttgtgatt	249, 251, 258, 262	5
Rh93	gctttgctgcatggttaggttg	ttctttttgtcgttctgggatgtg	251, 273, 275	5
RhAB38	gaggtggtcgattccatgtc	ttaccgttctacctaagtgactaac	149,173,190	5
Rh60	tctcttttcacggccaccact	tgaatccaaggccgtatagttaga	234, 240, 252	6
Rh85	acttttgggcgttcatcgcattacac	ggctatatgggctcaagtctagacaa	203, 207, 217, 221	6
Rh98	ggcctctagagtttgggatagcag	acgacgtcaataactccatcagtc	153, 170, 175, 121	6
Rh72	ccaaaagacgcaaccctaccataa	tcaaaacgcatgatgcttccactg	115, 276, 283, 285	7
Rh73	ggttagacgggtggaagaag	actgccgatagaagtatttcatca	160, 162, 172	7
RhAB28	gcagatgttattcatgttaa	ccaagtattttagtttcttc	171, 175	7

linkage analysis since the segregation distribution hardly effects the estimation of recombination frequency. The remaining markers with distorted segregation are labelled with asterisks on the map (Fig. 1).

Parental maps

A total of 520 markers including 469 newly generated AFLP, SSR, PK, RGA and 51 previously developed AFLP, SSR, RFLP, SCAR and morphologic markers were employed for the construction of the genetic linkage maps. Parental maps were first generated with uni- and (codominantly scored) bi-parental markers. Each map consisted of seven linkage groups, putatively corresponding to the seven rose chromosomes. Subsequently, the type 3 markers were added to the parental maps in a second mapping effort. Fixed marker orders based on 5–7 markers per linkage group from the first mapping attempt were used to give extra weight to the most informative markers. For the P119 map, 27 markers were eliminated and 44 were not assigned to linkage groups. Out of the segregating markers from P117, a total of 17 markers were excluded from mapping and 39 remained unmapped. The resulting parental maps covered a total length of 490 and 487 cM in the paternal P117 (A) and maternal P119 (B), respectively, with an average chromosome length of ~70 cM (Table 3 and Fig. 1).

Integrated map

Homologous linkage groups were identified with the help of common markers and integrated using the JoinMap program. Markers on the linkage map were checked and excluded when they did not meet the same criteria as those used for construction of the parental maps. The markers mapped in the integrated map tend to have the same marker order as in corresponding parental maps. Only a few cases of conflicting marker order were found, like those in linkage group 2. This was solved by using a ''fixed (marker) order''. The final outcome was a map with seven integrated linkage groups (Fig. 2) having a total length of 545 cM and an average chromosome length of 78 cM.

Distribution of markers

All parental linkage groups contained uni- and bi-parental markers, except for A6 which had only bi-parental markers. For the P117 map, 323 markers were employed in the linkage analysis and 271 (84%) of them could well be assigned to seven linkage groups, which had 14–56 markers, a chromosome length ranging from 51 to 91 cM and a marker density of 0.3– 0.9 markers per cM (Table 3). For the P119 map, 338 markers were used and finally 273 (81%) of these could be assigned to seven linkage groups, each containing 24–52 markers,

Fig. 1 Genetic linkage map of diploid rose progeny of parents P117 (*A*) and P119 (*B*). Linkage groups are numbered from A1 to A7 and from B1 to B7 according to Debener and Mattiesch (1999). Marker names are indicated at the *right*of each linkage group. Distances are given in Kosambi cM at the *left* of each linkage group. SSR markers are printed in *italic*. Segregation distortion is indicated with *asterisk(s)* for the significance level of the chi-square test: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.005$, ****$p \leq 0.001$

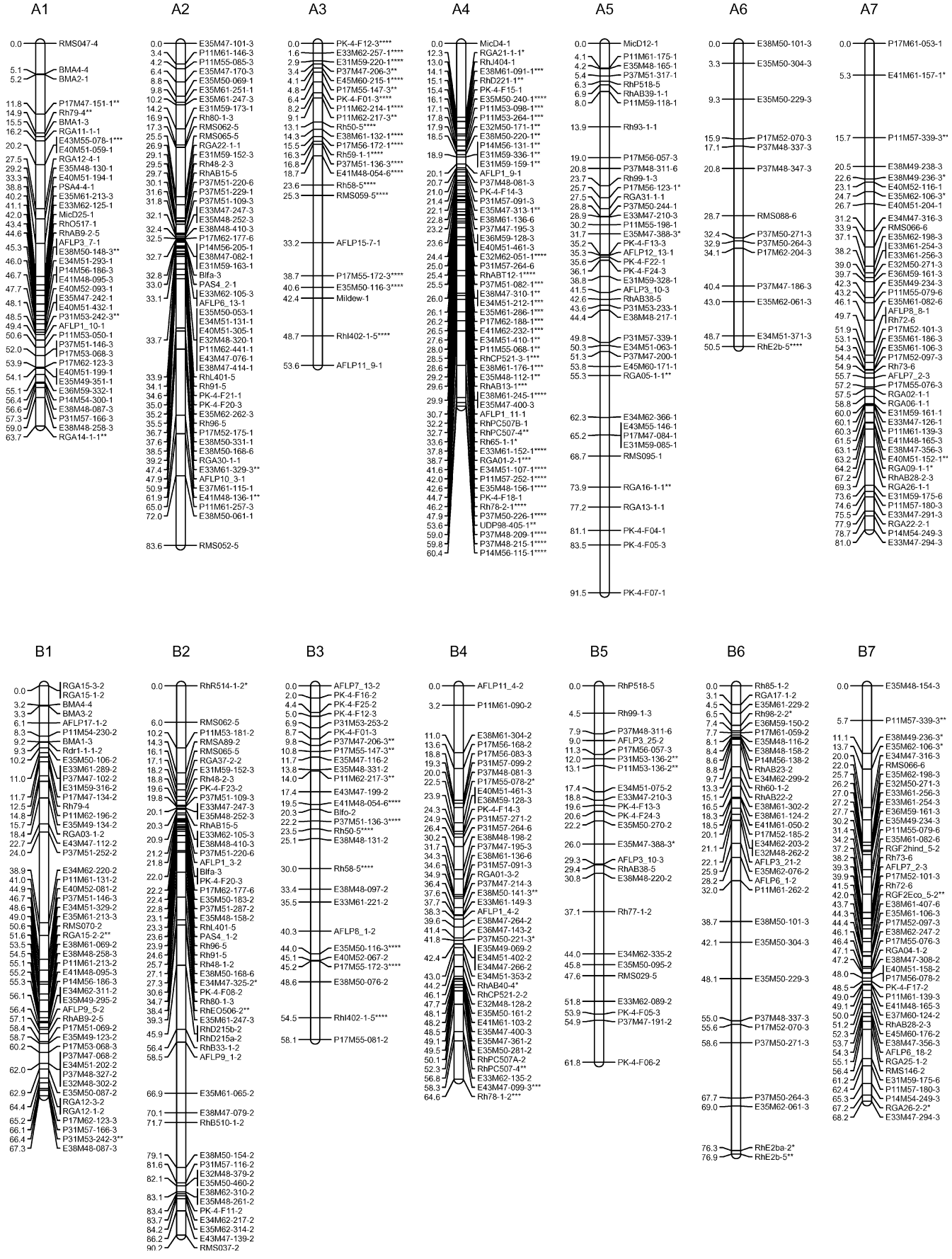

A1
0.0 RMS047-4
5.1 BMA4-4
5.2 BMA2-1
11.8 P17M47-151-1**
14.9 Rh79-4**
15.5 BMA1-3
16.2 RGA11-1-1
E43M55-078-1***
20.2 E40M51-059-1
27.5 RGA12-4-1
29.2 E35M48-130-1
33.3 E40M51-194-1
38.8 PSA4-4-1
40.2 E35M61-213-3
41.1 E33M62-125-1
42.0 MicD25-1
43.4 RhO517-1
44.6 RhAB9-2-5
AFLP3_7-1
45.3 E38M50-148-3**
46.0 E34M51-293-1
46.7 P14M56-186-3
E41M48-095-3
47.7 E40M52-093-1
48.1 E35M47-242-1
E40M51-432-1
48.5 P31M53-242-3**
49.4 AFLP1_10-1
50.6 P11M53-050-1
52.0 P37M51-146-3
P17M53-068-3
53.9 P17M62-123-3
54.1 E40M51-199-1
E35M49-351-1
55.1 E36M59-332-1
56.4 P14M54-300-1
56.6 E38M48-087-3
57.3 P31M57-166-3
59.0 E38M48-258-3
63.7 RGA14-1-1**
A2
0.0 E35M47-101-3
3.4 P11M61-146-3
4.2 P11M55-085-3
6.4 E35M47-170-3
8.8 E35M50-069-1
9.8 E35M61-251-1
10.2 E35M61-247-3
14.2 E31M59-173-1
16.9 Rh80-1-3
17.3 RMS062-5
25.5 RMS065-5
26.9 RGA22-1-1
29.1 E31M59-152-3
29.5 Rh48-2-3
29.7 RhAB15-5
30.1 P37M51-220-6
31.6 P37M51-229-1
31.8 P37M51-109-3
E33M47-247-3
32.1 E35M48-252-3
32.4 E38M48-410-3
32.5 P17M62-177-6
P14M56-205-1
E38M47-082-1
32.7 E31M59-163-1
32.8 Blfa-3
33.0 PAS4_2-1
33.1 E33M62-105-3
AFLP8_13-1
E35M50-053-1
E34M51-131-1
E40M51-305-1
33.7 E32M48-320-1
P11M62-441-1
E43M47-076-1
E38M47-414-1
33.9 RhL401-5
34.1 Rh91-5
34.6 PK-4-F21-1
35.0 PK-4-F20-3
35.2 E35M62-262-3
35.5 Rh96-5
36.7 P17M52-175-1
37.6 E38M50-331-1
38.5 E38M50-168-6
39.2 RGA30-1-1
47.4 E33M61-329-3**
47.9 AFLP10_3-1
50.9 E37M61-115-1
61.9 E41M48-136-1**
65.0 P11M61-257-3
72.0 E38M50-061-1
83.6 RMS052-5
A3
0.0 PK-4-F12-3****
1.6 E33M62-257-1****
2.9 E31M59-220-1****
3.4 P37M47-206-3**
4.1 E45M60-215-1****
4.8 P17M55-147-3**
6.4 PK-4-F01-3****
8.2 P11M62-214-1****
9.1 P11M62-217-3**
13.1 Rh50-5****
14.3 E38M61-132-1****
15.5 P17M56-172-1****
16.3 Rh59-1-1****
16.8 P37M51-136-3****
18.7 E41M48-054-6****
23.6 Rh58-5****
25.3 RMS059-5****
33.2 AFLP15-7-1
38.7 P17M55-172-3****
40.6 E35M50-116-3****
42.4 Mildew-1
48.7 RhI402-1-5****
53.6 AFLP11_9-1
A4
0.0 MicD4-1
12.3 RGA21-1-1*
13.0 RhJ404-1
14.1 E38M61-091-1***
15.1 RhD221-1**
15.4 PK-4-F15-1
16.1 E35M50-240-1****
17.1 P11M53-098-1***
17.8 P11M53-264-1***
17.9 E32M50-171-1**
18.5 E38M50-220-1**
P14M56-131-1**
18.9 E31M59-336-1**
E31M59-159-1**
20.1 AFLP1_9-1
20.7 P37M48-081-3
21.0 PK-4-F14-3
21.4 P31M57-091-3
22.1 E35M47-313-1**
22.8 E38M61-136-6
23.2 P37M47-195-3
23.6 E36M59-128-3
E40M51-461-3
24.4 E32M62-051-1****
25.0 P31M57-264-6
25.4 RhABT12-1****
25.5 P37M51-082-1***
26.0 E38M47-310-1**
E34M51-212-1***
26.1 E35M61-286-1***
26.2 P17M62-188-1***
26.5 E41M62-232-1***
27.6 E34M51-410-1**
28.0 P11M55-068-1**
28.5 RhCP521-3-1***
28.6 E38M61-176-1***
29.2 E35M48-112-1**
29.6 RhAB13-1***
29.9 E38M61-245-1****
E35M47-400-3
30.7 AFLP1_11-1
32.2 RhPC507B-1
32.7 RhPC507-4**
33.6 Rh65-1-1*
37.8 E33M61-152-1****
38.7 RGA01-2-1***
41.6 E34M51-107-1****
42.0 P11M57-252-1****
42.6 E35M48-156-1****
44.7 PK-4-F18-1
46.2 Rh78-2-1****
47.9 P37M50-226-1****
53.6 UDP98-405-1**
59.0 P37M48-209-1****
59.8 P37M48-215-1****
60.4 P14M56-115-1****
A5
0.0 MicD12-1
4.1 P11M61-175-1
4.2 E35M48-165-1
5.4 P37M51-317-1
6.3 RhP518-5
6.9 RhAB39-1-1
8.0 P11M59-118-1
13.9 Rh93-1-1
19.0 P17M56-057-3
20.8 P37M48-311-6
23.7 Rh99-1-3
25.7 P17M56-123-1*
27.5 RGA31-1-1
28.8 P37M50-244-1
28.9 E33M47-210-3
30.2 P11M55-198-1
31.7 E35M47-388-3*
35.2 PK-4-F13-3
35.3 AFLP12_13-1
35.6 PK-4-F22-1
36.1 PK-4-F24-3
38.8 E31M59-328-1
41.5 AFLP3_10-3
42.6 RhAB38-5
43.6 P31M53-233-1
44.4 E38M48-217-1
49.8 P31M57-339-1
50.3 E34M51-063-1
51.3 P37M47-200-1
53.8 E45M60-171-1
55.3 RGA05-1-1**
62.3 E34M62-366-1
E43M55-146-1
65.2 P17M47-084-1
E31M59-085-1
68.7 RMS095-1
73.9 RGA16-1-1**
77.2 RGA13-1-1
81.1 PK-4-F04-1
83.5 PK-4-F05-3
91.5 PK-4-F07-1
A6
0.0 E38M50-101-3
3.3 E35M50-304-3
9.3 E35M50-229-3
15.9 P17M52-070-3
17.1 P37M48-337-3
20.8 P37M48-347-3
28.7 RMS088-6
32.4 P37M50-271-3
32.9 P37M50-264-3
34.1 P17M62-204-3
40.4 P37M47-186-3
43.0 E35M62-061-3
48.7 E34M51-371-3
50.5 RhE2b-5****
A7
0.0 P17M61-053-1
5.3 E41M61-157-1*
15.7 P11M57-339-3**
20.5 E38M49-238-3
22.6 E38M49-236-3*
23.1 E40M52-116-1
24.7 E35M62-106-3*
26.7 E40M51-204-1
31.2 E34M47-316-3
33.9 RMS066-6
37.1 E35M62-198-3
38.2 E33M61-254-3
E33M61-256-3
39.0 E32M50-271-3
39.7 E36M59-161-3
42.3 E35M49-234-3
43.2 P11M55-079-6
46.1 E35M61-082-6
AFLP8_8-1
49.7 Rh72-6
51.9 P17M52-101-3
53.1 E35M61-186-3
54.3 E35M61-106-3
54.4 P17M52-097-3
54.9 Rh73-6
55.7 AFLP7_2-3
57.2 P17M55-076-3
57.5 RGA02-1-1
58.8 RGA06-1-1
60.0 E31M59-161-1
60.1 E33M47-126-1
60.3 P11M61-139-3
61.5 E41M48-165-3
63.1 E38M47-356-3
63.2 E40M51-152-1**
64.2 RGA09-1-1*
67.2 RhAB28-2-3
69.3 RGA26-1-1
73.6 E31M59-175-6
74.6 P11M57-180-3
75.5 E33M47-291-3
77.9 RGA22-2-1
78.7 P14M54-249-3
81.0 E33M47-294-3
B1
0.0 RGA15-3-2
RGA15-1-2
3.2 BMA4-4
3.3 BMA3-2
6.1 AFLP17-1-2
8.3 P11M54-230-2
9.2 BMA1-3
9.3 Rdr1-1-1-2
10.2 E35M50-106-2
E33M61-289-2
11.0 P37M47-102-2
E31M59-316-2
11.7 P17M47-134-2
12.5 Rh79-4
14.8 P11M62-196-2
15.7 E35M49-134-2
18.4 RGA03-1-2
22.7 E43M47-112-2
24.0 P37M51-252-2
38.9 E34M62-220-2
41.0 P11M61-131-2
44.9 E40M52-081-2
46.7 P37M51-146-3
48.6 E34M51-329-2
49.0 E35M61-213-3
50.6 RMS070-2
51.6 RGA15-2-2**
53.5 E38M61-069-2
54.5 E38M48-258-3
55.1 P11M61-213-2
55.2 E41M48-095-3
55.3 P14M56-186-3
E34M62-311-2
56.1 E35M49-295-2
56.4 AFLP9_5-2
57.1 RhAB9-2-5
58.4 P17M51-069-2
58.7 E35M49-123-2
60.2 P17M53-068-3
P37M47-068-2
62.0 E34M51-202-2
P37M48-327-2
E32M48-302-2
62.9 E35M50-087-2
64.4 RGA12-3-2
RGA12-1-2
65.2 P17M62-123-3
66.1 P31M57-166-3
66.4 P31M53-242-3**
67.3 E38M48-087-3
B2
0.0 RhR514-1-2*
6.0 RMS062-5
10.2 P11M53-181-2
14.3 RMSA89-2
16.1 RMS065-5
17.1 RGA37-2-2
18.2 E31M59-152-3
18.8 Rh48-2-3
19.6 PK-4-F23-2
19.8 P37M51-109-3
E33M47-247-3
20.1 E35M48-252-3
20.3 RhAB15-5
E33M62-105-3
20.9 E38M48-410-3
21.2 P37M51-220-6
21.8 AFLP1_3-2
22.0 Blfa-3
PK-4-F20-3
22.2 P17M62-177-6
22.4 E35M50-183-2
22.8 P37M51-287-2
23.1 E35M48-158-2
23.3 RhL401-5
23.6 PAS4_1-2
23.9 Rh96-5
24.6 Rh91-5
25.7 Rh48-1-2
27.1 E38M50-168-6
27.3 E34M47-325-2*
30.6 PK-4-F08-2
34.7 Rh80-1-3
38.4 RhEO506-2**
39.3 E35M61-247-3
45.9 RhD215b-2
RhD215a-2
56.4 RhB33-1-2
58.5 AFLP9_1-2
66.9 E35M61-065-2
70.1 E38M47-079-2
71.7 RhB510-1-2
79.1 E38M50-154-2
81.6 P31M57-116-2
E32M48-379-2
82.1 E35M50-460-2
E38M62-310-2
83.1 E35M48-261-2
83.4 PK-4-F11-2
83.7 E34M62-217-2
84.2 E35M62-314-2
86.2 E43M47-139-2
90.2 RMS037-2
B3
0.0 AFLP7_13-2
2.0 PK-4-F16-2
4.4 PK-4-F25-2
5.0 PK-4-F12-3
6.9 P31M53-253-2
8.7 PK-4-F01-3
9.8 P37M47-206-3**
10.8 P17M55-147-3**
11.7 E35M47-116-2
13.8 E35M48-331-2
14.0 P11M62-217-3**
17.4 E43M47-199-2
19.5 E41M48-054-6****
20.3 Blfo-2
22.2 P37M51-136-3****
23.5 Rh50-5****
25.1 E38M48-131-2
30.0 Rh58-5****
33.4 E38M48-097-2
35.5 E33M61-221-2
40.3 AFLP8_1-2
44.0 E35M50-116-3****
45.1 E40M52-067-2
45.2 P17M55-172-3****
48.6 E38M50-076-2
54.5 RhI402-1-5****
58.1 P17M55-081-2
B4
0.0 AFLP11_4-2
3.2 P11M61-090-2
11.0 E38M61-304-2
13.6 P17M56-168-2
18.8 P17M56-083-3
19.3 P31M57-099-2
20.0 P37M48-081-3
22.5 P17M55-078-2*
E40M51-461-3
23.9 E36M59-128-3
24.3 PK-4-F14-3
24.9 P31M57-271-2
26.4 P31M57-264-6
30.2 E38M48-198-2
31.7 P37M47-195-3
34.3 E38M61-136-6
34.6 P31M57-091-3
34.9 RGA01-3-2
36.4 P37M47-214-3
37.6 E38M50-141-3**
37.7 E33M61-149-3
38.3 AFLP1_4-2
39.6 E38M47-264-2
41.4 E36M47-143-2
41.8 P37M50-221-3*
E35M49-069-2
42.4 E34M51-402-2
E34M47-266-2
43.0 E34M51-353-2
44.2 RhAB40-4*
46.1 RhCP521-2-2
47.7 E32M48-128-2
48.1 E35M50-161-2
48.2 E41M61-103-2
48.5 E35M47-400-3
49.1 E35M47-361-2
49.5 E35M50-281-2
50.1 RhPC507A-2
52.3 RhPC507-4**
56.8 E33M62-135-2
58.3 E43M47-099-3***
64.6 Rh78-1-2***
B5
0.0 RhP518-5
4.5 Rh99-1-3
7.9 P37M48-311-6
9.0 AFLP3_25-2
11.3 P17M56-057-3
12.0 P31M53-136-2**
13.1 P11M53-136-2**
17.4 E34M51-075-2
18.8 E33M47-210-3
19.6 PK-4-F13-3
20.6 PK-4-F24-3
22.2 E35M50-270-2
26.0 E35M47-388-3*
29.3 AFLP3_10-3
29.4 RhAB38-5
30.8 E38M48-220-2
37.1 Rh77-1-2
44.0 E34M62-335-2
45.8 E35M50-095-2
47.6 RMS029-5
51.8 E33M62-089-2
53.9 PK-4-F05-3
54.9 P37M47-191-2
61.8 PK-4-F06-2
B6
0.0 Rh85-1-2
3.1 RGA17-1-2
4.5 E35M61-229-2
6.5 Rh98-2-2*
7.4 E36M59-150-2
7.7 P17M61-059-2
8.1 E35M48-116-2
8.4 E38M48-158-2
8.6 P14M56-138-2
8.8 RhAB23-2
9.7 E34M62-299-2
13.3 Rh60-1-2
15.1 RhAB22-2
16.5 E38M61-302-2
18.3 E38M61-124-2
18.5 E41M61-050-2
20.1 P17M52-185-2
E34M62-203-2
21.1 E32M48-262-2
22.1 AFLP3_21-2
25.9 E35M62-076-2
28.2 AFLP6_1-2
32.0 P11M61-262-2
38.7 E38M50-101-3
42.1 E35M50-304-3
48.1 E35M50-229-3
55.0 P37M48-337-3
55.6 P17M52-070-3
58.6 P37M50-271-3
67.7 P37M50-264-3
69.0 E35M62-061-3
76.3 RhE2ba-2*
76.9 RhE2b-5**
B7
0.0 E35M48-154-3
5.7 P11M57-339-3**
11.1 E38M49-236-3*
13.7 E35M62-106-3*
20.0 E34M47-316-3
22.0 RMS066-6
25.7 E35M62-198-3
26.2 E32M50-271-3
27.0 E33M61-256-3
27.2 E33M61-254-3
27.7 E36M59-161-3
30.2 E35M49-234-3
31.4 P11M55-079-6
34.2 E35M61-082-6
37.2 RGF2hind_5-2
38.2 Rh73-6
39.3 AFLP7_2-3
39.9 P17M52-101-3
41.5 Rh72-6
42.0 RGF2Eco_5-2**
43.7 E38M61-407-6
44.3 E35M61-106-3
44.4 P17M52-097-3
46.1 E38M62-247-2
46.4 P17M55-076-3
47.1 RGA04-1-2
47.2 E38M47-308-2
E40M51-158-2
48.0 P17M56-078-2
48.5 PK-4-F17-2
49.0 P11M61-139-3
49.1 E41M48-165-3
50.0 E37M60-124-2
51.2 RhAB28-2-3
52.3 E45M60-176-2
53.7 E38M47-356-3
54.3 AFLP6_18-2
55.1 RGA25-1-2
56.4 RMS146-2
61.2 E31M59-175-6
62.4 P11M57-180-3
65.3 P14M54-249-3
67.2 RGA26-2-2*
68.2 E33M47-294-3

Table 3 Distribution of markers on parental maps (*A*and *B*) and linkage group statistics

Marker type	Linkage group														Total
	A1	B1	A2	B2	A3	B3	A4	B4	A5	B5	A6	B6	A7	B7	
AFLP	26	35	36	26	13	17	38	33	23	13	12	23	33	32	360
PK	0	0	2	4	2	4	3	1	6	4	0	0	0	0	26
SSR	5	3	9	17	5	3	10	5	7	5	2	7	4	6	88
RGA	3	6	2	1	0	0	2	1	4	0	0	1	5	4	29
Others	6	6	3	4	3	3	3	2	2	2	0	2	2	3	41
Total	40	50	52	52	23	27	56	42	42	24	14	31	44	45	542
Length in cM	64	67	84	90	54	58	65	65	91	62	51	77	81	68	977
Marker density (markers/cM)	0.6	0.7	0.6	0.6	0.4	0.5	0.9	0.6	0.5	0.4	0.3	0.4	0.5	0.7	
Average distance between markers	1.6	1.3	1.6	1.7	2.3	2.1	1.2	1.5	2.2	2.6	3.6	2.5	1.8	1.5	
Largest gap in cM between markers	7.3	14.9	11.6	8.4	7.9	5.9	12.3	7.9	8.0	6.9	7.9	9.1	10.4	5.7	

with a map length of 58–90 cM and a marker density of 0.4–0.7 markers per cM (Table 3).

Markers were randomly distributed with high and moderate density on the 14 parental linkage groups, but with a number of gaps ranging from 5.7 to 14.9 cM (Table 3). The clustering of markers was most prominent in the centre of the linkage groups, especially on linkage groups A1, A2, A4, A7, B1, B2, B4, B6 and B7. These locations presumably coincide with the centromeric regions. Bi-parental (common) markers colocalized well on the homologous parental linkage maps except for small variations in the order of the markers, especially in the marker-dense regions.

Nearly 85% of the AFLP markers were mapped on the parental maps. The distribution of the AFLP markers generated with the different enzyme combinations on the linkage maps was well spread and quite similar (Fig. 1). The codominantly scored AFLPs were assigned to all linkage groups except on groups 1 and 6. More than 80% of the SSR markers could be mapped and were distributed over all parental chromosomes. About 75% of the PK markers were assigned to linkage groups 2, 3, 4 and 5. Half of the RGA markers mapped on linkage groups 1, 2, 4, 5 and 7. Some clustering of the PK and RGA markers was observed on some regions of linkage groups A2, A5, A7, B1, B3 and B5. Most RFLP and SCAR markers mapped on linkage groups 1, 2, 3 and 7. The morphological marker *Rdr*1 was localized on B1, *Blfa* on A2 and B2, *Mildew* on A3 and *Blfo* on B3, which was in line with the chromosomal locations on the maps published by Debener and co-workers (1999, 2001). Markers with distorted segregation ($P \leq 0.05$) were assigned to most of the linkage groups with the majority on A3, A4 and B3 (Fig. 1)

Alignment of the maps

The integrated maps were aligned with the parental maps and presented with markers at about 2 cM intervals (Fig. 2). A majority of the common markers, especially the SSRs, showed colinearity between the maps, indicating a high reliability of the constructed maps. The set of 21 SSR markers (Table 2) are well spread across the seven chromosomes (Figs. 1 and 2) and can be used as anchor points to enable alignment of the present map with other rose maps. Using the AFLP bridge markers, the present parental maps aligned well with the core maps of Debener and Mattiesch (1999) (data not shown).

Genome coverage

Statistical estimation of the genome coverage was performed with repeated sampling of the markers mapped on both parental maps. The asymptotic upper limit was approximately 500 cM for both parental maps (Fig. 3), indicating that the total length of the rose genome was estimated to be about 500 cM. Both of the present parental maps would cover more than 95% of the diploid rose genome.

Discussion

Marker analysis

AFLP technology is considered to be an efficient marker platform due to its high multiplex ratio of markers, reliability, reproducibility and locus specificity (Pejic et al. 1998; Haanstra et al. 1999). However, in most cases AFLP markers can only be scored dominantly, often making them less informative and limiting their use as anchor points for map alignment. Codominant scoring of AFLP markers, nevertheless, can be achieved based on quantitative assessment of the optical density of bands on a gel or from the fluorescence level in a gel-free marker assay (Piepho and Koch 2000; Jansen et al. 2001; Geerlings et al. 2003), but literature references on their use for genetic mapping are still scarce (Castiglioni et al. 1999; Bradeen et al. 2001). In the present study 320 reliable AFLP markers were generated by using 56 different primer combinations. A large proportion of the markers (31%) was found to be bi-parental; only 11 markers could be scored codominantly. Scoring of these markers should be maximized by taking special precautions, e.g. the normalized peak heights of the

heterozygous individuals should be similar and about half the peak heights of the flanking monomorphic markers. The mapping of codominant AFLP markers in this study yielded additional anchor markers for the alignment of the parental maps. Therefore, codominant scoring may be considered as a good procedure to obtain more of this kind of markers for mapping studies, taking into account map position and suitability to score the markers, even though the analysis is time-consuming and perhaps error prone. The latter can, however, be minimized by dominant scoring of certain plants in case of doubt.

The SSR analysis performed in this study revealed a high fraction (57%) of SSRs with only two marker alleles. In contrast, SSR markers in similar studies in rose-related species usually yielded at least three alleles. For example, more than 75% of the SSRs in apple (Maliepaard et al. 1998; Liebhard et al. 2002) and 73% in *Prunus* (Aranzana et al. 2003) were multi-allelic. The reason for the lower number of multi-allelic SSR markers is most likely due to the fact that the parents are half sibs. This implies that both parents theoretically have one quarter of all alleles in common (Debener and Mattiesch 1999). The common ancestor of the population also explains the presence of a large proportion (22%) of type 3 markers (Table 1).

Nearly 22% of the present markers showed distorted segregation ($P \leq 0.05$), of which the larger part was contributed by the loci from the male parent P117. The linkage analysis showed that a high proportion of markers with distorted segregation were found on linkage groups 3 and 4, especially on A3, B3 and A4. A high frequency of markers showing distorted segregation is common in outcrossing species like pine (Kubisiak et al. 1995), willow (Hanley et al. 2002), peach (Dettori et al. 2001) and apple (Liebhard et al. 2002). The common origin as well as the distortion pattern of the markers indicates the presence of gametophytic selection for sub-lethal genes, i.e. coding factors controlling the viability of pollen, zygote or seedlings, putatively located on one or more of these chromosomes. This is in line with the observation that reciprocal crosses with the parents of this population and backcrosses of individual plants to both parents indicated the presence of a self-incompatibility system (data not shown).

Parental maps

In comparison with the rose maps published to date (Debener and Mattiesch 1999; Rajapakse et al. 2001; Crespel et al. 2002), the presented parental maps are quite dense and are well-covered with the different types of markers. The rose genome is small. A mean chiasma frequency of 1.4 per bivalent (Lata 1982) and an average nuclear DNA content of 1.1 pg/2C (Yokoya et al. 2000) observed in diploid rose species would suggest that the average chromosome length is only about 70–80 cM with a total of about 500 cM. This estimation is in agreement with the result of statistical simulation by using the markers mapped on the present parental maps, i.e. the asymptotic upper limit is about 500 cM for both parental maps. This implies that the present maps, having an average length of 70.4 cM, may cover more than 90% of the rose genome.

Reliability of the integrated map

The integrated map presented here was generated with a two-step strategy. Parental linkage groups were first constructed using different types of segregation markers and then homologous linkage groups were merged using the JoinMap program. These maps, resulting from pre-grouping of uni-parental markers, are more reliable than maps generated with mixed markers since the estimates of the recombination rate and the determination of marker linkage phase for dominantly scored bi-parental markers were less accurate (data not shown).

The accessibility of common markers and especially codominant ones allows not only the identification of homologous linkage groups but also the integration of both parental maps (Qi et al. 1996). For most of the linkage groups, the order of the markers on the integrated map was consistent with the marker orders observed in the individual parental linkage groups, apart from some minor differences on some linkage groups, for example on two regions of linkage group 2. Inconsistencies like these are, however, not alarming since usually a number of almost equivalent marker orders exists, i.e. orders that fit the data equally well. As the algorithm for marker ordering of the JoinMap program does not guarantee the best solution to make integrated maps (Stam 1993), the ''fixed order'' option was used to solve the differences. As all the markers were mapped with high LOD scores (LOD $\geq$5.0), the chi-square value was low ($\chi^2 \leq 3.0$) and the orders of the majority of common markers were similar in all maps, we conclude that the integrated map has a high reliability.

Prospects for the maps

Genotyping of the mapping population with new markers and the integration of these with existing markers (Debener and Mattiesch 1999; Debener et al. 2001) in both parental and integrated maps in this study has led to a dense and reliable map of rose, which aligns well with the maps published by Debener and Mattiesch (1999). Both parental and integrated maps are useful for genetic analysis in rose. The parental maps will facilitate separate QTL analysis of the variation present in individual parents, while the integrated map allows a simultaneous analysis of QTLs from both parents (Maliepaard and Van Ooijen 1994; Hanley et al. 2002).

In the well-documented species *A. thaliana*, protein kinases and RGAs are large gene families involved in many biological processes in plants (*Arabidopsis* Gen-

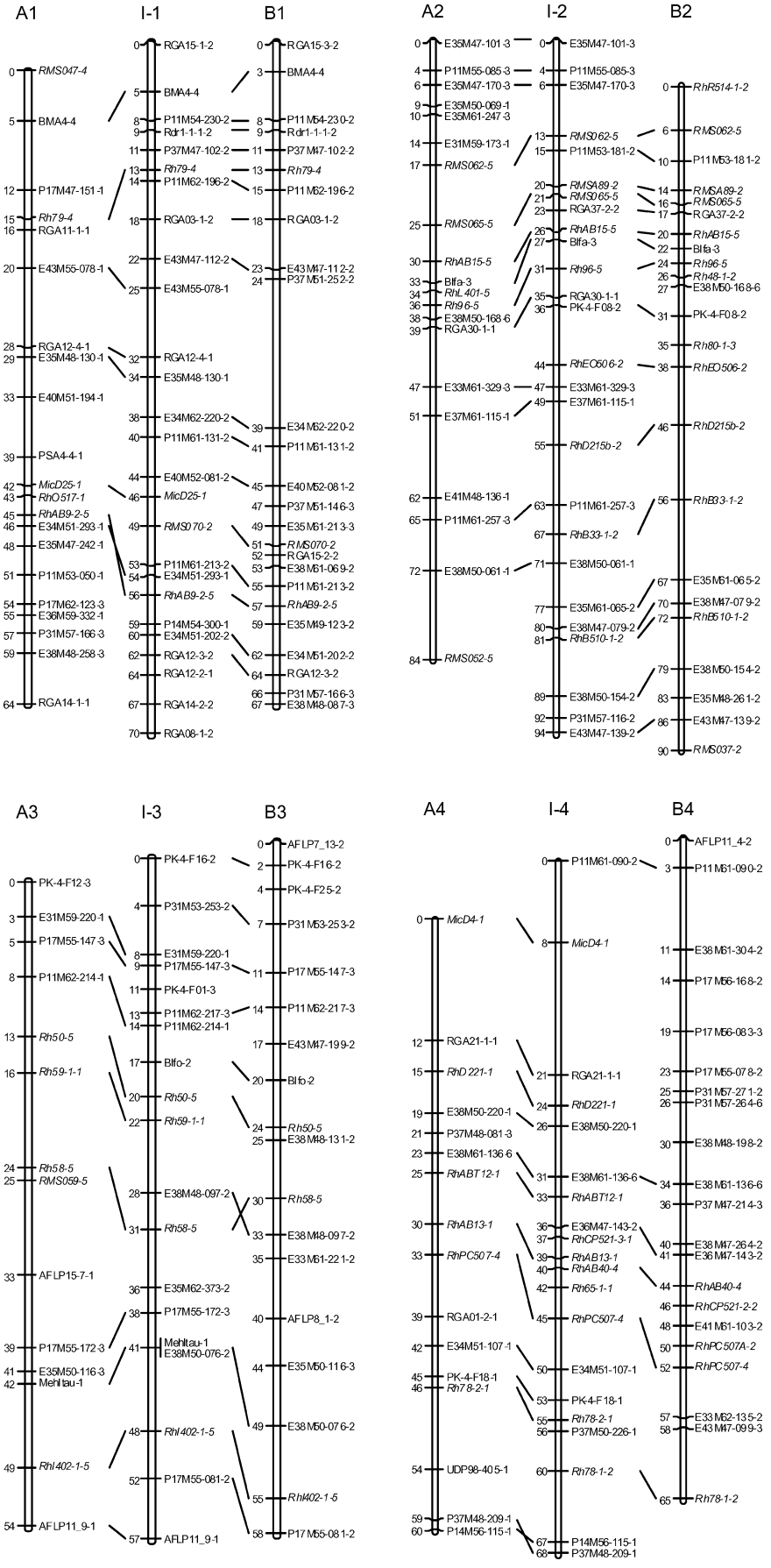
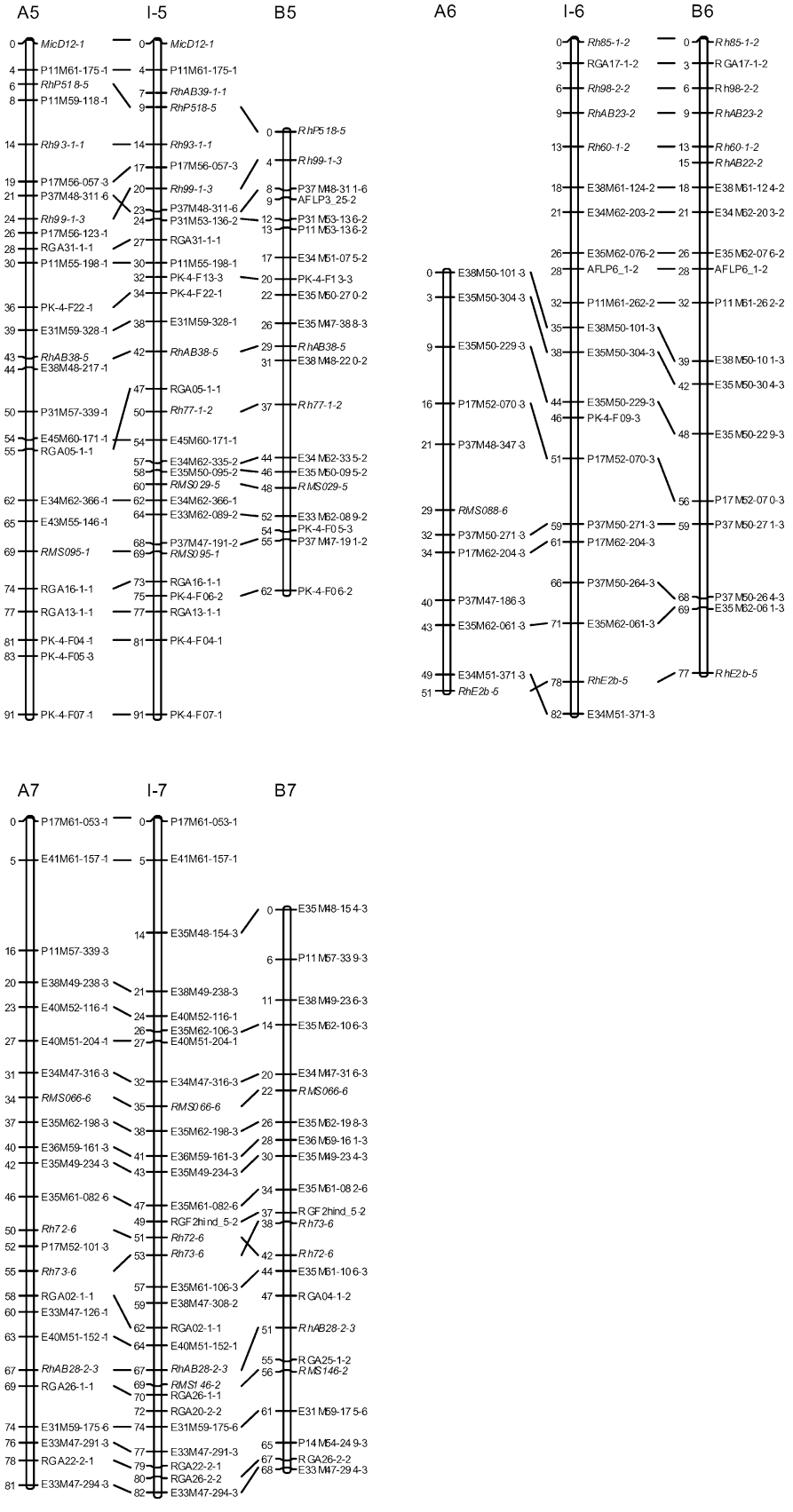


Fig. 2 Alignment of parental (*A* and *B*) and integrated (*I*) maps. For ease of survey only the markers at a distance of ~2 cM are shown on each linkage group. Marker names are indicated at the *right* of each linkage group. For details of the marker nomenclature see "Materials and methods". Distances are given in Kosambi cM at the *left* of each linkage group. SSR markers are printed in *italic*. Segregation distortion is indicated with *asterisk(s)* for the significance level of the chi-square test: $*p \leq 0.05$, $** p \leq 0.01$, $*** p \leq 0.005$, $*** p \leq 0.001$. Corresponding markers are indicated by *solid lines* between maps

Fig. 2 (Contd)



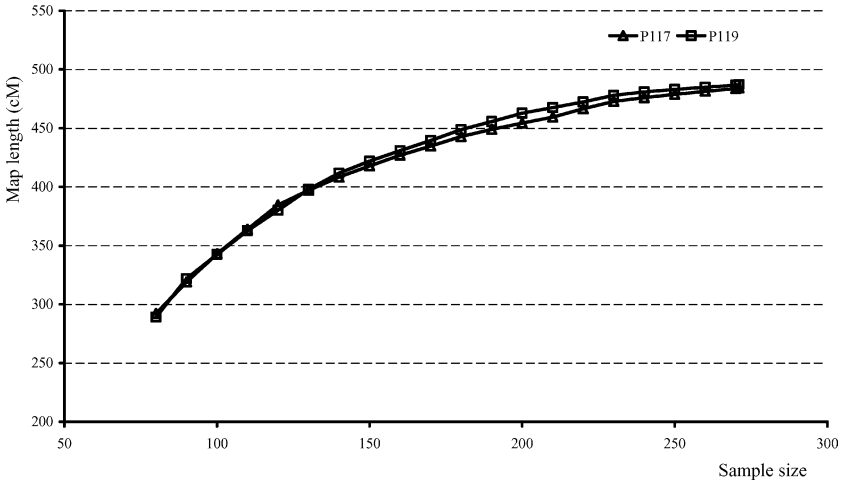


Fig. 3 Estimation of genome coverage with the parental maps. Genome coverage with the parental maps (P119 and P117) was estimated by repeated sampling of markers from the maps without replacement. The average map length covered by a single marker sample of a given size was first calculated. The average coverage of the maps was based on 20,000 samples. The asymptotic upper limit was estimated by increasing the sample size up to the actual number of markers in the maps and by fitting an exponential curve to the relation between sample size and average map length covered

ome Initiative 2000). For example, protein kinases play a crucial role in the self-incompatibility systems of plants (Nasrallah et al. 1994), plant hormone activation (Machida et al. 1997) and incompatible plant-pathogen interactions (Vallad et al. 2001). Therefore, the mapped PK and RGA markers in the vicinity of genes or QTLs for plant growth and defence especially for resistance to pathogens (Van der Linden et al. 2004) are interesting candidate markers for use in marker-assisted selection.

Currently, a co-operative effort is being made with several research groups acting in rose genetics to establish a consensus map for rose by future integration of the ongoing mapping studies using common SSR markers as anchor points. Finally, the addition of gene-based markers to the present map may provide a good starting point for comparative mapping with other rose related species like *Prunus*, *Malus*, *Fragaria* and well-documented species like *Arabidopsis*. This facilitates the research of genes associated with traits of interest for rose breeding and allows the identification of useful universal genes.

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