# Towards a unified genetic map of diploid roses

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**Abstract** We have constructed the first integrated consensus map (ICM) for rose, based on the information of four diploid populations and more than 1,000 initial markers. The single population maps are linked via 59 bridge markers, on average 8.4 per linkage group (LG). The integrated map comprises 597 markers, 206 of which are sequence-based, distributed over a length of 530 cM on seven LGs. By using a larger effective population size and therefore higher marker density, the marker order in the ICM is more reliable than in the single population maps. This is supported by a more even marker distribution and a decrease in gap sizes in the consensus map as compared to the single population maps. This unified map establishes a standard nomenclature for rose LGs, and presents the location of important ornamental traits, such as self-incompatibility, black spot resistance (*Rdr1*), scent production and recurrent blooming. In total, the consensus map includes locations for 10 phenotypic single loci, QTLs for 7 different traits and 51 ESTs or gene-based molecular markers. This consensus map combines for the first time the information for traits with high relevance for rose variety development. It will serve as a tool for selective breeding and marker assisted selection. It will benefit future efforts of the rose community to sequence the whole rose genome and will be useful for synteny studies in the Rosaceae family and especially in the section Rosoideae.

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## Introduction

Genetic linkage maps serve as structural frameworks to locate single genes, for QTL analyses, as starting point for map-based cloning of genes, and as versatile tools for genome sequencing in many plant and animal species. But the benefits from the linkage map information remain limited by the genetic background of the population that was mapped. To overcome this restriction consensus or integrated linkage maps have been constructed for many cultivated plant species including peach (Dirlewanger et al. 2004), apple (N'Diaye et al. 2008), grapevine (Doligez et al. 2006; Vezzulli et al. 2008), soybean (Cregan et al. 1999), rapeseed (Lombard and Delourme 2001), barley (Wenzl et al. 2006), red clover (Isobe et al. 2009), eucalyptus (Brondani et al. 2006), and loblolly pine (Sewell et al. 1999). Integrated maps not only increase the genome coverage but also make it possible to compare locations of major genes controlling important phenotypic traits or QTL positions between populations from multiple crosses. Although small chromosome rearrangements have been detected in

some closely related species of the Pinaceae (Pelgas et al. 2006) comparative mapping in the Rosaceae genus *Prunus* showed a very high degree of colinearity between the genomes of the diploid species peach, almond, apricot, and cherry (Dirlewanger et al. 2004). Furthermore consensus maps of various genera of the Rosaceae have been linked with a conserved orthologue set of sequence-based markers (Sargent et al. 2009).

In roses more than 20 major genes controlling various flower, plant or resistance traits have been mapped (reviewed in Byrne 2009), several BAC libraries (Kaufmann et al. 2003; Hess et al. 2007; Biber et al. 2010) have been constructed and multiple linkage maps have been created. The first map was constructed mostly with RAPD and AFLP markers in 1999, using the diploid population 94/1 (Debener and Mattiesch 1999), which was improved by adding a large number of markers comprising SSRs by Yan et al. (2005) 6 years later. This map used a *R. multiflora*-derived population with 88 $F_1$ plants and has 520 marker loci. The next published map was developed in a tetraploid population of 52 $F_2$ plants by Rajapakse et al. (2001). The third map (HW) was constructed with AFLP markers by using a diploid population of 91 individuals resulting from an interspecific cross between *R. wichurana* and the dihaploid hybrid H190 (Crespel et al. 2002). It was enriched by Hibrand-Saint Oyant et al. (2008) and Remay et al. (2009) with 45 rose SSRs, 38 rose EST SSRs, 6 Rosaceae EST SSRs and 30 flowering candidate genes, for both parental maps. In 2005 another mapping population was established based on a *R. wichurana* cross with 'Basye's Thornless' (Dugo et al. 2005). The population consisted of 96 $F_1$ individuals and the linkage map contained mainly RAPD markers. Linde et al. published in 2006 a linkage map for the diploid population 97/7, which was also *R. multiflora* derived. Genotyping for this map was done in 170 of the 270 phenotyped progenies. Currently under construction is a map in a tetraploid population in the Netherlands, which is segregating for powdery mildew resistance, based on 184 individuals using NBS markers and SSRs (Koning-Boucoiran et al. 2009). Major genes and QTLs for many phenotypic traits have been mapped in all these populations, but these are only useful in the particular genetic background of that specific population, even though they are of general interest for rose breeding (see Byrne 2009).

The aim of this study was to add transferable SSR markers from four genetic diploid rose maps to construct an integrated consensus map (ICM) for these populations. The SSRs that are shared between maps serve as bridge markers for the consensus map. Overall more than 100 already published SSR primers were tested for their segregation in the diploid populations 94/1 (Debener and Mattiesch 1999), 97/7 (Linde et al. 2006), HW (Hibrand-Saint Oyant et al. 2008) and OBxWOB26 (Tsai and Byrne, unpublished

data). Fifty-nine of these could be used for the ICM. Because of the importance of disease resistance genes in plants, we added markers based on NBS profiling (Van der Linden et al. 2004) and mapped 43 NBS-LRR markers. This integrated map allows a comparison of linkage groups (LGs) and the positions of major genes and QTLs among four rose maps. It will also validate the order of the anchor markers especially on the maps derived from smaller populations by using the more reliable genetic information from larger mapping populations. The map establishes the first consensus numbering of the LGs for roses, and will facilitate both comparative genetics within the Rosaceae and future genome sequencing of the rose.

## Materials and methods

### Plant origins

All populations used here have been described: 94/1 (93/1-117 × 93/1-119) by Yan et al. (2005), 97/7 (95/13-39 × 82/78-1) by Linde et al. (2006), HW (H190 × hybrid *R. wichurana*) by Crespel et al. (2002) and OBxWOB26 ['Old Blush' × (*R. wichurana* × 'Old Blush')] by Shupert et al. (2007). Except for the *R. multiflora* background of the 94/1 and 97/7 populations, the genotypes were not related to each other. The progenies 94/1, 97/7 and HW are $F_1$ populations and OBxWOB26 is a backcross of one genotype of the cross between *R. wichurana* 'Basye's Thornless' and 'Old Blush' to 'Old Blush'.

Plants were cultivated in green houses under semi-controlled conditions, or under field conditions. DNA extractions were performed as described in Yan et al. (2005) for 94/1, in Linde et al. (2006) for 97/7, in Hibrand-Saint Oyant et al. (2008) for HW and in Kiani et al. (2008) for OBxWOB26.

### Genetic markers

The AFLP, SSR and other markers used in map construction are described in various publications (Table 1). The sequences of the ''Rh''-SSR primers used in this study are available upon request from Plant Research International, The Netherlands. The "RMS"-SSR primers used in this study are published at http://www.wipo.int/pctdb/en/wo.jsp?wo=2003097869&IA=WO2003097869&DISPLAY=STATUS.

### Sequence-based markers

Several new markers for populations 94/1 and 97/7 were developed using sequences of genes with known functions. For this approach, primers for functional genes and for ESTs with sequence similarity to known genes (Spiller

**Table 1** Coding of AFLP, SRR and disease resistance-related markers is shown in the column "Designation" followed by the marker type, mapping population and reference

| Designation | Marker type | Population | References |
|---|---|---|---|
| ExMy.z | AFLP | HW | Crespel et al. (2002) |
| PxMy-z | AFLP | 94/1 | Yan et al. (2005) |
| ExMy-z | AFLP | 94/1 | Yan et al. (2005) |
| C*-**-39 or -Sp3 | AFLP | 97/7 | Linde et al. (2006) |
| Rh* | SSR | 94/1 | Yan et al. (2005) |
| RMS* | SSR | 94/1 | Yan et al. (2005) |
| Rw#E# | SSR | HW | Hibrand-Saint Oyant et al. (2008) |
| C# | SSR | HW | Hibrand-Saint Oyant et al. (2008) |
| CL# | SSR | HW | Hibrand-Saint Oyant et al. (2008) |
| HxFy | SSR | HW | Hibrand-Saint Oyant et al. (2008) |
| BFACT*/BPPCT* | SSR | HW | Remay et al. (2009) |
| CTG# | SSR | HW | Hibrand-Saint Oyant et al. (2008) |
| NBS* | NBS-LRR | 97/7 | Unpublished |
| PK-z-Fy | Protein kinase | 94/1 | Yan et al. (2005) |
| 1g1-Dx-Ky-* or D1-D1-* | RGA | 97/7 | Linde et al. (2006) |
| RGA#_# | RGA | 94/1 | Terefe and Debener (2010) |

x, y, z, #, * placeholders for any number or letter in series of markers

et al. 2010) were designed using Primer3 (http://frodo.wi.mit.edu/primer3/). These primers were tested with the parental genotypes and a few progeny plants of the mapping populations for the presence of SCARs or CAPS, and subsequently for the presence of sequence polymorphisms via SSCP gel analysis (Orita et al. 1989). SSCP analysis was performed as described in Yan et al. (2005). PCR products were verified by sequencing. These markers were named according to the internal EST database where they were derived from with the letters "T_", "G_" or "c". The development of sequence-based markers for candidate genes of floral initiation and development in the HW population (named "Ro-") was described in Remay et al. (2009). Some additional genes were added from the MASAKO gene family (Kitahara and Matsumoto 2000; Remay et al. 2009). Also, three markers were developed for sequences of EST BAC contigs of the *Prunus* T × E reference map (Dirlewanger et al. 2004). In these map, these BACs flank the *S*-locus. TE3-SSCP primer sequences (TE3f: GCCTTTTCAGCTTGCAAAAGA, TE3r: TTTACGATCCAAACCGACCAG9) were derived from the EST sequence PP_LEa0010K05f. Primers for TE22_2 (TE22f: TGATAGATGCGGCTTCTCAA, TE22r: GGCCTCATAATGCAGGGTAA) were based on sequence PP_Lea0009M17f, and TE39 primers were designed using PP_LEa0003D12f (TE39f: TTGGAGGACGAGCTTCAACT; TE39r: CCCGACAAACCAGATCAAGA).

NBS profiling in population 94/1

NBS profiling was performed in a two-step PCR procedure (Mantovani et al. 2006; Van der Linden et al. 2004). In brief, 400 ng DNA per individual were digested during 4 h with *Rsa*I or *Hae*III, and an adapter was ligated to the restriction fragments (Adapter long arm: 5′-CTCGATTCTCAACCCGAAAGTATAGATCCCA-3′; Adapter short arm: 5′-TGGGATCTATACTT-3′, with 3′-amino group). Three degenerate primers, NBS1 (5′-GCIARWGTWGTYTTICCYRAICC-3′), NBS3 (5′-GTWGTYTTICCYRAICCISSCATICC-3′), and NBS5A/6 (a mixture of 5′-YYTKRTHGTMITKGATGAYGTITGG-3′ and 5′-YYTKRTHGTMITKGATGATATITGG-3′) were designed on a part of the conserved P-loop motif to amplify DNA towards the 5′-end of the targeted genes, outside the NBS domain (Van der Linden et al. 2004). The first PCR was linear as it was carried out with only one primer in the reaction, namely one of the three degenerate NBS primers. The second PCR was exponential, i.e. it was performed with the same degenerate NBS primer and an adapter primer (5′-ACTCGATTCTCAACCCGAAAG-3′). Both PCR reactions were performed with an annealing temperature of 60°C, using the following cycling program: 30 cycles of 30 s 95°C, 1 min 40 s 60°C and 2 min 72°C. The final PCR products were labelled by primer extension, using [$^{33}$P]-ATP end-labelled NBS primers for ten cycles at conditions similar to that of the linear PCR. PCR products were separated on 6% polyacrylamide gels for 3 h at 110 W. Gels were then transferred to 3 MM paper covered with plastic wrap and exposed to Kodak Xomat films (New Haven, CT, USA). Results were scored using QuantarPro (Keygene, Wageningen, The Netherlands).

Mapping process

SSR patterns were scored (co)dominantly, and then tested for Mendelian segregation using a chi-square test. For all

mapping populations, markers with data for <80% of the individuals were excluded. Linkage analysis was carried out using JoinMap4 (Van Ooijen 2006) with the following settings: grouping with linkage LOD option, mapping with a recombination frequency threshold of 0.3 and LOD of 2 using the mapping function of Kosambi (1944). Overall grouping LOD was 7, except for the OBxWOB26 population for which a LOD of 3 was used. This was necessary due to the small number of markers in this dataset. The homologous LGs for all four populations and the consensus map were numbered LG1–LG7 to standardize the naming across populations. This order follows Yan et al. (2005) and Linde et al. (2006).

After calculating independent maps for each of the populations, the homologous LGs were integrated in a second step according to the *pseudo test cross* strategy (Stam 1993). The markers that disturbed the order of the consensus markers and those which were not located on the map during the first round of integration were excluded, except markers needed as bridge markers. The LGs for the BC$_1$ population OBxWOB26 were calculated in a single step.

To facilitate the integration of the maps we limited the marker density to one marker per cM for each single map. This was done to reduce the computing time needed for the calculation. Again, markers were excluded if they were not mapped during the first round of integration or severely disturbed the marker order. Charts were generated using Map-Chart Version 2.1 (Voorrips 2002).

For a better overview we only show (1) sequence-based markers including the bridge markers, (2) phenotypic traits, and, (3) AFLP markers every five cM if needed to close gaps (Fig. 1). Linkage maps of the single populations displaying all markers can be found in the supplementary material (Fig. S1).

## Results

We used the information of more than 1,000 markers to calculate seven integrated consensus LGs (Fig. 1). A large number of mostly dominant markers had to be removed from the single population datasets, either because they were evaluated for too few individuals (<80% of the population size) or because they could not be mapped during the first round of integration. We thus removed about 30% of the markers from the parental datasets of the populations 94/1 and 97/7 and 16 and 18% of the parental markers for the populations OBxWOB26 and HW, respectively (Table 2). As a prerequisite for the integration we needed sufficient markers shared between populations. We generated them by analysing the same sequence-based markers in all populations. For population 97/7 additional NBS markers were added as well. Due to this process, the map

**Fig. 1** Integrated consensus map (ICM) of roses. Map distances are ▶ shown in cM as a ruler at the *left page margin*. Indicated in *bold* are bridge markers which link at least two single population maps. Abbreviations of mapped genes and traits: RhACS, ACC-synthase; PAR, phenylacetaldehyde reductase; CCD, carotenoid cleavage dioxygenase; RoLHP, *TERMINAL FLOWER 2*; RoSOC, *SUPPRESSOR OF CONSTANS*; TPS, terpene synthase; RoLE, *LATE EMBRYOGENE-SIS*; RoGA20ox, gibberellic acid 20 oxidase; RGA, resistance gene analogue; Rd1, CAPS marker linked to *Rdr1*; RoTFL, *TERMINAL FLOWER*; RcOMT, caffeoyl-*o*-methyltransferase; RoAP, *APETALA*; RoGA3ox, gibberellic acid 3 oxidase; RoAXR, *AUXIN RESISTANT*; *Blfa*, petal colour; RhAAT, alcohol acyltransferase; gg358-F3H, flavonol-3-hydroxylase (Dani Zamir, Hebrew University of Jerusalem, Rehovot, Israel); RhOOMT, orcinol-*o*-methyltransferase; RhETR, ethylene receptor; SI, self-incompatibility; RoVIP, *VERNALIZATION INDEPENDENCE*; RoDELLA, repressor of gibberellic acid; RoSPIN-DLY, *SPINDLY*; RB, recurrent blooming; TE, Texas × Earlygold (see text); *Blfo*, double flowers; RoGID, *GIBBERELLIN INSENSI-TIVE*; RoIAA, *INDOLE-3-ACETIC ACID INDUCIBLE*; RoCOL, *CONSTANS-LIKE*; RoFT, *FLOWERING LOCUS T*; EOMT, eugenol-*o*-methyltransferase; AAT, alcohol acyltransferase; RoGA2, ent-kaurene synthase; pchcms3, a prunus BAC contig; RGAb, repressor of Ga1-3; RoGA2ox, gibberellic acid 2 oxidase; RhAADC, phenylethanol synthase; GDS, germacrene D synthase; RoGI, *GIGANTEA*; POMT, phloroglucinol-*o*-methyltransferase; RoEMF, *EMBRYONIC FLOWER*; RoSLEEPY, *SLEEP*; RoLFY, *LEAFY*. QTL positions are indicated for powdery mildew resistance, scent metabolites, petal number, days to flowering, prickles, and growth vigour

length for population 97/7 increased by about 10% whereas the mean marker distance decreased from 1.8 to 1.5 cM. The new calculated integrated map for the HW population had approximately the same length as the published parental maps from Hibrand-Saint Oyant et al. (2008) but also a decreased mean marker distance. In comparison to the map from Yan et al. (2005), the length of the 94/1 map decreased by 16% to 458 cM while the mean marker distance increased from 1.05 to 1.32 cM because 30% less markers were mapped. The size of the largest gaps in all the maps, which have been published before, decreased drastically by between 21 and 48%. With 89 sequence-based markers the map of the population OBxWOB26 includes the lowest number of loci resulting in a short map length of 286 cM (Table 2).

The ICM of diploid rose includes 597 markers, 206 of which are sequence-based, distributed on an overall length of 530 cM (Table 2). Therefore, this map is 20% longer than the average of the saturated maps of the populations 94/1, 97/7 and HW. The largest group is LG4 with 96 cM and 104 markers; the shortest is LG7 with 67 markers covering 61 cM (Fig. 1). The average marker distance is 0.88 cM with lower distances on LG2 with 0.6 cM/marker. Significantly higher distances of on average 1.9 cM/marker were observed on LG6, which has a consistently smaller number of markers compared to other LGs in all investigated populations. On most of the LGs the markers are distributed evenly with some clustering of markers on LG1 and LG2.
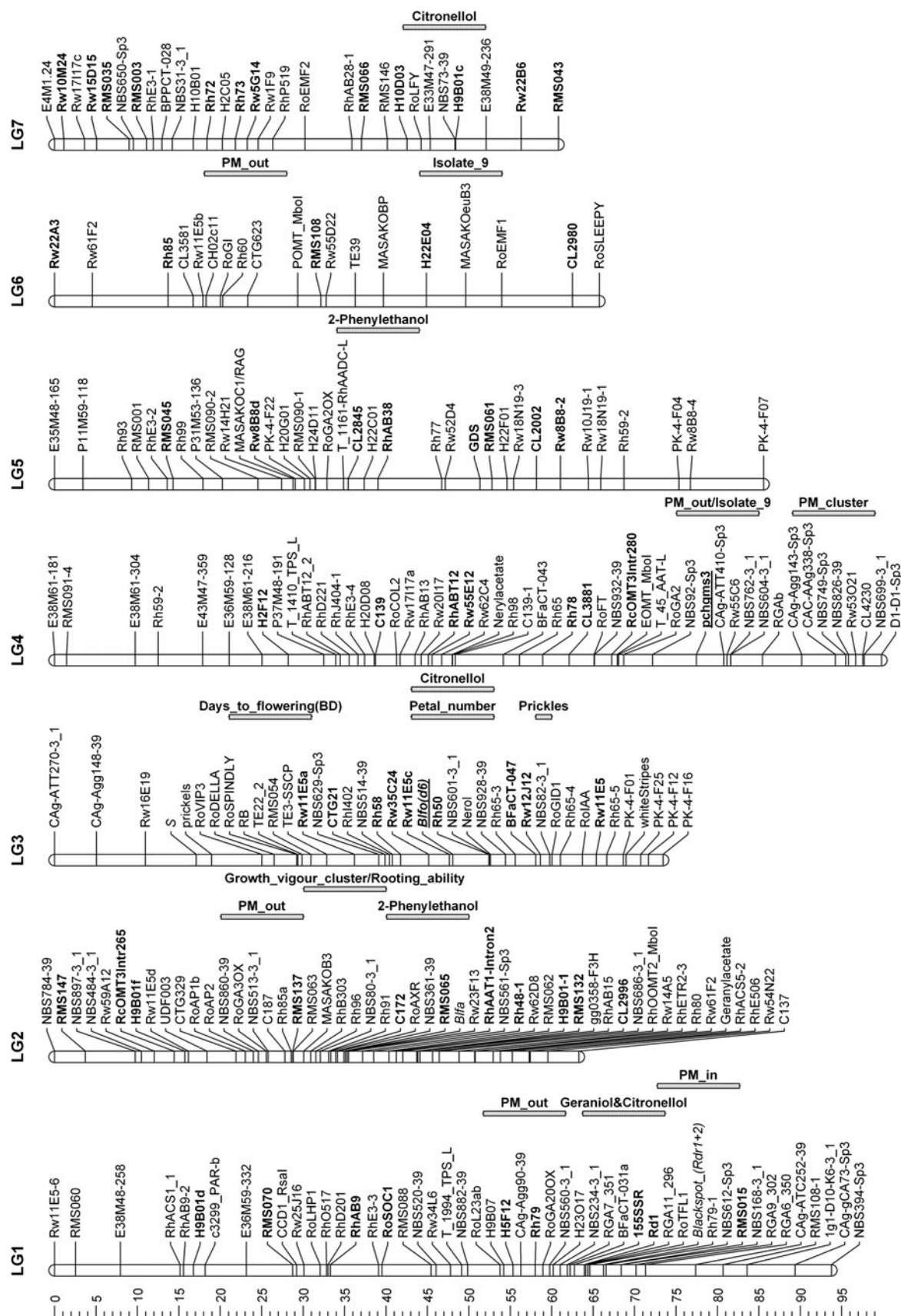
**Table 2** Summary data of the consensus map (ICM) and the currently calculated single population maps in comparison to the originally published maps

| Population | OBxWOB26 | 94/1 | | 97/7 | | HW | | ICM |
|---|---|---|---|---|---|---|---|---|
| Map version | Current | Yan et al. | Current | Linde et al. | Current | Hibrand-Saint Oyant et al., Remay et al. | Current | |
| Pop. size[a] | 99 | 88 | 88 | 170 | 170 | 91 | 91 | −/− |
| Marker no.[b] | 89 | 520 | 346 | 232 | 309 | 241 | 248 | 597 |
| Map length | 286 | 545 | 458 | 418 | 462 | 432/438 | 438 | 530 |
| Distance[c] | 3.21 | 1.05 | 1.32 | 1.80 | 1.50 | 3.0–4.0 | 1.77 | 0.88 |
| Largest gap[d] | 23 | 19 | 15 | 21 | 13 | 27 | 14 | 8 |

*ICM* integrated consensus map

[a] Size of population used in mapping

[b] Number of markers in the map

[c] Mean distance between markers in the map in cM/marker

[d] Largest distance between markers in cM

For 59 sequence-based bridge markers we could determine map positions in at least two populations. The ICM contains on average 8.4 anchor markers per LG (Table S1). Of the 59 bridge markers 23% (14 loci) were present in three of the mapping populations. Four connecting markers were present on all maps. Across all populations LG7 had the largest number of common markers (11). The LG with the smallest number of connecting markers was LG6, with only six SSRs bridging the maps. LG6 was difficult to identify in population OBxWOB26 and showed a low recombination frequency in all investigated populations.

Populations 94/1 and 97/7 shared 21 anchor points, 20 markers connected population 94/1 to population HW, and 17 to population OBxWOB26. Population 97/7 is linked via 14 bridge markers to population HW and via 9 markers to population OBxWOB26. Populations HW and OBxWOB26 are connected by 29 bridge markers. To increase the number of markers connecting the populations we tested 27 SSRs from the HW map of Hibrand-Saint Oyant et al. (2008) in the populations 94/1 and 97/7. Of these markers 11 could be mapped to the homologous LGs of these two populations. In parallel 18 "Rh-" and 12 "RMS-" microsatellite markers were tested on population HW, of which 17 were mapped. The OBxWOB26 dataset was created with SSR data for 102 sequence-based markers, taken from all available SSR pools (Rh-, RMS-, Rw-EST-sequence data). Using the location of these newly established markers we were able to identify the homologous LGs in the WOB population (Table S1). Among the 59 bridge markers only five showed segregation patterns indicating double- or multiple-loci. Only few changes in the order of the bridge markers were observed between the four separate population maps (Fig. S1). On LG5 RMS061 and germacrene D synthase (GDS) displayed reverse orientation between the 94/1 and 97/7 maps. On LG3 we detected

a difference in the order of the marker Rh50 and the *Blfo* locus between populations 94/1 and HW. On the LG with the highest marker density, LG2, we observed a distorted order for four bridge loci connecting the four single population maps.

Fifty-one ESTs and gene-based markers (Table 3) could be mapped on the ICM. We observed a concentration of ESTs related to floral identity and development on LG3 and 6. The 15 ESTs for floral scent volatile production were distributed over all LGs. On LG1 and 2, three genes for ethylene production or perception in roses [*RhACS1*, *RhACS5* and *RhETR2* (Mibus and Serek 2005)] were mapped.

We also located 43 NBS-LRR markers targeting putative resistance (R)-genes on the ICM. Remarkably no NBS markers mapped on LGs5 and 6, while six NBS markers clustered within 2 cM on LG2. We also could map 25 RGA markers (markers linked to resistance gene analogues), with 8 of them on LG1. In the lower part of LG1 the black spot resistance locus, derived from population 97/7, is flanked by 17 NBS markers and RGAs in close proximity. So, one quarter of all mapped resistance-related markers is located within a 50 cM region around the black spot resistance locus. Of these 17, 5 RGAs are derived from the Rd1LRR microsatellite marker (Terefe and Debener 2010) targeting the highly conserved LRR region of the 9 completely sequenced *Rdr1* paralogues. These five map within 10 cM around the black spot locus.

In addition to the black spot resistance locus, nine other single loci controlling different phenotypic traits could be located in the ICM (Table 3). The gene for pink flower colour, *Blfa*, was mapped on LG2 together with one locus for geranyl acetate production and one candidate gene for production of alcohol acetates. A second putative geranyl acetate locus was located on LG7. Loci controlling other floral traits such as the *Blfo* locus for double flowers and the locus

**Table 3** Phenotypic and genotypic traits, which are available on the consensus map via the homologous LGs of the single integrated rose maps

| Linkage groups consensus map | Linkage groups 94/1 Yan et al. (2005), Yan et al. (2007), Spiller et al. (2010) | Linkage groups 97/7 Linde et al. (2006), Spiller et al. (2010) | Linkage groups Hw Hibrand-Saint Oyant et al. (2008), Remay et al. (2009) | Linkage groups OBxWOB26 Tsai et al. (unpublished data) |
|---|---|---|---|---|
| LG1 | LG1: geraniol (QTL), RoSOC1, RhPAR, RhCCD1 | LG1: Rdr1 | LG1: RoLHP1, RoTFL1, RoGA20OX, RoSOC1 | Chr1 |
| LG2 | LG2: geranyl acetate, Blfa, RhAAT1, RcOMT3-1, RhOOMT1, RA (QTL, Langner, personal communication), main QTL cluster for growth vigour | LG2: RhAAT1, RcOMT3-1 | LG2: RoELF8, RoGA3OX, RoAP1b, MASAKOB3 | Chr2 |
| LG3 | LG3: SI, Blfo, nerol, $\beta$-citronellol (QTL) | LG3: SI, Blfo, prickles, whitestripes, PM (QTL), Rpp1 | LG4: Blfo, RB, PN (QTL), DF (QTL): RoVIP3, RoGID1, RoDELLA, RoSPINDLY | Chr3 |
| LG4 | LG4: neryl acetate, RcOMT3-2 | LG4: PM (QTL), RhEOMT (RcOMT1), RcOMT3-2 | LG3: RoCOL2, RoFT | Chr4 |
| LG5 | LG5: phenylethanol (QTL), GDS | LG5: GDS, RhAADC | LG5: RoEMF2, RoLFY, RoAP1a | Chr5 |
| LG6 | LG6: POMT | LG6: PM (QTL) | LG7: RoGI, MASAKOBP, MASAKOeuB3, RoEMF1, ROSLEEPY | Chr7 |
| LG7 | LG7: alcohol acetate production | | LG6: RoGAOX, RoRAG | Chr7 |

Designation of the LGs in the columns follows the original publications, thus providing a key to the consensus numbering of LGs

*Blfa* flower colour, *RA* rooting ability, *Blfo* double corolla, *SI* self-incompatibility, *Rdr1* black spot resistance, *Rpp1* powdery mildew resistance, *PM* powdery mildew, *RB* recurrent blooming, *PN* petal number, *DF* date of flowering (BD)

for the occurrence of white-striped flowers from population 97/7 were located on LG3. LG3 contains the locus for recurrent blooming (RB) cosegregating with the gibberellin signalling gene *RoSPINDLY* and close to the *RoDELLA* locus. The self-incompatibility-related loci *S*, which was phenotypically mapped, and TE3-SSCP, derived from the *S*-locus of the "Texas × Earlygold" reference map for *Prunus*, are located on LG3 as well.

## Discussion

### Single population maps

In order to construct an integrated rose consensus map we recalculated the four separate population maps. We added several new anchor markers to bridge every LG of the four investigated populations. Grouping for all mapping populations was done using a linkage LOD of 7, except for the OBxWOB26 caused by the smaller marker number of this population. To get a reliable marker order in the single population maps we excluded molecular markers that were not mapped in the second and third round of calculations in JoinMap 4 (Van Ooijen 2006), using a threshold of 0.3 for the recombination frequency and an LOD of 2. Therefore, we had to remove 30% of the molecular markers in the single population map for the population 94/1. This large percentage probably resulted from the very high number of markers (520) in this population combined with a quite low number of individuals (88). The exclusion of one-third of markers possibly reduced the inflated length of this map, which putatively was due to conflicting recombination data for single loci in the mapping process. In the populations 97/7, OBxWOB26 and HW the number of removed markers was lower, probably because of the better ratio of number of markers to number of progeny in these populations. The much larger size of the 97/7 (170 individuals) improved the stability of the marker order for the parental maps and also for the ICM. This coherence could also be shown by simulation studies using different population structures (BC, $F_2$ and RIL) with dominant and codominant markers and progeny sizes from 50 to 1,000 individuals by Ferreira et al. (2006), concluding that the reliability of a genetic map is strongly depending on the size of the experimental populations and therefore on the number of crossing over events which could be studied. Ferreira et al. (2006) also reviews, that marker order inversions in genetic maps of all population types are a general problem in population sizes of about 100 individuals, generated both by the too small size of the population and the marker saturation of the map. This effect of population size versus number of markers is also expressed in the reduction of gap sizes up to 48% in the 94/1, 97/7 and HW linkage maps compared to the

already published ones. Thus for our $F_1$ populations, the accuracy of marker order and distance strongly depends on the population size, especially when dominant markers are used. This has previously been reported for $F_2$ and RIL populations (Ferreira et al. 2006; Huhn and Piepho 2008). Currently most rose linkage maps depend on progeny sizes up to 96 individuals or even less but have marker numbers between 133 and 520 (reviewed in Debener and Linde 2009). Also in the largest existing mapping population, the 97/7 with 170 genotyped plants and now 309 markers, a stable marker order could not be resolved for dense map regions all the time. Inconsistencies in marker orders were also observed in other studies most likely due to the too small progeny sizes of the mapping populations (N'Diaye et al. 2008; Doligez et al. 2006), which means that few recombination events exist in large regions of the map, in combination with scoring errors and/or artifacts of the consensus mapping algorithm (Isobe et al. 2009). Based on these experiences, we are currently increasing the number of genotyped individuals to several hundred in the populations 97/7 and HW for the sequence-based markers and some of the AFLP markers. We expect that this will enable us to develop a much improved map, in which more markers can be combined with a smaller size in cM.

We detected differences in the transferability of the SSR markers between the different populations caused by various reasons. Only 50% of the SSR markers from Hibrand-Saint Oyant et al. (2008), originally mapped in HW, could be mapped in the populations 94/1 and 97/7, but more than 80% of the Rh- and RMS-markers from the 94/1 and 97/7 populations could be amplified and were successfully mapped in the HW population. The poor rate of mapping success of the SSR markers from the HW population may partly be due to technical reasons. To transfer the HW SSRs to the populations 94/1 and 97/7 a different method, the M13 labelling technique according to Schuelke (2000), was used, because this strategy saves costs for the PCR reactions. The Rh- and RMS-markers were tested as directly IRDye labelled PCR primers on the HW population in the French lab. Using rose DNA M13-PCRs according to Schuelke (2000) was known to perform somewhat worse, sometimes resulting in no or poor amplification even in repeated experiments, than working with directly labelled PCR primers. Another reason for the difference in success of transfer of the markers could be a difference in the genetic diversity of the populations. The diversity might be higher in the HW population, as it was derived from an interspecific cross of the dihaploid H190 (obtained from haploidisation of the tetraploid *Rosa hybrida* cv. Zambra, Meynet et al. 1994) and a hybrid of a diploid *R. wichurana* genotype. In contrast, the 94/1 and 97/7 both originate from intraspecific crosses (Yan et al. 2005; Linde et al. 2006). Also the origin of the SSR markers could play a role. The

H-, C- and CTG-markers are obtained from EST sequences, which may mean that they are less polymorphic than genomic SSRs, and hence the chance of them being not polymorphic in another population may be much higher.

A skewed segregation for the markers of the LGs3 and 4 was detected in all single population maps and therefore also resolved in the ICM. For these LGs segregation distortion was also reported for the previously constructed maps of populations 94/1 (Yan et al. 2005) and HW (Hibrand-Saint Oyant et al. 2008). This is most probably caused by the action of a gametophytic self-incompatibility system located on LG3. The effect of the gametophytic self-incompatibility system was recently shown in population 94/1 by backcross experiments with both parental plants (Debener et al. 2010) leading to the phenotypic marker *S* (mapped in Fig. 1). Additional molecular markers, TE3-SSCP and TE22_2, derived from the TEXAS × EARLYGOLD *Prunus* reference map (Dirlewanger et al. 2004) could be mapped on this LG. The EST sequences that were used to design these primer pairs are linked to the *S*-locus in *Prunus* without any recombination. All markers surrounding the *S*-locus in the ICM of rose possess a similar segregation distortion as the corresponding self-incompatibility-region in *Prunus*.

Features of the integrated consensus map

The aim of this study was to establish an ICM bridging four individual maps with a reliable order of anchor markers and consistent LG designation, but not the construction of an ultra dense linkage map for roses. Therefore, the marker number and density in the ICM is only slightly higher than in the 94/1 map of Yan et al. (2005) but the marker order is significantly more reliable, because a much higher number of individuals (Vezzulli et al. 2008) and higher number of recombination events was taken into account in the combined four populations. The length of the ICM is 530 cM, which is about 17% larger than the values from the recalculated single maps for 94/1, 97/7 and HW populations (Table 2). Between 10 and 30% increased map lengths have also been reported for integrated maps in other species. The integrated apple map (N'Diaye et al. 2008) is about 20% longer than three of the four single population maps. In grapevine Vezzulli et al. (2008) presented a 30% longer integrated map and Wenzl et al. (2006) a 10% increase for barley. The length of the integrated sorghum map (Mace et al. 2009) is in the range of the single population maps. Part of this increase may be due to an improved coverage of the ends of the chromosomes. Regions missing in some of the single maps, as for example, the upper half of LG4 in the 97/7 or the lower 20 cM of LG5 in populations 97/7 and 94/1, and the upper 20 cM on the same LG in the HW population are now covered in the ICM. Also LG6, which showed a low recombination rate in all populations, result-

ing in unstable marker positions because of a small number of informative loci in these groups, is much better resolved in the consensus map. The ICM LG6 contains 37 markers (23 shown in Fig. 1), 25% more than in the best saturated LG6 from all single maps (LG6 of population 97/7 with 29 markers).

With an overall length of 530 cM the ICM of rose is close to the value from Yan et al. (2005), who estimated genome coverage being 95% for diploid roses for such a map length. The genome coverage of the ICM has been improved in comparison to the single maps. The largest gap is only 8 cM and the mean distance of markers is 0.88 cM. This value is lower than that of any single map.

We observed a clustering of 30 markers originally mapped in populations 94/1 and 97/7 to a short interval from 33 to 38 cM in the middle of LG2 of the ICM and a minor one with 13 in a 2 cM region near the middle of LG5. The markers in the clusters are mostly AFLP and NBS markers. On all of the other LGs no significant marker clusters were observed. A clustering of markers, especially of AFLP markers, in centromeric regions of the chromosomes has been reported for many plant species and is sometimes attributed to AT-rich regions in combination with low recombination in pericentric parts of the chromosomes (reviewed in Saal and Wricke 2002). Therefore, the clustering may be caused by these factors or by the large number of markers mapped on a small number of individuals in these populations. A cluster of NBS markers may indicate a cluster of disease resistance genes. This could be the case for the lower part of LG4, in which two QTLs for powdery mildew resistance together with five NBS markers are located.

As in most other ICMs we observed a few inversions in the marker order of the consensus map as compared to the individual maps. This could be partly explained by different recombination events in the different species from which the populations are derived, especially when blocks of markers are inverted. But most probably the observed inversions are also due to the small progeny sizes in the separate populations, as these cause problems in the mapping of marker dense regions in these maps. Therefore, the marker order in the consensus map is estimated to be much more reliable than in the single population maps, because it results from a higher effective population size and a higher marker density, and so it provides a more reliable framework for precise mapping (Vezzulli et al. 2008).

During this study 28 new bridge markers were transferred between the populations, bringing the total number to 59 bridging markers. This has allowed to identify the homologous LGs of all four populations and to give them a similar designation. A lack of bridging markers in a region of about 15 cM from the telomeric ends of two of the LGs was detected in the ICM. In all other regions the bridge markers are quite evenly distributed over the LGs and support the correct order of the other markers. Missing of bridge markers at the ends of LGs was also observed by N'Diaye et al. (2008) in apple with 90 bridge markers for 17 LGs. The lack of bridge markers in telomeric regions was also observed in sorghum with as many as 251 bridge markers for 4 of the 10 chromosomes (Mace et al. 2009), and in red clover with 260 bridge markers on 7 LGs spanning 836.6 cM (Isobe et al. 2009). Therefore, the number of bridge markers may has to be very high in order to cover all telomeric regions of the LGs.

## Major genes and QTLs

Using the information available from the three populations 94/1, 97/7 and HW we were able to map 62 ESTs, gene-based markers and single loci for important phenotypic traits in one genetic map (Table 3). This provides reliable and detailed information about the possible linkage between these loci, which were previously mapped in different populations. Loci controlling floral development (*RoDELLA*, *RoSPINDLY*, *RoGID1*) and vernalization response (*RoVIP3*) are located on LG3 together with a locus controlling RB, and a QTL for the number of petals and for flowering date, as previously described in Remay et al. (2009). Interestingly these loci map in-between two loci involved in self-incompatibility (*S*, TE3-SSCP) and within 20 cM distance of the *Blfo* locus, which controls the switch from single to double flowers. A locus controlling the presence of double flowers in *Prunus* is mapped on an unknown position on LG G2 on the T × E reference map separated from the *S*-locus from almond and apricot on the lower end of group G6 on the same comparative map (Dirlewanger et al. 2004). In addition to the molecular bridge markers, the homologous LGs could also be verified by the co-localization of phenotypic traits, either QTL or single gene markers, also in cases where the original LG numbering had not revealed this. The presence/absence of prickles on LG3 (Linde et al. 2006) turns out to co-localize with the major QTL for number of prickles (on LG B4 from the map of Crespel et al. 2002), and major loci for the number of petals were mapped on LG3 in populations 94/1, 97/7, as well as HW (*d6* in Crespel et al. 2002; *NP* in Hibrand-Saint Oyant et al. 2008; *Blfo* in Debener and Mattiesch 1999; Dugo et al. 2005).

Disease resistance to different fungal pathogens like black spot, powdery and downy mildew are important traits for cultivated roses. Three major genes for resistance against *Diplocarpon rosae* Wolf have been described for roses. *Rdr1* and *Rdr2* (Von Malek and Debener 1998; Hattendorf et al. 2004) are probably located in the same cluster on LG1 of the ICM, whereas *Rdr3* (Whitaker et al. 2010) possibly represents a different locus but is not yet intro-

duced into a genetic map. A different approach beyond mapping single loci by resistance screens in mapping populations is the amplification of resistance gene-related sequences such as RGAs or NBS markers. In addition to the already established RGAs in the 94/1 (Yan et al. 2005) and 97/7 (Linde et al. 2006) populations we mapped 80 new NBS markers in the population 97/7. Integration into the consensus map was possible for 43 NBS markers and 25 RGAs. We observed some clustering of RGA and NBS markers on LGs1, 2 and 3. The largest cluster is located around the black spot locus (*Rdr1*) on LG1. This locus determines the resistance against *D. rosae* race DortE4 (Debener et al. 2001). Within 10 cM around the black spot locus, five RGA markers were mapped. These correspond to five of the nine *Rdr1* paralogues isolated from the *R. multiflora* hybrid 88/124-46 cluster within 200 kb of the BAC constructs, amplified with the Rd1LRR primers (Terefe and Debener 2010).

For the resistance to powdery mildew ten QTL regions (named PM) were identified by Linde et al. (2006) in the population 97/7 under six different environments. Seven of these intervals could be located on LGs1–4, 6, and 7 of the ICM using the molecular markers from the 97/7 population. Comparing the positions of these powdery mildew resistance QTLs with the currently mapped NBS markers, we detected ten NBS markers in these regions. This suggests that some of the QTLs for powdery mildew resistance may also be from the NBS-LRR class of disease resistance genes, as seen for the *Rdr1* resistance gene against *D. rosae*.

Exploitation of the integrated consensus map

In the current approach we have integrated the marker and phenotypic data available for the four populations 94/1, 97/7, HW and OBxWOB26 into one consensus map, providing the first consensus numbering of the LGs for roses. In addition, we propose a set of publically available SSR markers (Supplementary Table S1) that are polymorphic in various rose species and LG specific (Fig. 1). They can be used for all future genetic mapping studies, for diversity studies in rose species, or possibly for the characterization of rose varieties. This map also serves as a bridge to the published rose maps. We mapped eight anchor points to the molecular map by Zhang et al. (2006) from a tetraploid rose progeny. Also the LG D10-2 from Dugo et al. (2005) could be linked to our consensus LG3 by the SSRs Rh50 and Rh58 flanking the *Blfo* locus mapped in the populations 94/1, 97/7 and HW. Out of the seven LGs for *Rosa* six are already linked to LGs of other Rosaceae linkage maps (Table S3). Connecting markers were EST BACs from *Prunus*, *Fragaria* and *Malus* listed in the Genome database for Rosaceae (http://www.rosaceae.org). Homology searches using EST

data of roses in the completely sequenced *Fragaria* and *Prunus* genomes have additionally shown a high degree of conservation.

LG1 of the rose ICM is linked via BFaCT-031 to group 7 of the *Fragaria* and *Prunus* maps. Also, on this LG a block of six genes flanking the *Rdr1*-region on LG1 of the rose ICM was completely conserved in *Fragaria* and *Prunus* even in the order of the coding sequences (Terefe, unpublished results). This corresponds to loci for resistance against powdery mildew and root nematodes which are located on G7 of *Prunus*. LG2 containing the *Blfa* locus for pink/white flower colour of rose is only connected to the Fragaria group 5 by the EST UDF003.

Synteny studies with *Prunus* already conducted in the 94/1 population, have led to the identification of the homologous *S*-region (Debener et al. 2010) which is located on the rose ICM LG3. Homologous regions are mapped on group 6 of *Fragaria*, *Prunus* (almond, apricot, peach, and sweet cherry), and on group 17 of *Pyrus*. However, the *Blfo* locus for double flowers is located on this LG in roses but on group 2 of peach. This may be a consequence of several chromosomal rearrangements between the Rosoideae and the Prunoideae which have already been observed between *Fragaria* and *Prunus* (Vilanova et al. 2008).

Markers pchgms3 and BFaCT-043 connect the rose LG4 with the groups 1 of almond, peach, strawberry and the T × E reference map of *Prunus*. Whereas no species connecting markers are located on LG5 of the ICM, is LG6 linked via EST CH02c11 to the groups 10 of apple and pear, and LG7 of rose ICM is connected to B1 of apricot by EST BPPCT-028.

However, for a better comparison of the rose ICM to the other Rosaceae genomes it is necessary to map more anchor markers between species. Therefore, the next step is to use the information of the sequenced genomes of *Malus*, *Prunus* and *Fragaria* to develop further connecting markers.

Attempts to link the rose ICM to a strawberry linkage map using the *Fragaria* binset (Sargent et al. 2008) have already been started, but were hindered up to now by the poor amplification rate (only 10%) of the genomic rose microsatellites in strawberry DNA (data not shown).

Given the long term and cost-intensive process of rose breeding, the positioning of genes regulating important phenotypic traits and QTLs in comparison to transferable SSRs in the ICM, will be very helpful to facilitate marker assisted selection in this major horticultural crop. Additionally, a consensus map is a required prerequisite to prepare a complete sequencing of the whole genome of rose.

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