CONSTRUCTION OF THE DIPLOID, TETRAPLOID AND INTEGRATED DIPLOID-TETRAPLOID GENETIC LINKAGE MAPS IN ROSES USING SIMPLE SEQUENCE REPEAT (SSR) MARKERS

A Dissertation

by

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Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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December 2013

Major Subject: Horticultural Sciences

ABSTRACT

This study uses polymorphic microsatellites (SSR) to elucidate the similarities among the diploid and tetraploid rose genomes by comparing their maps and clarifying the predominant inheritance patterns (disomic versus tetrasomic) seen in the tetraploid population.

One hundred and eight out of 175 SSRs were polymorphic in both the OBxWOB26 (Old Blush x ('Basye's Thornless' x 'Old Blush') diploid backcross population and the GGFC ('Golden Gate' x 'Fragrant Cloud') tetraploid full-sib population. Of these 69 fluorescently labeled SSRs and 5 morphological traits were used which generated 107 loci and 5 trait loci with 99 diploid population progeny. The tetraploid map was constructed with SSRs and AFLPs with 131 tetraploid progeny using the single dose restriction fragment (SDRF) analysis. The degree of preferential chromosome pairing in the tetraploid population was examined by looking at the segregation ratios among the double-dose markers (DDMs) as well as the ratio of loci in repulsion vs coupling phase using single-dose markers (SDMs). These approaches showed that there was a combination of disomic and tetrasomic inheritance.

A diploid, a tetraploid and an integrated diploid-tetraploid genetic linkage map were developed from two populations using JoinMap 4 with the cross pollination option. In the diploid map, 7 integrated linkage groups covered a length of 352.3 cM with an

average chromosome size of 50.3 cM. The morphological traits, prickles on stem (*prickles*), recurrent bloom (*RB*)) and flower type (*Blfo*) were mapped on the Chr LG3 which matched with the ICM (Integrated consensus map) published by Spillers et al., (2010). Moreover, 5 out of the 69 SSR markers (RhJ404, H9_B01, RW11E5, RW8B8 and RhE3) were mapped to two or more loci each on different chromosomes of the diploid map. In the tetraploid map, 174 out of 346 (50%) loci of single-dose markers (SDMs) and double-dose markers (DDMs) were mapped on a length of 883.4 cM with 9 linkage groups. Sixty anchor SSR markers were used to join the diploid and tetraploid maps which included 215 loci with a map length of 632 cM. Synteny of common SSRs and morphological traits, *prickles*, *RB*, *Blfo*, powdery mildew resistance (*PM*) and petal number (*PN*) on the integrated diploid-tetraploid map with the ICM, the GGFC and the K5 map demonstrated the collinear alignment among these maps.

DEDICATION

I dedicate my dissertation work to my parents.

Without you, I would not be here.

ACKNOWLEDGEMENTS

I would like to express my deepest appreciation to my advisor, Dr. David Byrne, for his excellent guidance and support on my research and writing as well as his patience, encouragement and assistance when I faced the frustration in my life. I would like to thank my committee members, Dr. Hongbin Zhang, Dr. Stephen King, and Dr. Terri Starman for their support on my research as well as their professional knowledge and critical review of my dissertation. Heartfelt thanks to Dr. Patricia Klein in the Bourlaug Center for providing the software JoinMap 4 for the analysis of my mapping data.

Special thanks to my lab mates and friends, Natalie, Matthew, Mahnaz, Soni and Jeremy working in Rose Breeding and Genetics program at Texas A&M University. Thanks to my best friend, Yun-Hua, for her friendship and help in courses.

Finally, I wish to sincerely thank all of my beloved family members, my parents, my husband, John, my sweet son, Jerry, my sister, and brother for their unlimited support and love from the beginning to the end.

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CHAPTER I

INTRODUCTION AND

LITERATURE REVIEW

Rose as a species

The first fossil evidence of the rose dates back 35 million years ago in North America. Among the Rosaceae family, there are over 3000 species, which includes various domesticated fruit crops such as strawberry (*Fragaria vesca*), plum (*Prunus salicina*), peach (*Prunus persica*), almond (*Prunus dulcis*), apple (*Malus domestica*), pear (*Pyrus communis*), blackberry (*Rubus sp.*), and raspberry (*Rubus idaeus*), as well as ornamentals such as roses (*Rosa* x *hybrida*). The genus *Rosa* includes 130 recognized species in three subgenera, Eurosa, Hesperhodos and Platyrhodon. The subgenus Eurosa includes more than 95% of the rose species (Zlesak, 2006). However, only seven to ten species (*Rosa damascena, Rosa chinensis , Rosa foetida, Rosa gallica, Rosa gigantea, Rosa moschata, Rosa multiflora, Rosa phoenicia, Rosa rugosa, and Rosa wichurana*) were used to breed most modern roses. In the rose, chromosomes occur in basic sets of 7. Approximately one fourth of the rose species are diploids (2n=2x=14). Among the others, 3 triploid (2n=3x=21), 46 tetraploid (2n=4x=28), 24 pentaploid (2n=5x=35), 22 hexaploid (2n=6x=42), and 2 octoploid (2n=8x=56) species have been reported (Mastalerz and Langhans, 1969). Roses are broadly divided into three groups, wild roses (species roses), old garden roses, and modern roses. Breeders have worked on introgressing many desirable traits such as black spot resistance from wild rose species

into modern cultivars. Old garden roses introduced prior to 1867 and modern roses identified after 1867 are mostly tetraploids with a few triploids and diploids (Zlesak, 2009)

Commercial roses

As stated earlier, ploidy levels can range from the diploid to the octoploid level, however, most cultivated rose varieties are highly heterozygous tetraploid plants and recurrent flowering. In contrast, most diploid species are nonrecurrent flowering (Debener, 1999). The complex segregation patterns in the progeny of polyploids and the large number of genotypic groups that need to be resolved in tetrasomic as compared to disomic inheritance make the genetic analysis of polyploids more challenging than diploids (Barker et al., 2010). In tetraploids, the segregation of traits and difficulties of genetic analyses is much more complex than diploids. Therefore, expanded knowledge of the genetics of important rose traits together with the use of molecular markers would accelerate the introgression of genes from wild rose species into the genetic background of modern tetraploid roses (Byrne, 2003; Dugo et al., 2005). The purpose of making a genetic map of rose, as was done in many field and vegetable crops such as potato, legume, tomato, rice and corn is to assign traits to well defined regions on one or more of the 7 chromosomes (Rajapakse, 2003). In addition, roses have relatively small genome size among the flowering plants, comprising about 600 mega base pairs (Mbp) (Zhang et al., 2006). This combined with rose's diversity of morphological traits, ability to be transformed and vegetative propagation facilitates its use as a model plant to discover

and understand genes, which is important to the areas of the floral, ornamental and perfume industries.

Genetic maps in roses

To date the mapping in roses was done mostly using PCR based markers (Von Malek et al., 2000). Four pairs of maps are presently available for diploid roses and three pairs for the tetraploids (Table 1). The first molecular genetic linkage map for rose had 305 RAPD and AFLP markers (Debener and Mattiesch, 1999) on a diploid population derived from *Rosa multiflora* hybrids 93/1-117 and 93/1-119. Two maps of seven linkage groups each were constructed, one for each parent. The maps spanned 326 and 370 cM of the genomes with an average distance between markers of 2.4 and 2.6 cM, respectively. Among the seven groups, genes controlling double corolla (*Blfo*), pink flower color (*Bfla*), resistance to black spot (*Rdr1*) and resistance to powdery mildew (*Rsp1*) were mapped (Debener and Mattiesch, 1999; Von Malek et al., 2000).

The first set of diploid parental linkage maps were expanded by Yan (2003 and 2005). These two parental maps, consisting of 365 AFLP and SSR markers with the diploid rose population, 94/1, derived from a cross between two diploid half-sibs originating from open-pollinated progeny of a *R. multiflora* hybrid provided by Thomas Debener (Debener and Mattiesch, 1999). The two parental maps have eight (463 cM) and seven (491 cM) linkage groups with an average distance between markers of 2.9 cM and 3.7

Table 1. Genetic maps that have been constructed for diploid and tetraploid roses.

Population	**Progeny (no)**	**Molecular Markers**	**Morphological traits**	**Disease resistance**	**Reference**
Diploid population (94/1) derived from *Rosa multiflora* hybrids (93/1-119 and 93/1-117).	60	RAPD,AFLP (305 in total)	Petal number (*Blfo*) (double vs. single), flower color (*Blfa*) (pink vs. white)		Debener et al., 1999
A cross (94/1) of two diploid half-sib parents (P119 and P117) originating from open-pollinated progeny of a *R. multiflora* hybrid	88	AFLP, SSR (365 in total)	Leaf area, chlorophyll content		Yan et al., 2003
		AFLP, SSR, PK, RGA, RFLP, SCAR, morphological (520 in total)			Yan et al., 2005
			Number of internodes, stem thickness, shoot length, chlorophyll content, leaf area, leaf dry weight, stem dry weight, total dry weight, specific leaf area and growth rate (QTL analysis)		Yan et al., 2007
				*Rdr*1 is close to 155SSR and RMS015 in LG1	Biber et al.,2010

Table 1. Continued.

Population	Progeny (no)	Molecular Markers	Morphological traits	Disease resistance	Reference
See above page			Floral scent volatiles: nerol, neryl acetate and geranyl acetate (single trait); geraniol, beta – citronellol and 2-phenylethanol (QT L analysis)		Spiller et al., 2010
A tetraploid F_1 progeny crossed by male (95/1,95/2 and 95/3) and resistant female (91/100-5) parents segregating for the presence of the black spot resistance gene *Rdr*1		RAPD, AFLP, SCAR		Resistance to black spot (Rdr1)	Von Malek et al., 2000
Above population		AFLP, SCAR		Resistance to Powdery mildew (Rpp1)	Linde et al., 2004
A diploid progeny 97/7 crossed by the resistant diploid female parent 95/13-39 and the susceptible diploid male parent Sp3 (82/78-1)	270 for phenotypic trait analysis and 170 for marker analysis	SCAR, CAPS, RGAs and BAC end-derived marker (233 in total).	Prickles, white stripes, double flowers	Resistance to Powdery mildew and black spot (QTL analysis)	Linde et al., 2006

Table 1. Continued.

Population	Progeny (no)	Molecular Markers	Morphological traits	Disease resistance	Reference
See above page				*Rdr*1 is close to 155SSR and RMS015 in LG1	Biber et al.,2010
Population from an interspecific cross between diploid roses, 'Blush Noisette' and *R. wichurana* 'Basye's Thornless'	96	130 RAPD 2 SSR 1 morphological	Flower size, days to flowering, leaf size,	Resistance to powdery mildew	Dugo et al., 2005
A cross between a dihaploid rose, derived from the haploidisation of a modern cultivar *R. hybrida* cv Zambra, and a diploid species *R. wichurana.*	91	AFLP	Recurrent blooming , double corolla, thorn density of the shoots,		Crespel et al., 2002
		AFLP, 44 EST-SSRs, 20 genomic-SSRs 105 and 136 in maternal and paternal map, respectively)	Date of flowering, number of petals (QTL analysis)		Hibrand-Saint Oyant et al., 2008
		SSCP, CAPS and dCAPS (213 in total) and integrated map spans 482 CM	GA pathway in floral control (recurrent or non-recurrent blooming and date of flowering) (QTL analysis)		Remay et al., 2009

Table 1. Continued.

Population	Progeny (no)	Molecular Markers	Morphological traits	Disease resistance	Reference
Tetraploid F_2 selfed seedlings from F_1 plant 90-69, a hybrid between tetraploid 'Basye's Blueberry' (82-1134) x amphidiploid 86-7(male parent)	52	RFLP, RAPD		Resistance to black spot	Ballard et al., 1996
		AFLP, 3 restriction enzymes (167 and 171 in total, respectively)		Resistance to black spot	Rajapakse et al., 2001a
		675 AFLPs 1 isozyme 3 morphological 6 SSR	Prickles on the stem, growth habits prickles on the petiole malate dehydrogenase	Resistance to black spot	Rajapakse et al., 2001b
		AFLPs, 17 SSR(286 and 256 in paternal and maternal map , respectively)			Zhang et al., 2006
Cross between 'Golden Gate' (GG) and 'Fragrant Cloud' (FC)	132	AFLP, RFLP , SSR, CAPS and morphological traits	Anther color and flower color		Gar et al., 2011
A tetraploid population K5 (P540 x P867)	184	AFLP, NBS profiling, and SSR markers	QTL: Prickles on the stem, and petal number	Powdery mildew resistance	Koning-Boucoiran et al., 2012

cM, respectively. Two growth vigour-related quantitative traits, leaf area and chlorophyll content, were mapped with three and two QTLs, respectively (Yan et al., 2003; 2005).

The second set of diploid genetic maps was constructed with AFLP markers with a population of 91 individuals derived from a dihaploid rose, H190, originating from a modern tetraploid *R. hybrida* cv Zambra and the diploid species *R. wichurana* hybrid (*Rw*). The parental map of the dihaploid includes eight linkage groups covering 238 cM of it genome, while another map of *R. wichurana* comprises six linkage groups covering 287 cM. Two qualitative traits, recurrent blooming and double corolla as well as one quantitative trait, prickle density of the shoots controlled by a major and a minor QTL were located on the parental maps (Crespel et al., 2002).

The third genetic map developed with 96 F_1 diploid hybrids between 'Blush Noisette' (seedling of 'Champneys' Pink Cluster') and *R. wichurana* 'Basye's Thornless', which consists of 133 RAPD, SSR and morphological markers (Dugo et al., 2005). The parental map lengths for their 7 linkage groups were 300 and 260 cM, rspectively. Four quantitative traits (flower size, days to flowering, leaf size, and resistance to powdery mildew) were analyzed and multiple QTLs for each trait were located on the map (Dugo et al., 2005).

The fourth linkage map was developed for the diploid population 97/7. It is a cross between 95/13-29 (resistant against powdery mildew isolate 9) and 82/78-1 (susceptible

Sp3). This map used 233 markers including 172 AFLP, 50 RGAs, 4 SSRs, 4 morphological markers, 1 CAPS, 1 SCAR and 1 BAC end-derived marker to map 418 cM on 7 linkage groups. Twenty-eight QTLs were mapped which included 3 powdery mildew (PM) resistance QTLs which explained about 84% of the variability for this trait. Most PM resistance QTLs were detected on LGs 3 and 4 with one near the prickles locus on LG 3. Three morphological markers (prickles, double flowers and white stripes) were mapped on both ends of LG2 in the linkage map (Linde et al., 2006).

The first tetraploid rose map was developed with F_2 progenies produced by selfing 90-69 a hybrid between 87-6, an amphidiploid resistant to black spot, and 'Basye's Blueberry', a tetraploid moderately susceptible to black spot. The map of the amphidiploid consists of markers assigned to 15 linkage groups over 902 cM of the genome, while the map of 'Basye's Blueberry' consists of markers assigned to 14 linkage groups and 682 cM. Two genes, prickles on the leaf petiole (*Ppr*) and subunit of isozyme malate dehydrogenase (*Mdh-2*) are located on the amphidiploid map (Ballard et al., 1996; Rajapakse et al., 2001a; 2001b) (Table 1). Subsequently 17 new SSR loci were incorporated into the existing maps (Zhang, 2006).

The second tetraploid map was constructed with a mapping population of 132 progeny from a cross between a the pink and fragrant garden rose 'Fragrant Cloud' (FC) and the yellow cut rose cultivar 'Golden Gate' (GG). A total of 449 polymorphic markers including AFLPs, RFLPs, SSRs, CAPS and morphological markers were scored. Three

hundred and fifty-eight markers were used to construct the linkage map. The lengths of the GG and FC maps were 632 cM (259 markers) and 616 cM (210 markers) respectively over 7 linkage groups. Anther color, flower color and resistance to powdery mildew were also included on the map (Gar et al., 2011).

Recently, a third tetraploid map was created with a population K5, created by crossing two cut flower hybrid tea roses, the dark red flower maternal tetraploid, P540, and the pale salmon and resistant to powdery mildew paternal tetraploid, P867. Maternal linkage map of P540 includes 172 loci over 28 linkage groups spanning 1081 cM with 275 markers (143 uni-parental simplex markers and 132 bi-parental simplex markers); while paternal linkage map of P867 contains 209 loci over 30 linkage groups covering 1225 cM with 326 markers (194 uni-parental simplex markers and 132 bi-parental simplex markers). Quantitative trait locus (QTL), prickles on the stem, was mapped on P540 parental map and QTLs, petal number and powdery mildew resistance, were mapped on P867 parental map. In this article, according to the procedure of Wu et al. (1992), the mode of inheritance of the two parental linkage maps also was discussed and concluded that a tetrasomic inheritance or possibly a combination of disomic and tetrasomic inheritance fit the observations best (Koning-Boucoiran et al., 2012).

Synteny of diploids, diploid and tetraploid roses

A unified diploid genetic linkage map for roses was linked by 59 bridge markers from 4 different maps. This integrated consensus map (ICM) has 597 markers distributed over a

length of 530 cM on the 7 LGs (Spiller et al., 2010). The ICM provide information for the phenotypic traits with high relevance of rose variety development. In addition, diploid ICM map was compared with autotetraploid GGFC map manually (Gar et al., 2011).

Inheritance of rose traits

Among the approximately 130 different species of the genus *Rosa* (Zlesak, 2006), the ploidy level of genetic system can range from the diploid to the octoploid level. Given obstacles such as incompatibility among ploidy levels, self-incompatibility and fertility barriers between some species, and the highly heterozygous nature of genus *Rosa*, genetic studies of *Rosa* can be challenging (Debener 2003). As mentioned earlier, the difficulties of genetic analysis in tetraploids are more complex than diploids because they have more alleles per loci that are simultaneously segregating which necessitates the use of larger populations as compared to the diploid situation for the same trait resolution.

Over past few decades, the inheritance of morphological and physiological characters such as recurrent flowering (De Vries and Dubios, 1984; Crespel, 2002), resistance to the black spot fungus *Diplocarpon rosae* race 3 (Von Malek and Debener, 1998), resistance to powdery mildew (Linde and Debener, 2003), petal number (Hibrand-Saint Oyant et al., 2008), flower color (Debener and Mattiesch 1999), double corolla (Crespel, 2002), growth type (De Vries and Dubois, 1984; Dubois and De Vries, 1987), prickles

on petioles (Rajapakse, 2001b), and prickles on stems (Debener, 1999 and Rajapakse et al., 2001b) has been reported. QTLs conditioning prickle density of the shoots (Crespel, 2002), leaf area, chlorophyll content (Yan et al., 2003), flower size, flowering time, leaf size, and resistance to powdery mildew (Dugo et al., 2005) (Table 2) have been reported (Byrne, 2009).

Mutant types compared to wild types also play an important role in plant genetic research. Three out of five SSR markers were identified to distinguish polymorphism between the fragrant wild type ('Jinyindao') and its non fragrant mutant ('Wangriqinghuai') when screened with SSRs derived from an EST library from fragrant related genes of 'Jinyindao' and 'Wangriqinghuai' . In addition, these five SSRs developed from, 'Jinyindao' and 'Wangriqinghuai', were also identified as polymorphic among 18 fragrant and non-fragrant rose cultivars (Yan et al., 2009).

GA plays a key role in recurrent blooming in roses as indicated by the effect of its exogenous application on preventing flowering in non-recurrent blooming roses, but not in recurrent blooming roses (Roberts et al., 1999). A recurrent/nonrecurrent mutant pair ('Little White Pet' and 'Felicite&Perpetue') was used to study the effect of gibberellins (GA) on floral initiation and recurrent blooming via the analysis of transcript abundance. Subsequently the gibberellin-gene-relative primers were used to extend the existing QTL for date of flowering and single recessive locus, recurrent blooming (RB) (Remay et al., 2009). Molecular markers (SSCP, CAPS or dCAPS) of GA-influenced floral candidate

genes were designed from the similar genes of *Arabidopsis*, and 24 out of 25 candidate genes were assigned to genetic linkage map (Crespel et al., 2002; Hibrand-Saint Oyants, et al., 2008). Three flowering genes (*RoVIP3*, *RoSPY* and *RoDELLA)* which affect GA signaling, mapped closely to the two important morphological loci, date of flowering and recurrent blooming (RB) (Remay et al., 2009).

Table 2. Rose morphological and physiological characters genetically analyzed in the past three decades.

Character	**Mode of inheritance**	**Reference**
Recurrent flowering	Monogenic recessive	De Vries and Dubois, 1984 Crespel, 2002
Yellow flower color	Monogenic dominant	De Vries and Dubois, 1978
Miniature stature	Monogenic dominant	Dubois and De Vries, 1987
Pink flower color	Monogenic codominant	Debener, 1999
Double flowers	Monogenic dominant	Debener, 1999
Double corolla	Monogenic dominant	Crespel, 2002
Prickles on stems	Monogenic dominant	Debener, 1999 Rajapakse et al., 2001b
Prickles on petioles	Monogenic recessive	Rajapakse et al., 2001b
Moss phenotype	Monogenic dominant	De Vries and Dubois, 1984
Dwarf phenotype	Monogenic dominant	Dubois and De Vries, 1987
Resistance to black spot race Rdr1, Rbs, Race3, 8 and 9	Monogenic dominant	Von Malek and Debener, 1998; Yan, et al., 2005; Zlesak et al., 2010
Resistance to powdery mildew Race Rpp1, CRPM1, Rpm	Monogenic dominant	Linde and Debener, 2003

Table 2. Continued.

Character	**Mode of inheritance**	**Reference**
Anther color	Monogenic dominant	Gar et al., 2011
Winter hardiness	Quantitative	Svejda, 1979
Individual pigments	Quantitative	De Vries et al., 1980a Marshall et al., 1983
Flowering under low irradiance	Quantitative	De Vries et al., 1980b
Leaf area Chlorophyll content	Quantitative	Yan et al., 2003
Flower size, Flowering time, Leaf size	Quantitative	Dugo et al., 2005
Prickle density of the shoots	Quantitative	Crespel et al., 2002
Blooming date, petal numbers	Quantitative	Hibrand-Saint Oyant et al., 2008

Molecular markers

Several types of DNA markers have been widely used for map construction: restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), simple sequence repeats (SSR or microsatellites) and amplified fragment length polymorphisms (AFLPs). In recent years, RGA (resistance gene analog) analysis as well as NBS (nucleotide binding site) and LRR (leucine-rich repeat) profiling were utilized to design primers and to identify the markers linked to the resistance (R) gene, *Rdr*3 (Terefe and Debener, 2009; Whitaker et al., 2010). All types of DNA markers can detect sequence polymorphisms and can be used to monitor the segregation of a DNA sequence among a progeny to construct a linkage map. Potentially unlimited numbers of DNA markers can be analyzed in a single mapping population. Backcross and F_2 populations are more suitable than F_1 populations for DNA-based mapping. These types of populations are also better suited for the analysis of quantitative traits (Phillips and Vasil, 2001).

Selection of parents is a critical step in the gene mapping process because to construct a map, the parents must differ in their marker profiles. In this research, two rose species *Rosa wichurana* 'Basye's Thornless' (BTh) and *Rosa chinensis* 'Old Blush' (OB) were selected as the plant materials because they differ in growth habits, horticultural characteristics, and black spot resistance. The goal in the associated breeding program is to combine the desirable disease resistance and the absence of stem prickles from BTh with the everblooming trait and various horticultural traits from OB to develop disease

resistant and recurrent blooming commercial cultivars without prickles. Moreover, SSR markers tend to exhibit high levels of polymorphisms, which make them extremely usedful in the narrow crosses (Rongwen et al., 1995), or the hybrids from the distantly related parents as the plant materials we used in the experiments.

The development of a genetic map in rose will facilitate candidate gene identification, marker assisted selection and the comparison of the synteny among diverse taxonomic groups.

SSR markers (microsatellites)

SSRs (Simple sequence repeats) or microsatellites are ubiquitous sets of tandemly repeated DNA motifs. The repeat regions are generally composed of di-, tri-, tetra- and sometimes greater repeated nucleotide sequences (Tautz and Renz, 1984). Compound repeats composed of two or more repeat motifs are also frequently found (Phillips and Vasil, 2001). Microsatellites, although generally more expensive per data point, are especially attractive because they are frequently highly polymorphic as well as PCR based and generally inherited in a co-dominant manner (Morgante and Olivieri, 1993). Despite the high degree of polymorphism, microsatellite alleles also have a high transferability to other crosses and related species (Cipriani et al., 1999, Mnejja et al., 2004). For example, among the SSRs found in the rose genome, there were twice as many CT base pair repeats as GT base pair repeats, which was also found from characterization in the peach (Zhang, et al., 2006). In addition, SSR markers developed

from ESTs provide useful resources for mapping genes with putative functions (Jung et al., 2005). For instance, five EST-SSR markers were used to identify the polymorphism of the fragrant roses and non- fragrant roses in the study of floral scent (Yan, 2009). Therefore, due to the abundance of SSR in the rose genome, developing SSR markers for roses is an important tool for map construction. To date, over two hundred SSR markers have been developed and used for constructing genetic linkage maps of roses (Yan et al., 2005; Zhang et al., 2006; Hibrand-Saint Oyant et al., 2008; Yan et al., 2007; Biber et al., 2010; Spiller et al., 2010).

As the numbers of traits that have their genetic basis determined, our ability to develop breeding strategies to predict and minimize the number of progeny and generations needed for trait manipulation increases (Debener, 2003). In other words, reliable genetic markers for key horticultural traits in roses would accelerate the introgression of important traits from wild diploid rose species into the genetic background of diploid and tetraploid modern roses and would allow the pyramiding of desirable traits (especially disease resistance) leading towards the development of superior varieties (Byrne, 2003).

CHAPTER II

MATERIALS AND METHODS

Plant materials

Diploid population

The population studied is a rose backcross population of diploid (2n=2x=14) WOB26 hybrid (*Rosa wichurana* "Basye's Thornless" x *Rosa chinensis* 'Old Blush') backcrossed to 'Old Blush'. 'Basye's Thornless' (BTh) is a once blooming, ground cover rose with single white flowers, no prickles on the stem and high resistance to black spot. 'Old Blush' (OB) is a recurrent blooming, upright growing bush with double, pink flowers, prickles on the stem and susceptibility to black spot. The F_1 hybrid, WOB26, is a once blooming, low growing rose with single light pink flowers, few prickles on the stem and moderate resistance to black spot. A total of 99 progeny of this cross was used for the map development. This population was grown in the field in College Station (30°36′5″N 96°18′52″W), TX, USA, a subtropical mild winter, hot summer humid climate which has an average annual rainfall of 1000 millimeter, an average elevation of 112 meter above sea level and an average temperature of 20 °C.

Tetraploid population

The full-sib autotetraploid (double pseudo testcross) population is a cross between *Rosa x hybrida* cv. 'Golden Gate' bred by W. Kordes' Sohne and *Rosa hybrida* cv. 'Fragrant Cloud' bred by RosenWelt Tantau. This population progeny were grown in the pots with a peat: volcanic gravel (1:1, v/v) mixtures in a greenhouse under natural photoperiod and

28 ^{o}C day and 20 ^{o}C night controlled temperature in Rehovot, Israel (31°53′52.67″N 34°48′29.24″E). ‘Golden Gate’ (GG) is a modern cut-flower cultivar with caroteneids-containing yellow petals, faint fragrance and long vase life, whereas ‘Fragrant Cloud’ (FC) is an old garden cultivar with large red (anthocyanins) petals, strong scent and short vase life. A total of 131 individuals in the tetraploid population were used to construct the genetic linkage map.

DNA extraction and PCR amplification

DNA extraction

DNA extracted from young leaves from the three diploid parents (BTh, OB, and WOB26) and 99 WOB26 backcross progeny according to the modified procedures from Doyle and Doyle (1987) extraction protocol, whereas genomic DNA of 131 individuals in the tetraploid population and its parents (GG and FC) were extracted according to Roche et al. (1997).

The WOB26 backcross population was grown in the Texas A&M University research farm which is located south of the intersection of Harvey Michelle Parkway and University Drive near campus. Some progeny were planted on the bed Q next to the building of the Department of Horticultural Science on campus. The field samples were collected from the multiple-node stem cuttings with the younger leaves on the live plants and put into the clearly labeled plastic bags in an ice-filled container to maintain tissue fresh. In the laboratory, 100 mg of the youngest leaf tissue of each sample was put into a

1.5 mL micro-centrifuge tube. Approximately 100 mg of the youngest and unexpanded leaves of the live plants in bed Q was collected and put directly into the marked 1.5 mL micro-centrifuge tubes in an ice-filled bucket.

For DNA extraction, liquid nitrogen was poured into the micro-centrifuge tubes and then the frozen leaf tissues were crushed and stirred with a sterilized micro-centrifuge blue pestle on the cordless battery drill. After the tissues were thoroughly ground into a powder, seven hundred µL of 2X CTAB (cetyltrimethyl ammonium bromide) was added to each tube and mixed with vortex mixer (Vortex-Genie 2, Scientific Industries, Inc., Bohemia, NY) for approximately 10 minutes. All tubes were placed in a water bath at 65 °C for 2.5 hours and then removed and allowed to cool to room temperature (approximately 25°C). Seven hundred µL of CIA (chloroform : iso-amyl alcohol, 24:1) was added to each tube and vortexed until thoroughly mixed and then centrifuged at 13200 g for 10 minutes until the top layer of sample was clear and colorless. If this layer was not clear, the centrifugation step was repeated. Transfer the clear upper layer of a sample to a new1.5 mL micro-centrifuge tube and add another 700 µL of CIA. Next thoroughly mix the sample by inverting several times and vortexing briefly. And repeat the centrifugation step. Again remove the clear aqueous layer of the sample and put into a new micro-centrifuge tube. To this, add 1 volume of cold isopropanol (approximately 500 µL) to the sample to precipitate the DNA. The tubes were mixed by inverting several times and were placed in the -20 ^{o}C freezer overnight to help the DNA precipitation. The next day, the tubes were centrifuged at 6000 g for 10 minutes and the

supernatant poured off leaving the DNA pellet on the bottom of the tube. The tubes were then inverted on a paper towel and allowed to dry. The DNA pellet was twice washed with 70% ethanol and centrifuged at 6000 g for 1-2 minutes. The ethanol was gently poured out from each tube and the DNA pellet was allowed to air dry at room temperature. The DNA was then dissolved by vortexing for 10 minutes in 50-200 µL of TE. All DNA samples were stored at -20 ^{o}C.

PCR amplification

The PCR reaction mixture contained 1 µL 10 ng DNA, 0.5 µL HEX, TET, FAM, or NED fluorescently labeled forward primer or a non- fluorescently labeled forward primer, 0.5 µL reverse primer, 2.5 µL DNase-/RNase-free water, 5 µL GoTaq Green Master Mix [2X of GoTaq DNA Polymerase supplied in 2X Green GoTaq Reaction Buffer (pH 8.5), 400 µM dNTP and 3 mM $MgCl_2$] (Promega Corporation, Madison, WI) and 0.5 µL 25 mM $MgCl_2$. The PCR reactions were performed in a TECHNE TC-412 thermal cycler (Bibby Scientific Limited, UK) programmed for one step of denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 30 s, primer annealing at 55 °C for 45 s and primer extension at 72 °C for 1 min. A final extension step was carried out at 72 °C for 7 min and then held at 4 °C.

Molecular and morphological markers

AFLP markers

The analyses of identification and measurement of the complex AFLP band patterns was conducted by KeyGene N.V. as described by Vos et al (1995). Tetraploid genomic DNA was digested with EcoR1 (E) / MseI (M) enzyme combination. The polymorphic PCR amplification was generated by seven AFLP primer pairs: E33/M52, E35/M49, E35/M54, E33/M54, E33/M57, E35/M53 and E35/M61. One hundred fifty-five polymorphic marker amplicons were scored in the tetraploid population.

Microsatellite markers

Non-fluorescence SSR markers

The 3 diploid parents with 9 of their progeny and 2 tetraploid parents with 10 of their progeny (Figures 1 and 2; Table 3) were initially screened for polymorphisms with 175 non-labeled SSR markers (75 from INRA, France; 47 from Plant Research International, Netherlands; 32 from Clemson University, USA; 21 from University of Hannover, Germany) using 2% or 4 % MetaPhor agarose (Lonza Group Ltd, Switzerland) gels at 180 volts for 90 min. The gel was stained with ethidium bromide and photographed under UV light (Figures 1 and 2).

Ninety-nine progeny of the backcross population and the 3 parents were characterized with 32 out of 175 SSR markers.

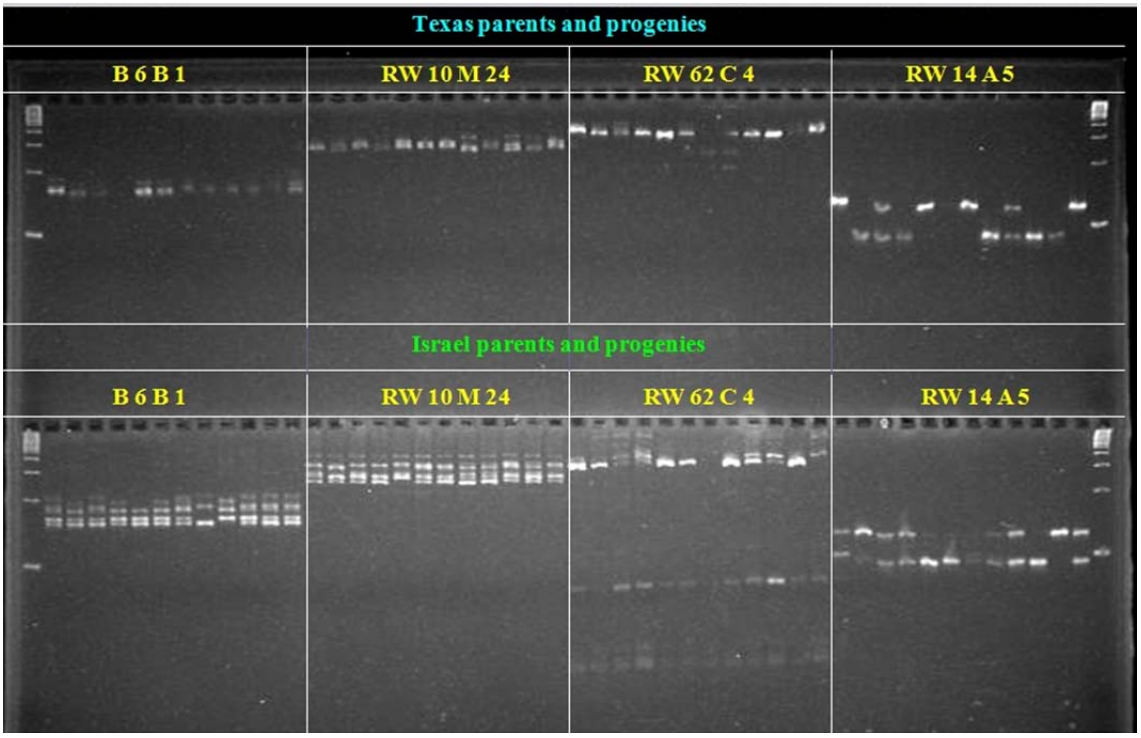


Figure 1. PCR products on 4% MetaPhor at annealing temperature 47.5°C with SSRs.

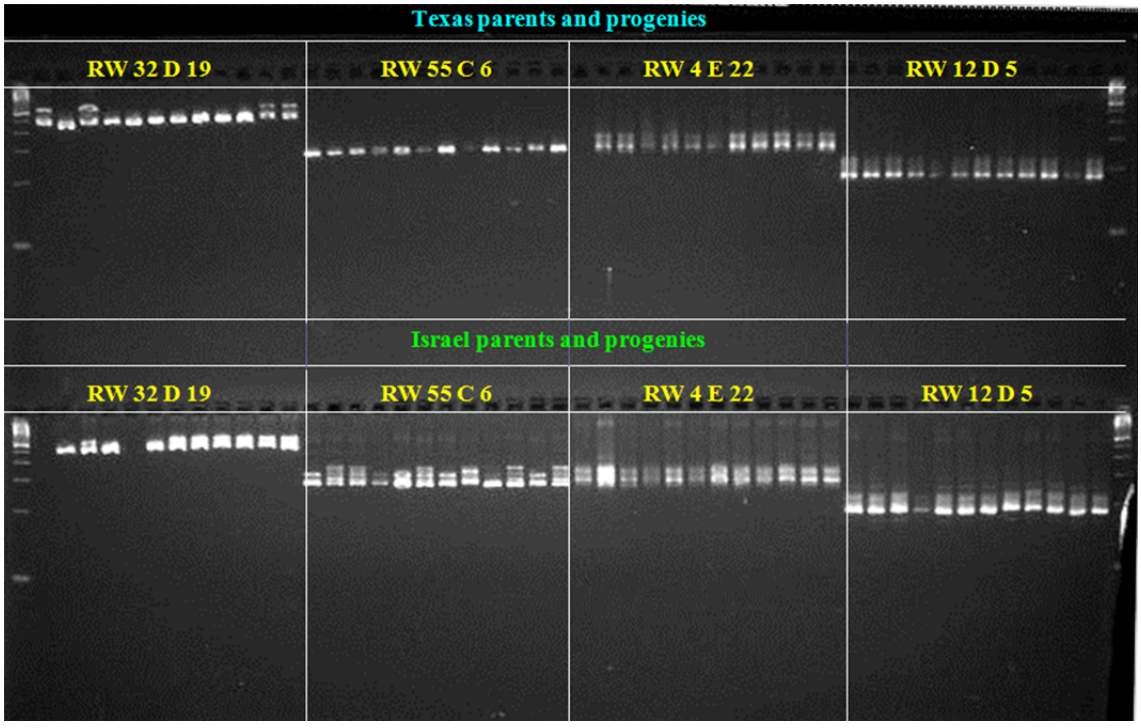


Figure 2. PCR products on 4% MetaPhor at annealing temperature 50°C with SSRs.

Table 3. 175 non-fluorescent SSR primers screened for rose mapping.

Annealing temp. (^{o}C)	Primer	Polymorphism in Texas population (Diploid)	Polymorphism in Israel population (Tetraploid)	Group[x]
75 French primers (Hibrand-Saint Oyant et al., 2008)				
55	Contig137	Yes	Yes	1
	Contig139	Yes	Yes / ?	1 / ?
	Contig172	Yes	No / ?	2 / ?
	Contig187	Yes	No	2
	Contig29	Yes	Yes	1
	H1_D05	Yes (2%)	No	2
	H1_F03	No	No	4
	H10_B01	Yes	Yes	1
	H10_D03	Yes	Yes	1
	H10_D04	Yes (2%) / ?	Yes(2%)	1 / ?
	H11_B02	No	No / ?	4 / ?
	H14_B02	No	No	4
	H14_D07	No	No	4
	H16_D05	No / ?	No / ?	4 / ?
	H17_C12	B	B	
	H18_B02	Yes (2%)	No (2%)	2
	H19_F04	No / ?	No / ?	4 / ?
	H2_C05	Yes	Yes	1
	H2_F12	B	B	
	H20_D08	Yes	No	2
	H20_G01	Yes / ?	No / ?	2 / ?
	H22_A02	Yes	No / ?	2 / ?
	H22_C01	Yes / ?	No / ?	2 / ?
	H22_E04	Yes	Yes	1
	H22_F01	No / ?	No / ?	4 / ?
	H23_O17	No	No	4
	H24_D11	Yes	Yes	1
	H24_F03	No / ?	No /?	4 / ?
	H3_G04	B	B	
	H4_F06	No	No	4
	H5_F12	Yes	Yes	1
	H6_G02	Yes	No	2
	H8_H05	?	No	
	H9_B01	Yes	Yes	1

Table. 3. Continued.

Annealing temp. (^{o}C)	Primer	Polymorphism in Texas population (Diploid)	Polymorphism in Israel population (Tetraploid)	Group[x]
	H9_B07	Yes	No	2
	H9_D03	No	No	4
	Rw4J4	No	No	4
	Rw5G14	Yes	Yes	1
	Rw15D15	Yes	Yes	1
	Rw16E19	Yes	Yes	1
	Rw20I17	Yes	No / ?	2 / ?
	Rw21F9	No	No	4
	Rw22C22	B	B?	
	Rw22F14	Yes / ?	No	2 / ?
	Rw23F13	Yes / ?	No	2 / ?
	Rw23F8	No	No	4
	Rw25J16	Yes	Yes	1
	Rw26G3	No	Yes	3
	Rw26H23	B	B	
	Rw27B12	B	B	
	Rw32K24	Yes	B?	
	Rw34L6	Yes	Yes	1
	Rw35C24	Yes	Yes / ?	1 / ?
	Rw37P7	Yes / ?	Yes / ?	1 / ?
	Rw38D11	B	B	
	Rw47J14	Yes	No / ?	2 / ?
	Rw53O21	Yes (light)	Yes (light)	1
	Rw55E12	Yes	Yes	1
	Rw59A12	Yes	Yes / ?	1 / ?
	Rw12J12	Yes	No / ?	2 / ?
	Rw19E15B	Yes	Yes / ?	1 / ?
	Rw52D4	Yes	No	2
	Rw55C21	Yes	Yes	1
	Rw62D8	Yes	No	2
	Rw54N22	Yes	Yes	1
	BFACT47	Yes	Yes	1
	CL2002	Yes	Yes	1
	CL2845	Yes	Yes	1
	CL2980	Yes	No / ?	2 / ?

Table 3. Continued.

Annealing temp. (°C)	Primer	Polymorphism in Texas population (Diploid)	Polymorphism in Israel population (Tetraploid)	Group[x]
	CL2996	Yes	Yes	1
	CL3881	Yes	Yes / ?	1 / ?
	CTG21	Yes	Yes	1
	CTG329	No	No	4
	CTG356	Yes	Yes	1
	CTG623	Yes	No	2
26 Dutch primers (Esselink et al., 2003)				
55	RhAB22	Yes	Yes	1
	RhB303	Yes	Yes	1
	RhO517	Yes	Yes	1
	RhP519	Yes / ?	Yes	1 / ?
	RhAB15	Yes	Yes	1
	RhM405	Yes	No / ?	2 / ?
	RhEO506	Yes / ?	Yes / ?	1 / ?
	RhD221	Yes	Yes	1
	RhP507	Yes	Yes	1
	RhD201	Yes / ?	Yes	1 / ?
	RhAB40	Yes	Yes	1
	RhE2b	Yes	Yes	1
	RhP518	Yes	Yes	1
	RhAB1	Yes	Yes / ?	1 / ?
	RhAB13	Yes	Yes	1
	RhAB26	Yes (light)	Yes (light)	1
	RhB19	Yes	No	2
	RhBK4	Yes	No	2
	RhD206	Yes	Yes	1
	RhE2a	Yes	No	2
	RhE3	Yes	Yes	1
	RhI402	Yes	Yes	1
	RhJ404	Yes	Yes	1
	RhP524	Yes	Yes (light)	1
	pchgms41	No	No	4
	Ch02c11	No	No	4

Table 3. Continued.

Annealing temp. (°C)	Primer	Polymorphism in Texas population (Diploid)	Polymorphism in Israel population (Tetraploid)	Group[x]
32 Clemson Primer (Zhang et al., 2006)				
47.5	B 6 B 1	Yes / ?	Yes	1 / ?
	RW 10 M 24	Yes	Yes	1
	RW 62 C 4	Yes / ?	Yes	1 / ?
	RW 14 A 5	Yes	Yes	1
50	RW 32 D 19	Yes	Yes	1
	RW 55 C 6	No	Yes	3
	RW 4 E 22	No	No	4
	RW 12 D 5	No	No	4
	RW 18 N 19	Yes	Yes	1
	RW 10 J 19	Yes	Yes / ?	1 / ?
	RW 3 K 19	Yes/ ?	Yes	1/ ?
	RW 11 E 5	Yes	Yes	1
55	B 10 H 3	No	No	4
	RW 3N 19	Yes / ?	Yes / ?	1 / ?
	RW 45 E 24	-	-	
	RW 27 A 11 B	-	-	
	RW 8 B 8	Yes	Yes	1
	RW 14 H 21	Yes	Yes	1
	RW 60 A 16	No	No	4
	RW 29 B 1	Yes	Yes	1
	RW 5 D 11	Yes	Yes	1
	RW 22 B 6	Yes	Yes / ?	1 / ?
	RW 49 N 14	Yes / ?	Yes	1 / ?
	RW 23 H 5	Yes / ?	Yes / ?	1 / ?
57	RW 50 N 23	No	No	4
	RW 55 D 22	No / ?	No	4 / ?
	RW 61 F 2	Yes	No / ?	2 / ?
	RW 1 F 9	No	Yes/ ?	3/ ?
	RW 22 A 3	Yes	Yes	1
	RW 48 N 6	Yes	Yes / ?	1 / ?
	RW 46 O 8	Yes	No / ?	2 / ?
	RW 1717	Yes	Yes / ?	1 / ?

Table 3. Continued.

Annealing temp. (^{o}C)	Primer	Polymorphism in Texas population (Diploid)	Polymorphism in Israel population (Tetraploid)	Group[x]
21 German primers (Biber, et al., 2010 and Spiller et al., 2010)				
55	RMS001	Yes	Yes	1
	RMS003	Yes	Yes	1
	RMS015	Yes	Yes	1
	RMS035	Yes	Yes	1
	RMS037	Yes	Yes	1
	RMS042	Yes	Yes	1
	RMS043	Yes	Yes	1
	RMS045	No	Yes	3
	RMS057	Yes	Yes	1
	RMS060	Yes	Yes	1
	RMS062	Yes	Yes	1
	RMS063	Yes	Yes	1
	RMS066	Yes	Yes	1
	RMS084	Yes	Yes	1
	RMS086	No	No	4
	RMS089	Yes	Yes	1
	RMS090	Yes (null)?	No/ ?	2/ ?
	RMS094	Yes	Yes	1
	RMS132	Yes	Yes	1
	RMS137	Yes	Yes	1
	RMS146	Yes	Yes	1
21 Dutch primers (Yan et al., 2005)				
55	Rh79	Yes	Yes	1
	RhAB9	Yes	Yes	1
	Rh48	Yes	Yes	1
	Rh80	Yes	Yes	1
	Rh96	Yes	Yes	1
	RhB510	Yes	Yes	1
	Rh50	Yes	Yes	1
	Rh58	Yes	Yes	1
	Rh59	Yes	Yes	1
	RhABT12	Yes	Yes	1
	Rh65	Yes	Yes	1

Table 3. Continued.

Annealing temp. (^{o}C)	Primer	Polymorphism in Texas population (Diploid)	Polymorphism in Israel population (Tetraploid)	Group[x]
	Rh78	Yes	Yes	1
	Rh77	Yes	Yes	1
	Rh93	Yes	Yes	1
	RhAB38	Yes	Yes	1
	Rh60	Yes	?	?
	Rh85	Yes	?	?
	Rh98	Yes	Yes	1
	Rh72	Yes	Yes	1
	Rh73	Yes	Yes	1
	RhAB28	Yes	Yes	1

[x]: group 1: polymorphisms between Texas and Israel populations; group 2: polymorphism only in Texas population; group 3: polymorphism only in Israel population; group 4: no polymorphisms between Texas or Israel populations. B: Blank, no PCR products. -: missing data. ?: doubtful data. Screening on 4% Agarose gel except where noted.

Fluorescence SSR markers

The five parents, 'Basye's Thornless', 'Old Blush', 'WOB26', 'Fragrant Cloud' and 'Golden Gate' of the two populations were characterized with 100 fluorescently labeled SSR primers (HEX, FAM, TET and NED) (Table 4). Seventy-eight out of 100 labeled SSR primers were run for the 99 diploid progeny and the 131 tetraploid progeny to generate the data for map construction (the examples of the labeled DNA peaks were given in the results section).

Morphological markers

Four morphological traits: stem prickles, bloom type (recurrent/non recurrent), flower type (single/double), flower color (white/pink) and black spot resistance were scored on the WOB population.

Electrophoresis

Metaphor gel electrophoresis

Metaphor gel electrophoresis was used for polymorphism screening of SSRs and analysis of marker inheritance.

According to the marker segregations by Yan (2005), the inheritance of markers were divided by two types, uni-parental and bi-parental markers. Fifteen SSRs were given the examples for the diploid population in the results section.

Table 4. One hundred labeled primers for DNA sizes of 5 parents. DNA sizes were double confirmed by their progeny with primer number 1 to 78 listed in the table.

No	Set	Primer name	Labeled	Wichurana	Old Blush	WOB26	Fragrant Cloud	Golden Gate
1	1	**H5_F12**	HEX	132	132,138	132	138	138,143
2	1	**RhAB22**	FAM	154,160	156,183	154,156	156,162	162,168
3	1	**RhI402**	FAM	207	210,216	207,210	197,210	210,216
4	1	**Contig137**	HEX	328,330	324,330	330	313,330,334	324,328,334,338
5	2	**RW15D15**	FAM	160,166	206	166,206	189	189,206
6	2	**CL2845**	FAM	301,304	295	295,301	295,304	292,295
7	2	**RW14H21**	HEX	137,145	118,122	118,137	118,122,126,128	116,118
8	2	**H2_C05**	HEX	222,231	215,228	228,231	215,228,234	228,234
9	3	**H10_B01**	FAM	203,204	203,225	204,225	199,203,212	203,225
10	3	**RhP524**	FAM	113	125	125	116,125	125,209
11	3	**RW14A5**	HEX	124	90	124	90	123
12	3	**RhAB13**	HEX	168,170	135	135,170	134,149	149
13	4	**RW34L6**	HEX	209,217	187,215	215,217	184,202,226	184,202,226
14	4	**RhP518**	FAM	140	140,163	140	163	140,163
15	4	**RW29B1**	HEX	353,355	342,349	342,355	342,349,363	342,349,363
16	4	**Contig139**	FAM	236,240	242,244,292	236,244,292	236,240,242,294	236,240,292
17	5	**H9_B01**	FAM	211,214,220,232	214,217,222,232	211,214,222,232	211214217222229 (234)	217,229,234
18	5	**RhE2b**	HEX	185	166,179	166,185	166,176,185	166,179
19	5	**Rh50**	FAM	312,318	303,332	312,332	302,332	302
20	5	**Rh93**	HEX	245	239,268	239,245	268	238,268
21	6	**RW5G14**	HEX	233	249,251	233,249	232,237,249,251	232,251
22	6	**Rh98**	FAM	141,143	143,157,163,218	141,143,163	147,157,(163),218	143,163,223
23	6	**RhO517**	FAM	262	259,265	259,262	257,258	257,262,265
24	6	**RhAB15**	HEX	104,114	112,137	104,112	124,130,137	112,127,137
25	7	**CTG21**	FAM	121,128	121,134	121(134)	122,131,134	122,134
26	7	**RW11E5**	HEX	150,164,172,177	168,172	150,164,172	152,164,168,172	152,157,164,168,172
27	7	**RhD201**	FAM	198,205	198,200?	198,200	193	193,201,237
28	7	**RW10M24**	TET	258,260	250,267	258,267	250,270,278,281	250,267,270
29	8	**RW8B8**	FAM	139,141,143,153	113,131,135	113,135,141,143	129,131,135,143	135,143,153
30	8	**RhP519**	FAM	231	238	231,238	234,238	228,231,234
31	8	**RhE3**	HEX	165,169,172,175	160,181	165,175,181	169,170,172,177,181	169,170,172,177,181,000
32	8	**RW10J19**	TET	242,261,362,365	253,259,340,365	259,261,362,365	259,283,365	251,259,357,365
33	9	**H22_E04**	FAM	236	236,243	236	236,239,245	236,240,242
34	9	**CL2996**	FAM	173,179	179,188	179,188	176,179,185,188	179,185

Table 4. Continued.

No	Set	Primer name	Labeled	Wichurana	Old Blush	WOB26	Fragrant Cloud	Golden Gate
35	9	**RhJ404**	HEX	126,135,161	123,129	126,129,135	123,129,160	126,129,146
36	9	**RW18N19**	TET	212,216	216,221,224	212,216,221	216,221,224,227	214,216,221,224
37	10	**RhB303**	HEX	119	121,146	119,121	125,129,146	119,121,129
38	10	**RW5D11**	HEX	246,253	236,242	236,246	222,236,244	226,242,255
39	10	**RhAB9**	FAM	98	95,114	95,98	103,112	95,109
40	20	**RhABT12**	FAM	162,202	151,165	151,202	151,165,174	174
41	11	**RW62C4**	HEX	350	321	321,350	321	NA
42	11	**RW3K19**	FAM	437	435,436	436,437	423,431,435	431,435
43	11	**RW22A3**	HEX	144,149	142,149	142,144,149	139,144,149	139,142,149
44	11	**RhAB26**	FAM	176,203	165,241	203,241	170,207,241	207,241,276
45	12	**RW22B6**	FAM	139,154	131,133	133,154	119,133	119,133
46	12	**RW32D19**	TET	510,538	510	510,538	497,510,518	497,510
47	12	**RhD206**	HEX	208	188,196	196,208	188,217,325	188,217
48	12	**RW19E15B**	FAM	247	247,253	247,253	238,249,253	249,251
49	13	**RMS001**	HEX	229,235,241	222,227,231,235	227,229,231,235,241	227,231,235,243	235,236,243
50	13	**RMS015**	HEX	173	154,172	154,173	132,162	132,162
51	13	**RMS063**	FAM	92	83,86	83	83,86	86
52	13	**RMS066**	FAM	194	197,201	194,201	183,201	198(201 ??)
53	14	**CL3881**	FAM	245	237,239	237,245	230,240	230,240
54	14	**RMS003**	HEX	159,164	151,171	164,171	145,148,171	143,145
55	14	**RMS043**	HEX	205,213	205,214	205	205,220	220,227
56	14	**RMS090**	FAM	198	190,null?	190,198	N/A	N/A
57	15	**Rh48**	FAM	106	97	97,106	136,145	90,136,145
58	15	**RW53O21**	HEX	166,172	156	156,166	156,159,166	156,159
59	15	**Contig29**	FAM	235	195,235	235	194196(233235)	196204(233235)
60	15	**RW35C24**	NED	248	245,258	248,258	245,258	245,258
61	16	**Contig172**	FAM	144	144,149	144	144,149	144,149
62	16	**RW55E12**	HEX	168,179	165,181	168,181	165,184	162,184
63	16	**Rms137**	FAM	228,232	222,224	222,228	212,214,224	212,228
64	16	**Rh58**	NED	259,280	247,289	247,280	240,247,263,289	289
65	17	**BFACT47**	NED	144,146	144,148	144	144,145,148	144,145,148
66	17	**RMS094**	FAM	161,164	156,161	156,161	161,168	159,161,168
67	17	**CL2002**	HEX	187,227	195	187,195	195	195,197

Table 4. Continued.

No	Set	Primer name	Labeled	Wichurana	Old Blush	WOB26	Fragrant Cloud	Golden Gate
68	17	**H10_D03**	FAM	235,241	222,233	222,241	216,222,225	208,216,222,235
69	18	**RMS089**	FAM	154	165	154	160,165	160,163,165
70	18	**RW25J16**	HEX	171,183	175,196	183,196	175	175,196
71	18	**Rh78**	FAM	201,379	211,288	201,211	211,288	211,288,322
72	18	**RhD221**	NED	227	221,227	221,227	221,227	221,227,270
73	19	**RhAB38**	FAM	157,167	141	141,167	123,141	141,163
74	19	**RMS132**	FAM	177,205	183,193	177,193	170,178,183	170,183
75	19	**RW54N22**	HEX		215,222	215	215,222	215,222
76	19	**RW59A12**	NED	215,224	213,225	213,224	213,219,247	213,247
77	20	**RMS062**	HEX	154,167,173	157165(174)	154,157	157,158,165	165,173,189
78	20	**RhEO506**	FAM	235,241	226,229	229,235	208,226,229	208,223,226,229
79		**RMS057**		158,165	173,177	158,173	161,171,173	161,171
80		**CTG623**			229,266		218,229,256	
81		**RMS060**			143,196	196	143,198,212	198,212
82		**CL2980**		225,228	222	222,228		222,261?
83		**RMS146**		172,184	166,175	166,172	166,175,190	164,190
84		**RW55C21**		204	217,234	204,217	217,224,236	217,231,234,237
85		**RMS084**		182,184,193,206,220	174	174,182,193,220	110,174,176	174,176,184,187
86		**RMS042**		173,196	175,242	173,175	180,(242)	148,242
87		**RMS045**		187,195	232	232	201	187,232
88		**RMS035**		195	188,193,196	193,195,196	188,193,202,219,226	188,196,207,209,228
89		**RMS037**		197,199	199,200	199	191,199,225	195,199,225
90		**RhAB40**		190,212	231,233	190,233	(231),233,(235),238	207,222,238
91		**RhP507**		86,159	92,159	92,159	159,182,214	182
92		**CTG356**		148,191	169,208	148,169	159,166,169	153,159,166,193,208,000
93		**H24_D11**		161,225,228	158,167,223	161,167,223,228	158,167	167,223
94		**RW16E19**			202		204	
95		**RW12J12**		167	144,170	144,167	170	170
96		**RW1F9**		298	NA	298	NA	NA
97		**RW55C6**		268	268	268	259,268	259,268,274
98		**RW3N19**		123,125,330	330	330	330	330
99		**Rh72**		261	250,259	259	No signal	250,259
100		**RMS086**		126,130	148	148	148	148

Capillary electrophoresis

One hundred fluorescently labeled SSR-primer PCR products were initially analyzed for the sizes with 5 parents. Twenty sets of 4 primer pairs were designed based on the use of two green (HEX and TET), two blue (FAM) and/or one blue and one black (NED) labels. In addition for similarly labeled primers, the DNA sizes of PCR products had non overlapping sizes. Every set of multiplex fluorescently labeled (HEX, TET, FAM or NED) PCR products (2 μL) generated from various SSR primer sets were added to 8.5 μL Hi-Di Formamide and 0.5 μL ROX400. This mixture was run through the capillary sequencer, ABI 3100 (Life Technologies Corporation, Carlsbad, CA). However, before DNA samples were run through ABI 3100, they were all tested by Metaphor gel electrophoresis to confirm the presence and concentration of the PCR products. The 78 labeled SSR primers were run using the capillary sequencer procedure (Figure 3) for all progeny (99 diploid progeny and 131 tetraploid progeny) and in their 5 parents in two populations (Table 4).

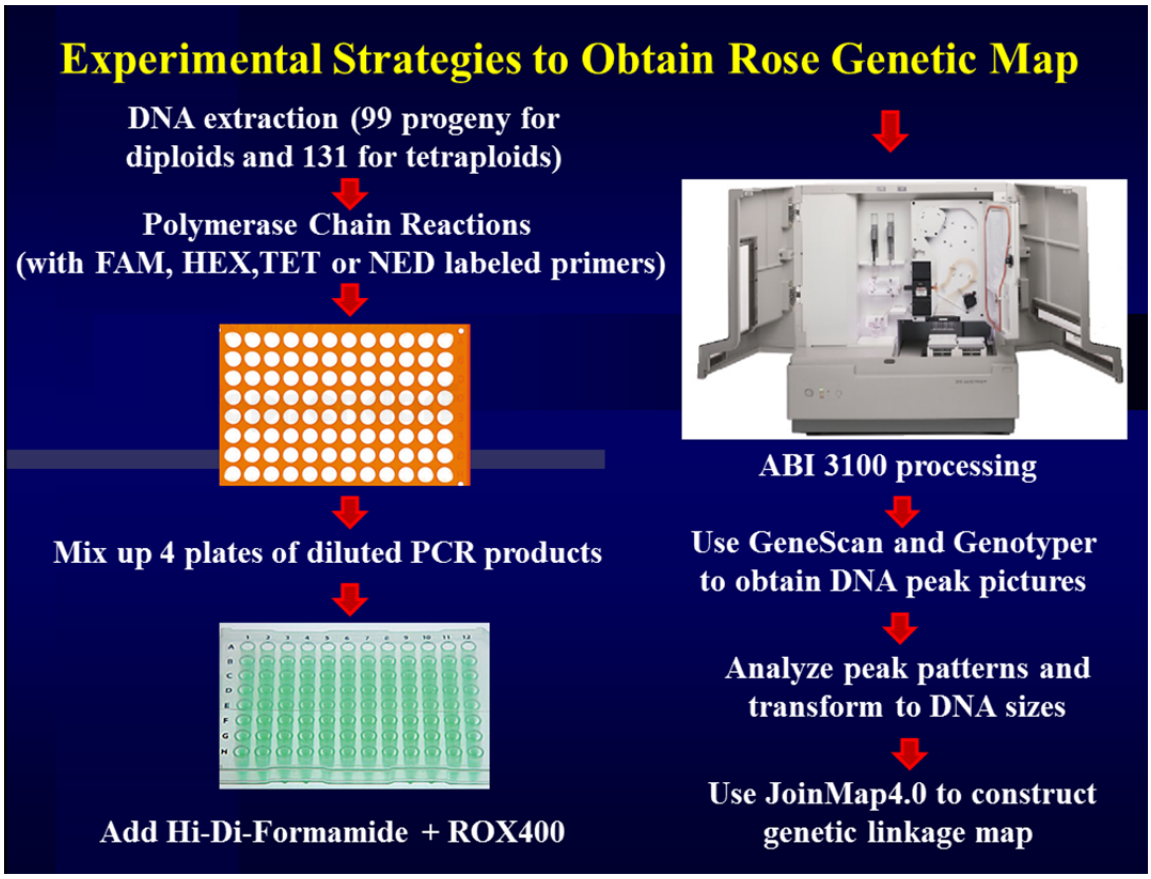


Figure 3. The procedure of capillary electrophoresis.

Fragment data analysis

The DNA peaks (sizes) separated on ABI 3100 were analyzed with the GeneScan and Genotyper software (Life Technologies Corporation, Carlsbad, CA).

GeneScan

Sample mixtures (DNA, fluorescently labeled PCR products, Hi-Di Formamide and ROX400) were processed by the genetic analyzer, ABI3100, which generates the sample files (.fsa). These .fsa files are then analyzed by the GeneScan software program that converts raw data to analyzed data which can be identified and read by Genotyper through the application of a size standard, ROX 400, a matrix file (fluorescently color separation) and specific parameter settings including analysis range and peak detection.

Genotyper

The Genotyper software converts GeneScan sized peaks into genotypes calls to provide defined results. First of all, through Genotyper, four categories were defined for the boundaries of the allele size range of 4 fluorescently labeled markers in a DNA sample. After categories were created, the categories also could be sorted by fluorescent dye color. Second, GeneScan data was imported to Genotyper document. Third, the allele peaks of each marker were labeled and unwanted labels were filtered. Four, the allele table was created, of which contents includes file name, sample information, category name, two columns for each allele in diploids, and an overflow column if more than two peaks occurred. Final, the table was saved as a Microsoft Excel form and exported from

Genotyper. More information is in the manual on the website and the link: http://www.nfstc.org/pdi/Subject06/pdi_s06_m01_03.htm.

Segregation analysis

MS-Excel

The table exported to Excel from Genotyper was used to transform data from allele size (bp) to JoinMap standard codes.

Diploid

The segregation patterns of marker alleles observed in the backcross mapping population (OBxWOB26) were tested for the goodness of fit (χ^2 at α=0.01) with the theoretical expected ratios 1:1,abxaa or aaxab, code lmxll or nnxnp in JoinMap; 3:1, abxab, code hkxhk in JoinMap; 1:2:1, abxab, code hkxhk in JoinMap and 1:1:1:1, abxac, code efxeg in JoinMap.

Tetraploid

Single-dose restriction fragment (SDRF) method (Wu et al., 1992) was used to analyze the segregation patterns (presence *vs* absence) for tetraploid population. Chi-square (χ^2) goodness-fit of test was calculated for the theoretical expected ratio of either 1:1, lmxll or nnxnp in JoinMap for the marker loci polymorphic between parents or 1:3, hkxhk in JoinMap for the marker loci shared between parents based on the segregation bands of marker alleles. The parental genotypes of marker loci in an autotetraploid are simplex (a-

--) by nulliplex (----) and simplex (a---) by simplex (a---) when segregating to 1:1 and 1:3, respectively, whereas in the case of allotetraploid, the genotypes will be heterozygous (a-) by homozygous (--) and heterozygous (a-) by heterozygous (a-) when segregating to 1:1 and 1:3, respectively, which is as in the diploid case (Barcaccia et al., 2003).

Mode of inheritance

In order to study the mode of inheritance, the ratio of coupling to repulsion linkage phase obtained from JoinMap was calculated for the goodness of fit (χ^2 at α=0.05) with the theoretical expected ratios 1:1 for allopolyploids as well as 1:0.25 for autotetraploids (Wu et al., 1992).

Linkage map construction

JoinMap 4 software (import from Excel) and MapChart 2.2

Pseudo-testcross strategy was used to develop the linkage maps for WOB26 diploid and GGFC tetraploid populations. Cross pollinators (CP) of population type codes in JoinMap 4 (van Ooijen 2006) is a population originating from a cross between a heterozygous (ab) and a homozygous (aa or bb) diploid parent with their linkage phases originally unknown. Marker alleles generated from each parent were scored according to the segregation type coding system using, efxeg, hkxhk, lmxll,and nnxnp within JoinMap 4. Chi-square (X^2) test of goodness-of-fit was used to check the segregation distortion. The maps were constructed with a REC = 0.4 and LOD (logarithm of the

odds) score of greater than 3 which is a reasonable value for the jump threshold between 3.0 and 5.0 (JoinMap 4 manual, van Ooijen, 2006). Mapping function selected was Kosambi's function. There were 107 SSR loci and 5 morphological trait markers with 99 diploid progeny, as well as 346 loci comprising 191 SSR and 155 AFLP amplicons with 131 tetrapoid progeny in the software calculation of genetic linkage maps. JoinMap text data of the linkage map could be imported and then revised in the software MapChart 2.2 (Voorrips, 2002).

CHAPTER III

RESULTS

Polymorphism screening of the 175 SSR markers

Gel electrophoresis

Nine selected diploid progeny, three diploid parents and two tetraploid parents were screened for polymorphism with 175 non-labeled SSR markers. One hundred and eight (61.7%) of the markers were polymorphic among the progeny and parents of both populations, whereas 26 (14.9%) were polymorphic in the Texas diploid population but not in the Israel tetraploid population. Four markers (2.3%) were polymorphic in the Israel but not in the Texas population. Only 24 (13.7%) markers were not polymorphic in either population (Table 3). The remaining 13 (7.4%) markers were non-scorable in either populations with either 2% and 4% MetaPhor agarose gel.

Upon characterizing 99 progeny and the 3 parents of diploid population with 32 non-labeled markers on MetaPhor agarose gels, problems were encountered with shadow bands and a lack of resolution (Figures 4, 5 and 6). To remedy these issues, the selected 100 polymorphic markers were run using fluorescently labeled primers and capillary electrophoresis.

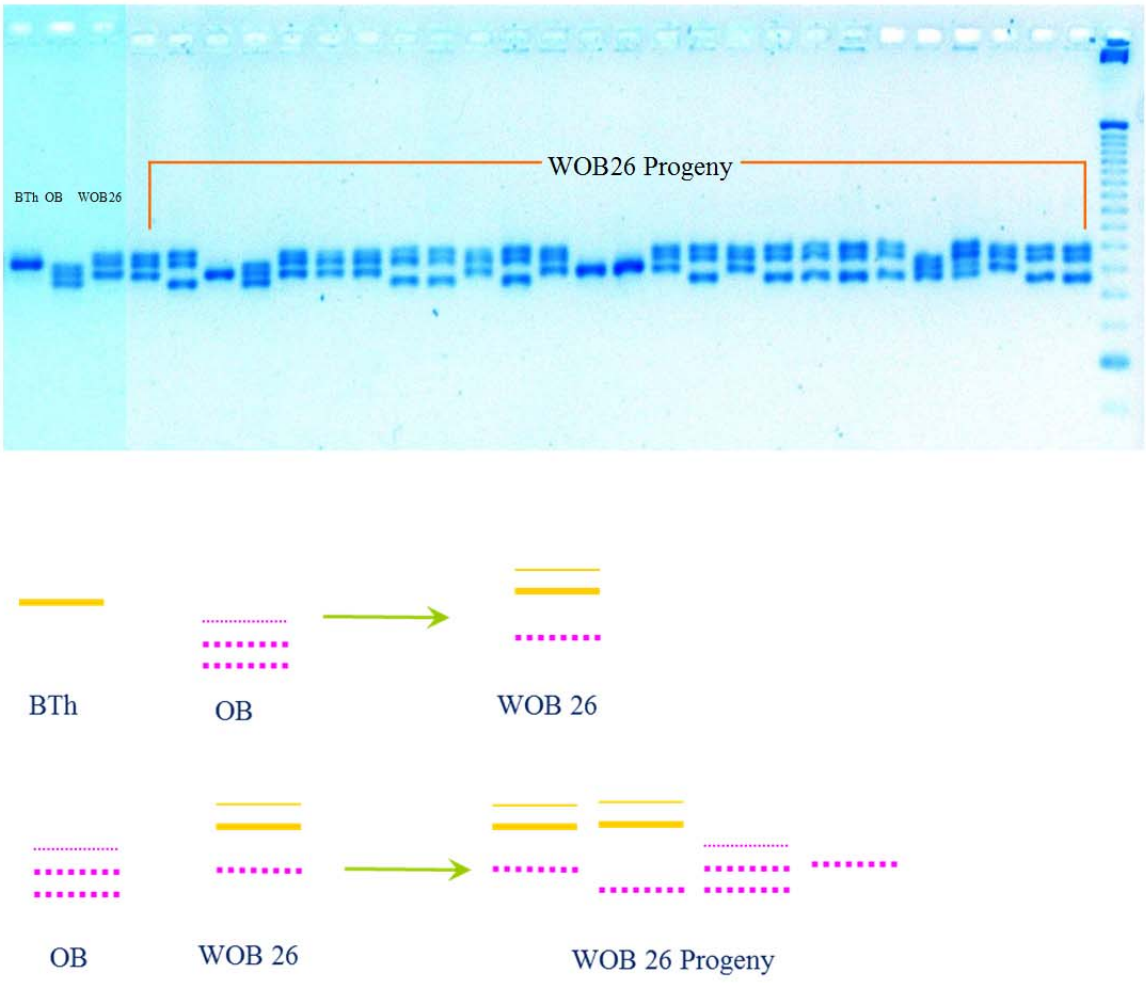


Figure 4. Marker RhD206 amplified one locus with three alleles from two parents and produced four-genotype progeny showed on the 4% Metaphor agarose gel (180-200Volt for 60-90 minutes).

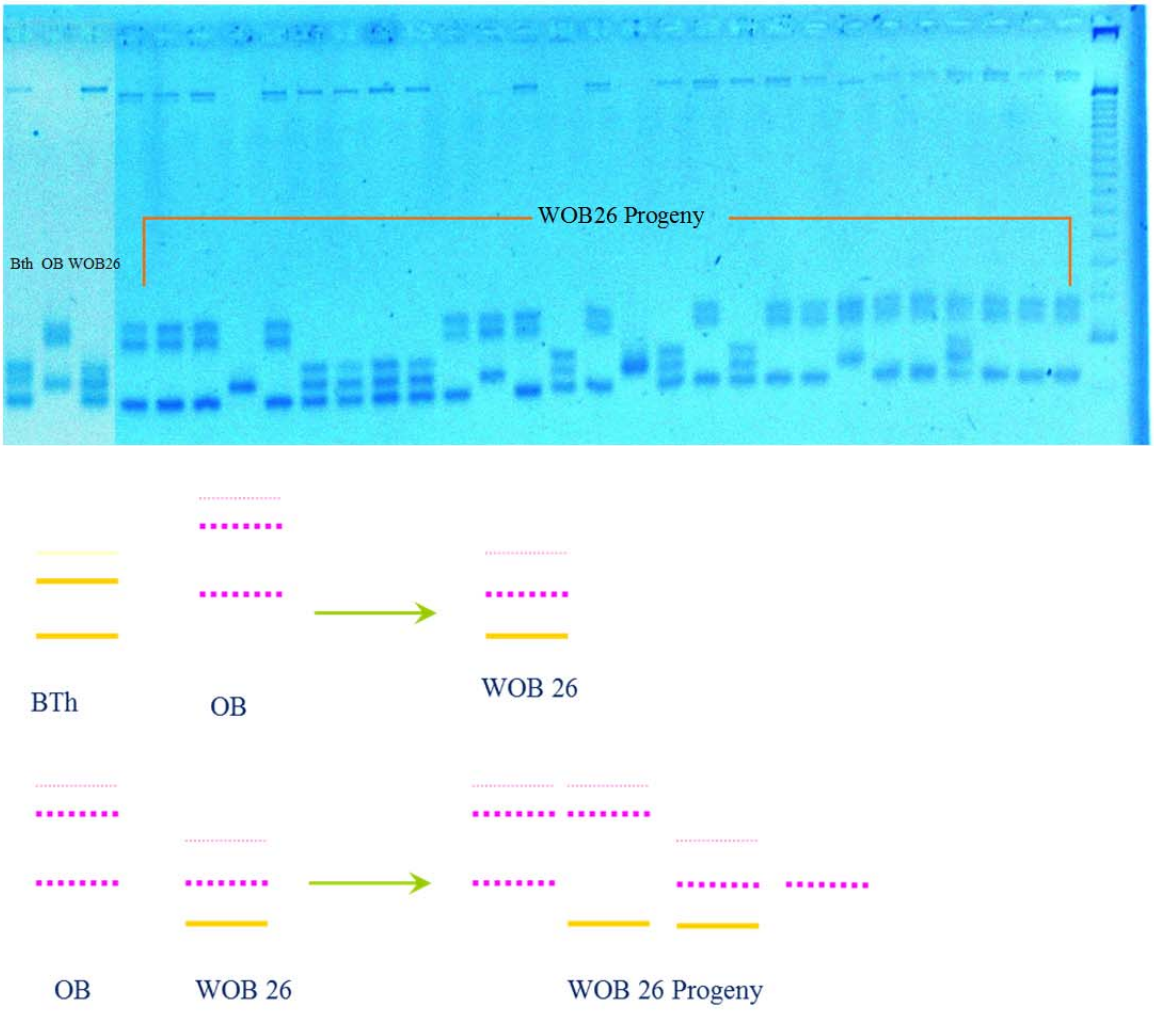


Figure 5. Marker RhAB 15 amplified one locus with three alleles from two parents and produced four-genotype progeny showed on the 4% Metaphor agarose gel.

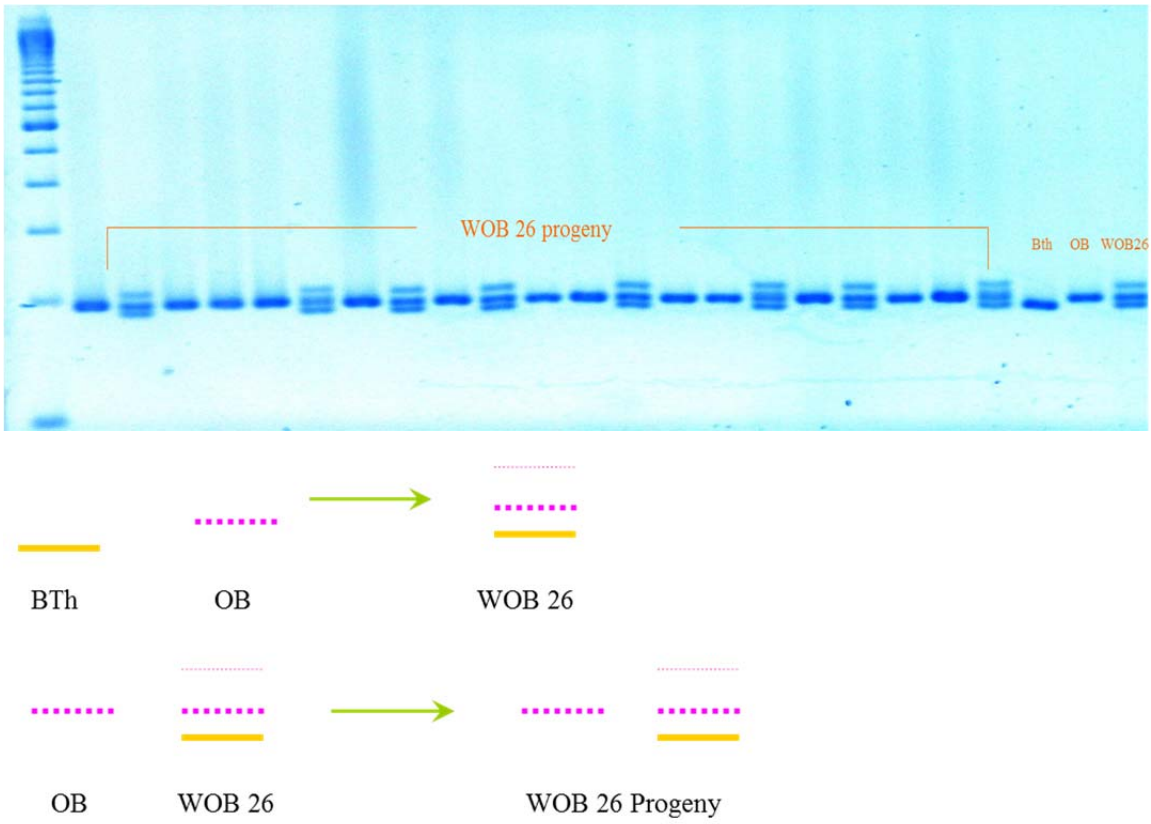


Figure 6. Marker CL2002 amplified one locus with two alleles from two parents and produced two-genotype progeny showed on the 4% Metaphor agarose gel.

Capillary electrophoresis

After characterizing the 5 parents from two populations for the 100 polymorphic SSRs (Table 4), 78 markers were selected to run with the 99 diploid progeny (Figures7 and 8) and the 131 tetraploid progeny (Figure 9) to analyze the segregation ratios using DNA sizes / peaks and subsequently to construct the linkage maps. From the 78 markers, 69 markers generated 107 loci which were ultimately assigned to seven linkage groups on the map, The segregation of 9 out of 78 markers were not useable and therefore, deleted from the dataset. Among the 69 SSR markers, 29 uni-parental markers and 40 bi-parental markers (the examples of 15 SSRs were given in Table 5) were used for the development of the female, male and integrated population diploid rose maps using JoinMap 4.

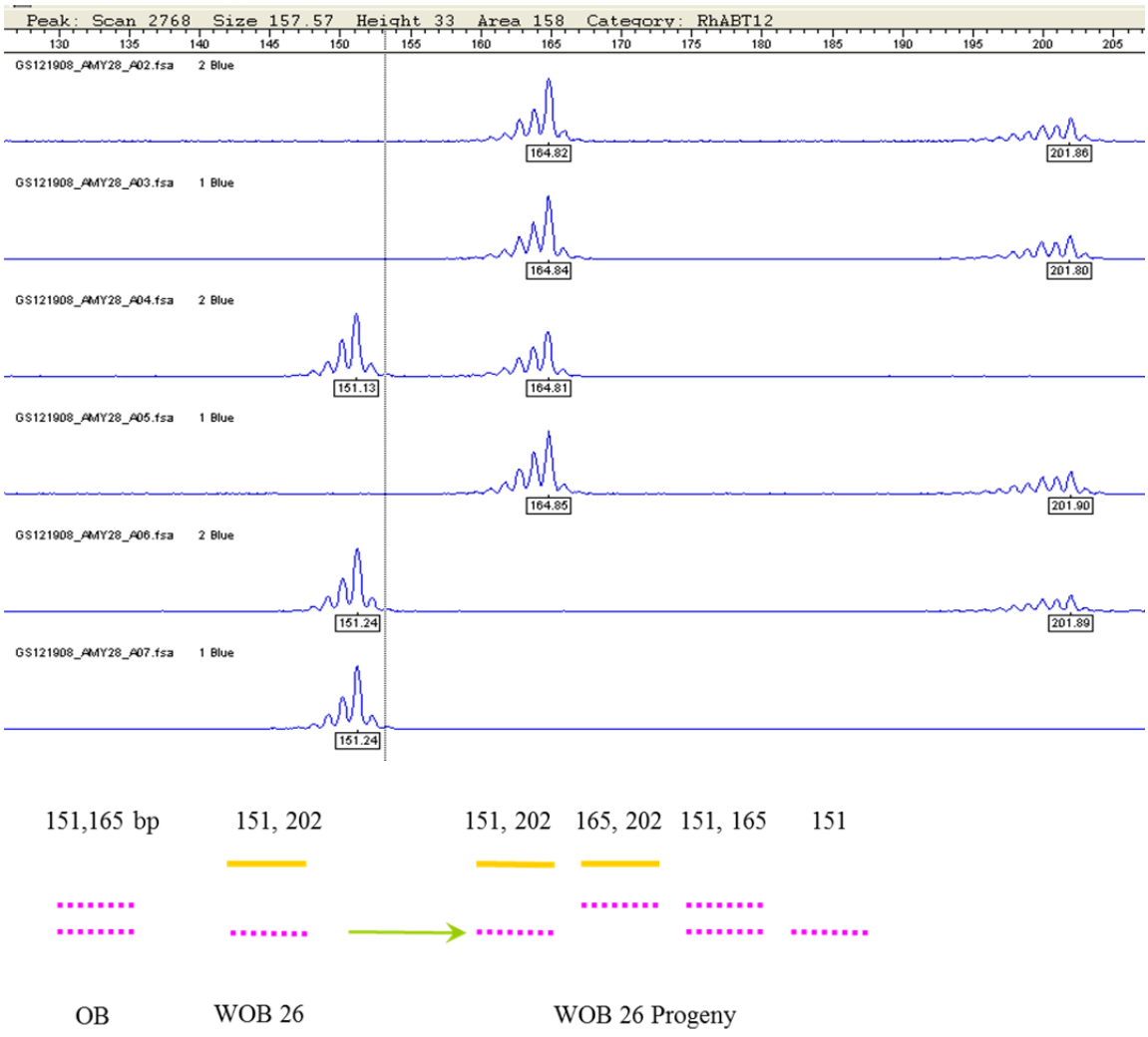


Figure 7. Marker RhABT12 amplified one locus with three alleles from two parents and produced four-genotype progeny analyzed by ABI3100 and Genotyper 3.7.

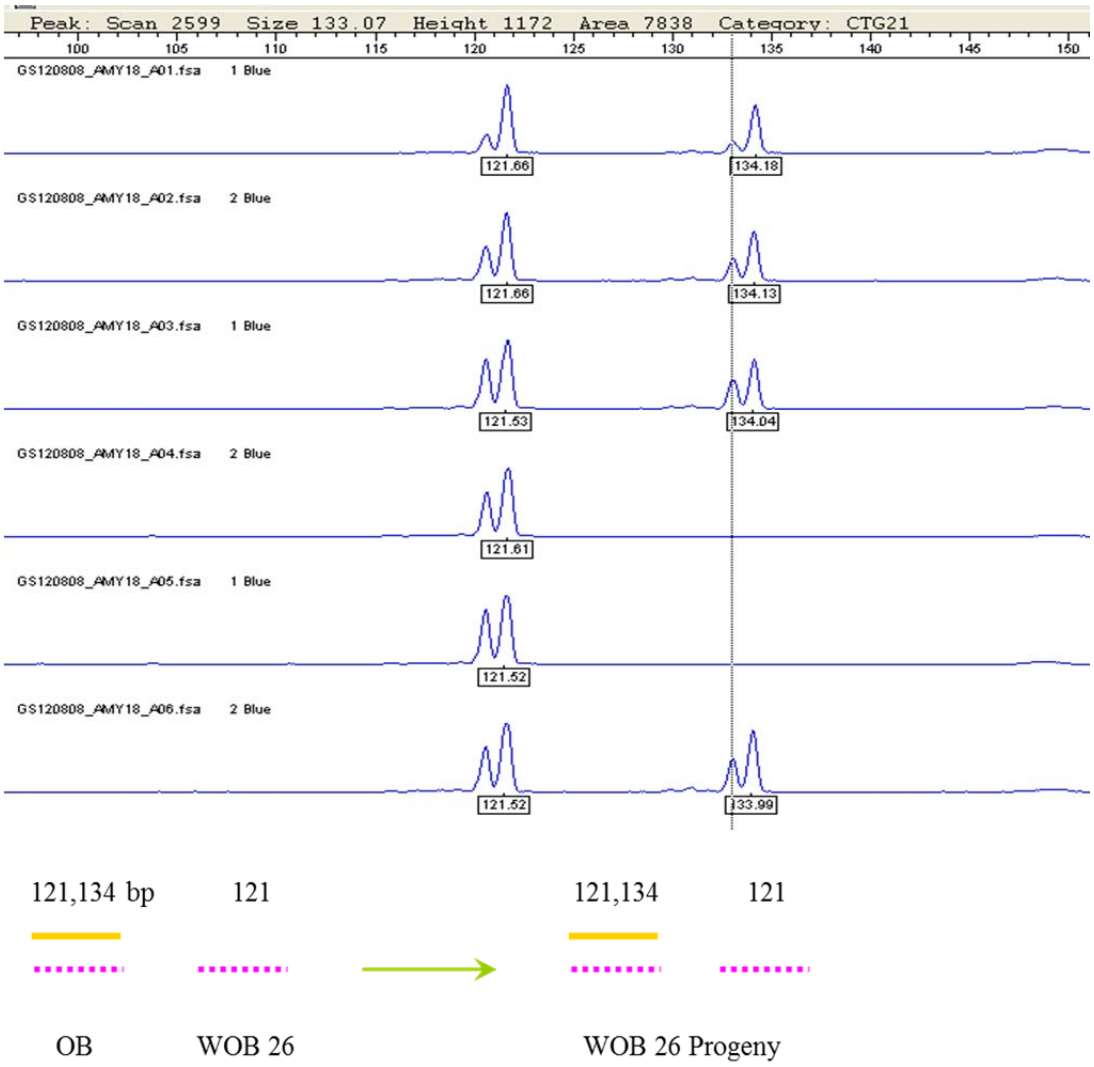


Figure 8. Marker CTG21 amplified one locus with two alleles from two parents and produced two-genotype progeny analyzed by ABI3100 and Genotyper 3.7.

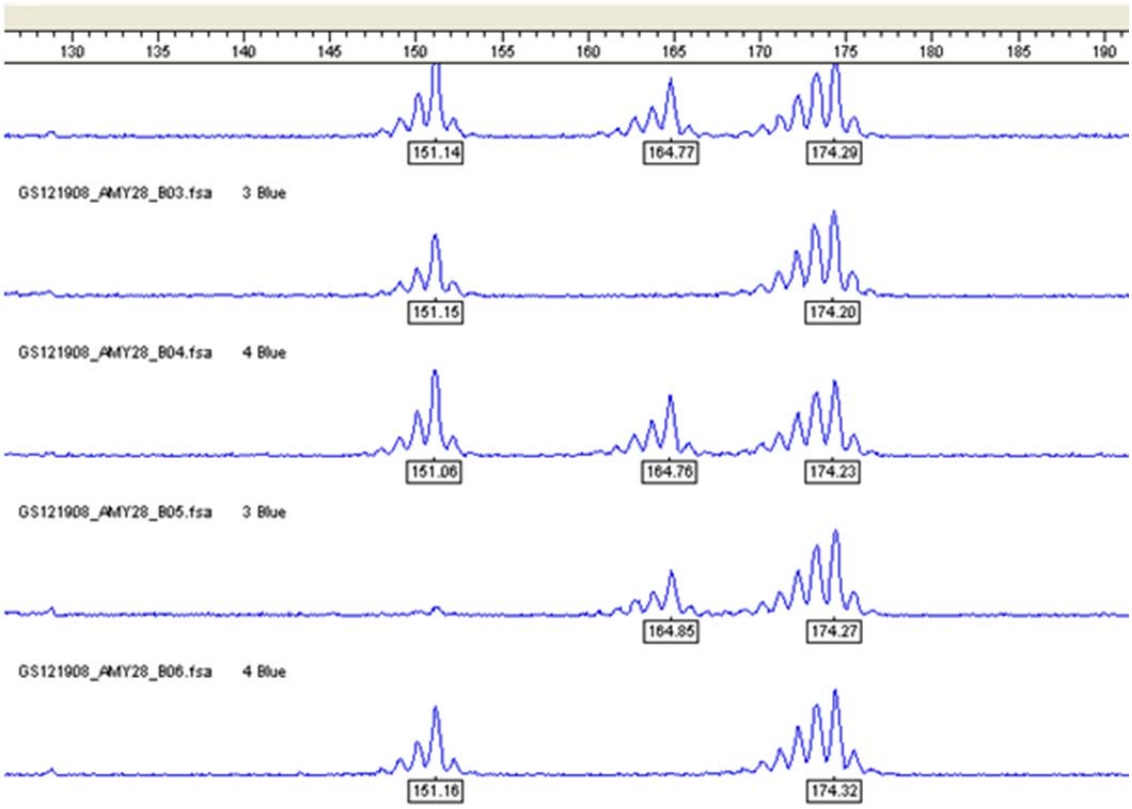


Figure 9. Marker RhABT12 (blue FAX labeled), RhD221 (Black NED labeled) and RW5D11(green HEX labeled) with tetraploid progeny analyzed by ABI3100 and Genotyper 3.7.

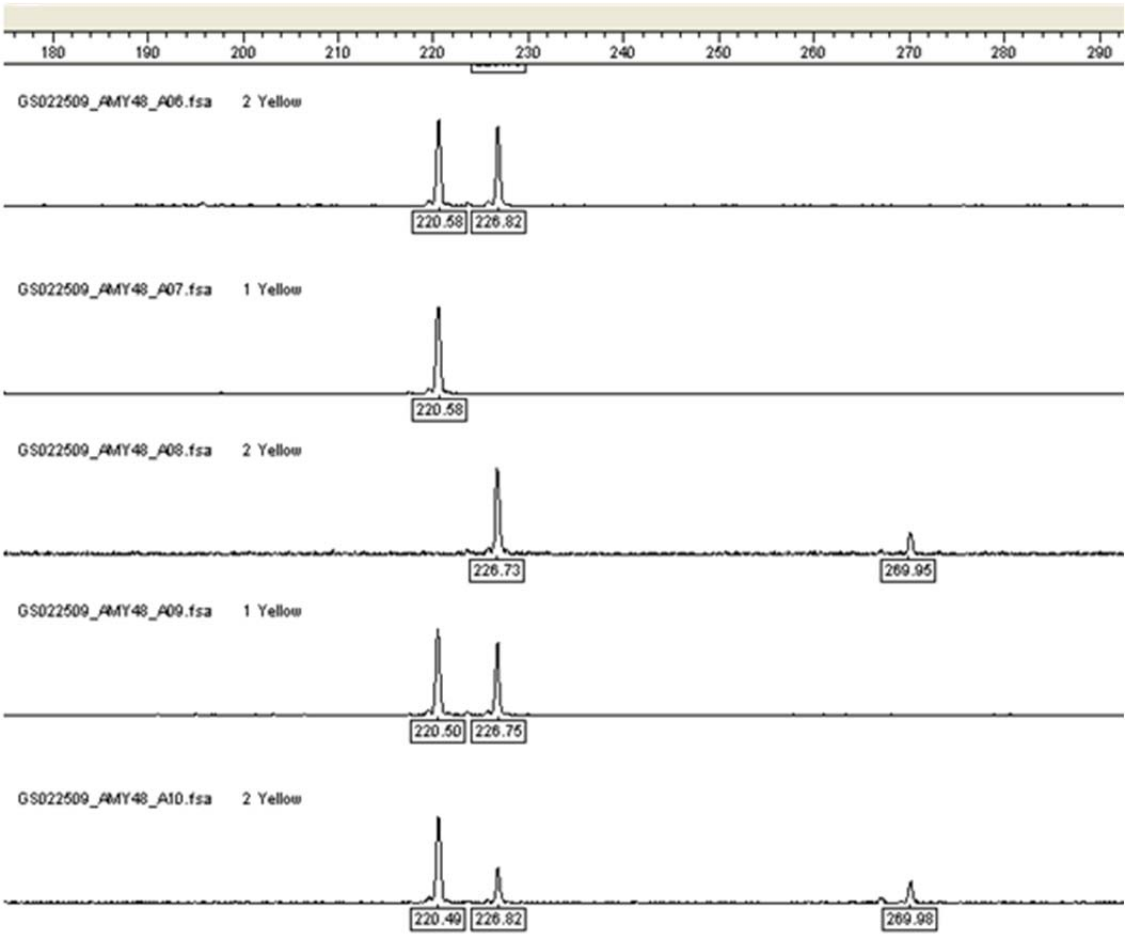


Figure 9. Continued.

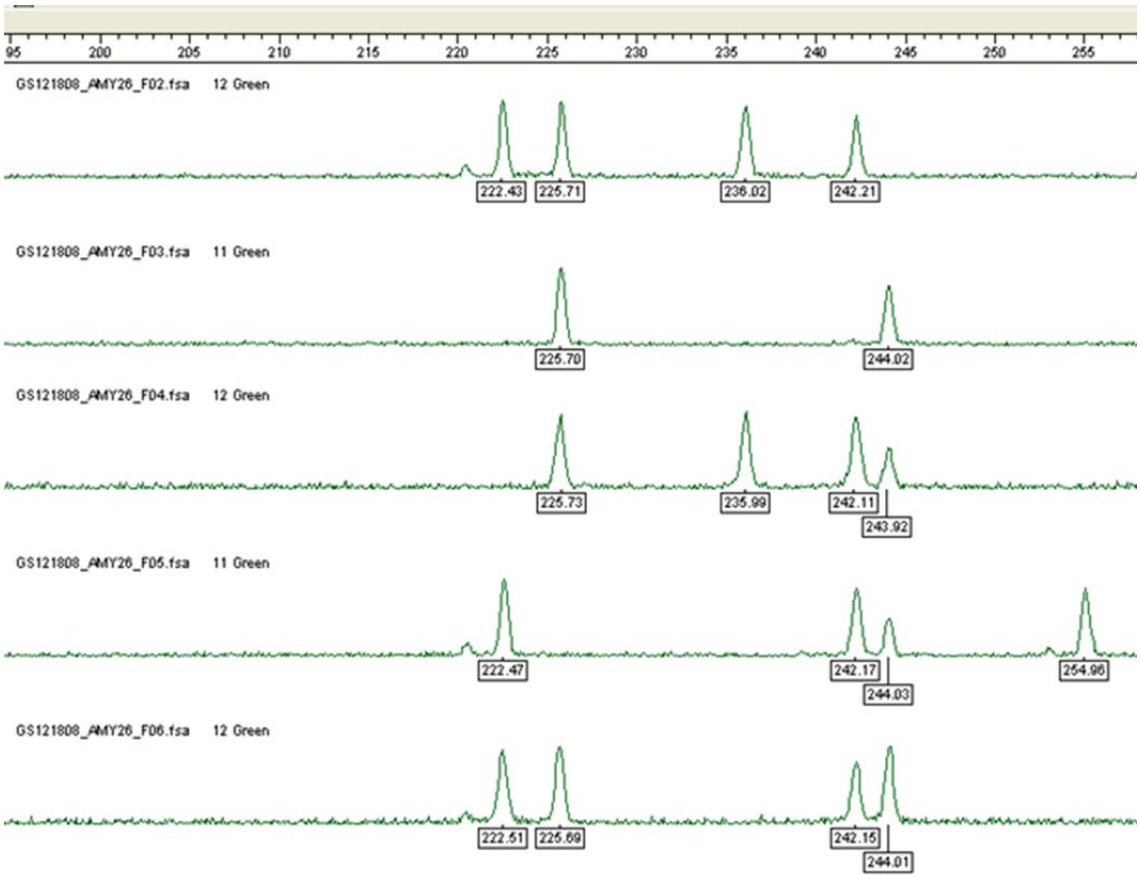


Figure 9. Continued.

Table 5. 15 SSR markers for DNA sizes (base pairs) and segregation types of the three parents of the diploid mapping population by the analysis of the DNA bands on the MetaPhor agarose gel and the DNA peaks from ABI 3100.

SSR marker	Basye's Thornless	Old Blush	WOB26	Segregation	Progeny genotype	Uni or bi-parental marker
RhAB15	104,114	112,137	104,112	<abxac>	aa, ac, ab, bc	Bi-parental marker
RhD206	208	188,196	196,208	<abxbc>	ab, ac, bb, bc	Bi-parental marker
RhABT12	162,202	151,165	151,202	<abxac>	aa, ac, ab, bc	Bi-parental marker
CL2996	173,179	179,188	179,188	<abxab>	aa, ab, bb	Bi-parental marker
RMS094	161,164	156,161	156,161	<abxab>	aa, ab, bb	Bi-parental marker
RhD221	227	221,227	221,227	<abxab>	aa, ab, bb	Bi-parental marker
RhAB22	154,160	156,183	**154,156**	<abxac>	aa, ac, ab, bc	Bi-parental marker
RW34L6	209,217	187,215	**215,217**	<abxbc>	ab, ac, bb, bc	Bi-parental marker
RhB303	119	121,146	**119,121**	<abxac>	aa, ac, ab, bc	Bi-parental marker
CL2002	187,227	195	187,195	<aaxab>	aa, ab	Uni-parental marker
RhAB38	157,167	141	141,167	<aaxab>	aa, ab	Uni-parental marker
RW53O21	166,172	156	156,166	<aaxab>	aa, ab	Uni-parental marker
RW54N22		215,222	215	<abxaa>	aa, ab	Uni-parental marker
Contig137	328,330	324,330	330	<abxbb>	ab, bb	Uni-parental marker
CTG21	121,128	121,134	121	<abxaa>	aa, ab	Uni-parental marker

Segregation analysis and marker inheritance

Segregation ratios in diploids

The segregation patterns of marker alleles observed in the backcross mapping population (OBxWOB26) were tested for the goodness of fit (χ^2 at α=0.01) with the theoretical expected ratios 1:1 (abxaa or aaxab), 3:1 (abxab and dominant), 1:2:1 (abxab and co-dominant) and 1:1:1:1 (abxac). In this diploid mapping population, the 69 SSRs markers represented 107 marker loci and 241 polymorphic amplicons. Of these 45, 26 and 36 marker loci were expected to segregate in a 1:1 ratio, 3:1 or 1:2:1 ratio, or a 1:1:1:1 ratio respectively. Of these only 23, 12, and 10 of the loci had the expected ratios. Thus only 47 of the 107 loci had the expected segregation: a 58% segregation distortion rate. Of the 3 mapped morphological traits, flower type (*Blfo* locus) and stem prickles (*prickles* locus) had distorted segregation whereas bloom type (RB locus) did not. All three of these traits map to linkage group three.

Segregation ratios in tetraploids

The construction of a genetic linkage map of a tetraploid with molecular markers is more difficult than with a diploid because of the greater number of genotypes produced in a tetraploid segregating population (Table 6), as well as the varying pattern of inheritance: disomic, tetrasomic or a combination in the tetraploids. To date, on the polyploid level, most researchers analyze the segregation of each fragment from markers based on its presence or absence in the progeny. This fragment that segregates in a single-dose (one allele) ratio (1:1) in the gametes of a heterozygous parent is called the single-dose

restriction fragment (SDRF) (Wu et al., 1992) while the marker is referred as a single-dose marker (SDM) (Barcaccia et al., 2003).

Table 6. Allelic composition per SSR locus in 5 parents of the OBxWOB26 diploid and GGFC tetraploid rose populations.

Population Parents	Diploid BTh [a]	OB[a]	WOB26[a]
Alleles per Locus	Number of SSR[b] Markers		
A_1A_1 or aa	32	18	19
A_1A_2 or ab	53[c]	69[c]	64[c]
≥2 Loci	11	9	14
Ambiguous bands	4	4	3
Population Parents	Tetraploid GG[a]	FC[a]	
Alleles per Locus	Number of SSR[b] Markers		
Nulliplex $A_1A_1A_1A_1$ or aaaa	10	15	
Simplex $A_1A_1A_1A_2$ or aaab	44	26	
Diallelic duplex $A_1A_1A_2A_2$ or aabb			
Trigenic $A_1A_1A_2A_3$ or aabc	27	38	
Tetragenic $A_1A_2A_3A_4$ or abcd	7	10	
≥2 Loci or > 4 bands	4	3	
Ambiguous bands	8	8	

[a]: BTh stands for parent 'Basye's Thornless', OB for 'Old Blush', WOB26 for F_1 hybrid of BTh and OB, GG for 'Golden Gate' and FC for 'Fragrant Cloud'.
[b]: SSR is representive for simple sequence repeat
[c]: Marker Rw14A5 and RMS089 include one null allele for each.

Chi-square (χ^2) goodness-fit of test was calculated for the theoretical expected ratio of either 1:1 for the marker loci polymorphic between parents or 1:3 for the marker loci shared between parents based on the segregation patterns (presence *vs* absence) of

marker alleles observed in the tetraploid full-sib mapping population. The parental genotypes of a marker loci in an autotetraploid are simplex (a---) by nulliplex (----) and simplex (a---) by simplex (a---) when segregating to 1:1 and 1:3, respectively, whereas in the case of allotetraploid, the genotypes will be heterozygous (a-) by homozygous (--) and heterozygous (a-) by heterozygous (a-) when segregating to 1:1 and 1:3, respectively, which is as in the diploid case (Barcaccia et al, 2003). However, the segregation ratios of 1:1 and 1:3 are only in the allotetraploid or autotetraploid condition with single-dose markers (SDMs) (Table 7).

The segregation patterns in an allotetraploid (aa) or an autotetraploid (aa--) with double-dose markers (DDMs) are different (Table 8). With DDMs, an allotetraploid with disomic inheritance will have ratios of 3:1 7:1 and 15:1 whereas an autotetraploid with tetrasomic inheritance would have the segregation ratios of 5:1, 11:1, and 35:1. The numbers of DDM loci fitted the expected segregation ratio in disomic inheritance (3:1 and 7:1) to tetrasomic inheritance (5:1) were 12 (11+1) to 16, and the numbers of SDM loci fitted the expected segregation ratio in either disomic or tetrasomic inheritance (1:1 and 3:1) were 202 (164 and 38). In addition, 13 marker loci were indistinguishable between ratios 3:1 (SDM) and 7:1 (DDM) (Table 8). Using the 28 DDM loci, the inheritance mode showed a combination of disomic and tetrasomic trending towards tetrasomic. In the tetraploid population the distortion rate was reduced from 38% to 8% if the segregation ratios calculated based on DDMs although 73% of them are indistinguishable among segregation ratios (Table 8).

Table 7. Segregation ratios of the polymorphic amplicons from 69 SSR markers and 5 morphological traits in diploid (OBxWO26) rose mapping population as well as from 191 SSR and 155 AFLP loci of SDMs (single dose markers) in tetraploid (GGxFC) rose mapping population.

Diploid (OBxWOB26)									
Marker		Segregation Ratio, observed/ fitted, P>0.01							
	Polymorphic Amplicons	1:1 (abxaa) [b]	1:1 (aaxab) [b]	3:1 (abxab) [b]	1:2:1 (abxab) [b]	1:1:1:1 (abxac) [b]	Total (loci)	X^2 not significant (α=0.01)	Distortion Rate
SSR marker	241[a]	18[g]/12[g]	27/11	22/12	4/0	36/10	107	45	58%
Morphologic al trait	10 (phenotypes)	2/0	3/2	0	0	0	5	2	
Tetraploid (GGxFC)									
Marker		Segregation Ratio (SDRF Analysis[c], Wu,1992), observed/ fitted, P>0.01							
	Polymorphic amplicons	1:1 [d] (S^fxN^f) [e]	1:1 [d] (NxS) [e]	3:1 [d] (SxS) [e]			Total (loci)	X^2 not significant (α=0.01)	Distortion Rate
SSR marker	256	68/53	58/36	65/28			191	117	38%
AFLP marker	203	65/45	42/30	48/23			155	98	

[a] Marker RW14A5 includes one null allele.
[b] abxaa and aaxab are uni-parental markers; abxab and abxac are bi-parental markers.
[c] SDRF analysis is to analyze the single dose restriction fragment based on its presence or absence among the population progeny (Wu et al., 1992).
[d] Expected segregation ratio belongs to disomic or tetrasomic inheritance using single dose marker analysis.
[e] SxN and NxS are uni-parental markers; SxS is bi-prental marker.
[f] N is for nulliplex (-- or ----) ; S is for simplex (a- or a---).
[g] Left number represents the number of loci evaluated for the expected segregation ratio, and right number is the number of loci that fit the expected segregation ratio.

Table 8. Segregation ratios of the polymorphic amplicons from 69 SSR markers and 5 morphological traits in diploid (OBxWO26) rose mapping population as well as from 191 SSR and 155 AFLP loci of **SDMs (single dose markers)** and DDMs (double dose markers) in tetraploid (GGxFC) rose mapping population.

	Diploid								
Marker		Segregation ratio, observed/ fitted, P>0.01							
	Polymorphic amplicons (OBxWOB26)	1:1 (abxaa)[b]	1:1 (aaxab)[b]	3:1 (abxab)[b]	1:2:1 (abxab)[b]	1:1:1:1 (abxac)[b]	Total loci /Trait	X^2 not significant (α=0.01)	Distortion rate
SSR marker	241[a]	18[m]/12[m]	27/11	22/12	4/0	36/10	107	45	
Morphological trait	10 (phenotypes)	2/0	3/2	0	0	0	5	2	58%

Tetraploid															
Marker		Segregation ratio (SDRF analysis[c], Wu,1992), fitted, P>0.01													
	Polymor-phic amplicons (GGxFC)	**1:1 [d] (S^jxN^j)[g]**	**1:1 [d] (NxS) [h]**	3:1 [e] (D^jxN) [g]	3:1 [e] (NxD) [h]	5:1 [f] (DxN) [g]	5:1 [f] (NxD) [h]	**3:1 [d] (SxS) [i]**	7:1 [e] (SxD) or (DxS) [i]	11:1 [f] (SxD) or (DxS) [i]	15:1 [e] (DxD) [i]	35:1 [f] (DxD) [i]	Total loci	X^2 not significant (α=0.01)	Distortion rate
SSR marker	256	53	36	4	3	5	3	23	*[k]	*	*	*	191	178[l]	8%
AFLP marker	203	45	30	2	2	6	2	15	1	*	*	*	155	141[l]	

[a] Marker RW14A5 includes one null allele.
[b] abxaa and aaxab are uni-parental markers; abxab and abxac are bi-parental markers.
[c] SDRF analysis is to analyze the single dose restriction fragment based on its presence or absence among the population progeny (Wu et al., 1992).
[d] Expected segregation ratio belongs to disomic or tetrasomic inheritance.
[e] Expected segregation ratio belongs to disomic inheritance.
[f] Expected segregation ratio belongs to tetrasomic inheritance.
[g] Uni-parental markers including 68 SSR and 65 AFLP loci were calculated for the segregation ratio fitted the expected ratio by chi-square test.
[h] Uni-parental markers including 58 SSR and 42 AFLP loci were calculated for the segregation ratio fitted the expected ratio by chi-square test.
[i] Bi-parental markers including 65 SSRs and 48 AFLP loci were calculated for the segregation ratio fitted the expected ratio by chi-square test.
[j] N is for nulliplex (-- or ----) ; S is for simplex (a- or a---) ; D is for duplex (aa or aa--).
[k] Total 55 SSR and AFLP loci were not significantly different between at least two out of four segregation ratios (7:1, 11:1. 15:1 and 35:1).
[l] The numbers includes 21 SSR and AFLP loci which were not significantly different between ratio 3:1(DDM) and 5:1 (DDM), 12 SSR and AFLP loci which were not significantly different between ratio 3:1 (SDM) and 7:1, 1 SSR and AFLP loci which were not significantly different among ratio 3:1 (SDM), 7:1 and 11 :1, and 55 SSR and AFLP loci were not significantly different between at least two out of four segregation ratios (7:1, 11:1. 15:1 and 35:1).
[m] Left number represents the number of loci evaluated for the expected segregation ratio, and right number is the number of loci that fit the expected segregation ratio.

Linkage phase and chromosome pairing analysis

To measure the preferential chromosome pairing among chromosomes in each linkage group, the observed ratio of SDRF pairs linked in repulsion versus coupling phases is expected to be 1:1 for allopolyploids and is 0.25:1 for autopolyploids (Wu et al., 1992). JoinMap 4 calculated the linkage phases of coupling and repulsion of the pairs of loci and then the hypothesis of preferential pairing was tested. Of the tetraploid linkage groups, 5 out of the 7 parental 'Fragrant Cloud' (FC) linkage groups and none of the parental 'Golden Gate' (GG) linkage did not differ from the 1:1 ratio with Chi-square (χ^2) goodness-fit of test ($\chi^2 \leq 3.881$, df=1 and $\alpha=0.05$) calculated by Excel. In contrast, when the hypothesis ratio of 1:0.25 was tested, four and three linkage groups rejected the hypothesis in the FC and GG maps, respectively (Table 9). As a result of the analysis for preferential chromosome pairing, the tetraploid population showed both disomic and tetrasomic inheritance indicating both allotetraploid and autotetraploid origin of the tetraploid rose.

Table 9. Linkage phase of marker loci belonging to ‘Fragrant Cloud’ and ‘Golden Gate’ in the tetraploid population.

Parent	Linkage group	Linkage phase	Linkage phase	Allotetraploid		Autotetraploid	
	chromosome	coupling	repulsion	1:1		1:0.25	
FC	1	5	9	1.14	ns	17.16	***
	2	18	11	1.69	ns	5.83	*
	3	5	4	0.11	ns	3.36	ns
	4	10	11	0.05	ns	13.76	***
	5	11	3	4.57	*	0.02	ns
	6	11	11	0.00	ns	12.38	***
	7	16	5	5.76	*	0.19	ns
GG							
	1	8	12	0.80	ns	20.00	***
	2	8	2	3.60	ns	0.00	ns
	3	4	5	0.11	ns	7.11	**
	4	11	4	3.27	ns	0.42	ns
	5	6	1	3.57	ns	0.14	ns
	6	9	8	0.06	ns	7.78	**
	7	7	4	0.82	ns	1.84	ns

Chi-square (χ^2) goodness-fit of test ($\chi^2 \leq 3.881$, df=1 and α=0.05) was performed to test 1:1 ratio coupling : repulsion and 1:0.25 ratio coupling : repulsion for 7 linkage groups each in two tetraploid parents, respectively.

ns : not significantly different from expected ratio; *, **, ***: significantly different from expected ratio at the 0.05, 0.01 and 0.001 levels.

Linkage map construction

Diploid map

The maternal and paternal population node were created using JoinMap 4 by selecting the following parameters: CP population, 107 SSR loci, 5 morphological traits and 99 diploid progeny. Seven linkage groups were obtained from grouping trees of both maternal and paternal population nodes with a minimum LOD score of 4.0 and 3.0, respectively. The maximum REC (recombination) was 0.4. The female (OB) map included 58 SSR loci spanning on a length of 356.9 cM and the male (WOB26) map contained 60 SSR loci along a length of 274.8cM. Therefore, the average distance between two loci on the OB map was larger than the WOB26 map. This was caused by 9 large gaps (>15cM) on the OB map compared to 3 large gaps on the WOB26 map. The sum of the seven largest gaps from each linkage group was 139.6 cM on OB map compared to 95.5 cM on WOB26 map.

Only 3 of 5 morphological trait markers were mapped. All three (stem prickles, flower type (Blfo) and recurrent blooming (RB) traits mapped to linkage group 3 as previously reported (Linde et al., 2006; Spiller et al., 2010; Hibrant-Saint Oyant et al., 2008).

Instead of joining female and male maps, a population node was also created using JoinMap 4 by employing the following options: a CP population, 107 SSR loci and 5 morphological trait markers with 99 diploid progeny. Six linkage groups were obtained from grouping tree of population node with a LOD score of 6.0 and maximum REC =

0.4. One of the six linkage groups finally was separated into two linkage groups after excluding a set of inefficient markers. Ultimately the seven linkage groups of the diploid population map were constructed with 96 SSRs and 3 morphological trait markers. These markers (80%) corresponded well with the linkage groups, LG1 to LG7, of the integrated consensus map (ICM) developed from four diploid rose genetic linkage maps (Spiller et al., 2010). All 3 morphological traits were mapped on the chromosome 3 of diploid population map. Moreover, among 69 SSRs, 5 markers, RhJ404, H9_B01, RW11E5, RW8B8 and RhE3, were mapped to two or more loci each on different chromosomes. RhJ404, Rw11E5, Rw8B8 were assigned to two loci on the chromosome 2 and 4, chromosome 3 and 6, and chromosome 5 and 7, respectively. H9_B01 was assigned to three loci on the chromosome 1, 2, and 7 and RhE3 was assigned to four loci on the chromosome 1, 4, 5, and 7 on the population map (Figure 10).

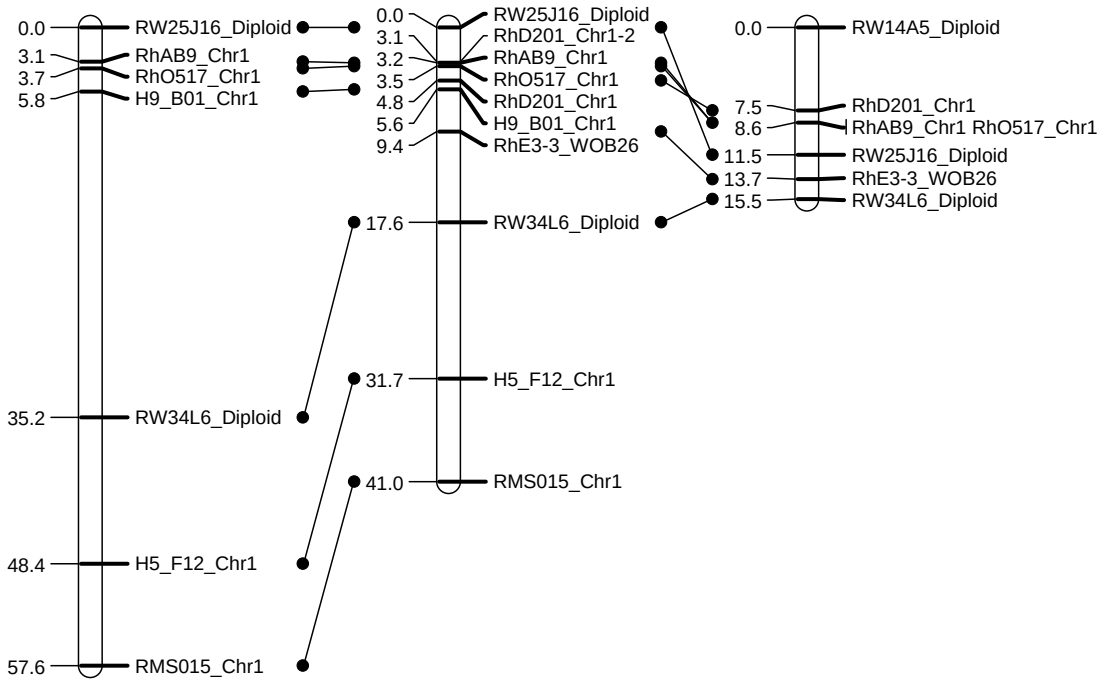
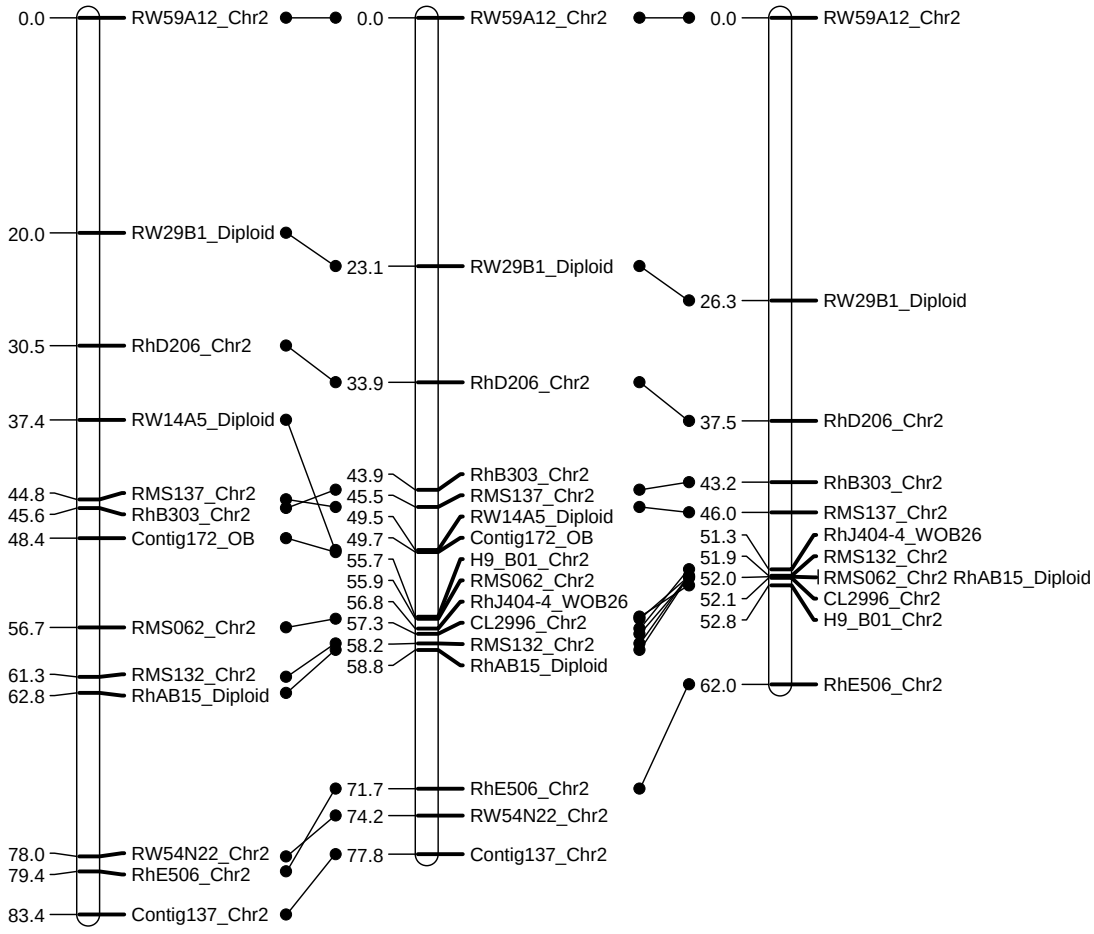


Figure 10. Diploid map for 'Old Blush' and WOB26 with their integrated map (population map) for 7 linkage groups (Chr1 to Chr7).



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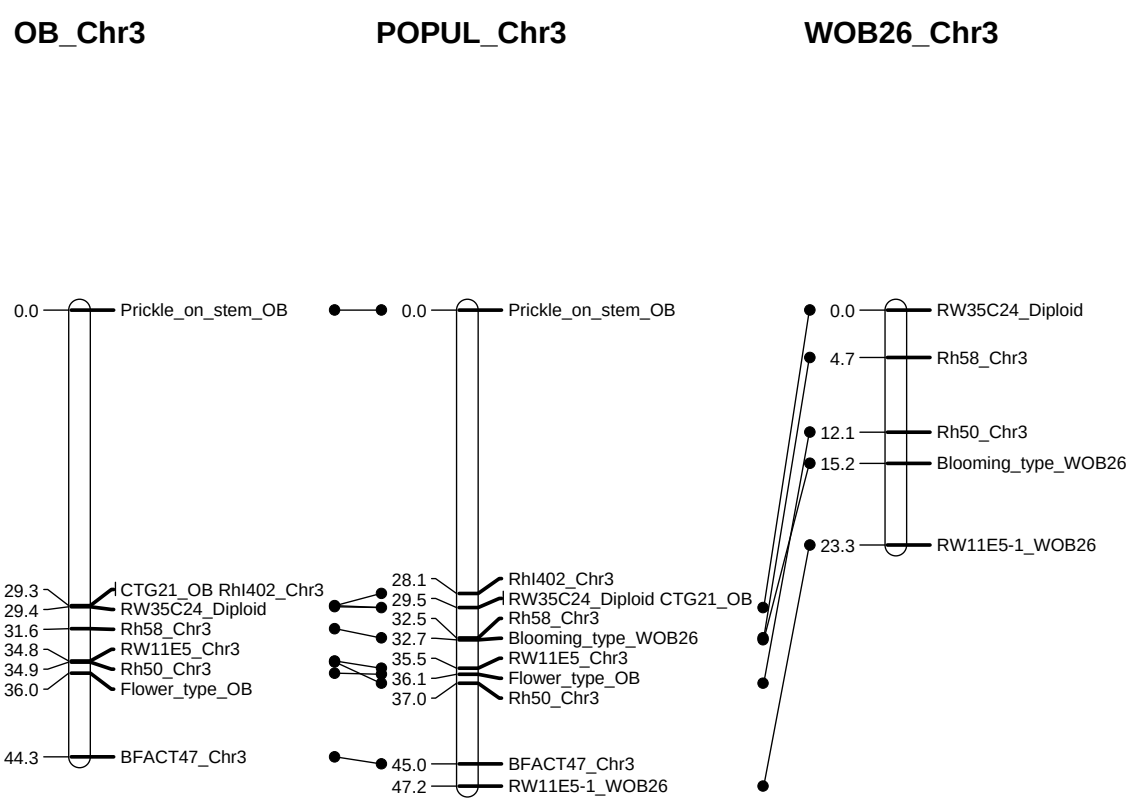

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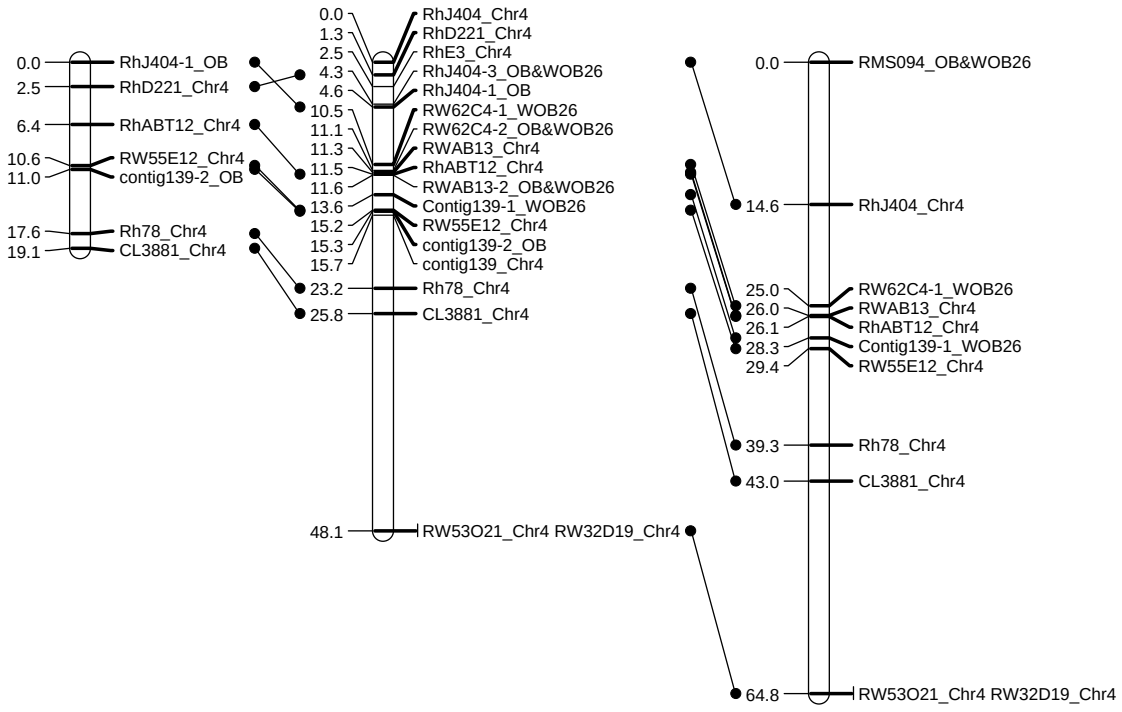

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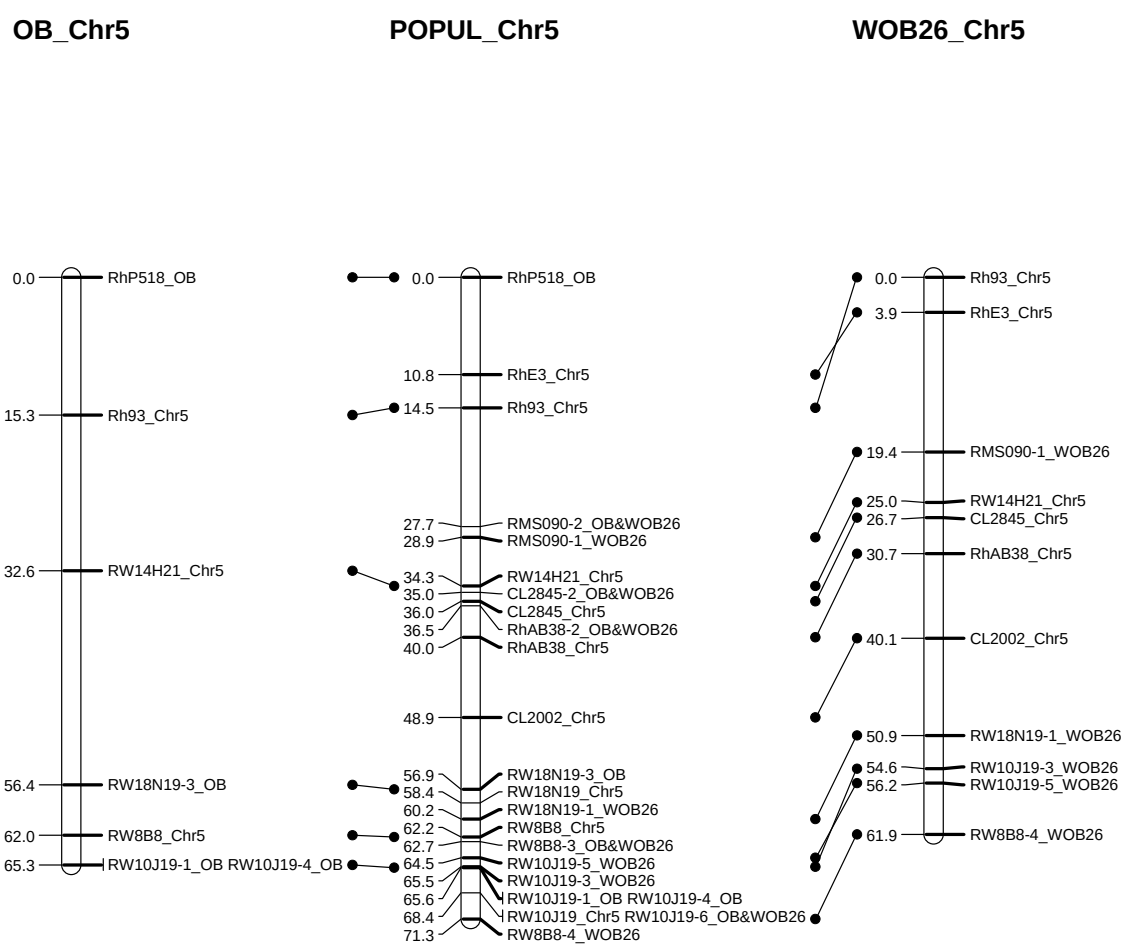

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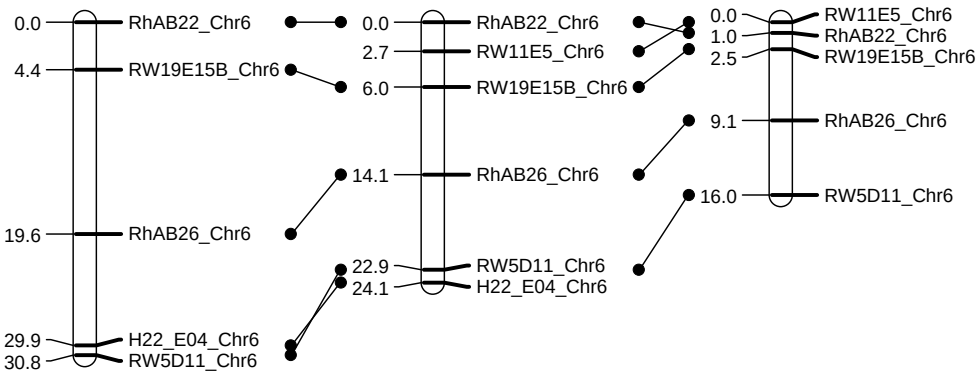

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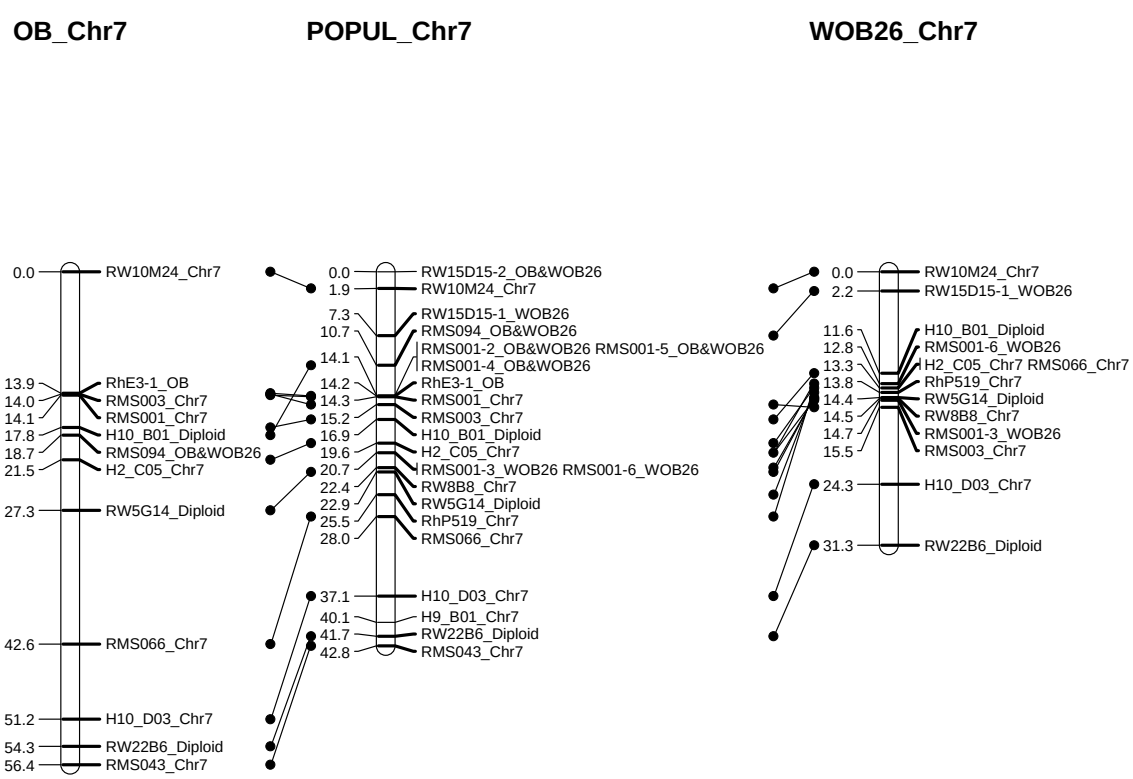


Figure 10. Continued.

Tetraploid map

The tetraploid population node was created using JoinMap 4 by selecting the following parameters: a CP population, 346 loci including 191 SSR and 155 AFLP amplicons with 131 tetraploid progeny. Twelve linkage groups were selected from grouping trees of the population nodes with a minimum LOD score of 3.0 and loci numbers ≥ 9 per linkage group. The maximum REC =0.4. Homologous pairs of linkage groups were identified by parallel, and multiple linkages of SSR markers in the diploid map (Figure 11), the integrated consensus map (Spiller et al., 2010) and tetraploid map (Gar et al., 2011). The loci which fit the expected segregation ratio were marked using a number with parenthesis followed by each marker name on the diploid and tetraploid map (Figure 11). The distorted marker loci not having numbers with parentheses were not excluded from the map if the marker showed on the integrated consensus map or the tetraploid map stated above. Ultimately, twelve linkage groups were all assigned to seven chromosomes. Chromosomes 1, 3, and 5 corresponded to one linkage group each. Chromosomes 2, 4, and 6 consisted of two linkage groups each. Chromosome 7 was comprised of three linkage groups (data not shown). For each chromosome which contained two or more linkage groups, one was selected as the main framework map, and then markers on another linkage group in the same chromosome were added to the framework map. The inefficient markers and markers with a negative distance were excluded from the map. Except for chromosome 2 and 5 with two linkage groups, respectively, other chromosomes had one corresponding linkage group each. Among 346 marker loci, 174 out of 346 (50%) loci of single-dose markers (SDMs) and double-dose markers (DDMs)

were mapped on the tetraploid linkage map. The 174 loci spanned a map length of 883.4 cM. Thus, there was an average of 24.9 loci on each linkage group. As the average length of a linkage group was 126.2 cM , the average distance between two loci was 5.4 cM. In addition, the number of map gaps with >15 cM was 1.9 per chromosome, and the largest gap was observed was on chromosome 5 with a distance of 21.8 cM (Table 10).

Integrated map of diploid and tetraploid roses

To date, 4 diploid (Yan et al., 2003 and 2005, Dugo et al., 2005, Crespel et al., 2002, Hibrand-Saint Oyant et al., 2008, Debener et al., 1999, and Linde et al., 2006) and 3 tetraploid (Rajapakse et al., 2001a, Zhang et al., 2006, Gar et al., 2011 and Koning-Boucoiran et al., 2012) rose genetic linkage maps have been created. One diploid integrated consensus map was also developed from 4 individual maps (Spiller, 2010). The integrated diploid-tetraploid map (Figure 11) is the first integrated rose map combining diploid and tetraploid maps. Sixty anchor SSR markers (Figure 12) were used to join the diploid (OBxWOB26) population map and the tetraploid (FFxGG) population map by JoinMap 4 function “combine groups for map integration”. Basically, the integrated linkage groups of individual chromosome were joined from one diploid linkage group and one tetraploid linkage group, but sometimes, also from one diploid linkage group and two tetraploid linkage groups. This integrated diploid-tetraploid map (Figure 11) consists of seven linkage groups covered by 215 loci with a map length of 632 cM (Table 10).

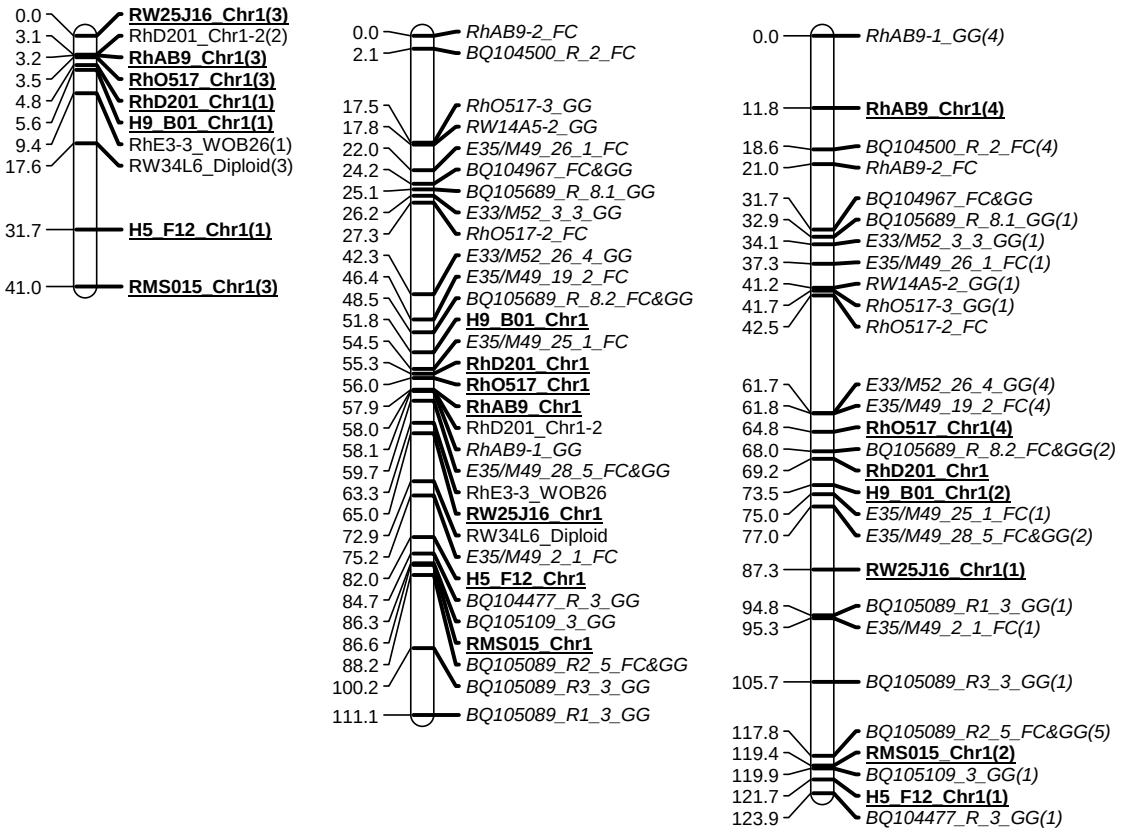
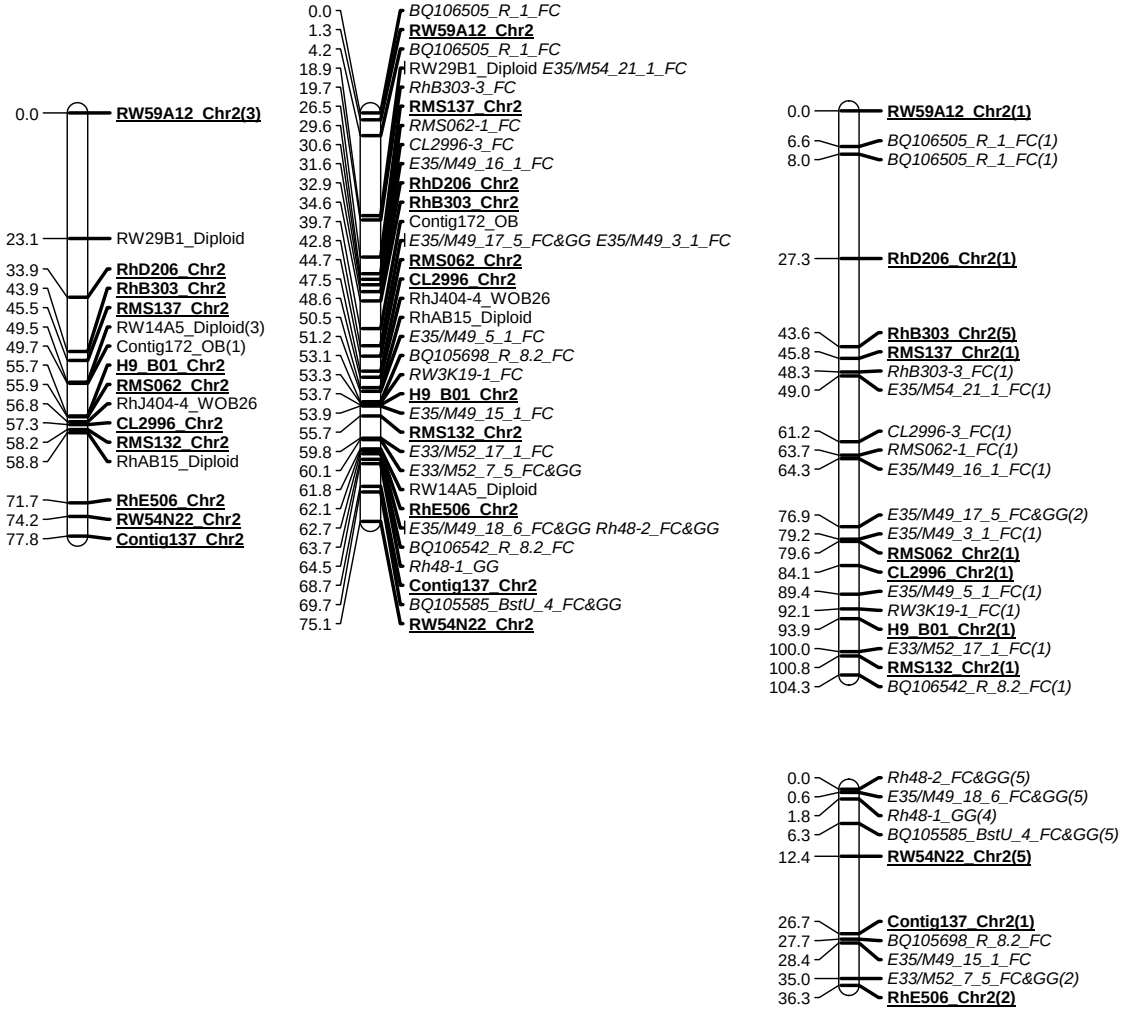


Figure 11. Integrated map of diploid and tetraploid roses. Di represents diploid, Te represents tetraploid, and chr represents chromosome /linkage group in Joinmap 4. Markers with normal font stand for SSR loci on diploid map, markers with italic font for SSR loci on tetraploid map, and markers with Chr, bold and underline font stand for anchor SSRs among 3 maps from chrosome 1 to 7, respectively. Marker names were followed by the alleles generated from parent's name, OB, WOB26, Diploid (OB&WOB26), FC, GG and FC&GG. The number with parenthesis (1) is representive for segregation ratio which fit the expected ratio 1:1, (2) for 3:1, (3) for 1:1:1:1, (4) for 3:1, 5:1 or both 3:1 and 5:1 using DDMs as well as (5) for at least following two ratios,7:1, 11:1 or 15:1 using DDMs.



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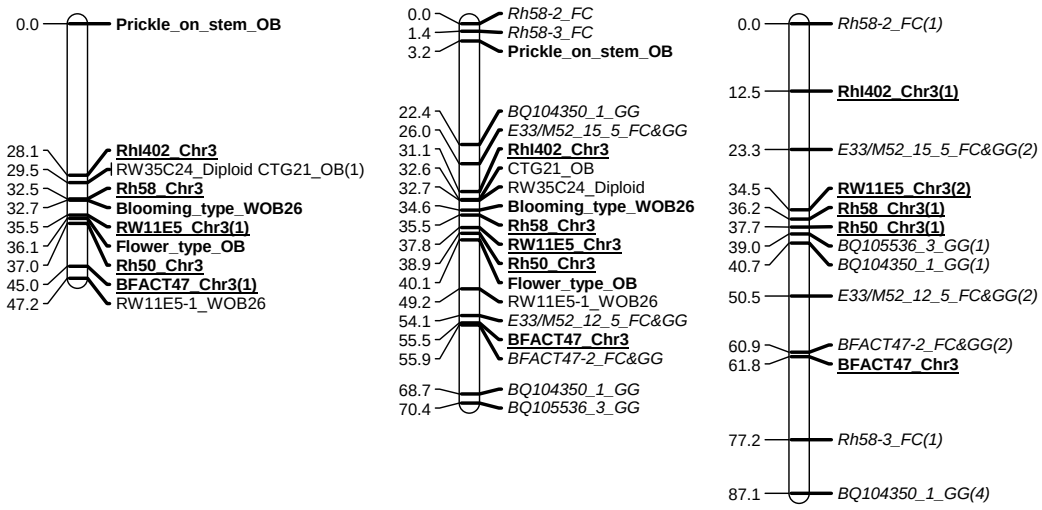

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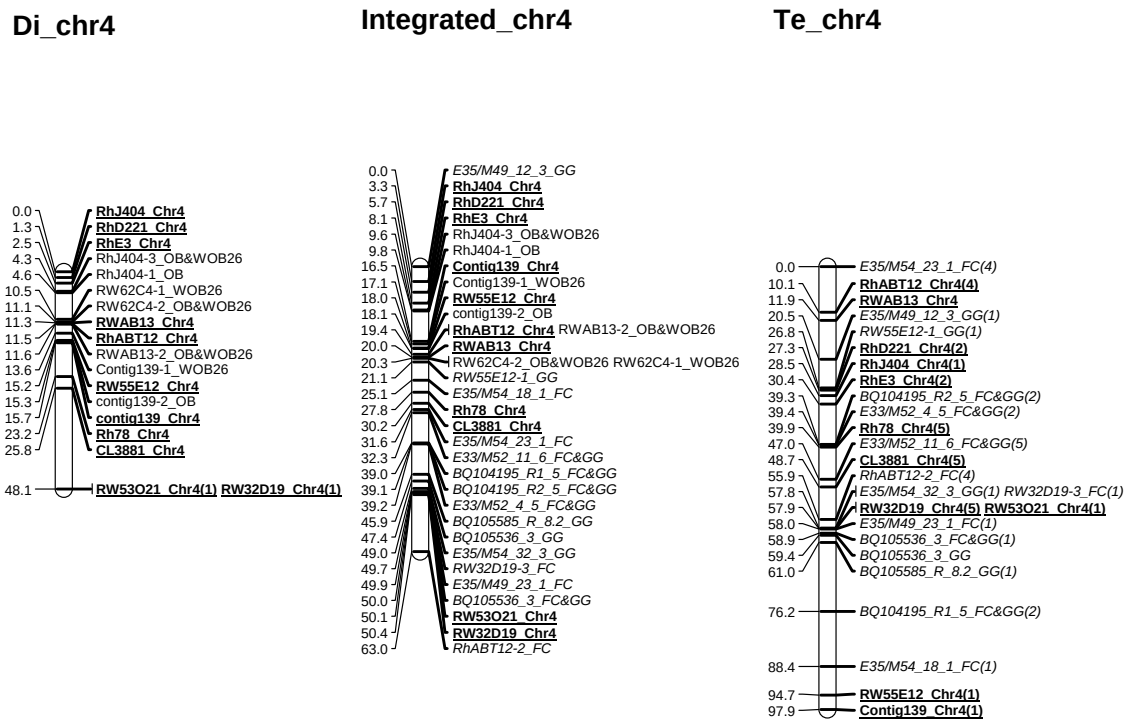

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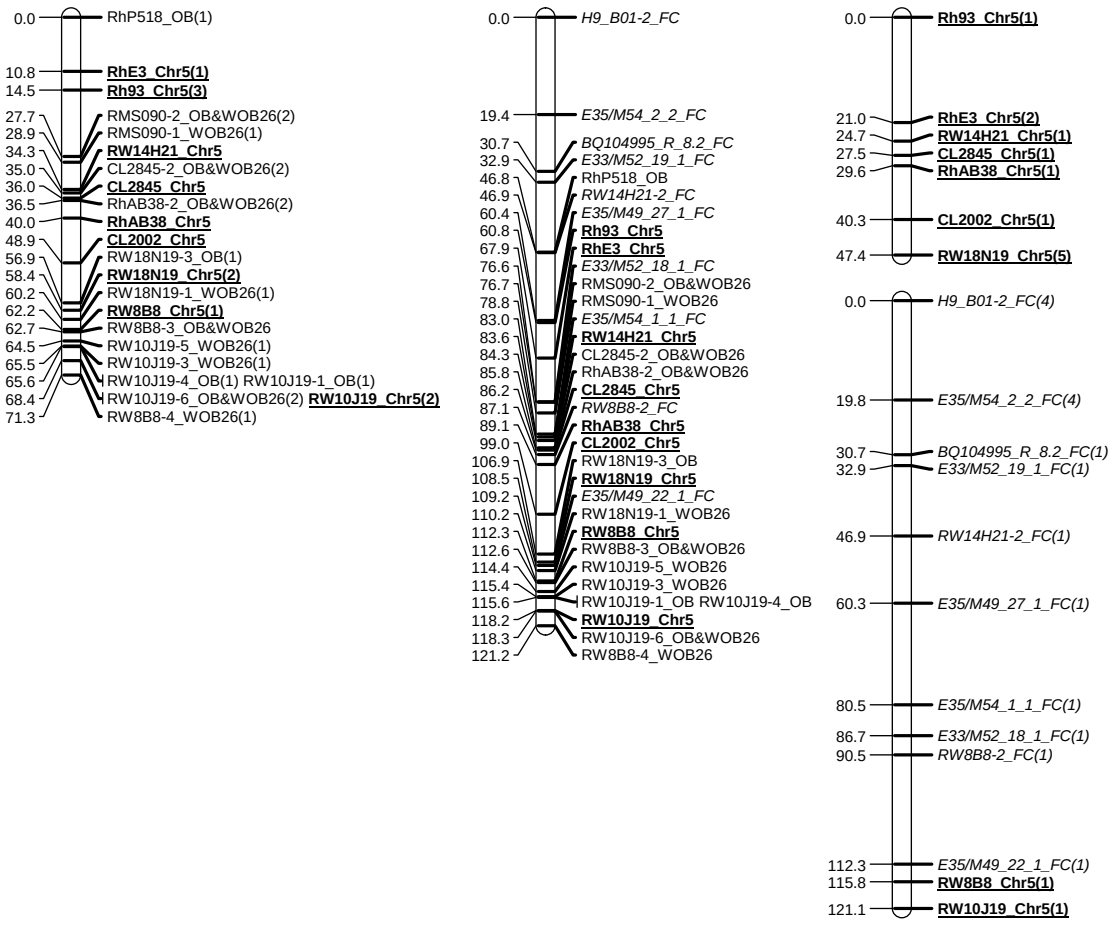

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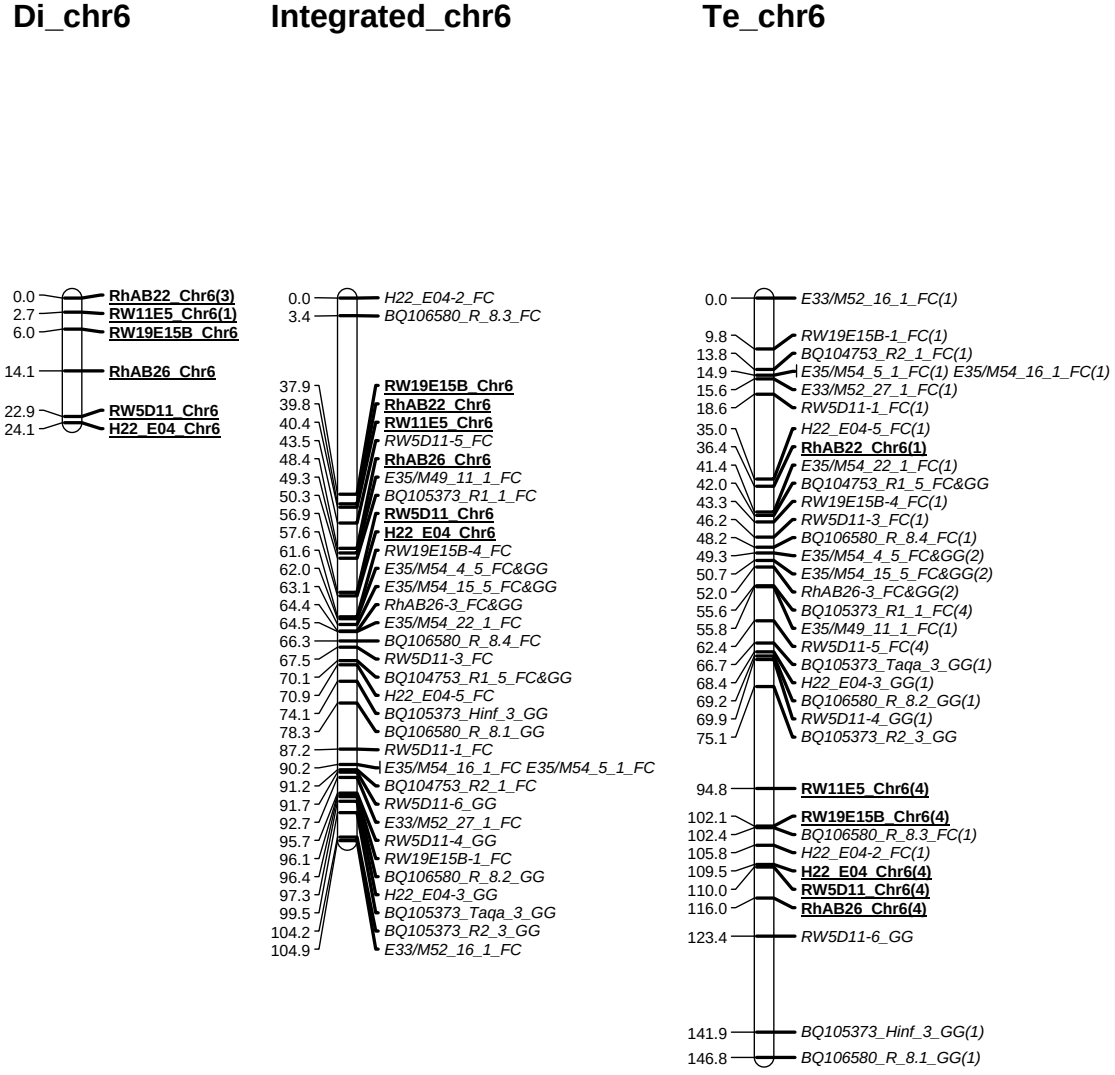

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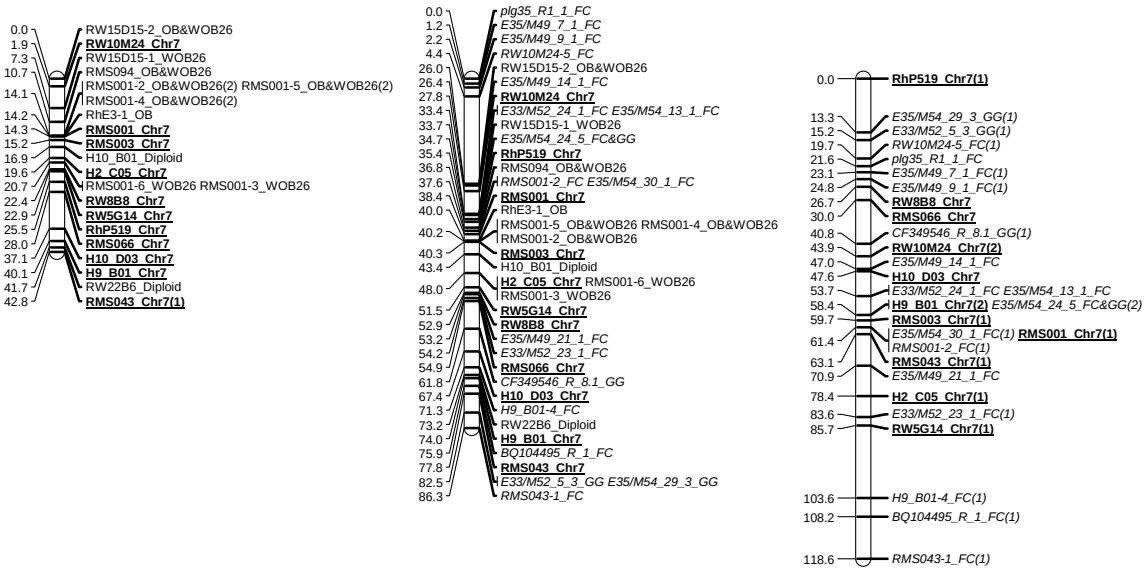

Figure 11. Continued.

Table 10. Loci composition of linkage groups in diploid, integrated diploid-tetraploid and tetraploid maps.

Linkage map	Chromosomes (Linkage groups)	Loci on map	SSR numb-ers	Anchor SSR numbers	Total length (cM)	Average distance (cM)	Number of gaps >15cM	Largest gap per LG
Diploid map	Chr1	10	9	7	41.0	4.1	0	14.1
	Chr2	16	16	11	77.8	4.9	1	23.1
	Chr3	10[a]	7	5	47.2	4.7	1	28.1
	Chr4	17	12	11	48.1	2.8	1	22.3
	Chr5	21	11	9	71.3	3.4	0	13.2
	Chr6	6	6	6	24.1	4.0	0	8.8
	Chr7	19	16	11	42.8	2.3	0	9.1
	Total	**99**	**77**	**60**	**352.3**		**3**	**118.7**
	Average	**14.1**	**11**	**8.6**	**50.3**	**3.7**	**0.4**	
Integrated map	Chr1	31	10	7	111.1	3.6	2	15.4
	Chr2	33	18	11	75.1	2.3	0	14.7
	Chr3	19	7	5	70.4	3.7	1	19.2
	Chr4	31	12	11	63.0	2.0	0	12.6
	Chr5	32	13	9	121.2	3.8	1	19.4
	Chr6	34	6	6	104.9	3.1	1	34.5
	Chr7	35	16	11	86.3	2.5	1	21.6
	Total	**215**	**82**	**60**	**632.0**		**6**	**137.4**
	Average	**30.7**	**11.7**	**8.6**	**90.3**	**3.0**	**0.9**	
Tetraploid map	Chr1	28	8	7	123.9	4.4	1	19.2
	Chr2	21+10[b]	13	11	104.3+36.3[b]	4.5	2	19.3
	Chr3	13	5	5	87.1	6.7	1	15.4
	Chr4	24	11	11	97.9	4.1	1	15.2
	Chr5	7+12[b]	11	9	47.4+121.1[b]	8.9	4	21.8
	Chr6	34	6	6	146.8	4.3	3	19.7
	Chr7	25	11	11	118.6	4.7	1	17.9
	Total	**174**	**65**	**60**	**883.4**		**13**	**128.5**
	Average	**24.9**	**9.3**	**8.6**	**126.2**	**5.4**	**1.9**	

[a]: Three out of 10 loci are morphological loci.
[b]:There are two linkage groups, that belong to chromosome 2 and chromosome 5 of the tetraploid map.

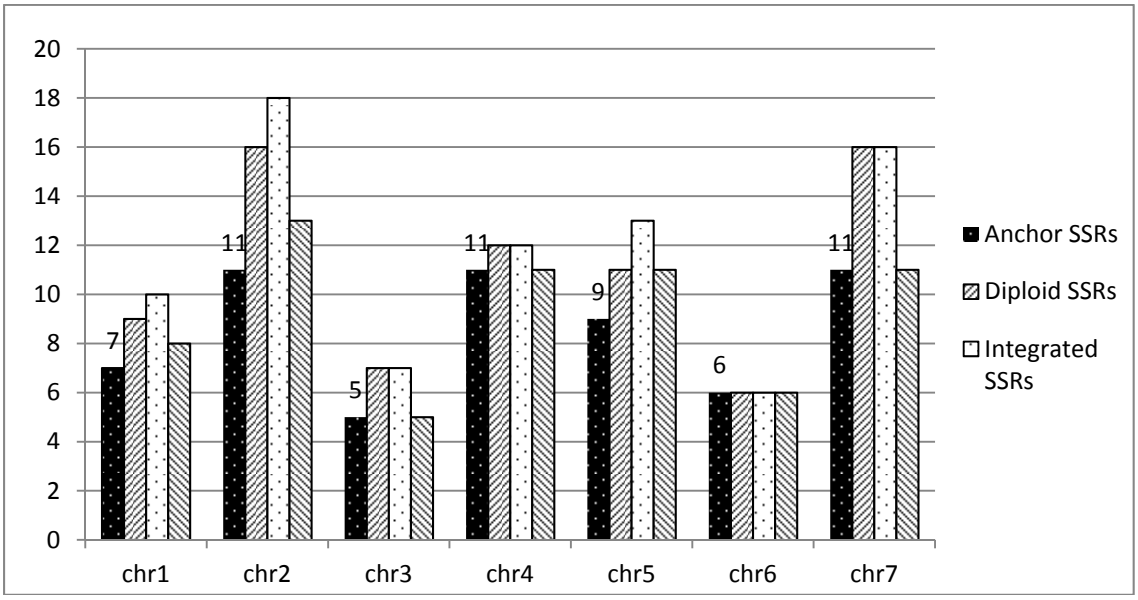

Figure 12. The numbers of anchor SSRs between diploid, integrated and tetraploid rose linkage maps. Anchor primers on each chromosome (Chr) as follows,

Chr1: H9_B01, RhD201, RhO517, RhAB9, RW25J16, H5_F12 and RMS015.
Chr2: RW59A12, RMS137, RhD206, RhB303, RMS062, CL2996, H9_B01, RMS132, RhE506, Contig137, and RW54N22.
Chr3: RhI402, Rh58, RW11E5, Rh50, and BFACT47.
Chr4: RHJ404, RhD221, RhE3, Contig139, RW55E12, RhABT12, RWAB13, Rh78, CL3881, RW53O21, and RW32D19.
Chr5: Rh93, RhE3, RW14H21, CL2845, RhAB38, CL2002, RW18N19, RW8B8, and RW10J19.
Chr6: RW19E15B, RhAB22, RW11E5, RhAB26, RW5D11, and H22_E04.
Chr7: RW10M24, RhP519, RMS001, RMS003, H2_C05, RW5G14, RW8B8, RMS066, H10_D03, H9_B01, and RMS043.

CHAPTER IV

DISCUSSION AND CONCLUSION

Mapping population in roses

The commercial rose is multispecies, mainly tetraploid, complex which has involved crosses among 5-10% of the existing rose species. In rose breeding today, the development of disease resistance has come to the forefront as in other crops such as potatoes, cucumber and grain (Song et al., 2003; Walters and Wehner, 2002; Feuillet et al., 2003). Excellent disease resistance has been found among the wild diploid rose species (Spethmann and Feuerhahn, 2003). However, this diploid to tetraploid introgression is obstructed by ploidy differences. This study seeks to elucidate the similarities among the diploid and tetraploid genomes by comparing their maps and clarifying the predominant inheritance patterns (disomic versus tetrasomic) seen in the tetraploid population.

Thus far, 5 diploid rose maps have been developed including WOB26 map. Of these, three involve *Rosa wichurana*, a species with high resistance to black spot (Dugo et al., 2005; Hibrand-Saint Oyant et al., 2008; this research), whereas, the other two involve the species *R. multiflora* (Yan et al., 2005; Linde et al., 2006) which was used to breed the Polyantha roses (Spethmann and Feuerhahn, 2003). The first reported tetraploid map also involves *R. wichurana* (Rajapakse et al., 2001b; Zhang et al., 2006) and the two latter tetraploid maps involve cut rose germplasm (Gar et al., 2011; Koning-Boucoiran et

al., 2012). Although the diploid maps have been integrated into a consensus map (Spiller et al., 2010), a diploid-tetraploid map integration does not exist.

Polymorphism of SSR markers

Single sequence repeats (SSRs) are abundant and highly polymorphic throughout plant genomes and thus were used for genetic analysis and map construction in polyploidy species, such as tetraploid alfalfa (Diwan, et al., 2000 and Julier et al., 2003), octoploid strawberry (Rousseau-Gueutin et al., 2008), tetraploid peanut (Hong et al., 2010) and tetraploid roses (Rajapakse, 2001b). In rose, SSRs developed from cDNA libraries (*R. chinensis* 'Old Blush', and *R. wichurana* 'Basye's Thornless') (Foucher, 2009), EST (expressed sequence tag) libraries (*R. hybrida* 'Fragrant Cloud' and *R. hybrida* 'Golden Gate') (Hibrand-Saint Oyant et al., 2008), various rose genes (Yan et al., 2005; Biber et al., 2010) and other sources of genomic DNA (Esselink et al., 2003; Yan et al., 2005; Debener, Germany, personal communication; Zhang et al., 2006 and Hibrand-Saint Oyant et al., 2008) have shown high levels of polymorphism ranging from 31 to 73% in various populations (Zhang, 2006; Hibrand-Saint Oyant et al., 2008 and this research).

Segregation analysis in diploid and tetraploid population

Bi-parental markers cdxcd (1:2:1) and abxac (1:1:1:1) scored co-dominantly generated the higher distortion rate than uni-parental markers abxaa (1:1), aaxab (1:1) and bi-parental markers abxab (3:1) scored dominantly in WOB 26 parents. Segregation distortion skews the genotypic frequencies from the expected ratios of Mendelian

inheritance. Segregation distortion could be caused by different physiological and genetic factors such as pollen tube completion, pollen lethals, preferential fertilization, and selective elimination of zygotes and gametophytic factors. In addition, chromosomal regions consistently associated with segregation distortion, called segregation distortion regions (SDRs), have been reported in several mapping population (Lu et al., 2002; Zamir and Tadmor, 1986). Segregation distortion is common during the development of genetic linkage maps and high distortion rates were discovered in populations of maize (65%) and tomato (68%) (Wendel et al., 1987; Paterson et al., 1988). Although the mean distortion rate of segregation ratio of WOB26 population (58%) was higher compared to 14.8%, 16%, and 38.8% among other diploid maps (Dugo et al., 2005; Debener and Mattiesch, 1999; Crespel et al., 2002), 66 out of 82 (80%) SSRs on the diploid-tetraploid integrated map aligned with anchor SSRs on the integrated consensus map (ICM) published by Spiller et al. (2010).

Given the complexity of a tetraploid genome which leads to uncertainty of parental genotypes and highly complex segregation patterns, single dose amplication fragments (SDAF) (presence vs absence) were predominantly used to construct the genetic linkage map and for segregation analysis on the polyploid level. These uni-parental single-dose fragments (1:1) and bi-parental single-dose fragments (3:1) were widely used for map construction in different polyploid crops such as potato, strawberry, alfalfa, switchgrass, bahiagrass and willow (Li et al., 1998; Rousseau-Gueutin et al., 2008; Diwan et al., 2000; Liu et al., 2012; Stein et al., 2007 and Barcaccia, 2003). Among the current 3 tetraploid

rose maps, 64 to 83% of the markers mapped were SSR and AFLP single-dose markers (SDMs) (Rajapakse et al., 2001b, this article and Koning-Boucoiran et al., 2012). Double-dose markers (DDMs) with segregation patterns 3:1 (duplex by nulliplex), 7:1 (simplex by duplex) and 15:1 (duplex by duplex) for disomic inheritance, and segregation ratio 5:1 (duplex by nulliplex), 11:1 (simplex by duplex) and 35:1 (duplex by duplex) for tetrasomic inheritance were excluded in one tetraploid rose map and contributed a lower percentage of loci (from 12.6% to 23%) in the other two tetraploid rose maps.

The distortion rate of 8 to 13.5% in 3 tetraploid rose maps is much lower compared to the rate in diploid maps. This is because SDMs were both calculated into marker segregation ratio in diploid and tetraploid mapping populations, while DDMs were calculated into the marker segregation ratio only in tetraploid mapping population subsequently to reduce the distortion rate. For instance, the distortion rate decreased from 38% to 8% in the case of this article (Tables 7 and 8).

Genomic structure of roses: autotetraploid or allotetraploid

Polyploids are very common in plants, with tetraploids being the most common. In nature, diploid roses could evolve to a fertile tetraploid rose via spontaneous chromosome doubling during mitosis in somatic cells or meiosis in gamete cells. The most common condition in nature appears to be alloploidy via hybridization and non reduction during meiosis leading to a doubling of chromosomes in a low fertility diploid

hybrid (Burnham, 1962). In rose breeding, several sterile diploids such as 'Max Graf' (*R. rugosa* and *R. wichurana)* and a diploid hybrid between *R. abyssinica* and *R. rugosa* (Spethmann and Feuerhahn, 2003) were converted to fertile tetraploids via doubling during meiosis. More commonly, breeders use colchicine and oryzalin applications to cause mitotic polyploidization of diploid germplasm to create tetraploids with which to work (Zlesak, 2006; Byrne et al., 1996).

Tetraploids range from allopolyploids (genomic combination) to autotetraploids (genomic duplication) (Wendel, 2000; Comai, 2005). As an autopolyploid has 4 copies of each homologous chromosome, it is expected to form tetravalents during meiosis whereas alloploidyploids being derived from differentiated genomes are expected to preferentially form bivalents during meiosis (Byrne and Crane, 2003). However, according to a survey in the polyploid plants, multivalent formation was observed in only 28.8% of the autotetraploids, which was less than expected and in 8% of the allopolyploid species which was higher than expected (Ramsey and Schemske 2002). In the evaluation of meiotic behavior in an allotetraploid developed from a sterile diploid hybrid among two rose species (*Rosa wichurana* x *R. rugosa*) and hybrids derived from crosses with it and a tetraploid rose indicated that multivalent formation involved from 15% to 74% of the chromosomes. This combined with the fact the chromosomes of the sterile diploid paired almost exclusively as bivalents with little univalent formation, indicated that the genomes of the rose species used in the development of commercial roses are only partially differentiated (Ma et al., 1997). The analysis of the segregation

ratio of the 28 DDM loci in GGFC population, gave a ratio of disomic to tetrasomic inheritance of 12 to 16. This appearance of both types of segregation patterns with a preference towards tetrasomic inheritance agrees with the conclusion by Gar et al., (2011) and Koning-Boucoiran et al. (2012). In contrast, when the thesis of preferential chromosome pairing was tested by looking at the proportion of coupling vs repulsion, the 1:1 hypothesis was rejected only in 2 out of 14 linkage groups of FC and GG map by Chi-square test, which showed a disomic inheritance as well as rejected 7 out of the 14 linkage groups for the tetrasomic hypothesis of 1:0.25 coupling versus repulsion arrangement. Thus in this analysis, the predominant inheritance appears to be disomic over tetrasomic. It is not clear why the conclusions are different although perhaps it might be in part due to low numbers of loci compared per chromosome. In any case, it is clear both disomic and tetrasomic segregation is operational in roses. Given the breeding history of the tetraploid rose as a multispecies complex which combines species with partially differentiated genomes (Ma et al., 1997; Ma et al., 2000), the relative proportion of disomic and tetrasomic segregation will likely vary some with the genetic background studied. To sum up, we can conclude that in these rose tetraploids, disomic and tetrasomic inheritance usually exists concurrently as the results showed in the GG x FC population and K5 population (Koning-Boucoiran et al., 2012).

Genetic linkage map in diploid and tetraploid roses

Anchor SSR markers can be used to integrate two or more genetic linkage maps manually or with JoinMap. In roses, JoinMap was used to align two parental maps 93/1-

119 and 93/1-117 to an integrated map (Yan et al., 2005), as well as to align 4 maps into an integrated consensus map Spiller et al (2010). In other crops, two examples are given. First, the genetic linkage maps of cultivated octoploid and diploid strawberry (*Fragaria* species) were compared manually and revealed a level of colinearity between them (Rousseau-Gueutin et al., 2008). Second, in peanuts (*Arachis* species), a SSR-based composite map was developed from an integration of three tetraploid populations and then compared with a SSR-based diploid map (Hong et al., 2010). An integrated map gives a general order and distance among the molecular markers, and thus provides some useful information to identify desirable traits for marker selection or to study genomic structure and gene function. A high percentage of anchor/common SSRs were shared between diploid map (78%) and tetraploid map (92%) (Table 10), which permitted the development of an integrated diploid-tetraploid map .This integrated diploid-tetraploid map when compared to ICM map (Spiller et al., 2010), GGFC population map (Gar et al., 2011) and K5 population map (Koning-Boucoiran et al., 2012) with numbers of the anchor SSRs and mutual morphological traits (Table 11) showed excellent collinearity among the maps.

Table 11. The comparison of the integrated diploid-tetraploid map with the ICM, and the two published tetraploid maps.

	ICM diploid map (Spiller et al., 2010)		Integrated diploid-tetraploid map		Full-sib tetraploid map (Gar et al., 2011)		K5 tetraploid map (Koning-Boucoiran et al., 2012)	
Linkage group (LG) or chromosome (Chr)	No. of Anchor SSRs	Mutual morphological traits	No. of SSRs	Mutual morphological traits	No. of Anchor SSRs	Mutual morphological traits	No. of Anchor SSRs	Mutual morphological traits
1	9	*Rdr1*	10		4		3	
2	14	*Blfa*	18		10		6	2 *Prickles* (QTL)
3	7	*Prickles*, *RB*, *Blfo*, *PM* (QTL), *PN*(QTL)	7	*Prickles*, *RB*, *Blfo*	5		2	*Prickles* (QTL), *PN*(QTL)
4	11	*PM* (QTL)	12		7	*Color_A*, Ag	3	*PM* (QTL)
5	10		13		9		2	
6	2	*PM* (QTL)	6		4	*PM*	0	
7	13		16		9		5	

Rdr1, black spot resistance; *Blfa*, flower color; *prickles*, stem prickles; *RB*, recurrent blooming; *Blfo*, flower type; *PM*, powdery mildew resistance; *PN*, petal number; *Color_A*, flower color; *Ag*, anther color.

The synteny among all the maps was excellent which should aid in the translation of genetic information from the diploid case to the tetraploid situation. Examples of this would be loci such as, Rdr1, black spot resistance, was closely linked to two markers 155SSR and RMS015 in LG1 (Biber et al., 2010) ,while RMS015 was also mapped on Chr1 (LG1) on the integrated map in this article. Therefore, using anchor marker, like RMS015 to look for/check whether *Rdr1* also located on the same locus on or near the same locus on the tetraploid map as diploid is a new research direction. Furthermore, the molecular markers of GA-influenced floral genes, *RoVIP3*, *RoSPY* and *RoDELLA,* designed from the similar genes of *Arabidopsis* were also successfully mapped to two important traits, date of flowering and recurrent blooming (RB) on the diploid rose map (Remay et al., 2009). This method provides an approach to explore more marker-related functional genes introduced from other species closely linked to the important traits in the future breeding program.

The great benefit of developing a genetic linkage map is to understand the gene linkages of desirable traits on chromosomes and the probability of gene recombination during meiosis. The diploid-tetraploid integrated map is the first step in comparing the similarity between the diploid and tetraploid genomes and to locate the putative anchor SSRs for some horticultural traits in roses which would accelerate the introgression of important traits from diploid roses into the genetic background of cultivated tetraploid roses.

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APPENDIX A TOWARDS A UNIFIED GENETIC MAP FOR DIPLOID ROSES*

Theor Appl Genet
DOI 10.1007/s00122-010-1463-x

ORIGINAL PAPER

Towards a unified genetic map for diploid roses

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Received: 10 June 2010 / Accepted: 24 September 2010

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.

Communicated by H. Nybom.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-010-1463-x) contains supplementary material, which is available to authorized users.

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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

Published online: 10 October 2010

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: GCCTT TTCAGCTTGCAAAAGA, TE3r: TTTACGATCCAAAC CGACCAG9) were derived from the EST sequence PP_LEa0010K05f. Primers for TE22_2 (TE22f: TGATAG ATGCGGCTTCTCAA, TE22r: GGCCTCATAATGCAG GGTAA) were based on sequence PP_Lea0009M17f, and TE39 primers were designed using PP_LEa0003D12f (TE39f: TTGGAGGACGAGCTTCAACT; TE39r: CCCG ACAAACCAGATCAAGA).

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′-CTCGATTCTC AACCCGAAAGTATAGATCCCA-3′; Adapter short arm: 5′-TGGGATCTATACTT-3′, with 3′-amino group). Three degenerate primers, NBS1 (5′-GCIARWGTWGTYTTICC YRAICC-3′), NBS3 (5′-GTWGTYTTICCYRAICCISSC ATICC-3′), and NBS5A/6 (a mixture of 5′-YYTKRTHGT MITKGATGAYGTITGG-3′ and 5′-YYTKRTHGTMITK GATGATATITGG-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′-ACTCGATTCTC AACCCGAAAG-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 MapChart 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 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.

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 EMBRYOGENESIS*; 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; RoSPINDLY, *SPINDLY*; RB, recurrent blooming; TE, TEXAS × EARLYGOLD (see text); *Blfo*, double flowers; RoGID, *GIBBERELLIN INSENSITIVE*; 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 ▶

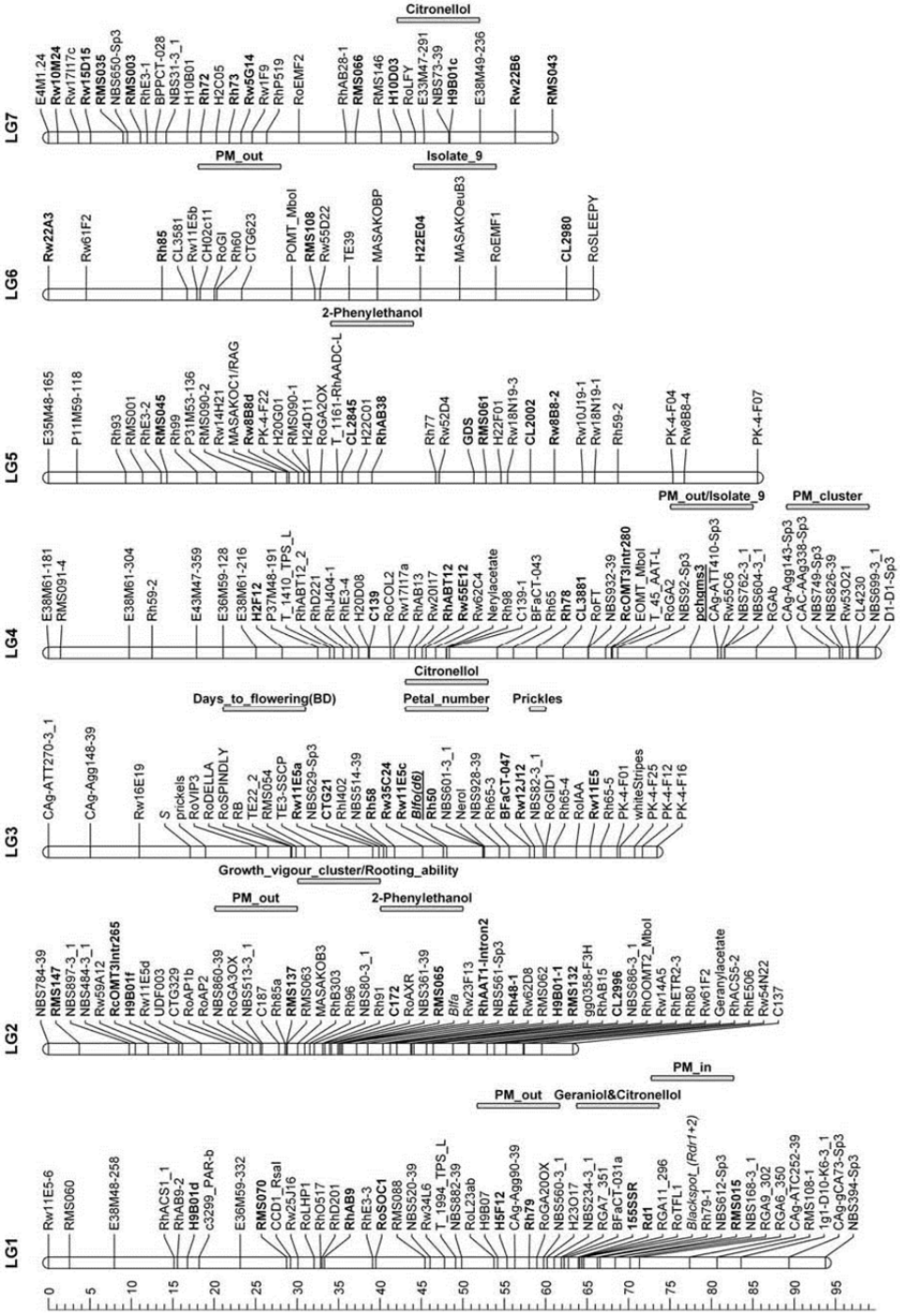

Table 2 Summary data of the consensus map (ICM) and the currently calculated single population maps in comparison to the originally published maps

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, *Bfa*, *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, *Bfo*, nerol, β-citronellol (QTL)	LG3: SI, *Bfo*, *prickles*, *whitestripes*, PM (QTL), *Rpp1*	LG4: *Bfo*, *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*	
LG7	LG7: alcohol acetate production	LG7	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

Bfa flower colour, *RA* rooting ability, *Bfo* 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.

Acknowledgments This study has been carried out in part with financial support from the Netherlands Ministry of Agriculture, Nature and Food Safety (KB-01) and from the Commission of the European Community [QLRT-2001-01278, Genetic evaluation of European rose resources for conservation and horticultural use (Generose)]. This

study does not necessarily reflect the Commission's views and in no way anticipates the Commission's future policy in this area. Additionally the Basye Endowment for Rose Genetics and the TDA-BARD funded by the Texas-Israel Exchange Fund supported this work.

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APPENDIX B AN AUTOTETRAPLOID LINKAGE MAP OF ROSE (*ROSA HYBRIDA*) VALIDATED USING THE STRAWBERRY (*FRAGARIA VESCA*) GENOME SEQUENCE*

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An Autotetraploid Linkage Map of Rose (*Rosa hybrida*) Validated Using the Strawberry (*Fragaria vesca*) Genome Sequence

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Abstract

Polyploidy is a pivotal process in plant evolution as it increase gene redundancy and morphological intricacy but due to the complexity of polysomic inheritance we have only few genetic maps of autopolyploid organisms. A robust mapping framework is particularly important in polyploid crop species, rose included ($2n = 4x = 28$), where the objective is to study multiallelic interactions that control traits of value for plant breeding. From a cross between the garden, peach red and fragrant cultivar Fragrant Cloud (FC) and a cut-rose yellow cultivar Golden Gate (GG), we generated an autotetraploid GGFC mapping population consisting of 132 individuals. For the map we used 128 sequence-based markers, 141 AFLP, 86 SSR and three morphological markers. Seven linkage groups were resolved for FC (Total 632 cM) and GG (616 cM) which were validated by markers that segregated in both parents as well as the diploid integrated consensus map. The release of the *Fragaria vesca* genome, which also belongs to the Rosoideae, allowed us to place 70 rose sequenced markers on the seven strawberry pseudo-chromosomes. Synteny between *Rosa* and *Fragaria* was high with an estimated four major translocations and six inversions required to place the 17 non-collinear markers in the same order. Based on a verified linear order of the rose markers, we could further partition each of the parents into its four homologous groups, thus providing an essential framework to aid the sequencing of an autotetraploid genome.

Citation: Gar O, Sargent DJ, Tsai C-J, Pleban T, Shalev G, et al. (2011) An Autotetraploid Linkage Map of Rose (*Rosa hybrida*) Validated Using the Strawberry (*Fragaria vesca*) Genome Sequence. PLoS ONE 6(5): e20463. doi:10.1371/journal.pone.0020463

Editor: Najib M. El-Sayed, The University of Maryland, United States of America

Received March 9, 2011; **Accepted** April 24, 2011; **Published** May 27, 2011

Funding: Support for this study was provided by the German-Israeli Foundation (www.gif.org.il) I-842-204.12/2004 and The Volkswagen Niedersachsen-Israel Foundation (www.mwk.niedersachsen.de) 11-76251-99-6/05ZN20209 grants to DZ. Additional support was provided by TDA-TIE/BARD (www.bard-isus.com) TB-8008-04 grants to DZ and DHB, and by the East Malling Trust and BBSRC (www.bbsrc.ac.uk) BB/E007074/1 grants to DJS. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

The theory and methodology for the construction of genetic maps in diploid species is well established, whilst mapping in autopolyploids lags behind [1,2]. Polyploidization has played a major role in plant evolution by increasing gene redundancy and morphological complexity [3,4,5,6,7,8]. As a result, polyploid species are often more adaptable and show increased tolerance to different environmental conditions [6,9,10]. Many crop species such as alfalfa, sugarcane, potato, sweet potato, tea and rose [11,12] amongst others, carry multiple copies of the same genome and are classified as autopolyploid.

Genetic segregation in autopolyploids is a reflection of meiosis with a combination of bivalent and multivalent pairing with multiple alleles per locus [13]. Adding to the complexity, multivalent pairing can lead to a unique situation in which the two chromatids originating from the same chromosome may be present together in the same gamete, giving exceptional progeny termed "double reduction" [14]. The complex segregation patterns in the progeny of autopolyploid crosses and the large number of genotypic groups that need to be resolved make it a challenge to construct autopolyploid linkage maps. In practice, sibling genotyping is used to determine the parental genotypes according to the segregation ratio of each marker genotyped, which allows an inference of marker dosage in the parental genotype to be made. The segregation ratio is determined from the ratio of offspring exhibiting the marker to those that do not [15]. Single-dose markers, also called simplex markers, are present with the allelic conformation (Aaaa), whereas double dose markers, also known as duplex markers, have the genotype (AAaa); triplex markers, the genotype (AAAa) and quadriplex markers, the genotype (AAAA). Nulliplex (aaaa) describes a parental genotype where the marker is absent [13] (Fig. S1).

Different theories and methods have been developed to overcome the difficulties associated with autopolyploid mapping. Initially, genetic maps were constructed for cultivated polyploid plant species according to linkage maps of diploid relatives, such as in potato [16]. Later Wu et al [17] proposed a general method for autotetraploid mapping using only simplex markers that was implemented in sugarcane [18]. The autopolyploid linkage map that published by Al-Janabi et al [19] in sugarcane was the first map constructed directly from a complex polyploid species without the aid of either diploid relatives or a classical linkage map. Subsequently da Silva et al [20] integrated this map with the simplex-based map of Sobral et al [18] and added duplex and triplex markers showing it is possible to use multi-dose markers if a

framework linkage map was available. More recently, sophisticated theories and methods have been developed for autopolyploid mapping by relying on the dosage identification for each marker and assigning chromosomes to homologous sets [1,21]- an issue unique to autopolyploids [1]. Statistical methods and theories using Markov Chain models were recently implemented by both Leach et al [2] for tetrasomic multilocus analysis, and by Baker et al [15] for allocating marker dosage in autopolyploids species.

"TetraploidMap" [22] is the only publically available software application that has been developed for autotetraploid mapping. The software performs calculations based on the simplest situation that can arise from tetrasomic inheritance, namely random pairing of four homologous chromosomes to give two pairs of bivalents at meiosis. In practice, many departures from this simple situation can occur, in particular: multivalent pairings and double reduction; lack of complete homology between chromosomes and hence departures from random pairing; and distorted segregation due to differential fertility and viability [23]. In spite of these considerations, the suitability of this software for linkage and QTL analysis in potato and alfalfa has been demonstrated [23,24,25] which led us to try to implement it for the construction of the autotetraploid maps of *Rosa hybrida*.

Due to its ubiquitous and long-standing popularity, the rose has become the most economically-important ornamental crop worldwide for cut flowers, garden ornamentals and potted flowering plants. Roses belong to the Rosaceae family and are therefore related to important fruit crops including strawberry, apple, peach and cherry. Wild rose species range from diploid to octoploid forms, whereas cultivated roses which are perennial are mostly highly heterozygous autotetraploids ($2n = 4x = 28$) with a small genome estimated at about 550 Mb (0.57 pg/1C) [26]. The major mapping efforts in the genus, recently reviewed by Spiller et al [27], have been concentrated at the diploid level, using a double pseudo testcross strategy (Fig. S2) which is suited for allogamous species with strong inbreeding depression [28]. Four different mapping populations allowed the construction of an integrated consensus map (ICM) consisting of 597 markers distributed across seven linkage groups, with an overall length of 530 cM [27]. The ICM facilitated the resolution of genes and QTL affecting flower morphology (double flowers, petal number, flower color and white-striped flowers), plant morphology (prickles and growth vigor), fertility (self incompatibility), flowering (days to flowering and recurrent blooming), scent metabolites, and disease resistance (black-spot and powdery mildew). However, as rose breeding is mainly performed at the tetraploid level, it is important to develop a tetraploid map that could be used for mapping QTL of value for rose improvement and for use in the development of tools and germplasm for marker assisted breeding [2,24].

Ten years ago we initiated a rose genomics project aimed at identifying genes for fragrance. Two rose varieties were selected as the basis of the research: "Golden Gate" (GG) and "Fragrant Cloud" (FC) (Fig. 1). The large peach red FC flowers possess a strong scent, accumulate anthocyanins, and have a short vase life, whereas the medium yellow flowers of GG accumulate carotenoids, have a long vase life, and lack a distinct odor [29]. It is interesting to note that although GG is nearly odorless to humans, insects are highly attracted to its scent [30]. The high level of scent polymorphism between these varieties allowed us to create an annotated petal EST database of ~2100 unique genes from both cultivars and to identify, and complement in bacteria, several scent-related genes [29].

To date, no available genome sequence exists for the *Rosa* genus with which to validate the positions of markers located to autotetraploid linkage maps. However, *Rosa* belongs to the Rosoideae subfamily of the Rosaceae [31], and is well-supported as the closest sister taxon to a clade containing the genus *Fragaria*. Recently, Villanova et al and Illa et al [32,33] reported a high degree of conservation of synteny between the distantly-related Rosaceous genera *Fragaria*, *Malus* and *Prunus* and demonstrated a large number of conserved syntenic blocks, some of which spanned whole chromosomes between genera. The genome of the diploid strawberry species *F. vesca* (FvH4) ($2n = 2x = 14$) was recently sequenced to 39× coverage and anchored to the diploid *Fragaria* genetic map [34]. The close genetic relationship between *Fragaria* and *Rosa* suggests that the FvH4 sequence could be used as a reference for which to validate markers mapped in the *Rosa* autotetraploid mapping progeny and elucidate the level of synteny between the *Rosa* and *Fragaria* genomes.

To further characterize the genetic basis of the differences between the FC and GG rose cultivated varieties, we have developed two autotetraploid maps for *Rosa hybrida* using large numbers of transferrable sequenced-based markers for a progeny of 132 siblings with the software application "TetraploidMap". We have validated the map through a comparison of linkage group marker placement and marker order on each of the parental linkage maps, and a comparison to the integrated consensus map of diploid *Rosa*. To characterize the genetic relationships between the genomes of *Rosa* and *Fragaria*, both

Figure 1. The *Rosa hybrida* L. cultivars used as parents of the segregating population (GGFC). A. *Rosa hybrida* cv. Golden Gate (GG) is a modern cut-flower cultivar, containing carotenoids that are responsible for its yellow color, with only faint odor and long vase life. **B.** *Rosa hybrida* cv. Fragrant Cloud (FC) is an old garden cultivar with large fragrant flowers, short vase life and peach red petals color due to the presence of anthocyanins (Short movie presenting the vase life behavior of these cultivars is available at http://www.youtube.com/watch?v = odOp92TKSXg - the movie composed of pictures that were taken every 60 minutes with total time lapse of 15 days).
doi:10.1371/journal.pone.0020463.g001

members of the Rosoideae sub-family of the Rosaceae, and to further validate marker order, we compared the positions of orthologous markers mapped to the autotetraploid map to their positions on the seven pseudo-chromosomes of the diploid *Fragaria* genome sequence.

Results

Marker segregation

The strategy for constructing an autotetraploid rose genetic map was to use a combination of conserved, sequence-characterized markers (RFLP and CAPS), to allow comparisons with sequenced Rosaceous genomes and other marker types (AFLP, SSR and morphological) to increase marker density. Markers were divided into uni-parental markers, showing heterozygosity (simplex or duplex dosage) in a single parent, and bi-parental markers, showing heterozygosity in both parents. The coding nomenclature method of marker segregation types, segregation ratios and scoring method is presented in Table 1. All the segregation types were assigned to markers after a χ^2 test with a null hypothesis according to their accepted segregation ratio (significance level of the χ^2 test $P>0.001$) to determine the parental genotypes. All markers were binary scored as "1"-present/"0"-absent.

Marker systems

Of the ~700 markers that we used to screen the GGFC population 449 polymorphic markers were scored. Out of those markers, 358 (80%) that could be associated with the parental genotypes were used for map construction.

AFLP. Using seven AFLP primer pairs, a total of 155 polymorphic markers were scored on the mapping population (Table 1). From those we were able to map, eighty-six (55%) segregated as simplex (1:1); 27 (17.5%) segregate as duplex (5:1); 28 (18%) double-simplex (3:1) and 14 (9%) of the markers showed segregation of (11:1) and were not used in map construction (Table S2).

RFLP. RFLP analysis was conducted mostly for candidate genes that may be associated with the production of fragrance compounds, as well as genes that could potentially affect flower morphology. Using 38 RFLP markers (Table 1), we scored 63 polymorphic loci (= alleles). Ten of the markers each hybridized to single loci in the rose genome while the remaining 28 belonged to small gene families and showed multiple banding. Nine (23.6%) were scored as codominant; 17 (44.7%) were scored as dominant, and for the remaining 12 markers the determination of the parental genotypes was not possible. Thus we mapped 26 RFLP markers.

SSR. More than 100 SSR primer pairs were previously used in generating the various diploid rose maps [27]. In order to associate the GGFC tetraploid maps with the existing diploid maps we used 63 labeled SSR primers out of those pairs in this study. With them 115 alleles (= bands) were scored but only for 102 could the parental genotypes be determined (Table 1). For 34% and 7% of these SSRs all the alleles from specific primer pairs were read together as codominant and dominant, respectively. Over the 35 polymorphic SSR loci with codominant segregation, the average number of alleles per locus in both parents was 2.3 of the potential 8 allelic positions in the two autotetraploid parental genotypes.

For the majority of the SSRs, it was impossible to determine the parental genotype when reading all alleles together for specific primer pairs. Thus, in these cases each allele was read separately enabling us to map 44 more alleles (= markers) giving a total number of 86 mapped SSR markers.

CAPS. We generated 323 CAPS markers based on the previously described EST database that was established using the population parents [29] and NCBI rose sequences. Out of those 323 markers, 137 CAPS markers were polymorphic (Table 1). For 100 markers (73%) we were able to determine the parental genotypes. For the remaining 37 markers when the marker was multiallelic each of the alleles amplified was scored separately producing 17 more alleles (= markers). Thus we were able to map 102 CAPS markers.

Morphological. We scored four phenotypic qualitative traits and then translated the phenotypic data into present/absent data. Integration with the marker data enabled us to treat each trait as a single marker, which allowed us to map the genomic region

Table 1. The markers used to construct the rose map.

	Uni-parental markers				Bi-parental markers				
Parent map	**FC**		**GG**		**FC & GG**				**Total**
Scoring method	Dominant	Dominant	Dominant	Dominant	Dominant	Dominant	Dominant	Codominant	
Parents genotype (FC X GG)	Aaaa X aaaa	AAaa X aaaa	aaaa X Aaaa	aaaa X AAaa	Aaaa X Aaaa	Aaaa X AAaa / AAaa X Aaaa	AAaa X AAaa	multiple alleles	
Segregation rate	01:01	01:05	01:01	01:05	01:03	01:11	01:35		
Segregation group type	1	2	3	4	5	6	7	8	
AFLP	63	10	23	17	28	14			**155**
RFLP	5	4	8	4	6	1	1	9	**38**
SSR	20	7	19	8	9	13	4	35	**115**
CAPS	48	21	38	15	3	1		11	**137**
Morphological	2	2							**4**
Total	**138**	**44**	**88**	**44**	**46**	**29**	**5**	**55**	**449**

Markers grouped by marker type and segregation ratios that were assigned after a χ^2 test. Markers from segregation type 6 (1:11) and in other cases where it was impossible to determine the parents genotypes were not used in the mapping.

doi:10.1371/journal.pone.0020463.t001

controlling each trait. Three traits were placed on the map: anther color (*Ag*) and flower color (*Color_A*) mapped to FC LG 6, and powdery mildew (*PM*) resistance mapped to FC LG 7 (Fig. 2). None of the siblings had yellow flower color (*Color_Y*) like the parent GG, suggesting monogenic tetrasomic inheritance in which the yellow flower color is recessive.

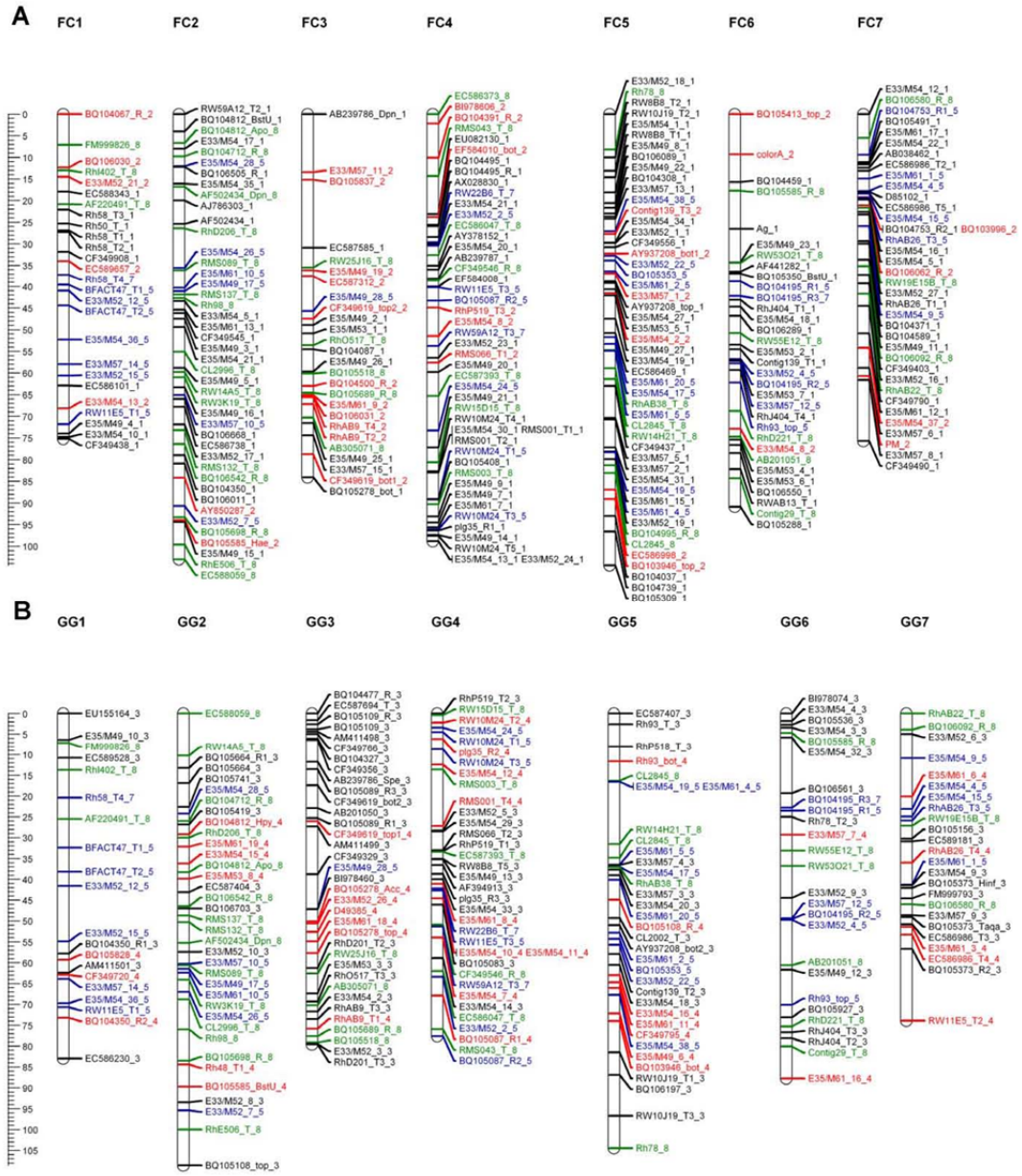

Figure 2. The parental linkage map. Each map consists of seven linkage groups. Map distances are shown in cM as a ruler at the left page margin. Marker names are assigned according to the nomenclature described in Table S1. Each color represents a different segregation ratio (black for 1:1, red for 5:1, blue for 3:1 and green for codominant markers). **A**. Map of Fragrant Cloud (FC). **B**. Map of Golden Gate (GG).
doi:10.1371/journal.pone.0020463.g002

Map construction

Map construction was performed using "TetraploidMap for windows"[25]. $^{2}\chi$ test was performed for all 449 markers in order to determine the parental genotypes on the basis of the segregation in their offspring. For 403 markers (dominant and codominant) the parental genotype could be determined, with a significance threshold larger than 0.001 ($^{2}\chi$ test) (Table S2) and were used to construct the maps. Despite passing the χ^2 test, the parental genotypes of 29 (7%) markers that segregated 11:1 could not be determined, and thus were excluded from the procedure. A further 16 (4%) of the markers showed distorted linkage patterns and thus these markers were excluded from the analyses.

Cluster analysis of all markers where parental genotypes were identifiable generated seven linkage groups corresponding to the basic chromosome number of the rose. To reveal possible discrepancies in the grouping, we performed a pre-ordering analysis by combining a two-point analysis with an initial-run and the ripple search. This facilitated the identification of ~3% of the markers that were wrongly placed, for which we re-checked their recombination frequency and LOD score values compared to the other markers in the group. If such markers did not fit in the particular linkage group they were excluded or moved manually to a different linkage group that generated lower recombination frequency and higher LOD scores. In the next step we reran the ordering analysis with the simulated annealing algorithm that explores the best possible orders and the maps were drawn. A total of 358 markers were placed on the maps (Table 2). For FC (Fig. 2A), the map length was 632 cM, with 259 markers with an average distance between markers of 2.4 cM and a largest gap of 14 cM (Table 2). The map of GG (Fig. 2B) covered 616 cM, with 210 markers positioned, an average distance between markers of 2.9 cM and a largest gap of 17 cM (Table 2).

Integrated map and comparison to diploid data

To validate the GGFC map we compared common markers on the FC and GG maps as well as common markers that were analyzed in diploid rose maps [27]. The accessibility of bi-parental markers, and especially those that were codominant, facilitated the identification of homologous linkage groups and the integration of both parental maps was done manually (Fig. 3; Fig. S5). Among the 111 common markers, the linear order was maintained for 88 (80%). Moreover, more than 95% of these markers appeared in the same linkage groups in both parents. Because the order of the majority of common markers was similar in both maps, we conclude that the positions of markers on the integrated map are reliable. A comparative analysis of the GGFC map with the recently published ICM for diploid rose [27] revealed that 51 of the 56 common markers (91%) were located on the same linkage group in both maps (Fig. 4). Additionally the total length of the ICM covers 85% of both GG and FC maps suggesting that their genome coverage is similar.

Synteny of *Rosa* and *Fragaria*

A total of 70 EST markers were used for comparison to the *Fragaria* FvH4 genome sequence assembly. The markers comprised those mapped to a single locus on the FvChr map (*F. vesca* Chromosomes map), corresponding to a single unambiguous position on the FvH4 genome sequence. The distribution of the 70 markers across the seven linkage groups of *Rosa* was relatively even (Fig. 5), ranging from 13 markers on RG2 (synonymous to Fragrant cloud linkage group), to seven markers on RG6 (average 10 markers per linkage group). Average marker densities ranged from 3.54 cM/marker on RG7 to 10.75 on RG6 (6.92 cM/marker average over the seven *Rosa* linkage groups). The map distance covered by the 70 markers was 484.71 cM, 77% of the coverage of the FC map constructed with all markers. Distribution of the 70 markers across the *Fragaria* pseudo-chromosomes was similar to *Rosa*, with a maximum of 14 markers on FvChr 6, 11 markers on each of FvChr 2, 3, 5 and 7, nine markers on FvChr 1 and three markers on FvChr 4.The total physical distance covered by the markers was 139.14 Mbp, 70% of the total genome sequence scaffolds anchored to the seven FvH4 pseudo-chromosomes.

The chromosomes, to which 54 markers (77%) were located, were conserved between *Rosa* and *Fragaria*. With the exception

Table 2. Distribution of markers on parental maps (GG and FC) and linkage group statistics.

Linkage Group	AFLP	RFLP	CAPS	SSR	Morphological	Total	Common Markers	Length (cM)	Average Distance (cM)
FC 1	8	1	7	10	0	**26**	11	75	2.88
FC 2	16	4	14	9	0	**43**	22	103	2.40
FC 3	9	2	11	4	0	**26**	12	84	3.23
FC 4	15	5	10	16	0	**46**	22	99	2.15
FC 5	26	1	15	7	0	**49**	18	104	2.12
FC 6	9	4	9	8	2	**32**	14	91	2.84
FC 7	16	5	9	6	1	**37**	12	76	2.05
Total (FC)	**99**	**22**	**75**	**60**	**3**	**259**	**111**	**632**	**2.44**
GG1	5	2	7	6	0	**20**	11	83	4.15
GG2	11	4	12	9	0	**36**	22	109	3.03
GG3	6	5	17	7	0	**35**	12	80	2.29
GG4	12	5	2	16	0	**35**	22	78	2.23
GG5	15	1	9	9	0	**34**	18	104	3.06
GG6	9	4	6	7	0	**26**	14	88	3.38
GG7	9	3	5	7	0	**24**	12	74	3.08
Total (GG)	**67**	**24**	**58**	**61**	**0**	**210**	**111**	**616**	**2.93**

doi:10.1371/journal.pone.0020463.t002

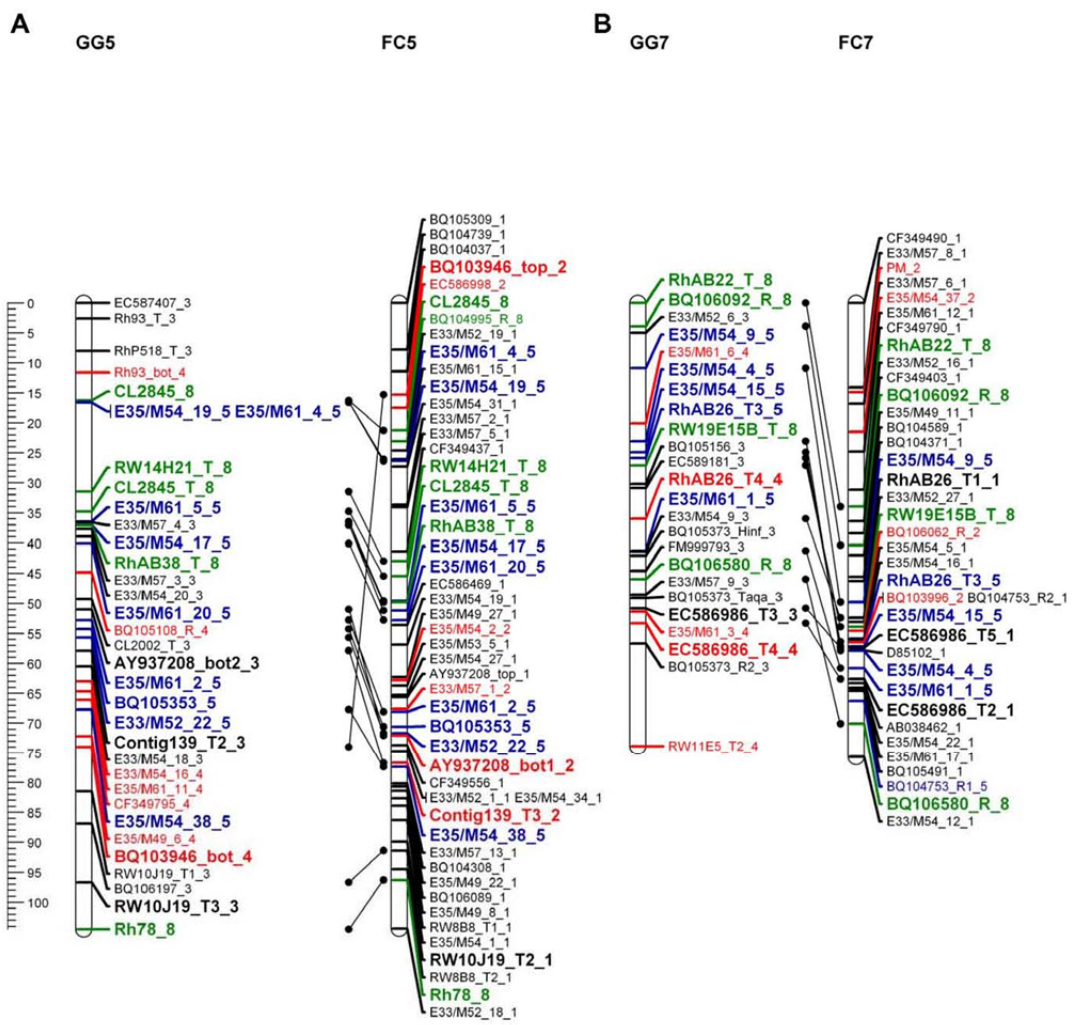

Figure 3. The linear order of common markers conserved in both parental maps. Each linkage group name contains the parental name and the linkage group number. Map distances are shown in cM as a ruler at the left page margin. Marker names indicated according to the nomenclature described in Table S1. Each color represents a different segregation ratio (black for 1:1, red for 5:1, blue for 3:1 and green for codominant markers). Common markers are indicated in bold and larger font. **A.** Linkage group 5. **B.** Linkage group 7. The remaining linkage groups are presented in Fig. S5.
doi:10.1371/journal.pone.0020463.g003

of FvChr 4, to which just three *Rosa* ESTs were located, all *Fragaria* pseudo-chromosomes contained sufficient markers to infer syntenic relationships between *Rosa* and *Fragaria* (Fig. 5). Conservation of macro-synteny was high between all *Rosa* linkage groups and *Fragaria* chromosomes, with the majority of markers on each linkage group in *Rosa* locating to a single *Fragaria* pseudo-chromosome. *Rosa* linkage group 3 corresponded to FvChr 7, RG4 to FvChr 5, RG5 to FvChr 3, RG7 to FvChr 2, whilst *Rosa* linkage groups 1 and 2 corresponded to *Fragaria* chromosomes 1 and 6. A tentative relationship between RG6 and FvChr 4 was inferred, although group FvChr 4 contained only two markers mapped to RG6, and the markers on RG6 displayed the least conservation of synteny with *Fragaria* (Fig. 6). Collinearity of markers between *Rosa* and *Fragaria* was high with an estimated four major translocations and six inversions required to place the 17 non-collinear markers in the same order on each genome. The most collinear groups were RG3 and FvChr 7, and RG5 and FvChr 3, whilst the least conserved were RG4 and FvChr 5. Markers that had been mapped to RG6 were distributed between five *Fragaria* chromosomes, with synteny observed between just two mapped markers on RG6 and FvChr 4.

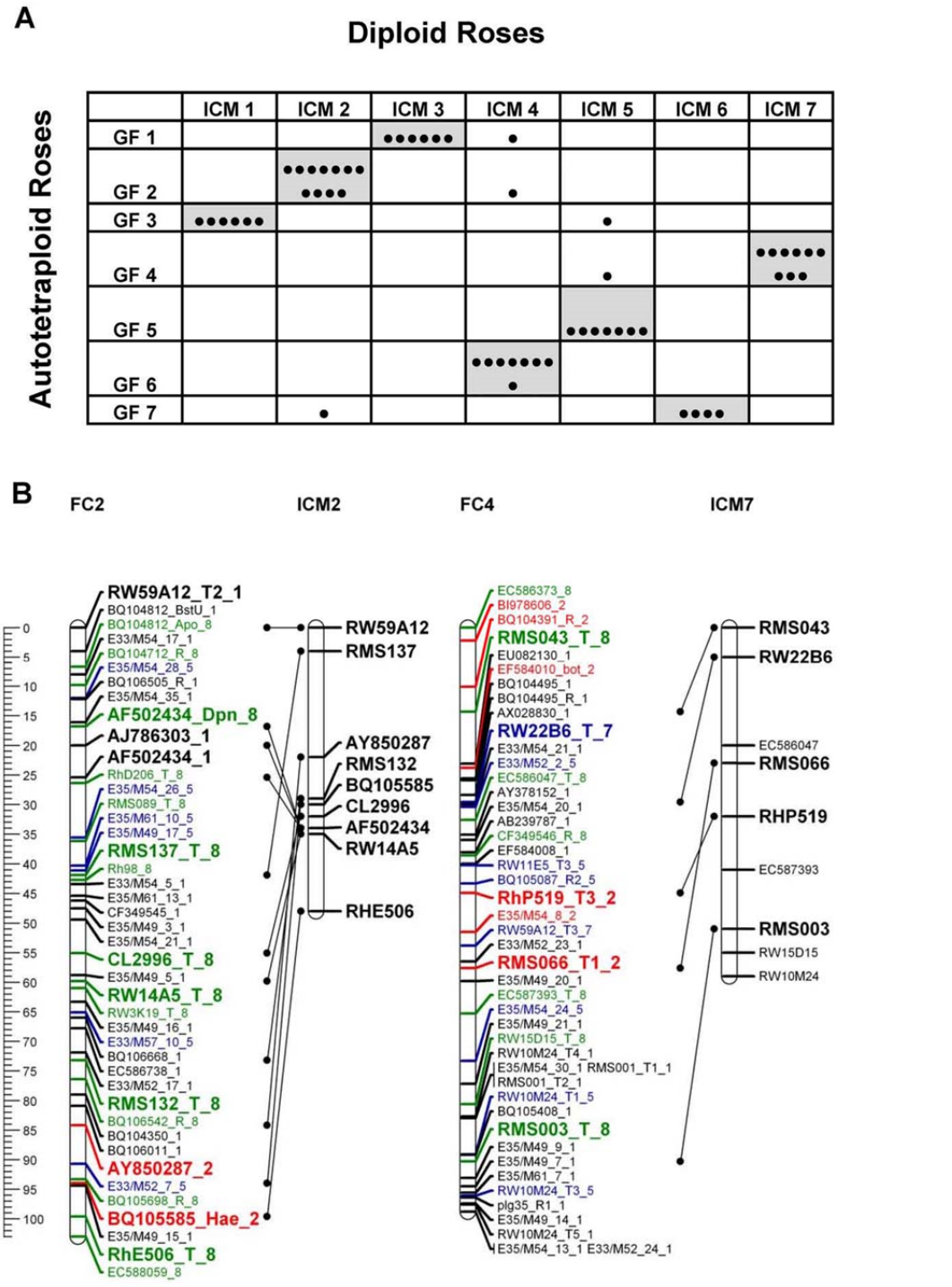

Figure 4. A comparison of the tetraploid GGFC maps with the diploid ICM. A. Number of markers of each diploid ICM linkage group that correspond to the autotetraploid linkage groups of the GGFC maps. Each marker is indicated by a black dot. Cells that contain more than one marker are noted with a grey background. **B.** The linear order of conserved markers between the FC map and ICM maps. Results are shown for FC2-ICM2 and FC4-ICM7. Map distances are shown in cM as a ruler at the left page margin. The linkage group numbers appear above each group. FC marker names are indicated according to the nomenclature described in Table S1. Each color represents a different segregation ratio (black for 1:1, red for 5:1, blue for 3:1 and green for codominant markers). The ICM marker names and positions are given according to Spiller et al [27]. Black lines connecting the common markers. The markers that present on the ICM but not connected to the FC map are corresponding to the GG map.
doi:10.1371/journal.pone.0020463.g004

Partitioning into homologous sets

The genetic map of an autopolyploid species has two components: linkage groups and homologous sets. After validating the first component we were able to tackle the second. Using the pairwise results, recombination frequency, LOD score and the coding of the simplex markers provided by the "TetraploidMap" software [25], we were able to manually determine the phase of each of the ordered markers enabling each of the seven linkage groups to be separated into four homologous chromosomes (Fig. 7). Importantly, this procedure is only available in autopolyploid designated software as homologous sets are unique to these species.

Discussion

In cut rose breeding, a common experience is that in a cross between two roses with classical flowers (http://www.youtube.com/watch?v = odOp92TK5Xg), the probability of recovering a progeny of high quality, at least as high as the parents is ~0.00001. One contributor to the complex genetics in roses is the strong inbreeding depression where often weak and albino progeny are derived. Thus homozygous lines are not available and therefore, we opted to construct an autotetraploid linkage map based on a cross of two unrelated heterozygous parents Fragrant Cloud (FC) which is a garden cultivar and Golden Gate (GG) a cut rose to create an F1 segregating population using a double pseudo testcross strategy [28] (Fig. S2). In the last 10 years, by exploiting the EST database previously established using these varieties, we were able to design and map 128 sequence-based markers (CAPS and RFLP) which are scarce in previously published rose genetic maps [27,35,36,37,38]. The sequences of these markers also permitted comparisons between the GGFC maps and sequenced rosaceous genomes, including that of *F. vesca*. Combining the EST markers with AFLP, SSR and morphological markers allowed marker density to be increased. Using these data, and employing the only available software suitable for the construction of genetic linkage maps of autotetraploid species, we have constructed an autotetraploid linkage map for rose.

The core issue in the construction of a derived map especially for an autopolyploid species is its validation. In order to confirm the fit of the GGFC map we initially used the basic character of the double pseudo testcross strategy that provides individual maps for each of the parents. Using both dominant and codominant markers with different dosage allowed us to compare and integrate the two parental maps (Fig. 3; Fig. S5); over 95% of the common markers group to the same LG in both parents. Moreover, an average of 16 markers per LG (80% of the total number of the common markers) showed a consistent collinear order between the parental maps, indicating that map construction and marker ordering was reliable. Comparing these results to the linkage maps in other autotetraploid species [23,24] shows that such high marker consistency between the parents is unique to this rose work.

The comparison between the GGFC map and the ICM for diploid rose [27] was performed using 56 common markers, of which 91% (4 11 common markers per linkage group) were located to the same linkage group in both maps. Moreover, in some linkage groups the collinearity of marker order was well conserved between the ploidy levels (up to 91% in FC LG2 in Fig. 4A). For the marker ordering it is noticeable that although it showed consistency it could be improved via an increase in marker

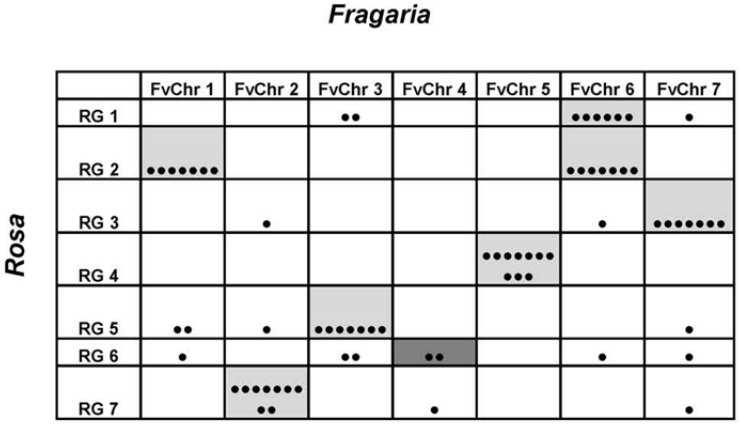

Figure 5. *Rosa* FC-*Fragaria* pseudo-chromosome comparison. The number of markers of each autotetraploid FC linkage group (RG) that correspond to the pseudo-chromosomes of the *Fragaria* FvH4 reference sequence (FvChr). Each marker is indicated by a black dot. Cells that contain more than three markers are shaded with a grey background. The tentative relationship between RG6 and FvChr4 (two markers) is shown with a dark grey background.
doi:10.1371/journal.pone.0020463.g005

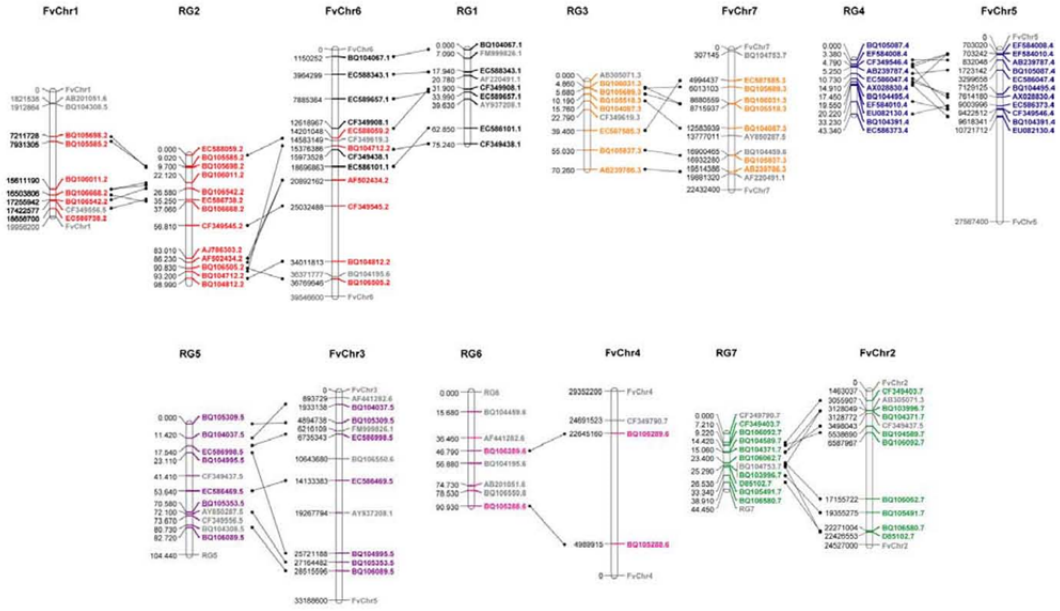

Figure 6. Comparison between the *Rosa* FC linkage map and the pseudo-chromosomes of *Fragaria* FvH4 genome sequence. Each group contains the map positions of the 70 orthologous markers used for comparison. Lines between *Rosa* linkage groups (RG) and *Fragaria* pseudo-chromosomes (FvChr) indicate marker positions within syntenic blocks. Map distances are given in cM, pseudo-chromosome positions are given in nucleotides. Marker names are given with the suffix according to the *Rosa* linkage group on which they are mapped. Markers common to syntenic blocks are given in the color of the *Rosa* linkage group; non-syntenic markers are given in grey. Delimiters defining the ends of the *Fragaria* pseudo-chromosomes and where necessary the *Rosa* linkage groups are given in grey with the pseudo-chromosome/linkage group name.
doi:10.1371/journal.pone.0020463.g006

density. Nevertheless, the similarity between the diploid and the autotetraploid rose maps is consistent with studies in other genera within the Rosaceae, such as between the diploid and the allooctoploid strawberry [39,40].

Synteny between *Rosa* and *Fragaria* genomes

A comparative analysis was performed using 70 EST-based markers mapped to the *Rosa* FC linkage map and physically-located to the FvH4 genome sequence [34] that fulfilled the criteria set out in the materials and methods. The markers represent good coverage of both the *Rosa* linkage map (77%) and the *Fragaria* genome sequence (70%). Average density of orthologous markers used for comparison was similar to that achieved in a comparative study between *Malus, Fragaria* and *Prunus* [33], and comparable to genomic comparisons based on linkage maps in species of other families [41,42,43].

Using a similar number of markers (71) to those used in this study, Villanova et al [32] revealed a high degree of synteny between the diploid *Fragaria* and *Prunus* linkage maps, showing that markers mapping to a single *Prunus* linkage group were located on just one or two *Fragaria* linkage groups. Similar patterns of synteny were revealed in a comparison of the reference maps of *Prunus* and *Fragaria* to the *Malus* × *domestica* cultivar "Golden Delicious" (MpGD) genome sequence [33]. Their study revealed large macro-syntenic blocks between the genomes of the three genera and validated the marker relationships revealed by Villanova et al [32], demonstrating that predictions about synteny of related genera can be made with a high degree of accuracy and precision using the numbers of markers we have employed in this investigation.

Rosa and *Fragaria* belong to the Rosoideae subfamily of the Rosaceae [31] with the genus *Rosa* well-supported as the closest sister clade to that containing *Fragaria* and *Potentilla*. Thus *Fragaria* and *Rosa* are closely related genetically, and this is reflected in the conservation of synteny between the structures of their respective genomes (Fig. 5), where most of the markers that mapped to a single linkage group in *Rosa* located on one *Fragaria* pseudo-chromosome, consistent with highly conserved syntenic genome blocks observed throughout the Rosaceae. Markers that were not located within syntenic regions may represent paralogous loci or translocation events that have occurred since the two genera diverged from a common ancestor, but this could not be determined with the density of common markers analysed in this investigation. Observed collinearity was high, with an estimated four translocations and six inversions required to put all syntenic markers in the same order on both genomes (Fig. 6). The comparisons presented here are extending the knowledge of comparative biology of the Rosaceae to a new clade- the Rosoideae, and will help elucidate the patterns of evolution that have occurred since the subfamily diverged from its common ancestor with the Spireaeoideae.

Our results indicate that there is sufficient synteny between the genomes of *Rosa* and *Fragaria* to allow the information from the FvH4 genome sequence of strawberry to inform genetics and genomics studies in *Rosa*. Recently, it has been demonstrated that a trait found in both *Rosa* and diploid *F. vesca*, perpetual

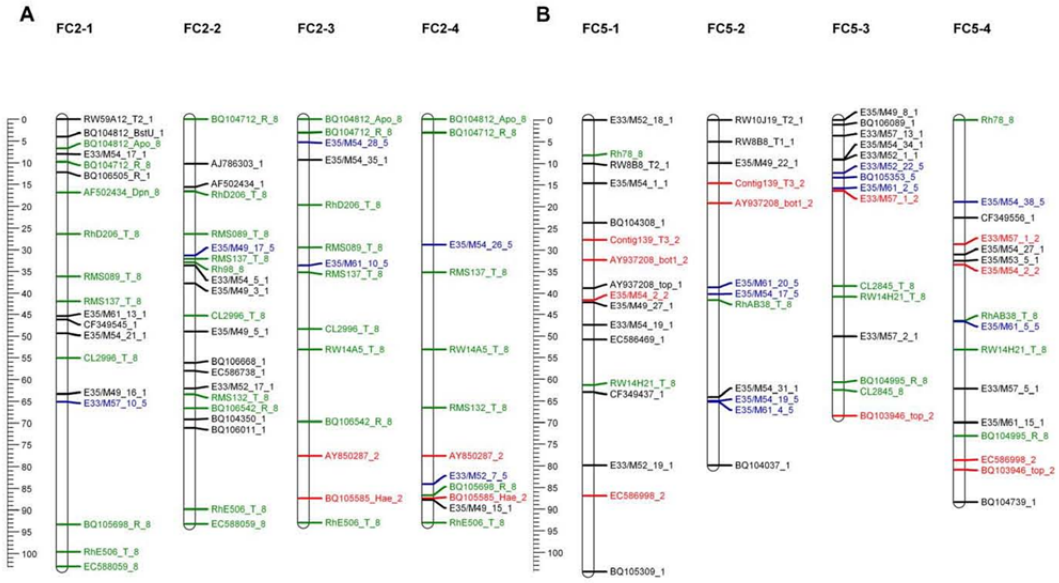

Figure 7. Two of the Fragrant Cloud (FC) linkage groups showing the four homologous chromosomes (1–4). Map distances are shown in cM as a ruler at the left page margin. Marker names indicated according to the nomenclature described in Table S1. Each color represents a different segregation ratio (black for 1:1, red for 5:1, blue for 3:1 and green for codominant markers). **A.** Linkage group 2. **B.** Linkage group 5.
doi:10.1371/journal.pone.0020463.g007

blooming, or *semperflorens* is governed in both species by a mutation in the same homologous gene (Fabrice Foucher, personal communication [Unpublished]). Moreover, as shown here, the morphological trait flower color peach red (*Color_A*) which mapped to the end of FC LG6 is similar to the *B* gene in *Prunus* (almond/peach petal color) which mapped to the end of LG1 in the more distantly-related *Prunus* reference map [44], a region shown to be syntenic to FvChr4 [32,33], which we have demonstrated here to be syntenic to FC LG6 (Fig. 6). These examples showing the potential benefit of our work to "translational genomics" studies in Rosaceae. Thus, it makes sense in the next phase to compare the multitude of QTL for common morphological and biochemical traits that were resolved for the rose GGFC with *Fragaria* and other Rosaceae.

Concluding remarks

The map for the cultivated autotetraploid rose that we present here, is a step towards understanding how multiple alleles interact genetically to control plant phenotypes. It was previously noted that the problem of constructing genetic maps in autopolyploids is twofold; loci must be ordered along individual chromosomes, and the chromosomes must be assigned to homologous groups [1]. The first problem can be solved with better ordering algorithms, and is also a common problem in the construction of diploid linkage maps, the latter problem however, is unique to autopolyploids. In this investigation, we were able to overcome both obstacles (Fig. 3 and Fig. S5 for ordering; Fig. 7 for homologous group). Moreover, by mapping sequence-based markers we have demonstrated highly conserved synteny between *Rosa* and *Fragaria* (Fig. 5; Fig. 6). Full mapping of the 28 chromosomes of the autotetraploid rose is an essential step towards QTL analysis. In the future we will present a large scale trait and QTL analysis for more than 400 ontology defined characters that were repeatedly measured on the GGFC population.

The advent of next-generation sequencing technologies has made whole genome shot-gun sequencing (WGSS) affordable and accessible to the entire biological research community and has thus enabled genome sequence data to be generated for virtually any species under investigation. However, the high degree of homology between the closely related genomes in autopolyploid species, coupled with an equally high degree of heterozygosity within those sub-genomes precludes the assembly of WGSS for autotetraploid *Rosa* species [45]. Here we present the development of a linkage map for *Rosa hybrida* from which we have characterised all 28 linkage groups. These maps, when populated with additional markers, can provide a framework for the development of a map-based resource to enable the sequencing, assembly and anchoring of a genome sequence for tetraploid rose. Additionally, as phenotyping is the rate limiting factor for discovery, the phenotypic traits measured over the past 10 years on the GGFC population make their parents "Golden Gate" and "Fragrant Cloud" attractive candidates for autotetraploid sequencing.

Materials and Methods

Plant material

A double pseudo testcross population (GGFC) of 132 individuals was generated from the crosses conducted in 2001 and 2002 between the parents "Golden Gate"® (GG) bred by W. Kordes' Söhne, and "Fragrant Cloud"® (FC) bred by RosenWelt Tantau (Fig. 1). Progeny of the cross were grown in pots filled with a peat:volcanic gravel mixture (1:1, v/v) in a greenhouse under controlled temperature (28/20°C day/night) and a natural photoperiod. Genomic DNA of each of the GGFC genotypes

was extracted according to Roche et al [46] and used for map construction.

Molecular markers

AFLP markers. Analyses were conducted by KeyGene N.V as describe by Vos et al [47] using the restriction enzyme combination of *Eco*R1 (E) / *Mse*I (M). Selective amplification was carried out with the primers: E33/M52, E35/M49, E35/M54, E33/M54, E33/M57, E35/M53 and E35/M61.

RFLP markers. The RFLP probes were generated using the sequenced clones constructed by Guterman et al [29]. A total of 20 µg genomic DNA from parental varieties and their progeny were loaded and separated on 1% agarose gels after digestion with one of four restriction enzymes; *DraI*, *Hind*III, *Eco*RI (New England Biolabs Inc., USA) *MvaI* (F. Hoffmann-La Roche Ltd., Switzerland), and blotted to positively charge nylon membrane Hybond XL (Amersham Biosciences, Sweden). Probes were radioactively labeled using the random primers method with $Dctp^{32}$ [48]. Electrophoresis, Southern blotting, hybridization and nick-translation of probes was performed according to Bernatzky and Tanksley [49].

SSR markers. A total of 63 SSR primers were analyzed. The PCR reaction mixture contained 1 µl DNA (10 ng), 0.5 µl HEX, TET, FAM or NED fluorescently labeled forward primer, 0.5 µl reverse primer, 2.5 µl DNase/RNase- free water, 5 µl GoTaq Green Master Mix (Promega Corporation, Madison, WI) and 0.5 µl $MgCl_2$ (25 mM stock solution). The PCR reactions were performed in a TECHNE TC-412 thermal cycler (Bibby Scientific Limited, UK) programmed for one step of denaturation at 94°C for 3 min. followed by 30 cycles of denaturation at 94°C for 30 sec., primer annealing at 55°C for 45 sec. and primer extension at 72°C for 1 min. A final extension step was carried out at 72°C for 7 min. and then held at 4°C. Multiplex fluorescently labeled PCR products (1 µl) generated from various SSR primers were added to 8.5 µl Hi-Di Formamide and 0.5 µl ROX400. This mixture was run through the capillary sequencer, ABI 3100 (Life Technologies Corporation, Carlsbad, CA). The DNA peaks (sizes) separated on ABI 3100 were analyzed with the GeneScan and Genotyper software (Life Technologies Corporation, Carlsbad, CA). The SSR names coding and primers sequences is according to Spiller et al [27].

CAPS (Cleaved Amplified Polymorphic Sequences) markers. These markers were mainly generated from the EST databases construct by Guterman et al [29]. PCR primers (Table S2) were designed with Primer3 software (http://frodo.wi.mit.edu/primer3) using the default settings.

A total of 19 previously characterized genes and markers [36,50,51,52] were also used as CAPS markers. Standard PCR reactions were performed with 50 ng of template DNA in a 25 µl PCR reaction containing 1x PCR buffer [53], 5 pmol of each primer, 2.5 mM dNTPs, and 2.5 U Taq polymerase (Gene Choice Inc., USA). PCR were conducted with a 90 sec. initial denaturation at 94°C, 35 cycles of 20 sec. denaturation at 94°C, 30 sec. annealing at a primer-specific annealing temperature, and 75 sec/kb product elongation at 72°C, followed by a 10 min. final elongation at 72°C. CAPS markers were generated by digestion of PCR products with 5 U of restriction enzyme for 3 hours at temperatures specified by the manufacturers. Polymorphism of the PCR products or digestion products for the CAPS markers were visualized on 3% agarose gel with ethidium bromide according to Sharp et al [54].

All types of markers were scored as "0" (fragment absent) or "1" (fragment present). In the case of codominant (multiallelic) markers each allele was first scored separately (e.g. "0" or "1") and then as a group to allow analysis of tetrasomic inheritance.

Morphological characters. Morphological traits segregating in the progeny include: anther color (Gramene Trait Ontology TO:0000187)- *Ag*; flower color (TO:0000572)- *Color_A* and *Color_Y*; and resistance to Powdery Mildew (TO:0000439)- *PM*.

Anther color phenotypes were determined in two different years (2005 and 2009) by visual inspection in the greenhouse (yellow/anthocyanic; Fig. S3). Yellow anthers are sometimes difficult to distinguish from pale anthocyanic colored anthers. For this reason we scanned the flower organs of the whole population using a Hewlett-Packard scanjet 4400c® and double-checked the anther color on the computer screen. (All photos are available at http://phnserver.phenome-networks.com).

Flower color phenotypes- peach red FC color (yes/no) and yellow GG color (yes/no) were determined in two years (2005 and 2006) by visual inspection in the greenhouse (Fig. 1 for the parental color).

Powdery Mildew disease in roses caused by the fungi *Sphaerotheca pannosa* (Wallr) Lev. var. *rosae* War. is one of the most common disease damaging both greenhouse and open field roses in Israel [55]. Powdery Mildew resistant and susceptible phenotypes were determined in two different years (2009 and 2010) by visual inspection in the greenhouse ("1"-resistance/"0"-susceptible; Fig. S4).

Map construction and comparisons

To construct a genetic linkage map for each of the parents we used the software "TetraploidMap for Windows" (http://www.bioss.ac.uk/knowledge/tetraploidmap) [22,25].

Five main steps were employed to construct the linkage maps using "TetraploidMap".

1) Analysis of single marker segregation ("FINDGENO") where the most likely dosage for each marker, conditional on the observed parent and offspring phenotypes, was identified, without or with double reduction. 2) Clustering into linkage groups ("CLUSTER") conducted on each parent for the markers identified as simplex, using the simple matching coefficient that is equivalent to the recombination frequency for simplex coupling linkages. This identified markers that mapped to the same chromosome. All simplex, duplex and multiallelic markers were then analyzed by group average cluster analysis to partition them into LGs, analyzing markers from the two parents separately. 3) Estimation of recombination frequency between all pairs of markers within a linkage group ("TWOPOINT") where for each LG recombination frequencies and LOD scores were calculated between every pair of markers for all possible phases using the EM algorithm. 4) Ordering, based on the pairwise data ("SIMANNEAL") where recombination frequencies and LOD scores from the phase with the highest likelihood were used to order the markers. A simulated annealing algorithm was used to identify the order with the minimum value of the weighted least squares criterion and to calculate map distances between the markers [22,23,25]. 5) In the final step, the chromosomes were assigned to homologous groups [1]. A panel of pairwise results that shows the most likely phase for any pair of ordered markers, together with their recombination frequency, LOD scores and the coding of the simplex markers enabled manual inference of the phase of the ordered markers [25]. With the phase information, each linkage group was reconstructed into four homologous chromosomes.

Comparisons between the parental maps were performed manually using the common markers. Comparisons between the ICM of diploid rose [27] and the autotetraploid maps were performed manually and were done only for the FC map which had a higher number of mapped markers. Linkage maps, the comparison of the parental maps, homologous chromosomes and

the FC map comparison to the ICM diploid map were presented using MAPCHART 2.2 for Windows [56].

Nomenclature of linkage groups and markers

The complexity of inheritance in autopolyploids leads to a greater number of segregation types in the population siblings than in diploid progenies [57]. Combining five different marker systems to generate marker data, using dominantly and codominantly scored markers, the presence of single to multiple alleles and the occurrence of uni-parental and bi-parental markers, prompted the use of detailed nomenclature to provide as much information as possible for a specific marker in a straight-forward manner on the map figures. The systematic marker nomenclature appears in Table S1. All marker names were composed from three components, describing the "serial number"; molecular type along with the scoring method and segregation ratio.

Comparative mapping and marker validation between *Rosa* and *Fragaria*

The seven pseudo-chromosomes of the *Fragaria vesca* (FvH4) genome sequence [34], were used to locate sequenced GGFC markers to the *Fragaria* genome. To evaluate the conservation of synteny between *Rosa* and *Fragaria*, 128 sequence-characterized markers from both the FC and GG *Rosa* linkage maps were used as queries for BLASTN, using a cut off E-value of 1e-15. A greater number of markers located to the FC map identified significant matches with orthologous sequences in the *Fragaria* genome sequence assembly, and thus a comparison was made between positions of markers from the FC map to the *Fragaria* pseudo-chromosomes. Markers were considered for comparison only if they mapped to a single discrete position on the FC *Rosa* linkage map, and matched to a single unambiguous position on the *Fragaria* genome, to which no other EST sequences were significantly aligned. A syntenic relationship between two sections of the *Rosa* and *Fragaria* genomes was defined when at least three orthologous markers were present in the same section of both genomes. *Rosa* linkage groups (RG) and *Fragaria* pseudo-chromosomes (FvChr) and links between homologous markers were plotted using MAPCHART 2.2 for Windows [56].

Supporting Information

Figure S1 Possible allelic constitutions in autotetraploids. The loci A–F illustrate the possible genotypes at one locus with two alleles (capital letter represent dominant allele). The terminology monogenic nulliplex, simplex, duplex, triplex and quadriplex describe the dosage of the dominant allele at the loci A, B, D, E and F respectively. Locus H shows codominant allele that contain up to four different alleles.
(TIF)

Figure S2 Double pseudo testcross strategy compare to classic pure line hybridization. A. Crossing two heterozygous parents results in a segregating sibling population that can be use for constructing individual maps for each of the parents. **B**. Crossing two homozygous parents (pure lines) results in uniform variety with specific characteristics from either or both parents.
(TIF)

Figure S3 Anther color (*Ag*) phenotype scoring. A. Yellow colored filament score as "0". **B**. Anthocyanic colored filament score as "1".
(TIF)

Figure S4 Resistance to Powdery Mildew (*PM*) phenotype scoring. A. Scored "0" for susceptible siblings. **B**. Scored "1" for resistant siblings.
(TIF)

Figure S5 The linear order of the common markers preserved in both parental maps. Each linkage group name contains the parent name and the linkage group number. Map distances are shown in cM as a ruler at the left page margin. Marker names are indicated according to the nomenclature described in Table S1. Each color represents a different segregation ratio (black for 1:1, red for 5:1, blue for 3:1 and green for codominant markers). The common markers are indicated in bold and larger font. **A**. Linkage group 1. **B**. Linkage group 2. **C**. Linkage group 3. **D**. Linkage group 4. **E**. Linkage group 6.
(TIF)

Table S1 Nomenclature of the markers which were used to construct the genetic linkage map in autotetraploid roses. For multiallelic markers that were scored codominantly, when the parental genotype identification failed each of the alleles amplified by the primer pairs were scored dominantly and separately (RFLP (II), SSR (II) and CAPS (II)). The detailed nomenclature makes it possible to infer the marker properties directly from the maps figures.
(XLS)

TableS2 Characteristics of the 449 polymorphic markers used us in this work. Marker type, total number of alleles, expected phenotype and genotypes of the parents as determined by "TetraploidMap" software, segregation ratio and the Chi-squared test (χ^2) its statistical significance (ratio_sig), double reduction coefficient (α) and its statistical significant (DR_sig) are given for each marker. Where possible the marker data also includes blast information, primers, restriction enzyme used and the band sizes for FC and GG.
(XLS)

Acknowledgments

We are grateful to Y. Avrahami for his assistance in the plant maintenance and KeyGene N.V. for the AFLP analysis.

Author Contributions

Conceived and designed the experiments: GS DZ. Performed the experiments: OG C-JT TP GS. Analyzed the data: OG DJS. Contributed reagents/materials/analysis tools: C-JT DHB. Wrote the paper: OG DJS DZ.

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