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The diploid origins of allopolyploid rose species studied using single nucleotide polymorphism haplotypes flanking a microsatellite repeat

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SUMMARY
The taxonomy of the genus *Rosa* is complex, not least because of hybridisations between species. We aimed to develop a method to connect the diploid *Rosa* taxa to the allopolyploid taxa to which they contributed, based on the sharing of haplotypes. For this we used an SNPSTR marker, which combines a short tandem repeat (STR; microsatellite) marker with single nucleotide polymorphisms (SNPs) in the flanking sequences. In total, 53 different sequences (haplotypes) were obtained for the SNPSTR marker, Rc06, from 20 diploid and 35 polyploid accessions from various species of *Rosa*. Most accessions of the diploid species had only one allele, while accessions of the polyploid species each contained two-to-five different alleles. Twelve SNPs were detected in the flanking sequences, which alone formed a total of 18 different haplotypes. A maximum likelihood dendrogram revealed five groups of haplotypes. Diploid species in the same Section of the genus *Rosa* contained SNP haplotypes from only one haplotype group. In contrast, polyploid species contained haplotypes from different haplotype groups. Identical SNP haplotypes were shared between polyploid species and diploid species from more than one Section of the genus *Rosa*. There were three different polymorphic repeat regions in the STR region. The STR repeat contained eight additional SNPs, but these contributed little to the resolution of the haplotype groups. Our results support hypotheses on diploid *Rosa* species that contributed to polyploid taxa. Finding different sets of haplotypes in different groups of species within the Sections *Synstylae* and *Pimpinellifoliae* supports the hypothesis that these may be paraphyletic.

A consensus taxonomy of the genus *Rosa* was updated by Wissemann (2003) following the system of Rehder (1940). The updated system consisted of four sub-genera: *Hulthemia* (one species), *Platyrhodon* (one species), *Hesperhodos* (two species), and *Rosa* (approx. 180 species). The sub-genus *Rosa* was further sub-divided into ten Sections: *Rosa*, *Pimpinellifoliae*, *Synstylae*, *Laevigatae*, *Caninae*, *Carolinae*, *Cinnamomeae*, *Banksianae*, *Bracteatae*, and *Indicae*. *Caninae* was the largest of these Sections and was further sub-divided into six sub-Sections. Considerable taxonomic confusion exists (Koopman *et al*., 2008), caused by extensive hybridisation between species (Wissemann and Ritz, 2005; Joly *et al*., 2006) leading to (i) segmental allopolyploids, (ii) an absence of clear morphological differences between many of the species (Wissemann and Ritz, 2005), and (iii) incomplete lineage sorting.

Several studies have used molecular markers and gene sequences to study genetic diversity and phylogenetics in the genus *Rosa*. Organellar DNA markers such as *matK* (Matsumoto *et al*., 1998; Wu *et al*., 2000; Leus *et al*., 2004) or *atpB* (Wissemann and Ritz, 2005), or nuclear DNA markers such as AFLP (Koopman *et al*., 2008) or short tandem repeat markers [STR; also called simple sequence repeat (SSR) or microsatellite markers; Esselink *et al*., 2003] have been used. An AFLP study by Koopman *et al*. (2008) provided the best-resolved dendrograms so far. AFLPs have also been used to clarify relationships in samples of dog-rose (De Cock *et al*., 2008). STRs have been used to estimate genetic diversity in varieties of hybrid tea rose (Smulders *et al*., 2009), *R. damascena* (Babaei *et al*., 2007), and *R. foetida* (Samiei *et al*., 2009), and to study taxonomic relationships among rose species (Scariot *et al*., 2006; Samiei *et al*., 2010). STR markers have some disadvantages for taxonomic studies, namely a high rate of mutation and the possibility that alleles back-mutate (homoplasy). However, Alvarez *et al*. (2001) concluded, from studies in tomato, that low-polymorphic STRs which have a lower mutation rate, and probably fewer cases of homoplasy, contain useful phylogenetic information at the species level.

SNPSTR markers combine the presence of an STR with at least one single nucleotide polymorphism (SNP) or an indel (Mountain *et al*., 2002). These have different mechanisms and rates of mutation; but, as they occur close together in the same stretch of DNA, they share a genealogy and can easily be genotyped together (Mountain *et al*., 2002; Payseur and Cutter, 2006; Sorensen and DaCosta, 2011). Ramakrishnan and Mountain (2004) showed the power of SNPSTR markers

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as estimates of the time of divergence of human populations leaving Africa, based on STR variations, were more accurate and precise when the SNP background was included in the analysis.

Various standard molecular markers have not resolved classification issues in taxa derived from hybridisations between diploid taxa, as hybridisation merely leads to new combinations of genomes in the allopolyploids, with mixtures of morphological characters as well as DNA polymorphisms. One way to clarify the taxonomy of polyploid rose species is to analyse which diploid species formed the parental taxa for the polyploid taxa, as for example Griffin *et al*. (2011) did in *Poa*, and Brassac *et al*. (2012) did in *Hordeum*. We wanted to connect certain DNA fragments (haplotypes or alleles) of polyploid rose species to the corresponding DNA fragments in a putative parental diploid rose species. To do this, the locus must be sufficiently polymorphic between diploid species and, at the same time, the haplotypes must be sufficiently stable (i.e., have a low level of homoplasy) to be traceable from a diploid rose species to the allopolyploid derived from it. For example, based on alcohol dehydrogenase (*ADH*) gene haplotypes, it was possible to conclude that the tetraploid *R. gallica* may have originated from an interspecific hybridisation between one *Cinnamomeae* species and one *Synstylae* species (O. Raymond, unpublished data; cited in Smulders *et al*., 2011). An SNPSTR marker may also fulfil these demands.

Our hypothesis was that SNPSTR alleles in diploid rose species could also be found in polyploids. If the same haplotype was found in diploid and polyploid species, this would support the contribution of a species from the sub-Section to which the diploid species belongs, to the polyploid genome. A proof-of-concept study was undertaken on the use of SNPSTR haplotypes to follow alleles in different taxa. Differences in STR lengths were convenient as uniquely-sized alleles that can be separated in polyacrylamide gels before sequencing, thus avoiding the need to clone all alleles.

MATERIALS AND METHODS

Plant material and DNA extraction

Fifty-five *Rosa* accessions, representing 20 diploid, 34 polyploid species, and one hybrid, from Iran, Switzerland, Germany, The Netherlands, and China, were included in this analysis (Table I). Genomic DNA was extracted from a single young leaf of each plant using the DNeasy Plant Mini Kit (Qiagen, Westburg, The Netherlands) and quantified using a Nanodrop spectrophotometer (Isogen, De Meern, The Netherlands).

SNPSTR marker amplification

An STR enrichment procedure was applied, as previously described (Esselink *et al*., 2003), directed at only trinucleotide repeat motifs in longer fragments (300 – 1,000 bp) instead of 250 – 700 bp fragments. Among the cloned fragments was a single polymorphic STR of 413 bp, called Rc06 (EMBL/GenBank Accession Number HE608872). Amplification of Rc06 in each *Rosa* accession was performed in 20 µl reaction mixtures, each containing 2.0 µl 10X PCR polymerase buffer, 0.4 µl 5 mM dNTP, 0.4 µl 10 pmol ml^{-1} of each primer, 0.3 Units Goldstar *Taq* polymerase (Eurogentec, Liege, Belgium), 1.5 µl $MgCl_2$, and 10 ng DNA sample. The primers (Rc06F 5'-CTGGATCAGCTATTGTAGACTGC-3'; and Rc06R 5'-TGCTTCGGATCAGGGTTC-3') were designed using DNASTAR software (DNASTAR, Madison, WI, USA). The PCR programme consisted of an initial denaturation step at 94ºC for 3 min, followed by 35 cycles of 30 s at 94ºC, a ramp to 50ºC (at 1ºC s^{-1}), 50ºC for 30 s, a ramp to 72ºC (at 1ºC s^{-1}), and 72ºC for 1 min, with a final extension at 72ºC for 7 min.

The PCR products were mixed with an equal volume of formamide containing 10 mM NaOH and 0.05% (v/v)

TABLE I
Fifty-five accessions of Rosa *species used in this study*

Accession Code No.¶	Species name	Location	Ploidy level
50	*R. acicularis* Lindl.	–	2
22	*R. arvensis*	Germany	2
23	*R. arvensis*	Germany	2
39	*R. arvensis*	–	2
44	*R. blanda* Aiton	–	2
18	*R. caesia* Nyman	Switzerland	5,6
2	*R. canina* L.	Iran	5
6	*R. canina* L.	Iran	5
11	*R. canina* L.	Switzerland	5
19	*R. canina* L.	Germany	5
27	*R. canina* L.	Netherlands	5
28	*R. canina* L.	Netherlands	5
51	*R. chinensis* '*spontanea*' Jacq	China	2
14	*R. columnifera*	Switzerland	4
34	*R. corymbifera*	Netherlands	5
20	*R. corymbifera* Borkh.	Germany	5
21	*R. corymbifera* Borkh.	Germany	5
4	*R. damascene* L.	Iran	4
15	*R. dumalis* Bechst.	Switzerland	5
26	*R. dumalis* Bechst.	Netherlands	5
5	*R. foetida* 'double' Herrm.	Iran	4
3	*R. foetida* Herrm.	Iran	4
9	*R. hemisphaerica* Herrm.	Iran	4
40	*R. hugonis* Hemsl.	–	2
1	*R. iberica* Steven ex M. Bieb.	Iran	5
7	*R. iberica* Steven ex M. Bieb.	Iran	5
13	*R. inodora*	Switzerland	5
46	*R. majalis* Herrm.	–	2
29	*R. micrantha* Borr. ex Sm.	Netherlands	4,5,6
41	*R. moschata* L.	–	2
45	*R. multiflora* '117'*	–	2
37	*R. multiflora* Thunb.	–	2
43	*R. nitida*	–	2
10	*R. orientalis*	Iran	5
48	*R. pendulina*	–	2
8	*R. pimpinellifolia* L.	Iran	4
42	*R. roxburgii*	–	2
35	*R. rubiginosa* L.	Switzerland	5
47	*R. rugosa* Thunb.	–	2
53	*R. sericea* Lindl.	China	2
54	*R. sericea* subsp. *omeiensis* (Rolfe) A.V. Roberts	China	2
55	*R. sericea* subsp. *omeiensis* (Rolfe) A.V. Roberts	China	2
49	*R. sertata* Rolfe	–	2
16	*R. sherardii* Davies	Switzerland	4,5,6
31	*R. sherardii* Davies	Netherlands	4,5,6
24	*R. spinosissima* L.	Germany	4
30	*R. spinosissima* L.	Netherlands	4
25	*R. tomentella* Léman	Netherlands	5
33	*R. tomentella* Léman	Netherlands	5
12	*R. villosa* subsp. *mollis*	Switzerland	4
17	*R. villosa* subsp. *mollis*	Switzerland	4
52	*R. wichurana* Crép.	–	2
36	*R. wichurana* Crép.	–	2
38	*R. woodsii* Lindl.	–	2
32	*R.* × *irregularis* Déségl. & Guillon§	Netherlands	Unknown

¶Accessions 1–19 were from Samiei *et al*. (2010). Accessions 11–35 were also used in Koopman *et al*. (2008).
**R. multiflora* 'hybrid 117' is a diploid rose from a cross between *Rosa multiflora* and an unknown garden rose.
§*R.* × *irregularis* is morphologically intermediate between *R. arvenis* and *R. canina* (Vander Mijnsbrugge *et al*., 2010).

bromophenol blue, and denatured for 5 min at 80ºC before being electrophoresed in standard 6% (w/v) polyacrylamide denaturing-sequencing gels at 110 W. The gels were silver-stained using the Silver Sequence DNA Sequencing System (Promega, Leiden, The Netherlands). For size estimation, a sequencing reaction on pGEM-3Zf(+), using the pUC/M13 forward 24-mer primer (Promega), accompanied the samples. Rc06 produced clear bands without stutter or shadow bands (quality 1, according to Smulders *et al*., 1997).

Allele sequencing

Individual alleles amplified from the 55 *Rosa* accessions were cut-out from the polyacrylamide gel using a razor blade and re-amplified using the same PCR conditions as for amplification of the SSR. Approx. 50 ng of each PCR product was used for sequencing in 4 µl 5x sequencing buffer, 0.5 µl 10 pmol ml^{-1} forward or reverse primer, in a total volume of 10 µl. The PCR programme consisted of 25 cycles of 20s at 94ºC, 50ºC for 15 s, and 60ºC for 1 min. The PCR products were sequenced directly on an ABI 3730 instrument (Life Technologies, Carlsbad, CA, USA).

Data analysis

After removal of the primer regions, all DNA sequences were aligned using Seqman (Burland, 2000) Version 8.10 (DNASTAR) and corrected manually. Haplotypes were defined manually, based on SNPs in the flanking regions. Subsequently, we determined which haplotype (or haplotypes) had been amplified in each accession. Some SNP-based haplotypes occurred in variants with a range of microsatellite repeat lengths. Some SNPs existed in the repeat region. The set of flanking SNP haplotypes, as well as the set of haplotypes based on all SNPs, were analysed using MEGA 5.04 software (Tamura *et al*., 2011) employing maximum parsimony (MP) clustering with 1,000 permutations. For this, the alleles were trimmed to the same length.

RESULTS AND DISCUSSION

SNPSTR development

In a preliminary analysis, and in contrast to Chatrou *et al*. (2009), we found that normal STR markers, including several with known map positions (Spiller *et al*., 2011; Koning-Boucoiran *et al*., 2012), had flanking sequences that were too short to contain sufficient SNP polymorphism outside the microsatellite repeat. This remained so, even when we redesigned the primers to amplify as much flanking sequence as possible, based on the genomic sequence available for the STR markers. We therefore performed a new STR enrichment, without size-limitation for the clones. This resulted in a single long, polymorphic STRSNP marker, Rc06 (EMBL/GenBank Accession Number HE608872).

SNPs flanking the STR

When we amplified and directly sequenced alleles of Rc06, most diploid species had only one allele, while the polyploid species had two-to-five different alleles. In total, 61 high quality sequences were obtained from the 55 *Rosa* accessions.

After aligning the resulting sequences, we focussed on the occurrence of SNPs. In total, 12 SNPs were detected in the regions flanking the repeat (Table II). They occurred in 18 different combinations (haplotypes; Table II). Of these, nine haplotypes were found in only one species, seven in two or three species, and two (H1 and H15) in as many as 12 different species.

The sharing of identical SNP haplotypes deserves closer attention. Haplotype H1 was found in diploid species from the sub-Sections *Cinnamomeae* (*R. majalis*, *R. rugosa*, *R. woodsii*, and *R. acicularis*) and *Carolineae* (*R. nitida*), together with polyploid species from the sub-Sections *Caninae*, *Rosa*, *Rubiginae*, *Pimpinellifoliae*, and *Vestitae*, suggesting that these diploid species had contributed to the tetraploid and pentaploid species. Halotype H15 was present in diploid *R. multiflora*, while *R. arvensis* contained halotype H7, which was only one SNP different from H15. Both species are from the Section *Synstylae*. All other accessions that had halotype H15 sequences were from polyploid accessions from most of the Sections in the genus *Rosa*.

These results may indicate the contribution of diploid species of Section *Synstylae* to various polyploid species of these Sections and sub-Sections. Of the haplotypes that we found less frequently, H4 was present in the

TABLE II

Overview of the 18 haplotypes based on the 12 SNPs that flanked the STR and the diploid (underlined) or polyploid species in which they were found

Haplotype	SNP‡	Species (followed by a number, if more than one allele was sequenced)
1	CTCAGGTATTTA	*R. acicularis*, *R. majalis*, *R. nitida*, *R. rugosa*, *R. woodsii*, *R. iberica*, *R. inodora*-2, *R. villos*a subsp. *mollis*-2, *R. canina*-4, *R. sherardii*-1, *R. orientalis*-1, *R. orientalis*-3
2	TCCAGATCTTTG	*R. foetida* 'double'-1, *R. foetida* 'double'-3, *R. hemisphaerica*
3	TCCAGACCTTTG	*R. foetida* 'double'-2
4	CTCAGGTCTTTA	*R. pendulina*, *R. pimpinellifolia*
5	CTCAGGTATTTG	*R. blanda*
6	CTCAGGTCTTTG	*R. sertata*
7	CTCAGATCTACG	*R. arvensis*
8	CCCGCATCTATG	*R. chinensis*, *R. moschata*, *R. canina*-1
9	CCCGCATCCATG	*R. tomentella*
10	CCCAGATCTTCG	*R. inodora*-1
11	CCCAGATCTATG	*R. wichurana*, *R. multiflora* 'hybrid 117', *R. canina*-3
12	CCCAGATCTACA	*R. villosa* subsp. *mollis*-3, *R. micrantha*-2, *R. caesia*
13	CCCAGATCTTTG	*R. foetida*
14	CCCAGATCTATA	*R. corymbifera*-2, *R. sherardii*-2
15	CCCAGATCTACG	*R. multiflora*, *R. micrantha*-1, *R. damascena*, *R. dumalis*, *R. villosa* subsp. *mollis*-1, *R. sherardii*-3, *R. columnifera*, *R.* × *irregularis*, *R. rubiginosa*, *R. spinosissima*, *R. orientalis*-2, *R. canina*-2
16	CCAAGATCTTTG	*R. sericea*, *R. sericea* subsp. *omeiensis*, *R. hugonis*
17	CCAAGATCTATG	*R. roxburgii*
18	CCCGGATCTATA	*R. corymbifera*-1

‡For SNP positions, see Figure 1.

diploid *R. pendulina* and the tetraploid *R. pimpinellifolia*, H8 was shared between the diploid *R. chinensis* and *R. moschata* and the pentaploid *R. canina*, while H11 was shared between the diploid *R. multiflora* and *R. wichurana* (both from sub-Section *Synstylae*) and *R. canina*.

STR repeat polymorphisms

The STR was structurally complex, as there were three different polymorphic repeat regions (Figure 1). The first region was a GXT repeat, for which the most frequent repeat length was $(GXT)_8$. This repeat existed in three sequence variants (Table III). We detected several copies of SNP haplotype H1 with a $(GXT)_4$ repeat, which was possibly a repeat contraction (deletion) from the more frequent $(GXT)_8$ repeat. It occurred in four polyploid species from two Sections: *Vestitae* (*R. sherardii* and *R. villosa* subsp. *mollis*) and *Caninae* (*R. iberica* and *R. orientalis*). These polyploid species may have received the allele from an ancestor in one of the diploid species that contained haplotype H1. The $(GXT)_4$ repeat also occurred in SNP haplotypes H2 and H3 in *R. foetida* and H15 in *R. caesia*. As H2 differed from H1 in five SNPs, plus the presence of an indel, this probably reflected an independent repeat length reduction. Halotype H3 was probably derived from H2 by a single mutation in *R. foetida*. *R. foetida* also had another unique allele, a length variant of SNP haplotype H2, with an expansion to $(GXT)_{12}$ in the first repeat.

There was a monomorphic region between the first and second repeat, except for a deletion of 12 nt in one of the two SNP haplotype H1 alleles of *R. orientalis* and *R. inodora*. The second repeat was always $(GXT)_{10}$, but it existed in six sequence variants. These variants appeared to represent independent mutations.

The third region was a $(GXT)_9$ repeat, except in *R. caesia* and *R. corymbifera* alleles 1 and 2, in which it was $(GXT)_6$ and $(GXT)_{15}$, respectively. A $(GXT)_6$ repeat occurred in SNP haplotypes H10, H14, and H18. A $(GXT)_{15}$ repeat was found once in SNP haplotype H12, in *R. caesia*.

In addition to the microsatellite region, there was a 9-bp insertion towards the 3'-end of the amplified allele, present in nine SNP haplotypes and absent in four haplotypes (H1, H4, and H5, and also in H10, which was quite different in sequence). Unfortunately, the sequences obtained for five haplotypes were not full-length; so, for these haplotypes, we do not know whether they did or did not contain the 9-bp insert sequence.

Phenetic relationships among haplotypes

We could distinguish 11 additional SNPs within the STR region (indicated in bold font in Table III). When these were added to the set of 12 flanking SNPs, to generate a maximum-likelihood (ML) tree (Figure 2), the signals of the SNPs from the repeat region contributed relatively little to the resolution of the dendrogram compared to that of an ML tree based only on flanking SNPs (data not shown). This suggests that the repeat region SNPs were more recent than some of the flanking SNPs.

Bootstrap values in the ML dendrogram were relatively low, probably due to the low number of informative sites. We tentatively distinguished five Groups of related haplotypes, but only haplotype Groups III, IV, and V were supported by somewhat higher bootstrap values (i.e., 70, 71, and 78 of 100 replications, respectively). Group III and Group IV haplotypes were found in species from the Section *Pimpinellifoliae*. Group III haplotypes were present in the tetraploid *R. foetida* (in various variants, see above) and in *R. hemispaerica*; while Group IV haplotypes were found in various diploid species from this Section, including *R. hugonis*, plus diploid *R. roxburgii* from the sub-genus *Platyrhodon*. *R. roxburgii* and *R. hugonis* were also the most similar species in the most parsimonous tree based on the AFLP data in Koopman *et al*. (2008). Therefore, our data support the conclusions of these authors and those of several others (Matsumoto *et al*., 1998; Wu *et al*., 2001; Wissemann and Ritz, 2005; Bruneau *et al*., 2007) that *R. roxburgii* was incorrectly classified into the separate sub-genus, *Plathyrodon*. The fact that Section *Pimpinellifoliae* haplotypes in Group IV were not found in any polyploid species may mean that these species did not contribute to the polyploid *Rosa* species, or that we did not include the polyploid species concerned. On the other hand, the fact that haplotypes from diploid and polyploid species did not resemble each other closely was consistent with the hypothesis (Matsumoto *et al*., 2001; Bruneau *et al*., 2007) that Section *Pimpinellifoliae* was a polyphyletic group.

Group V included haplotype H1, plus three other haplotypes. The group contained all haplotypes obtained from the seven diploid species of the Section *Cinnamomeae*, and a diploid species of the Section *Carolineae* that was included in this study, as well as haplotypes from various polyploid Sections, notably various species of the Section *Caninae*.

CONCLUSIONS

Haplotypes that occurred in polyploid *Rosa* species were shared with those in diploid *Rosa* species, indicating that these diploid species may have been involved in the formation of allopolyploid roses with higher ploidy levels. Nevertheless, our study should only be considered as a proof-of-concept, as we did not include a complete set of accessions from all diploid species, and used only a single SNPSTR locus. Multiple accessions per taxon may

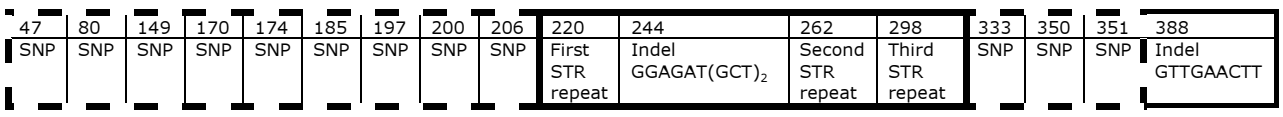

FIG. 1
Schematic overview of polymorphism in the STR (within solid box lines) and the 12 flanking SNPs (in dashed box lines), plus one distal indel, in RC06. Numbers represent the nucleotide co-ordinate at the start of each feature.

TABLE III
Sequences of the STR regions in 55 Rosa *accessions*

Species (followed by a number if more than one allele was sequenced)	SNP haplotype[¶]	STR sequences (SNPs in the repeats are underlined an in bold)				
		220-First STR	244-Indel	262-Second STR	298-Third STR	388-Indel
R.iberica	H1	GTTGCTGCTG**C**T	+	GCTG**T**TGTTG**T**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**T**TG**C**TGCTG**C**TG**T**TG**T**TGCT	–
R.inodora-2	H1	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	–	GCTG**T**TGTTG**T**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**T**TG**C**TGCTG**C**TG**T**TG**T**TGCT	–
R.villosa subsp. *mollis*-2	H1	GTTGCTGCTG**C**T	+	GCTG**T**TGTTG**T**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**T**TG**C**TGCTG**C**TG**T**TG**T**TGCT	–
R.canina-4	H1	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**T**TG**C**TGCTG**C**TG**T**TG**T**TGCT	–
R.sherardii-1	H1	GTTGCTGCTG**C**T	+	GCTG**T**TGTTG**T**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**T**TG**C**TGCTG**C**TG**T**TG**T**TGCT	–
R.orientalis-3	H1	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**T**TG**C**TGCTG**C**TG**T**TG**T**TGCT	–
R.majalis	H1	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**T**TG**C**TGCTG**C**TG**T**TG**T**TGCT	–
R.rugosa	H1	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**C**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**T**TG**C**TGCTG**C**TG**T**TG**T**TGCT	–
R.nitida	H1	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**C**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**T**TG**C**TGCTG**C**TG**T**TG**T**TGCT	–
R.orientalis-1	H1	GTTGCTGCTG**C**T	–	GCTG**T**TGTTG**T**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**T**TG**C**TGCTG**C**TG**T**TG**T**TGCT	–
R.acicularis	H1	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**C**TG**C**TGCTG**C**TG**T**TG**T**TGCT	–
R.woodsii	H1	GTTGCTGCTG**C**TG**C**TGCTGCTGCT	+	GCTG**T**TGTTG**C**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**T**TG**C**TGCTG**C**TG**T**TG**T**TGCT	–
R.foetida double-1	H2	GTTGCTGCTG**C**TG**T**TGCTGCTGCT GTTGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**A**TG**C**TGCTGCTGCTGCT	GTTGTTG**C**TG**T**TGCTG**C**TG**T**TG**T**TGCT	+
R.foetida double-3	H2	GTTGCTGCTG**C**T	+	GCTG**T**TGTTG**T**TG**A**TG**C**TGCTGCTGCTGCT	GTTGTTG**C**TG**T**TGCTG**C**TG**T**TG**T**TGCT	+
R.hemisphaerica	H2	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**A**TG**C**TGCTGCTGCTGCT	GTTGTTG**C**TG**T**TGCTG**C**TG**T**TG**T**TGCT	+
R.foetida double-2	H3	GTTGCTGCTG**C**T	+	GCTG**T**TGTTG**T**TG**A**TG**C**TGCTGCTGCTGCT	GTTGTTG**C**TG**T**TGCTG**C**TG**T**TG**T**TGCT	nd*
R.pendulina	H4	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**T**TG**C**TGCTG**C**TG**T**TG**T**TGCT	nd
R.pimpinellifolia	H4	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**T**TG**T**TGCTGCTGCTGCT	GTTGTTG**T**TG**C**TGCTG**C**TG**T**TG**T**TGCT	–
R.blanda	H5	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**C**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**T**TG**C**TGCTG**C**TG**T**TG**T**TGCT	–
R.sertata	H6	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**C**TGTTG**T**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**T**TG**T**TGCTG**C**TG**T**TG**T**TGCT	nd
R.arvensis	H7	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**C**TG**T**TGCTG**C**TG**T**TG**T**TGCT	nd
R.canina-1	H8	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**T**TG**T**TGCTGCTGCTGCT	GTTGTTG**C**TG**T**TGCTG**C**TG**T**TG**C**TGCT	+
R.chinesis 'spontanea'	H8	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**C**TG**T**TGCTG**C**TG**T**TG**T**TGCT	+
R.moschata	H8	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**C**TG**T**TGCTG**C**TG**T**TG**T**TGCT	+
R.tomentella	H9	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**T**TG**T**TGCTGCTGCTGCT	GTTGTTG**C**TG**T**TGCTG**C**TG**T**TG**T**TGCT	+
R.inodora-1	H10	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**T**TG**T**TGCTGCTGCTGCT	GTTGTTG**C**TG**T**TGCTG**C**T	–
R.canina-3	H11	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**T**TG**T**TGCTGCTGCTGCT	GTTGTTG**C**TG**T**TGCTG**C**TG**T**TG**T**TGCT	+
R.wichurana	H11	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**C**TG**T**TGCTG**C**TG**T**TG**T**TGCT	+
R.multiflora 'hybrid 117'	H11	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**C**TG**C**TGCTGCTGCTGCT	GTTGTTG**C**TG**T**TGCTG**C**TG**T**TG**T**TGCT	nd
R.villosa subsp. *mollis*-3	H12	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**C**TG**T**TGCTG**T**TG**T**TG**T**TGCT	+
R.micrantha-2	H12	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**C**TG**T**TGCTG**T**TG**T**TG**T**TGCT	+
R.caesia	H12	GTTGCTGCTG**C**T	+	GCTG**T**TGTTG**T**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**C**TG**T**TGCTG**T**TG**C**TG**T**TGCTG TTGCTGTTGTTGTTGCT	nd
R.foetida	H13	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**A**TG**C**TGCTGCTGCTGCT	GTTGTTG**C**TG**T**TGCTG**C**TG**T**TG**T**TGCT	+
R.corymbifera-2	H14	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**T**TG**T**TGCTGCTGCTGCT	GTTGTTG**C**TG**T**TGCTG**C**T	nd
R.sherardii-2	H14	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**C**TG**T**TGCTG**T**TG**T**TG**T**TGCT	+
R.micrantha-1	H15	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**C**TG**T**TGCTG**T**TG**T**TG**T**TGCT	+
R.damascena	H15	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**C**TG**T**TGCTG**T**TG**T**TG**T**TGCT	+
R.dumalis	H15	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**C**TG**T**TGCTG**T**TG**T**TG**T**TGCT	+
R.villosa subsp. *mollis*-1	H15	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**C**TG**T**TGCTG**T**TG**T**TG**T**TGCT	+
R.sherardii-3	H15	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**C**TG**T**TGCTG**T**TG**T**TG**T**TGCT	+
R.columnifera	H15	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**C**TG**T**TGCTG**T**TG**T**TG**T**TGCT	+
Rosa × *irregularis*	H15	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**C**TG**T**TGCTG**T**TG**T**TG**T**TGCT	+
R.rubiginosa	H15	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**C**TG**T**TGCTG**T**TG**T**TG**T**TGCT	+
R.spinosissima	H15	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**C**TG**T**TGCTG**T**TG**T**TG**T**TGCT	+
R.orientalis-2	H15	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**C**TG**T**TGCTG**T**TG**C**TG**T**TGCTG TTGTTGTTGCT	nd
R.canina-2	H15	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**C**TG**T**TGCTG**T**TG**C**TG**T**TGCTG TTGTTGTTGCT	nd
R.multiflora	H15	GTTGCTGCTG**C**TG**C**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**T**TG**C**TGCTGCTGCTGCT	GTTGTTG**C**TG**T**TGCTG**C**TG**T**TG**T**TGCT	+
R.sericea	H16	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**C**TG**C**TGCTGCTGCTGCT	GTTGTTG**T**TG**T**TGCTG**C**TG**T**TG**T**TGCT	+
R.omeiensis	H16	GTTGCTGCTG**A**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**C**TG**C**TGCTGCTGCTGCT	GTTGTTG**T**TG**T**TGCTG**C**TG**T**TG**T**TGCT	+
R.sericea subsp. *omeiensis*	H16	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**C**TG**C**TGCTGCTGCTGCT	GTTGTTG**T**TG**T**TGCTG**C**TG**T**TG**T**TGCT	+
R.hugonis	H16	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**C**TG**C**TGCTGCTGCTGCT	GTTGTTG**T**TG**T**TGCTG**C**TG**T**TG**T**TGCT	nd
R.roxburgii	H17	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**C**TG**C**TGCTGCTGCTGCT	GTTGTTG**T**TG**T**TGCTG**C**TG**T**TG**T**TGCT	nd
R.corymbifera-1	H18	GTTGCTGCTG**C**TG**T**TGCTGCTGCT	+	GCTG**T**TGTTG**T**TG**T**TG**T**TGCTGCTGCTGCT	GTTGTTG**C**TG**T**TGCTG**C**T	nd

[¶]SNP haplotypes based on 12 SNP loci (listed in Table II).
*nd, not determined, missing data (sequence was not full-length).

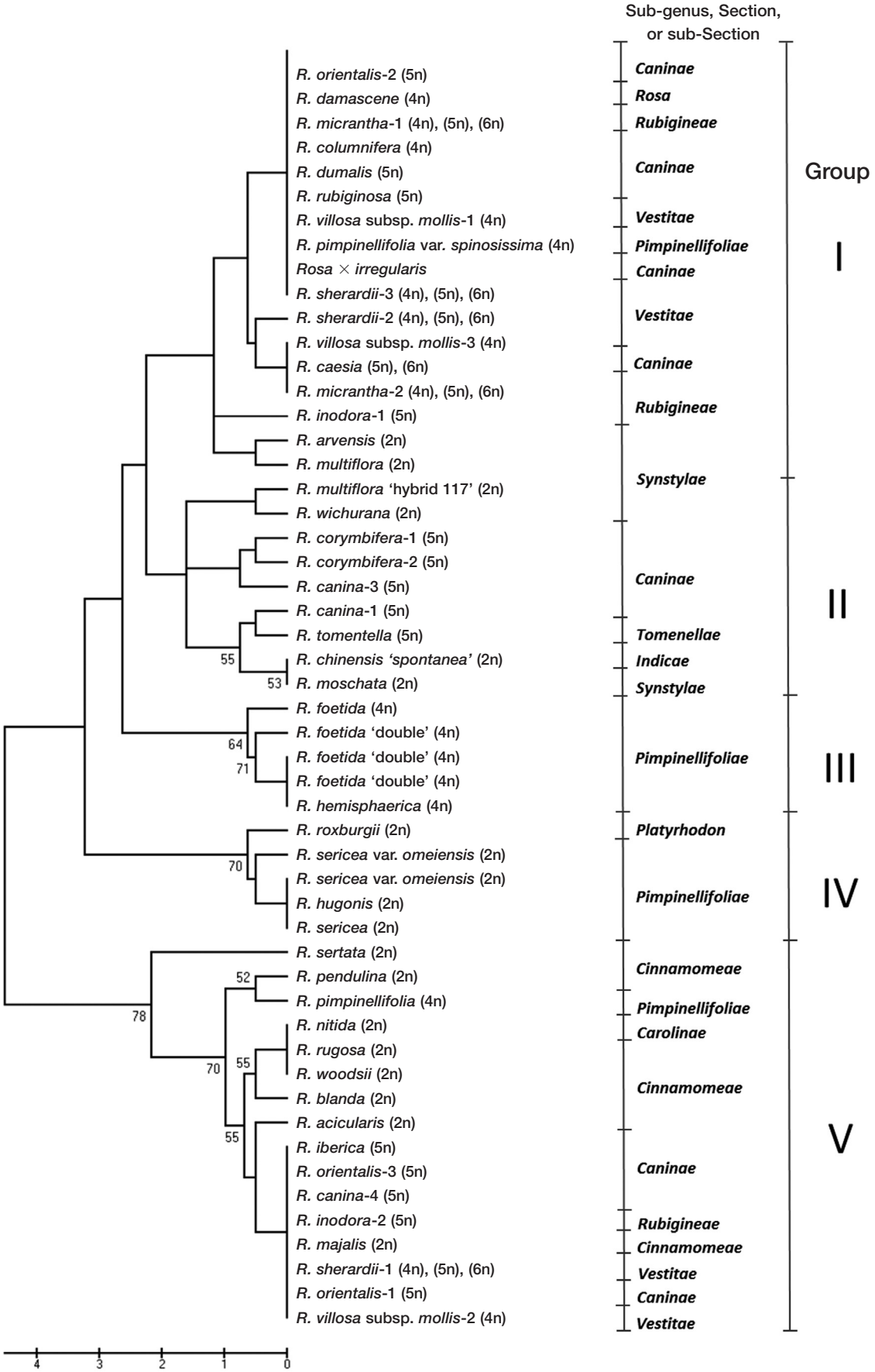

FIG. 2

Maximum-likelihood tree of all haplotypes found in the 55 accessions of *Rosa* studied here. Haplotypes were based on the 12 SNPs flanking the repeats (Table II) plus the eight SNPs present in the repeat region (Table III). Numbers at each node are bootstrap values based on 1,000 iterations. Only values ≥ 50% are shown. Ploidy levels are indicated next to the species name. Numbers next to the species name distinguish multiple haplotypes from the same accession. Groups I–V tentatively indicate major Groups of haplotypes. The scale bar indicates substitutions per nucleotide position.

be necessary if there is heterogeneity in the chromosomal segments present in polyploids. In that case, more loci would have to be included in order to cover the genomes involved. Next-generation sequencing will facilitate this, as it becomes cheaper to generate sequences from a large variety of samples without the need to clone the sequences. It is essential that multiple haplotypes with SNPs are obtained, as only this would generate the necessary resolving power.

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REFERENCES

ALVAREZ, A. E., VAN DE WIEL, C. C. M., SMULDERS, M. J. M. and VOSMAN, B. (2001). Use of microsatellites to evaluate genetic diversity and species relationships in the genus *Lycopersicon*. *Theoretical and Applied Genetics*, **103,** 1283–1292.

BABAEI, A., TABAEI-AGHDAEI, S. R., KHOSH-KHUI, M., OMIDBAIGI, R., NAGHAVI, M. R., ESSELINK, G. D. and SMULDERS, M. J. M. (2007). Microsatellite analysis of Damask rose (*Rosa damascena* Mill.) accessions from various regions in Iran reveals multiple genotypes. *BMC Plant Biology*, **10,** 12.

BRASSAC, J., JAKOB, S. S. and BLATTNER, F. R. (2012). Progenitor-derivative relationships of *Hordeum* polyploids (*Poaceae*, *Triticeae*) inferred from sequences of TOPO6, a nuclear low-copy gene region. *PLoS ONE*, **7,** e33808.

BRUNEAU, A., STARR, J. R. and JOLY, S. (2007). Phylogenetic relationships in the genus *Rosa*: New evidence from chloroplast DNA sequences and an appraisal of current knowledge. *Systematic Botany*, **32,** 366–378.

BURLAND, T. G. (2000). DNASTAR's Lasergene sequence analysis software. *Methods in Molecular Biology*, **132,** 71–91.

CHATROU, L. W., ESCRIBANO, M. P., VIRUEL, M. A., MAAS, J. W., RICHARDSON, J. E. and HORMAZA, J. I. (2009). Flanking regions of monomorphic microsatellite loci provide a new source of data for plant species-level phylogenetics. *Molecular Phylogenetics and Evolution*, **53,** 726–733.

DE COCK, K., VAN DER MIJNSBRUGGE, K., BREYNE, P., VAN BOCKSTAELE, E. and VAN SLYCKEN, J. (2008). Morphological and AFLP-based differentiation within the taxonomical complex section *Caninae* (subgenus *Rosa*). *Annals of Botany*, **102,** 685–697.

ESSELINK, G. D., SMULDERS, M. J. M. and VOSMAN, B. (2003). Identification of cut rose (*Rosa hybrida*) and rootstock varieties using robust sequence tagged microsatellite site markers. *Theoretical and Applied Genetics*, **106,** 277–286.

GRIFFIN, P. A., ROBIN, C. and HOFFMANN, A. A. (2011). A next-generation sequencing method for overcoming the multiple gene copy problem in polyploid phylogenetics, applied to *Poa* grasses. *BMC Biology*, **9,** 19.

JOLY, S., STARR, J. R., LEWIS, W. H. and BRUNEAU, A. (2006). Polyploid and hybrid evolution in roses east of the Rocky Mountains. *American Journal of Botany*, **93,** 412–425.

KONING-BOUCOIRAN, C. F. S., GITONGA, V. W., YAN, Z., DOLSTRA, O., VAN DER LINDEN, C. G., VAN DER SCHOOT, J., UENK, G. E., VERLINDEN, K., SMULDERS, M. J. M., KRENS, F. A. and MALIEPAARD, C. (2012). The mode of inheritance in tetraploid cut roses. *Theoretical and Applied Genetics*, **125,** 591–607 .

KOOPMAN, W. J. M., WISSEMANN, V., DE COCK, K., VAN HUYLENBROECK, J., DE RIEK, J., SABATINO, G. J. H., VISSER, D., VOSMAN, B., RITZ, C. M., MAES, B., WERLEMARK, G., NYBOM, H., DEBENER, T., LINDE, M. and SMULDERS, M. J. M. (2008). AFLP markers as a tool to reconstruct complex relationships: A case study in *Rosa* (*Rosaceae*). *American Journal of Botany*, **95,** 353–366.

LEUS, L., JEANNETEAU, F., VAN HUYLENBROECK, J., VAN BOCKSTAELE, E. and DE RIEK, J. (2004). Molecular evaluation of a collection of rose species and cultivars by AFLP, ITS, *rbcL* and *matK*. *Acta Horticulturae*, **651,** 141–147.

MATSUMOTO, S., KOUCHI, M., YABUKI, J., KUSUNOKI, M., UEDA, Y. and FUKUI, H. (1998). Phylogenetic analyses of the genus *Rosa* using the *matK* sequence: Molecular evidence for the narrow genetic background of modern roses. *Scientia Horticulturae*, **77,** 73–82.

MATSUMOTO, S., NISHIO, H., UEDA, Y. and FUKUI, H. (2001). Phylogenetic analyses of the genus *Rosa*: Polyphyly of the Section *Pimpinellifoliae* and the origin of *Rosa* × *fortuniana* Lindl. *Acta Horticulturae*, **547,** 357–363.

MOUNTAIN, J. L., KNIGHT, A., JOBIN, M., GIGNOUX, C., MILLER, A., LIN, A. A. and UNDERHILL, P. A. (2002). SNPSTRs: Empirically derived, rapidly typed, autosomal haplotypes for inference of population history and mutational processes. *Genome Research*, **12,** 1766–1772.

PAYSEUR, B. A. and CUTTER, A. D. (2006). Integrating patterns of polymorphism at SNPs and STRs. *Trends in Genetics*, **22,** 424–429.

RAMAKRISHNAN, U. and MOUNTAIN, J. L. (2004). Precision and accuracy of divergence time estimates from STR and SNPSTR variation. *Molecular Biology and Evolution*, **21,** 1960–1971.

REHDER, A. (1940) *Manual of Cultivated Trees and Shrubs*. 2nd Edition. McMillan Co., New York, NY, USA. 996 pp.

SAMIEI, L., NADERI, R., KHALIGHI, A., BUSHEHRI, A. A., MOZAFFARIAN, V., ESSELINK, D. and SMULDERS, M. J. M. (2009). In search of genetic diversity in *Rosa foetida* Herrmann in Iran. *Acta Horticulturae*, **836,** 25–30.

SAMIEI, L., NADERI, R., KHALIGHI, A., SHAHNEJAT-BUSHEHRI, A. A., MOZAFFARIAN, V., ESSELINK, G. D., KAZEMPOUR OSALOO, S. and SMULDERS, M. J. M. (2010). Genetic diversity and genetic similarities between Iranian rose species. *Journal of Horticultural Science & Biotechnology*, **85,** 231–237.

SCARIOT, V., AKKAK, A. and BOTTA, R. (2006). Characterization and genetic relationships of wild species and old garden roses based on microsatellite analysis. *Journal of the American Society for Horticultural Science*, **131,** 66–73.

SMULDERS, M. J. M., BREDEMEIJER, G., RUS-KORTEKAAS, W., ARENS, P. and VOSMAN, B. (1997). Use of short microsatellites from database sequences to generate polymorphisms among *Lycopersicon esculentum* cultivars and accessions of other *Lycopersicon* species. *Theoretical and Applied Genetics*, **94,** 264–272.

SMULDERS, M. J. M., ESSELINK, D., VOORRIPS, R. E. and VOSMAN, B. (2009). Analysis of a database of DNA profiles of 734 hybrid tea rose varieties. *Acta Horticulturae*, **836,** 169–174.

SMULDERS, M. J. M., ARENS, P., KONING-BOUCOIRAN, C., GITONGA, V., KRENS, F., ATANASSOV, A., ATANASSOV, I., RUSANOV, K., BENDAHMANE, M., DUBOIS, A., RAYMOND, O., CAISSARD, J. C., BAUDINO, S., CRESPEL, L., GUDIN, S., RICCI, S. C., KOVATCHEVA, N., VAN HUYLENBROECK, J., LEUS, L., WISSEMANN, V., ZIMMERMANN, H., HENSEN, I., WERLEMARK, G. and NYBOM, H. (2011). *Rosa*. In: *Wild Crop Relatives: Genomic and Breeding Resources Plantation and Ornamental Crops*. (Kole, C., Ed.). Springer-Verlag, Berlin, Heidelberg, Germany. 243–275.

SORENSON, M. D. and DACOSTA, J. M. (2011). Genotyping HapSTR loci: phase determination from direct sequencing of PCR products. *Molecular Ecology Resources*, **11,** 1068–1075.

SPILLER, M., LINDE, M., HIBRAND-SAINT OYANT, L., TSAI, C.-J., BYRNE, D. H., SMULDERS, M. J. M., FOUCHER, F. and DEBENER, T. (2011). Towards a unified genetic map of diploid rose. *Theoretical and Applied Genetics*, **122,** 489–500.

TAMURA, K., PETERSON, D., PETERSON, N., STECHER, G., NEI, M. and KUMAR, S. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, **28,** 2731–2739.

VAN DER MIJNSBRUGGE, K., DE COCK, K., COX, K. and BREYNE, P. (2010). Conservation measures for *Rosa arvensis* Huds. in Flanders (Belgium) based on congruent genetic and phenotypic population differentiation. *Conservation Genetics*, **11,** 2243–2253.

WISSEMANN, V. (2003). Conventional taxonomy of wild roses. In: *Encyclopedia of Rose Science*. (Roberts, A., Debener, T. and Gudin, S., Eds.). Elsevier, London, UK. 111–117.

WISSEMANN, V. and RITZ, C. M. (2005). The genus *Rosa* (*Rosoideae*, *Rosaceae*) revisited: molecular analysis of *nrITS-1* and *atpB-rbcL* intergenic spacer (IGS) *versus* conventional taxonomy. *Botanical Journal of the Linnean Society*, **147,** 275–290.

WU, S., UEDA, Y., HE, H., NISHIHARA, S. and MATSUMOTO, S. (2000). Phylogenetic analysis of Japanese *Rosa* species using *matK* sequences. *Breeding Science*, **50,** 275–281.

WU, S., UEDA, Y., NISHIHARA, S. and MATSUMOTO, S. (2001). Phylogenetic analysis of Japanese *Rosa* species using DNA sequences of nuclear ribosomal internal transcribed spacers (ITS). *Journal of Horticultural Science & Biotechnology*, **76,** 127–132.