WAGENINGEN
UNIVERSITY & RESEARCH

# Rosa

## Wild Crop Relatives: Genomic and Breeding Resources

Smulders, M.J.M.; Arens, P.; Koning-Boucoiran, C.F.S.; Gitonga, V.W.; Krens, F.A.

https://doi.org/10.1007/978-3-642-21201-7_12

This article is made publicly available in the institutional repository of Wageningen University and Research, under the terms of article 25fa of the Dutch Copyright Act, also known as the Amendment Taverne. This has been done with explicit consent by the author.

Article 25fa states that the author of a short scientific work funded either wholly or partially by Dutch public funds is entitled to make that work publicly available for no consideration following a reasonable period of time after the work was first published, provided that clear reference is made to the source of the first publication of the work.

This publication is distributed under The Association of Universities in the Netherlands (VSNU) 'Article 25fa implementation' project. In this project research outputs of researchers employed by Dutch Universities that comply with the legal requirements of Article 25fa of the Dutch Copyright Act are distributed online and free of cost or other barriers in institutional repositories. Research outputs are distributed six months after their first online publication in the original published version and with proper attribution to the source of the original publication.

You are permitted to download and use the publication for personal purposes. All rights remain with the author(s) and / or copyright owner(s) of this work. Any use of the publication or parts of it other than authorised under article 25fa of the Dutch Copyright act is prohibited. Wageningen University & Research and the author(s) of this publication shall not be held responsible or liable for any damages resulting from your (re)use of this publication.

For questions regarding the public availability of this article please contact openscience.library@wur.nl

# Chapter 12
# *Rosa*

M.J.M. Smulders, P. Arens, C.F.S. Koning-Boucoiran, V.W. Gitonga, F.A. Krens, A. Atanassov, I. Atanassov, K.E. Rusanov, M. Bendahmane, A. Dubois, O. Raymond, J.C. Caissard, S. Baudino, L. Crespel, S. Gudin, S.C. Ricci, N. Kovatcheva, J. Van Huylenbroeck, L. Leus, V. Wissemann, H. Zimmermann, I. Hensen, G. Werlemark, and H. Nybom

## 12.1 Basic Botany of the Species

### 12.1.1 *Basic* Rosa *Taxonomy*

The genus *Rosa* has attracted considerable attention from taxonomists and numerous species have been described. Presently, about 100–250 species are usually recognized. Many of these species are now thought to have arisen by hybridization, often accompanied by polyploidization. In addition, extensive anthropogenic impact has led to the development of many new semi-wild and/or cultivated rose varieties. Consequently, taxonomy is not straightforward! Although much criticized in, e.g., numerous recent DNA-based analyses (see below), the classification system of Rehder (1940) or variations thereof (e.g., Wissemann 2003) still constitute the standard taxonomic treatment of this genus. Rehder (1940) recognized four different subgenera: *Hesperhodos* (two species), *Hulthemia* (one species), *Platyrhodon* (one species), and *Rosa*. Subgenus *Rosa* is furthermore divided into ten sections: *Pimpinellifoliae*, *Carolinae*, *Cinnamomeae*, *Synstylae*, *Caninae*, *Gallicanae*, *Indicae*, *Banksiae*, *Laevigatae,* and *Bracteatae*, but the five latter sections have only one to three species each. According to the nomenclatural code, it is advised to change sect. *Cinnamomeae* into sect. *Rosa* (McNeill et al. 2006) due to the generic type designation of *R. cinnamomea*. However, before this designation in 2006, the generic type was *R. centifolia* phylogenetically located in sect. *Gallicanae*, thus this name has also been much used for sect. *Gallicanae* (e.g., Wissemann 2003). To avoid confusion based on nomenclatural reasons, it is therefore avoided altogether in the following treatise to use sect. *Rosa*.

The wild ancestors of our domesticated ornamental roses are found mainly in the sections *Synstylae* (*R. moschata*, *R. wichurana,* and *R. multiflora*), *Gallicanae* (*R. gallica*), *Indicae* (*R. chinensis* and *R. gigantea*), and *Pimpinellifoliae* (*R. foetida*) (Wylie 1954). A smaller but still noticeable contribution has been made by, e.g., *R. spinosissima* in sect. *Pimpinellifoliae* and by *R. cinnamomea* and *R. rugosa* in sect. *Cinnamomeae*. *R. damascena* (sect. *Gallicaneae*) is also worth mentioning due to its considerable influence both as an ornamental and for the commercial production of rose oil. This rather small set of species has thus been instrumental in producing the enormous cornucopia of shape, color, and fragrance that we now enjoy in gardens and parks, and as pot plants and cut flowers. In addition, wild or semi-wild genotypes in, e.g., sect. *Caninae* (dogroses) are used as rootstocks and landscape plants. A possibly expanding area is the production of rose hips for culinary and medicinal purposes based mainly on species in sections *Caninae* and *Cinnamomeae*, and the chestnut rose *R. roxburghii* in subgenus *Platyrhodon*.

### 12.1.2 *Morphometry*

Traditionally, rose species have been defined according to quantitative and qualitative morphological characters like shape, size, and color of petals, sepals, and hips; inflorescence architecture; length of pedicel; presence or absence of glandular hairs; shape and size of leaves, leaflets, and leaflet indenture; and

M.J.M. Smulders (✉)
Wageningen UR Plant Breeding, P.O. Box 16, NL-6700 AA Wageningen, Netherlands
e-mail: rene.smulders@wur.nl

C. Kole (ed.), *Wild Crop Relatives: Genomic and Breeding Resources Plantation and Ornamental Crops*,
DOI 10.1007/978-3-642-21201-7_12, © Springer-Verlag Berlin Heidelberg 2011

number, shape, and color of prickles. Quantitative (morphometric) characters show a continuous variation and are likely to be polygenically controlled whereas qualitative (descriptive) characters produce major discrete categories and are more likely to be monogenic in nature. Descriptive characters are often easier to evaluate in naturally growing populations and/or on herbarium sheets but may overemphasize the underlying genetic differentiation. Moreover, strong linkage among a few such genes may lead to parallel combinations of characters as evidenced in dogrose taxonomy (Nilsson 1999). Recent taxonomic studies have made use of morphological characters evaluated by hand or by automated image analysis, sometimes also involving electron microscopy (e.g., pollen and seed morphology). Most of these studies have, however, targeted either a restricted number of closely related taxa and/or a restricted geographic area. Several of these studies have concerned the allopolyploid and taxonomically very controversial sect. *Caninae*. In one study on morphometric variation in Nordic dogroses, only 65% of all evaluated plants could thus be referred to a single species according to a canonical variates analysis (Nybom et al. 1996). Another study on Nordic dogroses using Fourier coefficients of leaflet shape also detected some differentiation among six taxa but without clear-cut boundaries (Olsson et al. 2000). Similarly, much overlapping was found among seven investigated dogrose taxa in Belgium, investigated with a set of 17 morphometric and descriptive characters (De Cock et al. 2007, 2008). In all of these studies, considerable differentiation was, however, found between taxa belonging to different subsections (subsections *Caninae*, *Rubigineae,* and *Vestitae*) suggesting that these entities are rather well defined.

Allopolyploid speciation has also taken place in sect. *Cinnamomeae*. When Joly and Bruneau (2007) studied five diploid and three tetraploid taxa in eastern North America, they could not discern distinct groups when all samples were studied together using a set of 25 morphometric characters. Analysis of each ploidy level separately did, however, provide evidence of differentiation between some of the described taxa.

A general finding in the above-mentioned morphology-based studies is that single characters generally cannot discriminate completely among species. A multivariate approach is needed, and preferably both ordination and cluster analyses. Still, considerable overlapping is usually encountered, and many samples cannot be unambiguously classified into discrete species.

### 12.1.3 DNA-Based Taxonomy

Numerous DNA-based analyses have been applied to investigations of species differentiation and relationships in *Rosa*. The division into four different subgenera does not have much support in the DNA-based data. The first major study was published by Jan et al. (1999) who used random amplified polymorphic DNA (RAPD) markers and demonstrated that *Platyrhodon* and *Hesperhodos* should be placed within subgenus *Rosa*. In another study, Koopman et al. (2008) applied amplified fragment length polymorphism (AFLP) markers and showed that *Hulthemia* and *Platyrhodon* should be included in subgenus *Rosa*. The lack of a sound genetic basis for a subgeneric division has been demonstrated also in studies based on the sequencing of regions in the chloroplast-DNA (Matsumoto et al. 1998; Wisseman and Ritz 2005; Bruneau et al. 2007) as well as in the nuclear-DNA (Wisseman and Ritz 2005).

Division of species among the different sections in subgenus *Rosa* has also been problematic. The largest section in the genus is sect. *Cinnamomeae* (approximately 80 species), which should be merged with sect. *Carolinae* (approximately five species) according to analyses based on RAPD (Jan et al. 1999), AFLP (Joly and Bruneau 2007; Koopman et al. 2008), simple sequence repeat (SSR or microsatellites markers, Scariot et al. 2006), and sequencing data (Wisseman and Ritz 2005; Bruneau et al. 2007). In addition, sect. *Pimpinellifoliae* (approximately 15 species) is clearly polyphyletic (Matsumoto et al. 1998; Wisseman and Ritz 2005; Koopman et al. 2008), and some of its species apparently belong together with sections *Carolinae* and *Cinnamomeae* (Scariot et al. 2006; Bruneau et al. 2007).

Two major clades have been identified in the genus, with *Carolinae*, *Cinnamomeae,* and parts of *Pimpinellifoliae* in one clade and most of the other sections in the other clade (Jan et al. 1999; Bruneau et al. 2007). In this second major clade, sect. *Synstylae* (approximately 25 species) shows considerable similarities with sect. *Gallicanae* (Koopman et al. 2008) and

with sect. *Indicae* (Wu et al. 2000; Wisseman and Ritz 2005). Removal of the only European species in section *Synstylae*, *R. arvensis*, would result in an apparently monophyletic section (Matsumoto et al. 2000; Wu et al. 2000; Koopman et al. 2008). However, the largest member in this second clade is the sect. *Caninae* (approximately 50 species, also known as dogroses). Early in the last century, several hundreds of taxa were described but more critical evaluations, both in the field and in herbaria, have later prompted a reduction to approximately 50 dogrose species (Wissemann 1999, 2002, 2003). DNA analyses suggest that this section constitutes a well-circumscribed monophyletic group (Matsumoto et al. 2000; Wisseman and Ritz 2005; Scariot et al. 2006; Koopman et al. 2008). Although sharing some internal transcribed spacer (ITS) sequence types with species in other sections, thereby confirming their hybridogenous origin, the *Caninae* species also have one unique ITS sequence type, which is further evidence of their monophyly (Ritz et al. 2005; Kovarik et al. 2008). Of the five described subsections in sect. *Caninae*, only subsection *Rubigineae* is well defined according to AFLP data (De Cock et al. 2008; Koopman et al. 2008).

Interestingly, almost all of the horticulturally important species belong to this second clade, thus suggesting that the wealth of cultivated roses has a surprisingly narrow genetic basis (Matsumoto et al. 1998).

Only a few in-depth studies of species delimitations have been carried using DNA markers. In sect. *Caninae*, ordination analyses based on RAPD and AFLP, respectively, produced fewer but larger groups of samples compared to results obtained with morphological characterization, and several commonly recognized taxa overlapped completely in their DNA profiles (Olsson et al. 2000; De Cock et al. 2008). A set of five diploid and three tetraploid and probably hybridogenous species in sect. *Cinnamomeae* were studied using AFLP and morphometry (Joly and Bruneau 2007) as well as sequencing of the nuclear *GAPDH* gene (Joly et al. 2006). Similar patterns for species delimitations were found, but evidence for the exact origination of the allopolyploid species was rather inconclusive.

The relationships among cultivated and wild material have been investigated in several studies. AFLP analysis thus showed that most cultivars grouped either into a European cluster related to *R. damascena* and *R. gallica*, or into an Oriental cluster related to *R. moschata*, *R. wichurana,* and *R. multiflora* (Koopman et al. 2008). The first cluster contained mainly European cultivars belonging to the Alba, Centifolia, Damask, Gallica, Moss, and Portland cultivar groups. These groups are derived from the old European garden roses in sect. *Gallicanae*, but often with some contribution also from the Hybrid China roses. This European cluster also showed affinity with sect. *Caninae*, which may contain the seed parent of the Alba roses. The Oriental cluster instead contained cultivars that belong mainly to the Bourbon, Moschata, Multiflora, Noisette, Polyantha, and Tea groups together with *R. moschata*. Another cluster with *R. wichurana* and *R. multiflora* was sister to this group. The Hybrid perpetuals, which are derived from crosses between Hybrid China roses and *R. gallica*/*R. damascena* hybrids, appeared to be closer to the latter.

Similarly, SSR analysis of both wild species and cultivars produced a large and mainly European cluster, which also contained the Hybrid China roses and one Noisette cultivar (Scariot et al. 2006). This cluster showed affinities to both the dogroses in sect. *Caninae* and to the Alba roses. Species and cultivars involving the remaining sections, *Indicae*, *Carolinae* and *Cinnamomeae*, and *Synstylae*, were further apart in the dendrogram.

### 12.1.4 Phylogeny

Both morphological and DNA-marker differentiation among rose species involve mainly novel character combinations caused by gene flow instead of novel character states caused by the amassing of mutations over a long time period. Similarly, the extremely low levels of DNA sequence divergence found across the genus (Matsumoto et al. 1998; Wisseman and Ritz 2005; Bruneau et al. 2007) suggest that this is a young genus where much speciation has taken place after the last glaciation. Poor phylogenetic resolution and commonly occurring contradictions between chloroplast and nuclear gene phylogenies also suggest that hybridization has been a strong driving force in the evolution of roses.

In contrast, the fossil record points to an old origin of *Rosa*. Fossil record including hips date back into the

Miocene and Oligocene, so approximately 30 million years ago (Kvacek and Walther 2004). It is therefore conceivable that the origin of *Rosa* is quite ancient, but the radiation and diversification process is recent. Clearly, further research into the age of *Rosa* is required.

So far, the wealth of genomic data has not been successfully used to produce a comprehensive phylogeny of *Rosa* species. As mentioned earlier, one of the reasons is that divergence is recent, thus making it difficult to use coding gene sequences for the production of well-supported trees. Nonetheless, at least one partial phylogenetic tree has been published based on *OOMT* 1 and 2 (Scalliet et al. 2008), reflecting the history of a gene duplication in Chinese and European roses. Because they evolve faster than coding sequences, non-coding sequences (intronic or promoter regions of genes) should be more useful for phylogenetic inference in *Rosa*. Characterizing haplotypes could also be useful to understand past hybridization and/or polyploidization events; for example, based on alcohol dehydrogenase (*ADH*) haplotypes, it was possible to conclude that the tetraploid *R. gallica* may have originated from an interspecific hybridization between one *Cinnamomeae* and one *Synstylae* species (O. Raymond unpublished data). Future research should focus on the evolution of the regulatory regions of some key genes to morphological and/or architectural innovation such as the transcription factors controlling floral organ identity (e.g., Kitahara and Matsumoto 2000).

## 12.2 Conservation Initiatives

Cultivated roses have a very ancient history and the first selections were reported already in the early sixteenth century. Later on, artificial crossing led to what is today perceived as the "modern rose cultivars." However, the genetic basis on which these modern rose cultivars are established is poorly understood. It is thought that only between 8 and 20 species out of about 200 have contributed to the origin of our present cultivars (de Vries and Dubois 1996; Reynders-Aloisi and Bollereau 1996; Gudin 2001).

Martin et al. (2001) tried to clarify the domestication history by DNA analysis with RAPD markers of 100 cultivars of old roses. They showed that selection resulted in the retention of only a small number of alleles during the process of rose domestication. These alleles probably originate from *R. chinesis* for characters concerning color and recurrence, and from European groups for those concerning hardiness and flower complexity. Hence, genetic diversity in wild species may be used to increase the diversity for specific traits in cultivated roses. This has already been done to some extent but there are many more traits that could prove very valuable for cut and garden rose improvement. It is one of the reasons why genetic diversity in these wild species should be conserved.

For in situ conservation, we would need to know how large the variation is in wild *Rosa* populations. For *Rosa canina*, Jürgens et al. (2007) found a high level of genetic variation within populations whereas also population differentiation between regions was very high, as can be expected given the breeding system of this species. Hence, populations should be conserved across a large region. De Cock (2008) and De Cock et al. (2008) describe the genetic diversity within and between populations of various *Rosa* species in Flanders and in some western European countries, as determined using AFLP. They showed that the European *R. spinosissima*, *R. gallica*, *R. majalis*, *R. pendulina*, *R. arvensis,* and *R. sempervirens* populations showed strong geographical genetic differentiation. However, in many cases there was no consistent differentiation based on taxon or on geographical pattern. For instance, the three taxa *R. canina*, *R. corymbifera*, and *R. balsamica* showed a higher interspecific similarity when sampled at the same location compared to their congeners sampled at other localities in Flanders. Apparently, for these taxa the locality is a more accurate predictor of genetic relatedness than the taxonomical determination. This is perhaps not so surprising considering the taxonomic problems in the genus, but it means that in situ conservation efforts should try to cover as many populations as possible.

Given that the taxonomy is not always clear, it is not straightforward to predict how much genetic variation resides within the wild species. The good cross-species transferability of SSR markers across the genus *Rosa* (another indication that it is a young genus) will enable the study of genetic diversity across the whole genus (Scariot et al. 2006) and a reasonable balanced assessment of the levels of diversity in the different species groups.

Ex situ conservation takes place by collecting plants and maintaining them in botanical gardens and, typical for roses, in rose gardens. There are many rose gardens in the world. However, they contain relatively large collections of cultivated roses. The wild material has been described taxonomically, but there is no overview of how accurately this has been done. As rose gardens exchange material, just as botanical gardens and genebanks, they collectively may conserve only a tiny amount of the variation present in the wild.

## 12.3 Role in Elucidation of Origin and Evolution of Rose

### *12.3.1 The Origin of Damask Roses*

Damask roses are well known for their strong fragrance (Widrlechner 1981). From a historical and geographical perspective, the Damask roses are considered to originate from Persia (today Iran). By the fourteenth century, the Damask roses were already available in West Europe (Beales et al. 1998). Some Damasks have been maintained in West European rose collections as garden roses ("York and Lancaster," "Quatre Saisons," "Quatre Saisons Blanc Mousseux," "Kazanlik," and others). During the nineteenth century, the Damask roses are thought to have played a substantial role in the improvement of the modern European hybrid roses. The most significant Damask rose from a commercial point of view is the 30-petalled *R. damascena* "Trigintipetala" which is cultivated for rose oil production in Bulgaria, Turkey, Iran, India, China, and northern Africa.

In 1941, Hurst classified the Damask roses into two groups according to their flowering time: Summer Damasks that bloom only in early summer and Autumn Damasks that have a second blooming in the autumn. This classification was based on morphological and general botanical observations, which can often be misleading. In-depth investigations of the actual existing genetic diversity in this group of roses based on DNA genotyping have only recently been conducted. Iwata et al. (2000) analyzed two Summer Damask varieties ("Kazanlik" and "York and Lancaster") and two Autumn Damasks ("Quatre Saisons" and "Quatre Saisons Blanc Mousseux") and found no difference in their DNA profile using 24 RAPD primers. Agaoglu et al. (2000) found no difference among accessions of *R. damascena* plants in Turkey using RAPD markers. Baydar et al. (2004) demonstrated that 15 *R. damascena* plants brought from 15 different plantations in Isparta province, which is the main rose growing region in Turkey, possess identical genotypes based on AFLP markers and nine microsatellite loci. Rusanov et al. (2005a) characterized a total of 40 Damask rose accessions of which 25 originated from Bulgaria (the collection of the Institute of Roses and Aromatic Plants, Kazanlak) using microsatellite markers derived from *Rosa wichurana* and *Rosa hybrida*. The results showed that all analyzed "Trigintipetala" accessions and the old garden Damask rose varieties "York and Lancaster" and "Quatre Saisons" (in confirmation of Iwata et al. 2000) possess identical genotypes. In Iran more than one genotype was found, but the genotype in the main production area was identical to "Trigintipetala" (Babaei et al. 2007). In conclusion, it appears that the industrial production of rose oil in Bulgaria, Turkey, and to a great extent in Iran is based on a single genotype (and mutants thereof). An interesting observation in this study was the reported high somatic stability of the analyzed microsatellite loci as the allele sizes of the 33 assayed SSR loci had remained unchanged in accessions, which have been vegetatively propagated for centuries in different geographical regions.

The studies of Babaei et al. (2007) and Kiani et al. (2008) identified non-"Trigintipetala" genotypes, mostly in the mountainous northwestern part of Iran, with microsatellite alleles that are not present in the Bulgarian and Turkish genotype. They are therefore not the result of self-pollination. This may suggest that the center of diversity may be in Iran, but detailed sampling of wild populations has not been carried out in the whole distribution area of the species.

So far the only in-depth DNA based study on the parental origin of the Damask roses was published by Iwata et al. (2000). They compared the sequences of the ITS of the ribosomal DNA and the *psb*A–*trn*H spacer sequence of the chloroplast genome of four Damask varieties possessing an identical genotype ("Kazanlik," "York and Lancaster," "Quatre Saisons," and "Quatre Saisons Blanc Mousseux") with those from the species that had been suggested by Hurst (1941) as parents of the Damask roses: *R. gallica*,

*R. phoenicia,* and *R. moschata*. The results rejected *R. phoenicia* as a potential parent in the initial crossing. The authors further included *R. fedschenkoana* in their analysis and proposed that the actual crossing that led to the formation of the genotype found in all four Damask varieties is (*R. moschata*♀ × *R. gallica*♂) × *R. fedschenkoana*♂. On the other hand when Rusanov et al. (2005b) analyzed the genetic similarity among various oil-bearing roses, they found that *R. damascena* differs in all alleles at several microsatellite loci from the profiles of the analyzed accessions of *R. moschata* and *R. gallica*. As microsatellites are polymorphic within species this does not immediately preclude these species as parents. It will be necessary to assay a number of genotypes that are closely related to *R. damascena* and its putative ancestors with molecular markers allowing easy allele scoring.

## 12.4 Ploidy Levels and How to Manipulate Them

### 12.4.1 Ploidy Levels

Almost all presently grown rose cultivars are tetraploid and usually interfertile. Most of them are derived from several generations of spontaneous or man-made crosses and no doubt contain several different wild species in their ancestries. By contrast, most of the wild rose species are diploid and have a regular meiosis with seven bivalents. Polyploid species are found mainly in sections *Cinnamomeae* and *Carolinae*, which have only made minor contributions to the cultivar gene pool. Some polyploids are, however, found also in other sections like *R. chinensis* (sect. *Indicae*), which has been reported as 2*x*, 3*x,* and 4*x*, and the tetraploid *R. gallica* (sect. *Gallicanae*). Interfertility among wild species is generally high as long as the crosses are undertaken between species at the same ploidy level. Still, the prevalence of tetraploidy in cultivars suggests that hybridization has been more successful at this higher ploidy level although other desirable traits like increased plant vigor may also have played a part.

One section deviates from the remainder; all species in sect. *Caninae* are characterized by the peculiar *canina* meiosis (Lim et al. 2005). Regardless of ploidy level (usually 5*x*, but some 4*x* and 6*x* taxa also occur), only seven bivalents are formed in the first meiotic division. The remaining chromosomes form univalents and are not included in viable pollen grains, which therefore contain only the seven divided bivalent chromosomes. All univalents are transmitted to one of the daughter cells in the female meiosis, and are finally included in the viable egg cells, which therefore contain 21, 28, or 35 chromosomes depending on ploidy levels. SSR-based analyses of different species and offspring from controlled crosses suggest that bivalent formation involves one biparentally inherited, highly homozygous diploid genome, whereas the remaining 2, 3, or 4 haploid and often highly differentiated genomes are transmitted only from the seed parent (Nybom et al. 2004, 2006). Interfertility is very high among dogroses, and they can also be used in crosses with species on other levels, behaving as a polyploid species when used as seed parent, and as a diploid species when used as pollen parent.

Hybridization between diploid and tetraploid roses results in triploid hybrids that are generally sterile or have very low fertility. Two different strategies can be envisaged to overcome this ploidy barrier: haploidization of tetraploid cultivars and polyploidization of wild, mostly diploid genotypes.

### 12.4.2 Haploidization

Up to now, the only successful haploidization method has resulted from in situ induction of parthenogenesis using irradiated pollen and subsequent in vitro culture of immature seed.

#### 12.4.2.1 In Situ Parthenogenesis in Roses

Dihaploid derivatives from tetraploid rose cultivars are produced by in situ parthenogenesis induced after pollination by irradiated pollen and subsequently in vitro embryo rescue (Meynet et al. 1994). For this, anthers are collected from flower buds of the male parents 1–2 days prior to flower opening. The anthers are dried for 2 days in an incubator at 30°C. The pollen is sifted and stored in a desiccator at 4°C for the duration of the hybridization period, e.g., from April to June in European countries. All the flower buds on

the maternal plants are emasculated 2 days prior to anthesis by removing the calyx, petals, and anthers with a forceps. Dry pollen samples are exposed to gamma irradiation from a $Cobalt^{60}$ source for a total exposure of 600 Gy. Pollinations with irradiated pollen are made on the day of irradiation or the following day. After pollination, the flowers are protected with paper bags.

Eight weeks after pollination the hips formed are collected and achenes are extracted from each hip. The enlarged achenes are plunged into water. The probability that achenes contain an embryo is approximately 15 times higher in achenes of density >1 than in floating ones. The achenes that sink are disinfected in 30 g/l $CaCl_2$ for 20 min, and subsequently rinsed three times for 5 min in sterile distilled water. Embryos are aseptically removed from the endocarp. Embryos are placed on a solid culture medium in darkness at 4°C for 4 weeks, and then transferred to a 16 h photoperiod provided by daylight fluorescent illumination [90 μmol/($m^2$ s)] and at 22°C for 2 weeks to obtain rooted plantlets. At the end of incubation, the embryos germinate and the rooted plantlets are planted into a classical horticultural medium and grown under greenhouse conditions. The ploidy level of the plants obtained is then determined by flow cytometry (FCM) or vegetative meristem chromosome counts.

#### 12.4.2.2 Characteristics of Dihaploid Roses

Characteristics such as the guard cell length, chloroplast number, stem length, leaf and flower size are reduced in dihaploids as compared with their tetraploid donors. Male fertility of the dihaploids is usually very low; 76% of them were characterized by pollen viability lower or equal to 5% (El Mokadem 2001). However, three dihaploids showed pollen viability greater or equal to that of their tetraploid donors. Female fertility of the dihaploids was variable.

Many progenies have now been obtained from a hybridization program between dihaploids of rose cultivars, used as female parents, and diploid wild species (e.g., *R. gigantea*, *R. roxburghii*, *R. rugosa*, and *R. wichurana*), used as male parents (El Mokadem 2001). Although some of these dihaploids were fertile, their gametogenesis often revealed abnormalities and resulted in the frequent production of 2*n* gametes, i.e., gametes with the somatic chromosome number (El Mokadem et al. 2002a, b). Unreduced gametes or 2*n* gametes are mainly formed in two ways (1) an incomplete first meiotic division (first division restitution, FDR), or (2) an incomplete second meiotic division (second division restitution, SDR). Unreduced gametes via FDR are comprised mainly of the non-sister chromatids of each homologous pair of chromosomes, whereas in SDR the sister chromatids are included in the same gametes. As a result, 2*n* gametes formed by FDR transmit more of the parental heterozygosity into progenies than those formed by SDR.

The formation of 2*n* gametes can be detected by analyzing ploidy level (e.g., with FCM) of progenies resulting from crosses between dihaploids and diploid species, as well as by analyzing the size of the pollen grains produced by a dihaploid rose. The nature of the mechanisms underlying male and female 2*n* gametes produced by the dihaploids was determined by a cytological study of male meiosis and by estimating the heterozygosity level transmitted by female and male 2*n* gametes into the triploid progeny resulting from crosses made with diploid species, using AFLP markers (Crespel et al. 2002b). Among meiotic abnormalities leading to 2*n* pollen production, triads (containing a 2*n* microspore at one pole and two *n* microspores at the other) resulting from abnormal spindle geometry were frequently observed (El Mokadem et al. 2002a). There were various types of meiotic nuclear restitution leading to 2*n* pollen production: second division restitution with crossing-over (SDR-CO), first division restitution without crossing-over (FDR-NCO), and first division restitution with crossing-over (FDR-CO) transmitting ±40%, 100% and ±80% of the parental heterozygosity, respectively. The proportion of different 2*n* gamete types produced was mainly genotype dependent with some seasonal effects (Crespel et al. 2002b, 2006; Crespel et al. 2003). Since the ability to produce 2*n* gametes was transmitted to the offspring, a return to tetraploid level can be envisaged by meiotic polyploidization via 2*n* gametes.

### 12.4.3 Polyploidization

Polyploidization can be obtained via three different methods: mitotic, meiotic, and somatic polyploidization.

#### 12.4.3.1 Mitotic Polyploidization

For mitotic polyploidization, a chemical substance is applied that transiently blocks mitotis, resulting in DNA replication without chromosome separation during anaphase. Amphidiploids, i.e., allotetraploids, are more useful than autotetraploids in such procedure, notably to restore the fertility of diploid interspecific hybrids, but also to avoid the typical infertility of primary autotetraploids. There are two practical ways to produce amphidiploids from diploid rose species (1) to double the diploid species and cross the resulting autotetraploids; and (2) to cross the diploid species and double the resulting diploid interspecific hybrid.

Since the middle of the twentieth century many authors have tried to improve the chromosome doubling technique, with varying success depending on genotypes, type and concentration of chemicals used, duration of application, and type of exposed explant. Both in vivo and in vitro assays have been made in roses. The most frequently used chemical is colchicine, an alkaloid obtained from *Colchicum autumnale*. Dinitroaniline compounds, which are herbicides, have also been tested: oryzalin, amiprophosmethyl (APM), and trifluralin. Chromosome doubling has been successfully performed using three applications of 0.5% colchicine solution at alternating days (Semeniuk and Arisumi 1968), or application of 0.06% colchicine solution each day during 4 days on the top lateral bud in active growth (4–8 leaf stage) of seedlings (Basye 1990; Byrne et al. 1996), on different genotypes (e.g., *R. laevigata* and *R. laevigata* hybrids, *R. roxburghii* and *R. roxburghii* hybrids, *R. bracteata* or *R. wichurana*). However, the results remain erratic. Trifluralin assays were also performed in vivo, by dropping 0.086% trifluralin solution on the shoot apex of *R. rugosa* hybrid germinating seedlings (Zlesak et al. 2005). These assays resulted in some polyploids, but with a quite low success rate (around 5% doubled plants) and a high level of chimerism.

In vitro assays have also been performed using colchicine. However, in this case an in vitro multiplication phase of plantlets before treatment, and an in vitro regeneration phase of treated explants afterwards, is necessary. Optimal application was achieved by growing plantlets on medium containing 1.25 mM colchicine (Ma et al. 1997), or by soaking nodal sections in 1.25 mM or 2.5 mM colchicine solution before transfer to solid culture medium (Roberts et al. 1990; Ma et al. 1997). Soaking assays on *R. wichurana* in vitro plantlets gave up to 30% of doubled cells, but the number of doubled plants was not reported (Roberts et al. 1990). Amphidiploids from the hybrids *R. wichurana* × *R. roxburghii* and *R. wichurana* × *R. setigera* were obtained at the end of the 1980s in these ways, but overall success rate was lower than 5% (Basye 1990; Byrne et al. 1996). A similar study was performed in the middle of the 1990s on five interspecific diploid hybrids involving the diploid species *R. wichurana*, *R. roxburghii*, *R. banksiae*, *R. rugosa rubra*, and *R. setigera* (Ma et al. 1997). Some amphidiploids were detected, but success rates remained below 5%.

Faced with the low efficiency of colchicine and its carcinogenic nature, other anti-microtubule chemicals have been explored as an alternative for polyploidization since the early 1990s. Oryzalin, APM, and trifluralin have actually been shown to have greater affinity for binding to plant microtubule, and so are used at micromolar concentrations, a thousand times lower than colchicine (Zlesak et al. 2005). Compared to colchicine, the efficiency of such herbicides in vitro for polyploidization of plants has been variable but promising. The first attempts with oryzalin were performed on in vitro plantlets from diploid *R. hybrida* "Thérèse Bugnet" (Kermani et al. 2003). Treatment of the shoot apex in liquid medium at 5 μM during 24 h, followed by 13 days on semi-solid medium at the same concentration, resulted in 40% doubled plants. The best results were obtained by exposing nodal section to oryzalin on solid medium at a concentration of 5 μM during 24 h, with a success rate of 66.6% doubled plants and a survival rate of 20%.

The use of oryzalin, APM, or trifluralin in vitro treatments on triploid *R. hybrida* "Iceberg" nodal sections showed similar efficiency (Khosravi et al. 2008). Application of the chemicals was done on a two-phase (liquid and semi-solid) shoot proliferation medium, at 6 μM concentrations, during 24 h. The same treatment on diploid *R. persica* and tetraploid *R. hybrida* "Akito" explants resulted in 6.3% and 0% chromosome doubling, respectively, which suggests that chromosome doubling is genotype dependent (Khosravi et al. 2008).

The above-mentioned approaches are limited to rose genotypes that can easily be grown in vitro. Concentrations of spindle inhibitor and exposure time are critical factors to success, but whatever chemical is used, chromosome doubling is genotype dependent,

and standard conditions that work for all rose species cannot be specified.

#### 12.4.3.2 Characteristics and Use of Amphidiploids

Induced tetraploidy is visually detected by "gigas" characteristics: slower growth, thicker leaflets with a greater width-to-length ratio, greater overlapping of the leaflets, and larger flower size (Byrne et al. 1996; Ma et al. 1997; Kermani et al. 2003; Allum et al. 2007). Other indicators are a larger guard cells and larger pollen grains. These two parameters are typically used for ploidy level estimation of rose genotypes, due to the easiness of use in comparison to chromosome counts, but FCM analyses are currently performed for ploidy level assessment.

Since antimitotic agents work on a single cell level, the original plant is likely to be a chimera, a plant with sectors of doubled and non-doubled tissue. Thus, care needs to be taken to examine the plant thoroughly and isolate the doubled sector.

Fertility of amphidiploids depends on fertility of the initial diploid hybrid. The general rule is that the less fertile the interspecific diploid hybrid, the more fertile (and so usable in breeding program) the amphidiploid derived from it (Byrne and Crane 2003). The most widely used amphidiploid in modern rose breeding is *R. kordesii*, a spontaneous tetraploid seedling of the sterile diploid hybrid (*R. rugosa* × *R. wichurana*) "Max Graf," which gave rise to the Kordesii hybrid roses in European and Canadian breeding programs. Some of Basye's (1990) amphidiploids have also been hybridized with commercial rose germplasm in order to transfer general blackspot resistance to the breeding population.

#### 12.4.3.3 Meiotic Polyploidization

Sexual polyploidization is the process by which a polyploid zygote is formed by natural fertilization, involving $2n$ gametes. Two cases are possible (1) unilateral polyploidization occurring in interploidy crosses ($2x \times 4x$), in which case one $2n$ gamete (from the diploid parent) fertilizes a reduced gamete (from the tetraploid parent); (2) bilateral polyploidization occurring by fusion of two $2n$ gametes coming from two diploid parents (in a $2x \times 2x$ cross). Production of $2n$ gametes in rose was first shown on the dihaploids obtained via in situ parthenogenesis using irradiated pollen (see above), but some tetraploid cultivars have been found to produce $2n$ gametes as well (Crespel and Gudin 2003).

In roses, $2n$ gametes produced by dihaploid rose cultivars and their diploid hybrid are used to obtain new tetraploid genotypes through unilateral and bilateral polyploidization. Crosses between dihaploids of rose and rose cultivars have been realized. The percentage of tetraploid progenies obtained varied from 14.2 to 100% according to the dihaploid parent used. A strong gametic selection is observed, in favor of $2n$ gametes, especially when a tetraploid partner is involved (Crespel and Meynet 2003).

#### 12.4.3.4 Somatic Polyploidization

Finally, it is also possible to perform polyploidization by fusing somatic cells as protoplasts. This approach is described later, together with other biotechnological approaches.

## 12.5 Role in Crop Improvement Through Traditional and Advanced Tools

### *12.5.1 Role in Crop Improvement*

Rose is the most ancient ornamental species. There is evidence that roses were already cultivated 5,000 years ago in China, western Asia, and northern Africa. The first acts of rose domestication corresponded to the cultivation and multiplication of wild rose species. Spontaneous hybridization between these collected species formed the start of new genetic variation (Gudin 2000). Some of these spontaneous hybridizations were only possible because of human activity as the original species involved were collected by plant hunters in distant parts of the world and brought together in gardens.

The voyages of discovery from Europe to Asia brought together the species that have formed the genetic basis of the modern cultivated rose assortment. Controlled rose breeding really started in the nineteenth

century and is nowadays practiced by 25–30 international commercial companies and by many amateur breeders. The activities of controlled and formerly spontaneous hybridizations have led to the modern rose cultivars that correspond to a complex artificial species, often referred to as *R. hybrida* (Gudin 2003).

Some of the important characteristics that were introduced from the progenitors of the modern cultivars in the rose cultivar gene pool were recurrent flowering from *R. chinensis* (around 1800), cold resistance from *R. wichurana*, and yellow flower color from *R. foetida* (around 1900). Recurrent flowering was also introduced from *R. rugosa*, *R. fedschenkoana,* and *R. bracteata*. For specific usages other species have been involved during the twentieth century. For instance, ramblers are mostly hybrids from *R. arvensis*, *R. wichurana*, *R. multiflora*, *R. pendulina,* and *R. sempervirens*. Species used in ground cover roses are *R. wichurana*, *R. davidii elongate,* and *R. bella* (Wylie 1954). For shrub roses, *R. rugosa* has attracted attention from breeders for over 100 years, with recent renewed interest. Also *R. moschata*, *R. multiflora*, *R. spinosissima*, *R. rubiginosa*, *R. moyesii,* and *R. multibracteata* have been used (Wylie 1954).

An extensive overview of interspecific hybrids from natural and artificial crosses that are mentioned in the literature has recently been provided by Spethmann and Feuerhahn (2003). Species belonging to the same section are easier to cross with each other than species from different sections. Sometimes unilateral incongruity is observed. In spite of the specialized cytology of the pentaploid species belonging to the section *Caninae*, these species might be successfully crossed both with species of the same section and with species belonging to other sections (Spethmann and Feuerhahn 2003). Also in nature several interspecific hybridizations between these species have been observed.

Interspecific crosses between species and cultivars are hampered by several factors, including taxonomic distance and ploidy differences. Another bottleneck is that hybrid seeds obtained from crosses between modern cultivars and wild species often show seed dormancy. Therefore germination starts only in the second year. The obtained $F_1$ progenies also frequently show some dominant characteristics of the wild parent: no flowers are formed during the year of germination, and the growth habit is in general more wild (Van Huylenbroeck et al. 2007). Thus, incorporating wild species in a breeding program implies that several backcross generations are made to obtain a "modern rose" with an additional "wild" characteristic. As a consequence breeding programs with wild species are usually enormously time-consuming and expensive. Especially for cut roses the gap between the desired high quality level of a commercial cultivar and the morphological features of the wild relative is enormous. These morphological differences make it difficult to use wild species in the breeding program. As a result the genetic background in these commercial breeding programs becomes narrow. For garden roses, the genetic and morphological distance between modern cultivars and the wild species is still narrower, which makes the use of wild species more feasible.

### 12.5.2 Desirable Agricultural Traits

Depending on the trait, different wild species might be valuable to broaden the genetic base of cultivated roses. Examples of interesting traits in species are the yellow flower pigmentation of *R. hugonis*, *R. ecae,* and *R. primula*; thornlessness in populations of *R. blanda*, or, quite the contrary, the ornamental prickles of *R. sericea* ssp. *omeiensis* var. *pteracantha*. Winter hardiness exists in *R. laxa* from central Siberia, *R. suffulta* possesses drought resistance, while *R. arvensis* is known for its shade tolerance (Spethmann and Feuerhahn 2003). Several species as *R. moschata*, *R. rubiginosa*, *R. canina*, *R. gallica,* or *R. damascena* could improve hip quality for specific medicinal or food uses (see below) or add aesthetical value to garden roses and to cut rose stems for flower arrangements. Evergreen rose species from the *Bracteatae* section might be of interest for improvement of shelf-life, i.e., sustained flowering of pot rose plants under room conditions (De Vries 2003).

The highest priority in rose breeding research is the development of disease resistant roses. The two major diseases in roses are black spot (*Diplocarpon rosae*) and powdery mildew (*Podosphaera pannosa*). Different resistance mechanisms for both black spot (Blechert and Debener 2005) and powdery mildew have been described in roses (Dewitte et al. 2007). Interesting fungal resistance is found in wild rose species. Since the discovery of resistance genes in several crops a lot of effort in breeding research has

been directed towards this monogenic or so-called qualitative or vertical resistance. However, an easy and systematic combination of traits as in diploids cannot be expected in tetraploid rose cultivars.

Resistance to powdery mildew varies among rose species and cultivars, and is often pathotype specific (Linde and Debener 2003; Leus et al. 2006). Results of natural and artificial infections with this pathogen show that only few cultivars are highly resistant. In wild species, there is no distinction between sections concerning resistance. *R. agrestis*, *R. glutinosa,* and *R. omeiensis* var. *pteracantha* are mentioned as highly resistant (Linde and Shishkoff 2003). Most modern cultivars are susceptible to black spot, but several species show resistance: *R. banksiae*, *R. carolina*, *R. laevigata*, *R. multiflora*, *R. rugosa*, *R. roxburghii,* and *R. wichurana* (Drewes-Alvarez 2003). A concern while using wild species for ameliorating disease resistance is that these traits are often genotype specific. As a consequence, resistance for a specific pathogen should be tested on individual plant genotypes. Results of interspecific crosses between wild species and resistances for black spot (*D. rosae*) and powdery mildew (*P. pannosa*) of obtained progeny are presented by Spethmann and Feuerhahn (2003).

Resistances to populations of the root-knot nematodes *Meloidogyne arenaria*, *M. incognita,* and *M. javanica* can be found in *R. multiflora* and *R. indica*. For *M. hapla* resistances found in rootstocks of the mentioned rose species were more variable. Experiments on *R. multiflora* indicate that resistance to *M. hapla* is polygenic (Wang et al. 2004b). *R. manetii* appears to be highly resistant, whereas the often-used *R. canina* rootstocks have, in general, a good nematode resistance. For root-lesion nematodes (*Pratylenchus penetrans*) resistances were identified in *R. multiflora* "K1," one accession of *R. virginiana*, *R. laevigata anemoides* and some accessions of *R. glauca*. Resistances in these species were higher compared to the resistance found in the popular rootstock *R. corymbifera* "Laxa" (Peng et al. 2003).

### 12.5.3 Improving Damask Rose

Currently the only Damask rose, which has a significant commercial and industrial importance and is widely grown in Bulgaria, Turkey, and Iran for production of rose oil, rose water, rose absolute, and rose concrete is *R. damascena* "Trigintipetala." The quality and composition of the distilled rose oil is controlled by implementation of an international standard (ISO 9842:2004), which corresponds to the oil from "Trigintipetala." Breeding of *R. damascena* has been mostly limited to clonal selection of best performing plants in the rose fields, e.g., the cultivars "Eleina" and "Janina," which show elevated tolerance to freezing and rust (*Phragmidium mucronatum*), were created by chemical and radiation mutagenesis, respectively (Raev 1984). Although crosses with other rose species such as *R. gallica* have been implemented by some rose breeders (Staikov and Kalaijiev 1980) the obtained hybrid roses never made their way to industrial cultivation for the perfumery and cosmetics industry because of the altered composition of the distilled rose oil from their petals.

According to Iwata et al. (2000) the old varieties of *R. damascena* (genotypically identical to "Trigintipetala") have a triparental origin. "Trigintipetala" generally produces no or just a few seeds with low germinating potential when forced to self- or cross-pollinate. However, Rusanov et al. (2005c) found that 24 seeds collected from "Trigintipetala" plantations in Bulgaria are the result of self-pollination or cross-pollination with neighboring plants (which possess the same genotype). On the basis of SSR allele inheritance they concluded that *R. damascena* "Trigintipetala" is most probably a segmental allotetrapoid species and that the type of inheritance (tetrasomic or disomic) depends on the chromosomal location. This suggests that it would be relatively easy to create a self-pollinated segregating population through collecting seeds from oil rose plantations, but that creating a genetic map and identifying quantitative trait loci (QTL) in "Trigintipetala" may not be straightforward.

During the last decade a number of studies have been published involving the elucidation of genes that are responsible for the rose scent formation (Guterman et al. 2002; Lavid et al. 2002; Scalliet et al. 2002, 2006, 2008; Shalit et al. 2003). Databases including sequences of thousands of petal-expressed genes have been established providing a solid ground for development of single nucleotide polymorphism (SNP) markers (Channelière et al. 2002; Guterman et al. 2002; Jung et al. 2004, 2008; http://www.bioinfo.wsu.edu/gdr/). Recently, at the AgroBioInstitute a strategy for development of SNP markers related to key genes involved in the rose scent formation was implemented.

The results (K. Rusanov unpublished data) demonstrated that data obtained through sequencing of PCR products derived from genomic DNA could easily be converted into gene-specific SNP markers. The developed markers could be used in a breeding program in order to follow the segregation of alleles of key genes that are responsible for the composition of rose oil. When applied to a population of self-pollinated "Trigintipetala," the developed markers might be used to identify a minimum set of allele configurations that can be used to screen for genotypes with genes for improved rose oil quality.

The most desirable traits for "Trigintipetala" breeding include flower yield, rose oil content, and resistance to diseases. Drought tolerance has also been recognized as a desirable trait due to the insufficient and irregular rainfall in rose plantation regions over the last decade (Gunes 2005). As was recently reported (Pirseyedi et al. 2005; Babaei et al. 2007; Tabaei-Aghdaei et al. 2007; Kiani et al. 2008) Iran represents a center of genetic diversity of the Damask roses. One of the main tasks would be to evaluate the reported non-"Trigintipetala" genotypes in Iran as well as other naturally occurring genotypes of oil-bearing roses such as "Stambolska" in Bulgaria (Rusanov et al. 2005b) for both their rose oil production potential and as a natural source of desirable traits.

### 12.5.4 Expanding the Gene Pool of Cultivated Rose by Biotechnology

In case of combinations between species that do not form hybrids readily, tissue culture-based techniques, e.g., embryo rescue, can be used to overcome post-fertilization barriers, such as premature embryo abortion. Embryo rescue has been applied in rose (El Mokadem et al. 2000), as described earlier. In this section, the focus is on two other methods based on modern biotechnology, namely somatic hybridization (or cell fusion) and genetic modification (through transformation).

#### 12.5.4.1 Somatic Hybridization

The full procedure of obtaining hybrid plants through cell fusion is composed of several, equally important steps. For each step good-working protocols need to be established. Protocols for sterilization, protoplast isolation, cell fusion, cell division, callus growth, regeneration, rooting, and transfer to soil followed by hybrid identification are all required before thinking of using somatic hybridization. In rose, nearly all these requirements are met.

Increasing vigor, introducing fungal resistance, and improving fertility were the aims of Mottley et al. (1996) and Squirrell et al. (2005) in their attempts to produce hybrids from intergeneric fusions of three rose cultivars with a cherry rootstock *Prunus avium* × *pseudocerasus* "Colt" or a blackberry *Rubus laciniatus* "Thornless Oregon." They also performed self-fusion with a wild rose hybrid, *Rosa persica* × *xanthina* to increase the ploidy level to tetraploid. Hybrid callus lines were obtained and from them plants were regenerated. Some of those plants showed aberrant phenotypes, however, and further cytogenetic and molecular screening could not confirm the true hybrid nature of the plants. The aberrant phenotypes can be explained by somaclonal variation induced by the in vitro procedure.

The introduction of resistance to blackspot, *D. rosae*, was the target of Schum and Hofmann (2001) and Schum et al. (2002). Wild rose species that were studied for suitability to use in somatic hybridization were *R. canina*, *R. caudata*, *R. corymbifera*, *R. indica*, *R. majalis*, *R. multiflora*, *R. nutkana*, *R. roxburghii*, *R. rubiginosa*, *R. spinosissima*, *R. sweginzowii macrocarpa*, and *R. wichurana*. Recipients were two *R. hybrida* cultivars and the hybrid *R. persica* × *xanthina* in the ultimate fusions that were done with diploid accessions of *R. multiflora*, *R. roxburghii,* and *R. wichurana*. FCM and AFLP analyses on callus lines obtained after fusions indicated that hybrids between the cvs. "Heckenzauber" and "Pariser Charme" (+) *R. wichurana* had been formed. Unfortunately, the final step of shoot regeneration turned out to be difficult. Plants were obtained only from the combination "Pariser Charme" (+) *R. wichurana* (Schum et al. 2002), but FCM showed loss of chromosomes during culture and upon regeneration. No further details were given about continued testing on what remained of the chromosomes of the wild parent, on applications or on disease assays.

To widen the gene pool of *R. damascena,* Pati et al. (2008) fused this species with *R. bourboniana*. They reported on obtaining hybrid calli, as confirmed by molecular analysis, but not on hybrid plants.

The conclusions for somatic hybridization in *Rosa* are:

- Protoplast isolation, culture, and regeneration are possible.
- Parent knock-out treatments work (IOA, R6G, and irradiation).
- Cell fusion is possible, as are divisions and hybrid callus formation.
- Plants were obtained but there is no proof for hybrid nature.
- Phenotypical aberrations were probably caused by somaclonal variation; no proof for transfer of traits.

#### 12.5.4.2 Genetic Modification

In genetic modification, genes from other organisms can be used to expand the pool of available genes in breeding and improvement of crops. In the production of genetically modified (GM) plants, two major processes are involved, i.e., gene transfer and regeneration. The plant cell, usually present within a multicellular explant (plant part), has to be amenable to accepting foreign DNA either transferred by *Agrobacterium tumefaciens* or by biolistics, and subsequently integrating it into its own nuclear genome. The preferred result is integration of one copy of transferred foreign DNA in a site allowing desired expression patterns and stability without negative interference on the expression of resident genes. This has to be ascertained in later stages. After successful integration of foreign DNA, this particular, altered cell needs to be capable of entering a stage of sustained cell divisions and development into a plant.

In rose, several groups have tested many explant types and dedifferentiation and regeneration media combined with *Agrobacterium* inoculation or particle bombardment, however without much success. Ming et al. (2007) used nodal and leaf explants for direct regeneration in their gene transfer experiments. They reported on transient GUS expression as a reporter system aimed at optimization of conditions for gene transfer, but they did not obtain plants that could be molecularly analyzed. Earlier, Derks et al. (1995) used stem slices from the rose rootstock "Moneyway." Here, plants were obtained and tested. Recently, rose petals were used to test a transient expression system for gene function analysis. The expression of the reporter gene *gus* was checked after agroinfiltration (Yasmin and Debener 2010). This transient expression system avoids the necessity to obtain fully regenerated new plants.

All other literature reports on the use of regeneration through somatic embryogenesis. Embryogenic callus material, either obtained after induction on roots, leaf explants, petioles, or filaments, is used primarily in transformation of rose. Somatic embryogenesis is a long and tedious technique entailing an induction phase, often of several months, a maintenance phase for growth and multiplication, a maturation phase and a germination phase followed by the outgrowth of rooted plants. However, because of the lack of efficient, more direct alternatives, somatic embryogenesis is the main regeneration method in rose.

Protocol development for gene transfer and GM plant production using reporter genes such as *gus* (glucuronidase), *gfp* (green fluorescent protein), or *luc* (luciferase) has been reported by Firoozabady et al. (1994), Derks et al. (1995), van der Salm et al. (1996), Marchant et al. (1998a), Li et al. (2002), Condliffe et al. (2003), Kim et al. (2004), Ming et al. (2007), and Vergne et al. (2010). The reports vary in the cultivars that were used, in the tissue source of the embryogenic callus, the reporter gene, or the gene delivery system. In all cases except for Ming et al. (2007), regeneration of transgenic rose plants was achieved and plants could be assayed for gene presence and expression.

The goals that were aimed for in rose improvement by genetic modification were quite diverse. Impairing bacterial growth in stems in vases was achieved by the introduction of the gene coding for cecropine (derived from the giant silkmoth *Hyalophora cecropia*; Derks et al. 1995). Improvement in yield in stem production was obtained by the introduction of *rol* genes from *Agrobacterium rhizogenes* in the rootstock "Moneyway" (van der Salm et al. 1997, 1998), however overall flower quality was poorer (De Jong and Visser 2000). Changing plant architecture was the aim of introducing the *ipt*-gene from *A. tumefaciens* involved in cytokinin biosynthesis (Condliffe et al. 2003). Transgenic plants carrying the gene have been produced; however, no obvious change in phenotype could be observed (F. Krens personal observations). Disease resistance, particularly resistance against

blackspot or powdery mildew was the goal of introducing chitinase or glucanase genes (derived from rice or from barley) or of genes coding for the T4 lysozyme (from T4 phage) or for a Type I ribosome inhibiting protein (RIP from barley) by Marchant et al. (1998b) and Dohm et al. (2001, 2002). Marchant et al. (1998b) found reductions of 14–43% in lesion sizes indicative of a reduction in the severity of blackspot symptom development, and Dohm et al. (2002) demonstrated a reduction in susceptibility to blackspot of up to 60% after introduction of the barley RIP. The antimicrobial protein of *Allium cepa* (Ace-AMP1) was successfully used by Li et al. (2003) to introduce resistance against powdery mildew (*Spaerotheca pannosa*) in rose. A significant reduction in disease symptoms could be demonstrated in multiple transgenic plant lines both in a detached leaf assay as well as in the greenhouse.

Another very important trait in ornamental crops is flower color. Souq et al. (1996) and Katsumoto et al. (2007) introduced genes involved in anthocyanin production or constructs aimed to silence endogenous anthocyanin production-related genes, and succeeded in altering the color of transgenic rose flowers. The origin of the genes used was respectively rose itself (the full length of the rose chalcone synthase [*CHS*] gene in antisense orientation) or rose (siRNA *DFR*) and iris/viola (*DFR*/*F3′5′H*) and plants expressing the genes did contain altered anthocyanin patterns and color.

None of the GM roses mentioned earlier has reached the market, but it is clear that genetic modification as a technology opens up possibilities to introduce new traits in tetraploid rose cultivars. The origin of the genes that were used varied from *Agrobacterium* to silkmoth and to plant species such as rice and onion. However, broad spectrum resistance genes from non-related species might be far less efficient to the specific diseases attacking rose than specific resistance genes aimed at the specific rose pathogens from wild *Rosa* species, especially when multiple genes can be pyramided within elite cultivars. Acceptance of such GM roses, carrying rose species derived "cisgenes" (Schouten et al. 2006), by growers, retailers, and European consumers may also be higher. With the full genomes of Rosaceae family members, *Prunus*, *Fragaria,* and *Malus*, sequenced and perhaps in the near future the genome of a diploid rose itself, the identification and availability of wild rose-derived new genes or better alleles coding for interesting traits will increase, thus contributing significantly to the improvement of cultivated *R. hybrida*.

## 12.6 Role in Classical and Molecular Genetic Studies

### 12.6.1 Use in Classical Genetic Studies

The basic chromosome number ($x$) of *Rosa* is 7, with the DNA content varying from 0.78 pg/2C in diploids ($2n = 2x = 14$) to 3.99 pg/2C in octaploids ($2n = 8x = 56$; Yokoya et al. 2000; Roberts et al. 2009). This genome is small in comparison to many other crops but still large compared to the *Arabidopsis* genome (0.085–0.215 pg). Despite the low chromosome number and small genome size, relatively little is known about the genetic basis of important traits (Gudin 2000; Hibrand Saint Oyant et al. 2008). The rose breeders, nevertheless, have managed to combine many favorable specific plant characters and were able to produce highly heterozygous, vegetatively produced cultivars with the desired combinations (Rajapakse et al. 2001). However, the changing global climate, the growing conditions in state-of-the-art greenhouses, together with demands for control of diseases in order to produce with a lower environmental impact all necessitate more insight into genetics of traits and into the interactions between genes and environment.

### 12.6.2 Molecular Marker Maps and Physical Maps

The main applications of molecular markers are genotype or cultivar identification, phylogenetic studies, construction of chromosome maps, and mapping of morphological and physiological characters (Debener et al. 2003). The commercially used hybrid rose and garden rose germplasm are tetraploid, but the inheritance of traits and the generation of a molecular map are much easier studied at the diploid level. For that reason mapping started using crosses between diploid parents, and still more marker–trait associations are

being analyzed at the diploid than at the tetraploid level.

The first diploid linkage map in rose was constructed by Debener and Mattiesch (1999) using over 300 RAPD and AFLP makers. A population of 60 $F_1$ hybrids from a cross between the diploid rose genotypes 93/1-117 and 93/1-119 was used as mapping population. This $F_1$ population showed segregation for double versus simple flowers and pink versus white flower color, and perpetual flowering. The parental genotypes were half-sibs from an open-pollination of the diploid genotype 81/42-15. The genotype 81/42-15 was derived from a breeding program in which diploid hybrids between *R. multiflora* and garden roses were produced (Reimann-Philipp 1981).

Debener and Mattiesch (1999) were able to map two morphological traits: double versus single flower (*Blfo*) and pink versus white flower color (*Blfa*). The blackspot resistance gene *Rdr1* was mapped in the same population by von Malek et al. (2000). This map was further saturated by Debener et al. (2001a, b) with additional AFLPs, SSR, restriction fragment length polymorphisms (RFLPs), and sequence characterized amplified regions (SCARs). Linde et al. (2004) mapped the dominant resistance gene for powdery mildew (*Rpp1*), and identified several AFLP makers closely linked to *Rpp1*. Yan et al. (2005) added AFLP, SSR, protein kinase (PK), resistance gene analog (RGA), RFLP, and SCAR markers to this map, as well as four morphological makers: pink flower color (*Bfla*), double flower (*Bflo*), resistance to black spot (*Rdr1*), and resistance to powdery mildew (*Mildew*). Thus, they constructed a map with a total of 520 molecular markers and a total length of 490 cM for P117 and 487 cM for P118, with an average chromosome length of ca. 70 cM. They concluded that both maps may cover more than 90% of the rose genome. Moreover, both parental maps were integrated as a map with seven linkage groups having a total length of 545 cM and an average chromosome length of ca. 78 cM.

A second genetic linkage map based on a diploid population was constructed using AFLP markers by Crespel et al. (2002a, b). A population of 91 $F_1$ hybrids from a cross between H190, a dihaploid rose, and the diploid species *R. wichurana*, was used as mapping population. H190 resulted from the haploidization of the 4*x R. hybrida* "Zambra." H190 is a recurrent blooming, double-flowered, and thornless rose, whereas *R. wichurana* is single blooming, single flowered, and thorny rose. In H190, 68 AFLP markers were mapped in eight linkage groups with an average length of 29.8 cM and a total length of 238.4 cM for the map. In *R. wichurana*, 108 AFLP markers were mapped on six linkage groups with an average length of 47.8 cM and a total length of 287.3 cM for the map. The average marker interval was 3.4 cM for both parental maps, which were not integrated since they did not include biparental multiallelic markers. They identified genes underlying a major QTL (*t4*) and minor QTL (*t4b*) for number of thorns on the stem, and located the QTL for recurrent blooming (*r4*) and double corolla (*d6*) that was previously known as *Blfo*.

A third diploid population was analyzed with RAPD and SSR markers by Dugo et al. (2005). A population of 96 $F_1$ plants from an interspecific cross between diploid roses, "Blush Noisette" (D10), one of the first seedlings from the original "Champneys' Pink Cluster," and *R. wichurana* (E15), was used as mapping population. The maternal parent (D10) is a pink double-flowered (more than five petals), thorny, and spreading rose with recurrent blooming, and susceptibility to powdery mildew. The paternal parent (E15) has white flowers with five petals, and is a ground covering thornless bush rose with single blooming and resistance to powdery mildew. A total of 133 markers (mainly RAPDs) were mapped on seven linkage groups for both parental maps. Four linkage groups could be integrated since they contained common biparental markers. The map length was 388.3 cM for D10 and 260 cM for E15. The average marker density was one every 5.7 cM for both parental maps. The map of the maternal parent D10 contains almost twice as many polymorphic markers as the one of E15, probably due to the presence of a China rose and *R. moschata* genetic background. Moreover a low number of biparental markers were included, but more microsatellite markers will be tested. They were able to putatively map QTL loci controlling flower size, days to flowering, leaf size, and resistance to powdery mildew.

The first two tetraploid linkage maps were produced by Rajapakse et al. (2001) using the $F_2$ population from a cross between "Basye's Blueberry" (82-1134), a moderately susceptible tetraploid plant, and 86-7, a black spot resistant amphidiploid, and AFLP and SSR markers. The maternal parent of the cross, 82-1134, is a spreading bush with pink flowers

consisting of ten or more petals. This tetraploid is free of prickles on both stems and petioles. Amphidiploid 86-7 contains genomes of two highly black-spot-resistant species, *R. wichurana* "Basye's Thornless" and *R. rugosa* var. *rubra* (Byrne et al. 1996). The 86-7 bush is a sprawling ground cover with white flowers of five petals. Its stems and petioles have prickles, traits inherited from the *rugosa* parent. From the $F_1$ hybrid 90-69 an $F_2$ mapping population of 52 individuals was obtained by open-pollination, showing a high level of field resistance to black spot, pink flowers, and prickles on stems and petioles. Simplex uni-parental markers were used to construct both parental maps separately. Resistance to black spot, growth habit, absence of prickles on the stem petiole (*Petiole Pr*) and the locus for malate dehydrogenase (*Mdh-2*) were mapped.

The maps were later expanded and integrated by Zhang et al. (2006) with the help of additional SSR markers. The final maps for 82-1134 consist of 256 markers assigned to 20 linkage groups with a total length of 920 cM, and for 86-7, 286 markers were mapped along 14 linkage groups with a total length of 770 cM. Based on shared SSR markers, consensus order maps for four out of seven rose chromosomes were generated. However, map distances could not be calculated yet since the homeologous linkage groups originating from different species may not be of the same size.

Yan et al. (2005, 2006) identified QTLs for powdery mildew resistance in a segregating tetraploid population with 181 genotypes derived from a cross between two tetraploid cultivars, P867 and P540. The map requires more markers in order to be integrated into other tetraploid and diploid maps. Koning-Boucoiran et al. (2009) are currently saturating Yan's genetic linkage map with nucleotide binding sites (NBS) profiling and SSR markers.

Spiller et al. (2011) recently published the first integrated consensus map for diploid rose. It was based on the information of four diploid maps, which were linked via 59 bridge markers. The integrated map comprised 597 markers distributed over 530 cM on seven linkage groups, which is close to the value from Yan et al. (2005), and about 17% larger than the values from the recalculated single maps. The map established a standard nomenclature for the rose genetic map. It also included the location of ten monogenic traits, including recurrent blooming, double flowers, pink flower color, prickles, self-incompatibility, and black spot resistance.

The integrated consensus map will be useful in synteny studies with related Rosaceae genomes. This is particularly interesting, as the genomes of apple, peach, and strawberry have now been sequenced. As for establishing the genome sequence of rose as well: using next generation sequencing technology it is not a large task to generate sufficient DNA sequences to cover several fold the complete rose genome. However, a major technical challenge in assembling the rose genome will be the heterozygosity.

### 12.6.3 Characterization of Disease Resistance Genes

Markers tightly linked to QTLs will allow an early selection of progeny having the desired trait. Because it is well known from breeders that first generation hybrids between highly developed rose varieties and wild roses rarely comprise genotypes suitable for variety development, Debener et al. (2003) showed the advantages of marker-assisted background selection in rose breeding to reduce the genetic background of wild rose species in introgression programs, when they introgressed the gene *Rdr1* conferring resistance to black spot into the genetic background of cultivated tetraploid roses. *Rdr1* originated from a diploid *R. multiflora* hybrid whose chromosomal set was doubled via colchicine treatment. It was subsequently crossed to tetraploid hybrid tea varieties according to a modified backcross strategy. Each new generation was screened for disease resistance, and the molecular marker fraction originating from the original donor was determined in order to select interesting progeny for further breeding. This strategy proved to select interesting genotypes, which would not have been identified by scoring of morphological characters alone or in conventional breeding programs.

Yan et al. (2005) identified QTLs for powdery mildew resistance in a segregating tetraploid population with 181 genotypes derived from a cross between two tetraploid cultivars, P867 and P540, showing partial resistance to powdery mildew. Multiple marker loci were found to be associated with powdery mildew resistance, which suggests that quantitative resistance

to powdery mildew is controlled by multiple genes in rose.

Linde et al. (2006) mapped QTL for resistance to powdery mildew in six different environments over 3 years. They included AFLP, RGA, SSR, SCAR markers, the *Rdr1* locus (Hattendorf et al. 2004; Hattendorf and Debener 2007), and four morphological markers (*prickles t4* and *t4b*, *double flowers d6*, and *white stripes*). The map was made on a diploid population that resulted from a cross of the diploid line 95/13-39 (resistant against powdery mildew isolate 9) and the susceptible diploid male parent Sp3 (82/78-1). Both genotypes are open-pollinated seedlings from a breeding program aimed at the introgression of genes from tetraploid garden roses into *R. multiflora* (Reimann-Philipp 1981). This enabled them to detect resistance QTLs that were stable over different environments and pathogen races as well as race- and environment-specific QTL. Highly significant QTLs (LOD scores of 18 and 12.5) were mapped near a RGA marker on linkage group 4. However, they could not be detected again on year three while a new QTL (LOD of 5.4) was detected on linkage group 7 where no significant gene action could be located in the previous 2 years. The authors speculated that in year three the infection pressure was lower hampering the detection of the QTL on group linkage 4. There could also have been a change of pathogenic strain allowing the detection of a new QTL on linkage group 7.

In parallel to genetic mapping, a bacterial artificial chromosome (BAC) library was constructed to serve as a tool for the physical mapping and positional cloning of rose genes (Kaufmann et al. 2003). By high resolution mapping of the *Rdr1* locus previously identified and mapped (von Malek et al. 2000) and the use of the BAC library, both genetic and physical maps for the *Rdr 1* genomic region have been aligned.

### 12.6.4 Characterization of Genes for Other Traits of Interest

Debener (1999) analyzed the inheritance of important morphological and physiological characters in diploid roses. He concluded that the presence of pink flower color, double flowers, and prickles are inherited as dominant genes whereas recurrent flowering is inherited as a recessive gene. He also mentions earlier studies that showed that recurrent flowering phenotype, dwarf character, and the moss character are inherited monogenically. Debener et al. (2001a, b) confirmed this in tetraploid populations. In addition, resistance to blackspot was also found to be inherited as a monogenic dominant character. They also concluded that when pink flower color was visually scored versus white, a dominant inheritance could be inferred, but when the total anthocyanin content in the pink flowers was measured using a photometric assay, two classes of genotypes were identified confirming a codominant inheritance in the pink flower.

Baudino (2003) identified a molecular marker linked to fragrance intensity in the progeny of a cross between tea hybrids. Scalliet et al. (2002) identified phenolic methyl ether 3,5-dimethoxytoluene as a major compound responsible for tea scent of many of the modern roses varieties, which have lost most of their scent, whereas the phenolic methyl ether pathway is restricted to Chinese roses.

Hibrand Saint Oyant et al. (2008) showed that petal number was controlled by a major gene and QTL as previously proposed by Debener (1999). Recently, Dubois et al. (2010) used a candidate gene approach to demonstrate that the double flower phenotype is associated with a deregulation of expression of the rose ortholog of AGAMOUS (RhAG). Furthermore, this deregulation of RhAG expression appears to have occurred in double flower roses during rose domestication, suggesting that man has tinkered with the regulation of a unique regulatory gene to obtain double flowers (Dubois et al. 2010). A strong QTL controlling blooming date was also located, but on a different linkage group from the two (*Df1* and *Df2*) detected by Dugo et al. (2005). Thus, the process of blooming may be different from one population to another.

## 12.7 Genomics Resources Developed

Traditional genetic mapping has been successful so far to identify QTLs and even loci responsible for a given phenotype. However, it is not always easy to find adequate segregating $F_1$ populations for given traits. Furthermore, the establishment of a genetic experiment is a time- and money-consuming process, as very often relatively large $F_1$ and backcross

populations are required, and phenotyping can be tedious (the plants need to be adult, and the phenotype studied for three consecutive years). Global "omics" approaches combined with traditional genetics could prove to be a powerful approach.

From an economic point, a small number of rose traits are very important. These include plant architecture, flower development, function and senescence, scent biosynthesis, reproduction and resistance to biotic and abiotic stresses. There is no or very little information available on the molecular mechanisms that control these traits. This dearth of information is a handicap, limiting the scope of rational selection for improvement of ornamental plants. Furthermore, several aspects (i.e., scent production, recurrent blooming, color) cannot be addressed using model species such as *A. thaliana*, or at least only in a limited manner. Rose represents an ideal ornamental model species to address some of these aspects.

Here we focus on the recent advances in molecular tools and their potential use to improve our understanding of the molecular bases of rose traits as well as to help in addressing and identifying molecular markers associated with different traits.

## 12.7.1 "Omics" Resources

### 12.7.1.1 Expressed Sequence Tags Libraries

Before 2002, only about 180 rose gene sequences were available in the public databases. During the past few years, several research groups have initiated projects for expressed sequence tag (EST) sequencing in order to obtain a good representation of genes expressed in roses. Channelière et al. (2002) and Guterman et al. (2002) provided the first global overview of genes in the rose genome through EST sequencing. These EST sequences represent mainly genes expressed in petals because for centuries, the economic importance of the rose relied on flower architecture and on natural fragrances, mainly determined by the petals. These studies provided the first annotated rose EST database comprising 2,977 unique sequences. Recently, Foucher et al. (2008) sequenced 5,000 new ESTs corresponding to about 2,336 genes expressed in vegetative and floral meristems of *Rosa* sp. Presently, there are about 9,289 ESTs, representing about 4,834 uni-sequences, available in the different databases (URGI: http://urgi.versailles.inra.fr/; GDR: http://www.bioinfo.wsu.edu/gdr/).

Rose ESTs have enabled the identification of many genes with potential roles in flower development and senescence, recurrent flowering, scent and pigment biosynthesis (Channelière et al. 2002; Guterman et al. 2002; Scalliet et al. 2002; Foucher et al. 2008). Furthermore, these studies provide a starting point for understanding some of the molecular, genetic, and biochemical processes associated with traits that greatly influence rose quality, especially flower quality. Furthermore, the above studies demonstrated the advantages of global EST sequencing approaches to identify genes of interest in non-model plants, such as the rose.

The available EST sequences (about 5,000 genes) represent only about a fifth of the expected number of expressed genes in rose. We are far away from having information on all *Rosa*-expressed genes. Recently, a French consortium performed a large-scale targeted rose EST sequencing program using novel technologies such as 454 pyrosequencing technology. cDNAs from a range of rose organs (root, leaves, flower, etc.) at different developmental and physiological stages (biotic and abiotic stresses) were used in this sequencing program (M. Bendahmane and collaborators unpublished data). The newly obtained EST database is expected to provide sequence information on the majority of genes expressed in rose. It also represents a resource to identify genes whose expression correlate with certain physiological characters as well as developmental and morphological characters. Furthermore, such resource can also serve as a base for the rose genome sequencing.

### 12.7.1.2 Proteomics

Proteomics has been also used to increase knowledge on rose petal development (Dafny-Yelin et al. 2005). In this study, authors generated stage-specific petal protein maps. They studied nearly 1,000 protein spots in closed buds, mature flower, and flower at anthesis and found that 30% of these proteins were development stage specific. Interestingly, they obtained 15 proteins with unknown function and seven novel proteins.

## 12.7.2 Identification and Functional Characterization of Genes Associated with Important Traits in Rose

### 12.7.2.1 Usefulness of Rosa Resources

ESTs were used as source to identify novel genes whose expression is associated with several rose traits. They have enabled the identification of a few rose scent-associated genes such as *O*-methyltransferases and rose alcohol acetyltransferase encoding genes (David et al. 2002; Scalliet et al. 2002, 2006, 2008; Shalit et al. 2003; Guterman et al. 2006). Recently, Foucher et al. (2008) used an EST approach to identify genes associated with recurrent blooming in rose. EST sequences were also used to generate the first rose DNA microarray comprising 2,100 uni-sequences (Guterman et al. 2002). The use of this microarray led to the discovery of several novel flower scent-related candidate genes (i.e., germacrene D synthase and *O*-methyltransferases encoding genes; Guterman et al. 2002). A new rose microarray chip comprising about 4,800 genes was recently developed and used to identify genes whose expression is associated with several flower characters such as double flower (M. Bendahmane's group unpublished data) and scent (S. Baudino's group unpublished data).

Application of suppression subtractive hybridization (SSH) to cloning differentially expressed cDNA enabled the isolation of eight novel scent-associated genes (RhMYB, OOMT, a geranylgeranylated protein-encoding gene, and five ESTs with no sequence homology; Jirong et al. 2008). Ahmadi et al. (2008) used a cDNA differential display approach to isolate an ethylene-induced cDNA homologous to a laccase whose expression is associated with abscission of petioles and flowers.

### 12.7.2.2 Identification of Rose Genes Using a Candidate Gene Approach

In *Rosa* a candidate gene approach was used to identify homologs of genes known to be involved in pathways, which are crucial for understanding the molecular mechanisms of several traits. These studies have targeted a few major rose traits, such as flower development and longevity. Homologs of genes associated with flower organ initiation and development have been identified (i.e., *MASAKO C1*, *MASAKO D1*, and *Rh-PIP2*; Kitahara and Matsumoto 2000; Ma et al. 2006). Post-harvest quality, including a long vase-life of cut flower roses, has always been a major selection criterion for rose breeders. For that reason, several genes putatively involved in senescence of rose petals have been isolated, for example ACC synthase and ethylene receptors (Wang et al. 2004a; Ma et al. 2006; Pan et al. 2005; Xue et al. 2008), protein kinases (Müller et al. 2002), lipoxygenase (Fukuchi-Mizutani et al. 2000), and delta-9-desaturase (Fukuchi-Mizutani et al. 1995).

## 12.7.3 Perspectives

One of the major tasks to be accomplished within the coming few years will be the rose genome sequencing. A complete rose genome sequence is now conceivable, even realistic and necessary in order to improve genomics research in the field of woody ornamentals. Rose represents an ideal model species for genome sequencing because of (1) its economic importance in the ornamental plant sector accounting for approximately 30% of the market, (2) the small size of its genome (approximately 560 Mbp or four times the size of the *A. thaliana* genome), (3) its amenability to transformation, and (4) its well-documented genetic history.

Sequencing technologies have made enormous progress in the recent years. As each genome sequencing technique has its specific drawbacks, in terms of sequence length and fragment assembly as well as sequence quality, a combination of different sequencing technologies will allow sufficient genome coverage and sequence quality, at a reasonable cost. It should be noted that heterozygosity is an important issue in the perspective of rose genome sequencing. Previously, de novo genome sequencing of a hybrid grapevine cultivar, Pinot Noir (Velasco et al. 2007) and an inbred Pinot Noir cultivar (Jaillon et al. 2007) was undertaken at about the same time. Better and faster results were obtained from sequencing the inbred line rather than the hybrid. This indicates that a wild diploid species, rather than a tetraploid cultivar, with low heterozygosity will be more suitable for

a rose genome sequencing project. Another option would be to create a dihaploid from a diploid hybrid cultivar. Both solutions are labor-intensive but necessary steps to provide good starting material for a successful genome sequencing project in rose.

## 12.8 Scope for Domestication and Commercialization

The chemistry of rose flowers involves highly diversified secondary metabolites that participate in their interactions with the environment and contribute to reproductive success through pollinator attraction. This diversity of molecules has also been harnessed during the domestication process, ultimately resulting in scented and colorful varieties displaying valuable ornamental flowers. For this reason, the chemistry of floral pigments and volatile compounds in roses has been extensively characterized.

### 12.8.1 Polyphenols

Anthocyanins are the main pigments of red *Rosa* species. In this family of molecules sharing the same biosynthesis pathway, flavonols act as copigments in order to protect anthocyanins against hydrophilic attacks. In wild *Rosa* species, these polyphenolic pigments and copigments are produced from four molecular skeletons: cyanidin and paeonidin for the anthocyanins and quercetin and kaempferol for the flavonols. Chromatography techniques (TLC and HPLC) have been used to determine the polyphenolic profiles of wild and cultivated species. The quantitative data produced by HPLC have been analyzed with multivariate methods, such as PCO, resulting in the definition of chemotypes that could be correlated to the taxonomy of *Rosa* species (Mikanagi et al. 1995).

The *Cinnamomeae* species display almost the whole range of existing chemotypes, in good agreement with the notion that diversification of *Rosa* has been especially pronounced in this large section. Three major chemotypes can be distinguished: paeonidin 3,5-diglucoside associated with quercetin and kaempferol 3-sophorosides as in *R. rugosa* and *R. canina*; cyanidin 3,5-diglucoside associated with quercetin and kaempferol 3-glucoside as in *R. chinensis* and in *R. gallica*; and quercetin and kaempferol 4-glucosides as in *Pimpinellifoliae*.

### 12.8.2 Carotenoids

A complete and highly diversified carotenoid metabolism provides the characteristic yellow color in petals of *Pimpinellifolia*e roses such as *R. foetida* (Eugster 1985). This ability to accumulate yellow pigments was introduced in hybrid cultivars around 1900 when a cross between a Hybrid Tea and *R. foetida* cv. "Persian Yellow" produced the cultivar "Soleil d'Or." Carotenoids are not only important for petal pigmentation, they are also related to the synthesis of minor volatile compounds, such as beta-damascenone, which is an oxidative cleavage product of beta-carotene, and – although it is produced in extremely low quantities – provides the typical fragrance found in *R. damascena* flowers and in the essential oil derived from them (Auldridge et al. 2006).

### 12.8.3 Volatiles

Volatile molecules are important cues for pollinators and therefore contribute to reproductive success in entomophilic plant species. In roses, these molecules are produced by the petal epidermis and/or by pollen (Dobson et al. 1999; Bergougnoux et al. 2007). The major scent components of roses include 2-phenyl ethanol, monoterpenes, and phenolic methyl ethers such as 3,5-dimethoxytoluene (DMT) or 1,3,5-trimethoxybenzene (TMB). However, characteristic odors can be due to minor compounds: in *R. damascena*, beta-damascenone accounts for most of the scent but only comprises 0.1% of the weight of volatiles. The peri-Mediterranean progenitors of modern hybrid rose varieties (*R. gallica*, *R. damascena*) mainly emit 2-phenylethanol and monoterpenes, whereas the Chinese progenitors (*R. chinensis*, *R. gigantea*) mainly produce DMT and/or TMB (Flament et al. 1993; Joichi et al. 2005). The ability to synthesize DMT or TMB is restricted to Chinese roses, since these, due to a recent gene duplication in the clade of *R. chinensis*, possess

a unique combination of two orcinol *O*-methyl transferases (OOMT) able to achieve this enzymatic sequential biosynthesis (Scalliet et al. 2008).

### 12.8.4 Rose Oil and Other Components from Damask Roses

Although some Damask roses have been maintained for centuries in West European garden rose collections, commercial cultivation of Damask roses is undertaken mainly for production of rose oil and rose water, obtained by steam distillation of rose petals, and "rose absolute" and "rose concrete" produced by solvent extraction. Since rose oil mixes well with other odors and prolongs the duration of the perfume aroma, it is used as a basic component in a number of products in the perfumery and cosmetics industry. The main rose oil producing countries are Bulgaria, Turkey, and Iran while India, China, and the countries from northern Africa produce mainly rose water and a minor fraction of the rose oil used by the perfumery industry. The world production in 2006 consisted of approximately 3,000 kg rose oil (of which 1,900 kg was produced in Bulgaria), 5,000 kg absolute and dozens of tons of rose water (http://www.biolandes.com/biolandes-rose-2007-lettre-65-gb.pdf). The price of rose oil has gradually increased during the last years, and reached €5,000 per kg in 2007.

Rose oil consists of a small number of major compounds including citronellol, geraniol, nerol, phenethyl alcohol, linalool, farnesol, eugenol and eugenol methyl ether, and more than 275 minor constituents. A major part of the rose oil odor is derived from two minor constituents, beta-damascenone and beta-ionone (Kovats 1987; Ohloff 1994). The composition of the distilled rose oil can vary significantly from year to year depending on the climatic conditions and the geographic regions where cultivation takes place (Nikolov et al. 1977, 1978; Topalov 1978). Therefore, rose oil is produced in only a few areas with favorable climatic conditions. In Bulgaria, the rose plantations are situated in the so-called Rose valley between the Balkan Mountains and the Sredna Gora Mountains where winters are mild and summers only moderately hot. In Turkey and Iran, the main rose growing regions are the provinces of Isparta and Isfahan, respectively.

In addition to the application of rose oil and rose water in the perfumery and cosmetics industry, these products are also included as minor components in a number of foodstuffs like gelatines, sweets, dairy desserts, etc. Dry petals from Damask roses are included in various preparations of traditional herbal medicine and homeopathic products.

Rose oil and other flower extracts have potential as a source of therapeutic compounds and dietary supplements. Rose water obtained from petals has been known for its soothing effect and was also found to be beneficial in ophthalmopathy (Kiritikar and Basu 1987; Biswas et al. 2001).

Aridogan et al. (2002) analyzed the antibacterial activity of *R. damascena* essential oil as a composite mixture against *Staphylococcus aureus*, and the antimicrobial activity of some components of the essential oil such as citronellol, geraniol, and nerol against *S. aureus* and *Escherichia coli*. They found that the tested compounds have more potent antimicrobial activity individually than in the mixture form of the rose essential oil. Basim and Basim (2003) determined the antibacterial effects of rose essential oil against three strains of *Xanthomonas axonopodis* ssp. *vesicatoria* and found that the essential oil may be a potential control agent in the management of the disease caused by *X. a. vesicatoria* in tomato and pepper plants.

Ozkan et al. (2004) determined the antibacterial activities of fresh and spent *R. damascena* flower extracts. The authors determined the antibacterial activity of the extracts against 15 species of bacteria, and found both extracts to be effective against all tested bacteria except *E. coli* O157:H7, the fresh flower extract being more effective than the spent flower extract.

Achuthan et al. (2003) reported that fresh juice of *R. damascena* flowers exhibits promising in vitro antioxidant potential and may protect against carbon tetrachloride (CCl4)-induced hepatotoxicity, possibly through its free radical scavenging activity. Boskabady et al. (2006) observed a potent relaxant effect of ethanolic extract and essential oils of *R. damascena* on guinea pig tracheal chains. Mahmood (1996) even observed that water and methanol extracts of *R. damascena* petals exhibited moderate anti-HIV activity.

### 12.8.5 Useful Dogroses

In contrast to their flashy cousin, *R. damascena*, species of *Rosa* section *Caninae*, the dogroses, have single short-lived flowers, not so much fragrance and are seldomly used for ornamental purposes. They are long-lived woody plants growing in woodland margins and disturbed habitats such as roadsides and open pastures. The plants are upright or climbing with more or less prickly and bristled shoots. During autumn these plants show their real value: an abundance of rosehips containing various bioactive contents. Unfortunately, scientists performing studies on the chemical contents of rosehips are seldom aware of the taxonomic confusion within this section. Expressions like, e.g., "dogrose" or "*canina* rose" or even "*R. canina*" are often used for any dogrose species, or at least any species within subsection *Caninae*. It is also almost impossible to compare reported concentrations between different studies, since the concentration of various compounds in the plant may depend on horticultural procedures and on environmental factors (Kovacs et al. 2004; Ercisli 2007). In addition, the extraction efficiency differs considerably between different laboratory protocols and may depend on the technical equipment used.

#### 12.8.5.1 Rose Hip Components

Dogrose rosehips have high levels of vitamin B and C, carotenoids, polyphenols, and the minerals K and P. Polyphenols, vitamin C (ascorbic acid), and carotenoids are powerful antioxidants. In a study by Halvorsen et al. (2002) in which different fruits and berries were screened for total antioxidants, the dogroses were in the top with 39.46 mmol/100 g FW (fresh weight). The second best, crowberry (*Empetrum hermaphroditum*), had only 9.17 mmol/100 g FW.

Vitamin C level is estimated to be 300–4,000 mg/100 g DW (dry weight) (Ercisli 2007) depending upon species, genotype, and environmental factors. Vitamin C is an essential ingredient to our bodily functions, but has also been reported as a protection against cardiovascular diseases and atherosclerosis. However, vitamin C may not be the only component responsible for prevention and eventually apoptosis of, e.g., cancer cells; a synergistic action of several other compounds or metabolites in berries and fruits has also been suggested (Eichholzer et al. 2001; Vecchia et al. 2001; Olsson et al. 2004; Halliwell 2006).

Vitamin $B_9$ or folate is an essential vitamin required for DNA and RNA formation, and for cellular replication. It is recommended that pregnant women increase their daily dose of folate to prevent neural tube defects in their babies. Rosehips and strawberries are rich sources of this vitamin, with levels of 100–180 μg/100 g FW and 70–90 μg/100 g FW, respectively.

Polyphenols are the most abundant antioxidants in the human diet. The main dietary sources are fruits and berries and plant-derived beverages such as juices, tea, and red wine. The polyphenols commonly occur as complex mixtures in plants, such as the flavonoid anthocyanin (provides red and purple color in fruits) and tannins. The antioxidative effect of rosehips is caused mainly (up to 90%) by polyphenols (Gao et al. 2000). The tannin in rosehips is ellagic acid, also present in strawberries, which is said to have antimutagenic and anticarcinogenic effects (Mertens-Talcott et al. 2005).

Rosehips also contain large amounts of carotenoids of which the carotenes are the most interesting. Here, we find beta-carotene (precursor of vitamin A) and lycopene. Lycopene is usually associated with tomatoes and tomato products, but Hornero-Méndez and Mínguez-Mosquera (2000) found up to 390 mg/kg DW in rosehips from Chile, which is more than in most fresh tomatoes. They suggested that rosehips could be used as an alternative to tomatoes in the food industry.

The seeds of rosehips have often been regarded as a waste product, but they do contain the unsaturated oils alpha-linolenic and linoleic acid (omega-3 and omega-6), which can be used for medicinal applications (Szentmihályi et al. 2002). In South America this oil has been used for centuries as a cure for different skin diseases. Now, it is being exported all over the world as a treatment for eczema and as cosmetic oil. However, there are so far no scientific reports on the action or results of this treatment.

Rosehips are very seldomly eaten raw; they must be processed one way or the other. Many of the bioactive compounds are sensitive to light and heat, and many are also water soluble, and can therefore easily be

destroyed by the processing, or leech out into the surrounding water used for canning or cooking. It is vital that the processing is performed as fast as possible to retain the valuable compounds.

#### 12.8.5.2 Dogrose Hip Uses

The dogrose plant has been used since the Middle Ages as a medicinal plant. All parts of the plant were then used – leaves, flowers, hips, and roots – in the form of concoctions and infusions as a cure for all sorts of ailments. The main use was for various stomach troubles and as a remedy for all sorts of infections. Rosehips from section *Caninae* have a very special aroma and taste, and they have therefore been used also for various foodstuffs. Today they are used as jam, jelly, marmalade, and teas, and they are also ingredients in, e.g., ice cream and yogurt. In Sweden, rosehip soup has been a well-known dessert and snack for hundreds of years. The soup is made from dried rosehips and it is served either warm or cold, often with whipped cream and sweet biscuits. The current interest in dogroses is, however, not directed to their culinary properties. Rosehips are instead being commercialized for their potential in functional food, i.e., food products that contain biologically active components with a potential of enhancing human health or reducing risk of disease, and nutraceuticals with a medicinal effect on human health. A nutraceutical is normally ingested as a capsule or powder in a prescribed dose.

In Turkish medicinal folklore, rosehips have been used as a remedy for all kinds of stomach problems, and several of the present day studies also focus on the gastrointestinal tract. Rats that were fed extracts from *R. canina* showed a 100% protection from ulcerogenesis caused by ingestion of 96% ethanol (Gürbüz et al. 2003). In a recent study, extracts from *R. canina* combined with a *Lactobacillus* bacterial strain decreased the ROS (reactive oxygen species) activity in injured rat colons (Håkansson et al. 2006). The authors suggested that this combination could be useful as a treatment in colonic and vascular surgery and also in organ transplantations. The polyphenols in rosehips from *R. canina* have shown to be effective ROS scavengers in human blood (Daels-Rakotoarison et al. 2002). In yet another study, extracts of *R. canina* (fruit flesh and seeds) showed an inhibitory effect on body weight gain in mice (Ninomiya et al. 2007). The authors have now applied for a patent with this extract as the active ingredient of a method for improving fat metabolism in biological tissues (http://www.freepatentsonline.com/y2008/0003312.html).

The best-studied effect of rosehips is the influence on osteoarthritis. Patients with diagnosed osteoarthritis have been subjected to double-blind, randomized, and placebo-controlled clinical tests as they orally ingested a powder made from ground rosehips containing seeds and dried fruitflesh, called Litozin® (or LitoMove®). The patients improved overall mobility as well as the capacity of knee and hip joints. Several of these patients were also able to reduce their pain-killing medication (Warholm et al. 2003; Winther et al. 2005). The effect was apparently mediated by the galactolipid "GOPO" ((2S)-1,2-di-*O*-[9Z,12Z,15Z]-octadeca-9,12,15-trienoyl-3-*O*-beta-D-galactopyranosyl glycerol). A more recent study has shown that the observed effect on osteoarthritis by rosehips might also be due to fatty acids in the seeds: alpha-linolenic and linoleic acids (Jäger et al. 2008). There are also indications that rosehip extracts together with minor amounts of blueberry, blackberry, and grapevine extracts may diminish the risk of cardiovascular diseases by lowering the levels of inflammatory substances associated with early heart infarctions (Kornman et al. 2007).

## 12.9 Some Dark Sides and Their Addressing

Roses have a long history as horticultural plants and have as such been introduced into several countries outside their native range. Unfortunately, some of these *Rosa* species escaped gardens or parks and invaded natural habitats and threatened the native flora. Four *Rosa* spp. are so far known to be invasive globally (*R. canina*, *R. multiflora*, *R. rugosa,* and *R. rubiginosa*). Management is cost-intensive, since once established as alien plants *Rosa* spp. are difficult to eradicate due to their ability to resprout after cutting, browsing, or even fire.

### 12.9.1 Invasive Species of the Genus Rosa

While the *Rosa* genus has its origins north of the equator, these four species have already invaded habitats in the southern Hemisphere (Weber 2003). With their ability to grow root suckers and well armed spiny shoots and branches they form impenetrable thickets in their invasive range (Epstein and Hill 1999; Amrine 2002; Yates et al. 2004; Bruun 2005).

*R. multiflora* and *R. rugosa* are both native to temperate Asia, yet nowadays they are invasive in Europe and they have both been introduced to several countries in the southern Hemisphere as well as in North America. *R. canina* is native to Europe, temperate Asia, and northern Africa. *R. rubiginosa* is native to Europe and temperate Asia as well and both species have likewise been introduced to several countries in the southern Hemisphere as well as in North America (Fig. 12.1). Another wild rose from Asia, *R. bracteata*, is noted as being invasive in North America, but this is yet to be recognized on a global scale (ISSG 2008).

### 12.9.2 Management Strategies: The Example R. multiflora

Formerly planted as a windbreaker or as a natural fence, *R. multiflora* became the most detrimental weed in the 1960s in the eastern United States (Amrine 2002). From then on it spread rapidly throughout the USA and is expected to become even more abundant in the future (Robertson et al. 1994; Hunter and Mattice 2002; Merriam 2003). *R. multiflora* stands cause severe degradation of land used for grazing or recreation through the formation of dense multi-crowned thickets (Epstein and Hill 1999). It is also locally common in four provinces of Canada, where it was cultivated as a garden plant and nowadays grows in old fields, forest clearings, and along shores and roadsides (Darbyshire 2003). Furthermore, *R. multiflora* has been reported for northern Europe, New Zealand, southern Africa (Bean 1951; Weber 2003), and central Argentina (H. Zimmermann unpublished data).

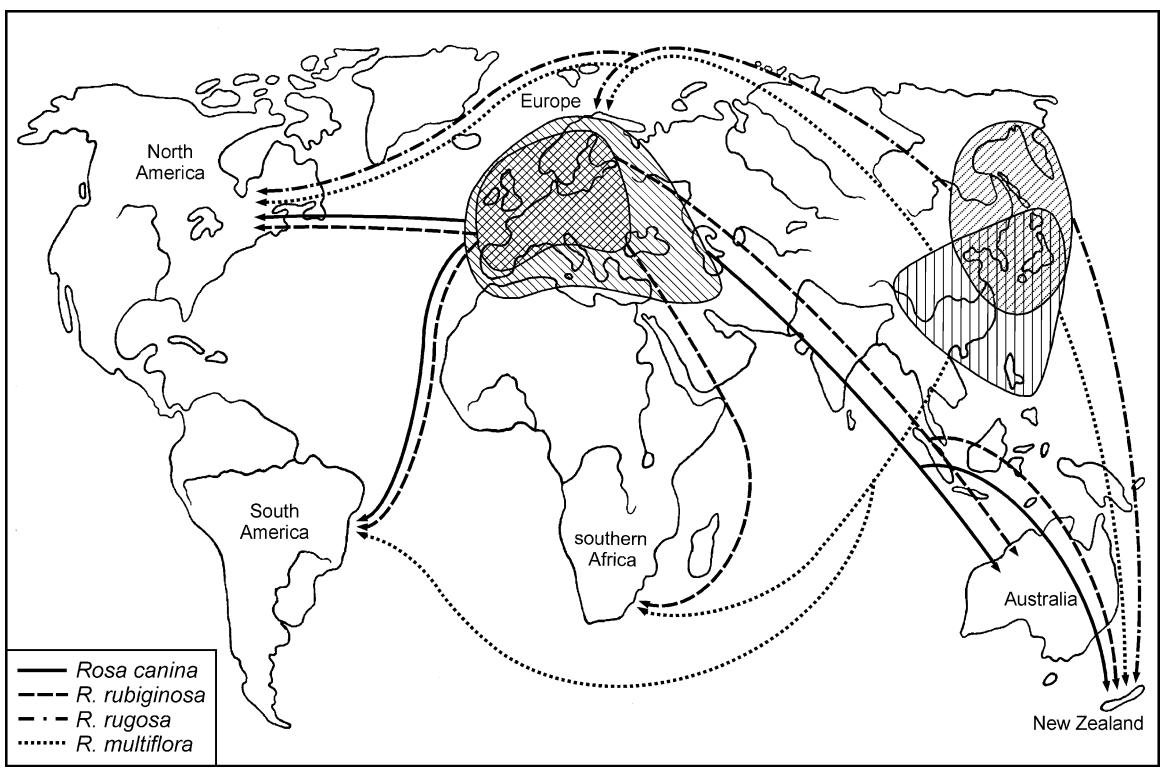


**Fig. 12.1** Four rose species are currently invasive worldwide, *Rosa canina*, *R. rubiginosa*, *R. multiflora,* and *R. rugosa*. The native ranges are indicated by *shaded areas*, and represent only a rough outline, rather than an exact distribution range

In North America, numerous studies have investigated possible methods to eradicate *R. multiflora*. Loux et al. (2005) provide an overview of several control methods. As an alternative to biocontrol agents, herbicides, and mechanical control, they also propose a well-balanced grazing management by goats. They do, however, emphasize that the control of *R. multiflora* is a long-term task and that any success achieved in 1 year will be largely negated by reinfestation within the following 2 or 3 years. Amrine (2002) estimated that a herbicide-driven eradication in West Virginia alone would cost about $80 million. Since herbicide applications are cost- and labor-intensive, and herbicides pollute the environment, research in the USA has mainly focused on biocontrol agents for *R. multiflora*. The rose rosette disease (RRD) and the seed chalcid, *Megastigmus aculeatus*, seem to be the most promising candidates. So far the causal agent of RRD is not known but it can be transmitted with a 100% success by grafting (Amrine et al. 1988). Epstein and Hill (1999) propose the RRD as a biocontrol agent since it is native to North America, is lethal to *R. multiflora*, seems to be restricted to the genus *Rosa*, and implementation of this management method is feasible by landowners. No symptoms on ornamental roses in the surroundings were found in the 5 years following application (Epstein and Hill 1999), however, since a comprehensive screening of all available rose cultivars has not been conducted the risk of RRD is of concern to the rose industry (Jesse et al. 2006). Moreover, Hartzler (2003) objects that RRD alone will not provide a permanent solution, since plants in shaded habitats are less susceptible to infection and thus may act as new source populations. The eradication of *R. multiflora* plants alone cannot be deemed successful, since persistent *R. multiflora* seeds might still be in the soil ready to replace the recently removed adults. The ample production of rose hips as food for wildlife was one reason for planting this species in the USA (Epstein and Hill 1999). The second promising biocontrol agent, the seed chalcid (*M. aculeatus*), could resolve this problem. *M. aculeatus,* to which *R. multiflora* seems to be especially susceptible (Amrine 2002), lays its eggs in the receptacle and the developing larvae consume and kill the rose seeds.

### 12.9.3 R. rugosa: *A Threat to Nature Conservation*

*R. rugosa* has invaded habitats in northwestern Europe, northeastern North America, and New Zealand (Darbyshire 2003; Weber 2003; Bruun 2006). Its expansion is well demonstrated on the British Isles (Bruun 2005), where it was introduced in 1845 (Bean 1951). The species' preference for sandy coastline habitats (Ohba et al. 1973) made it a preferred candidate in the nineteenth-century shoreline protection schemes (Schlätzer 1974; Dubra and Olšauskas 2002). Shortly thereafter its invasive potential rendered it uncontrollable along the coast of the Baltic-, North-, and Atlantic Seas (Bruun 2006). In particular, the long distance dispersal of fruits and seeds by sea currents may have contributed to its success (Bruun 2005; Isermann 2008b). Moreover, it can be found inland in forest fringes and along railroad tracks (Brandes et al. 2003).

The presence of *R. rugosa* on coastal dunes represents a severe conservation problem as species richness, and more so the number of rare species, declines; thereby reducing species diversity of the dunes both on the local and regional scales (Isermann 2008b). Comparable dune shrub-communities, e.g., colonized by *Hippophae rhamnoides* or *Empetrum nigrum*, contain higher numbers of species (Isermann 2008a). Thus, this Asian rose is possibly a stronger competitor compared to *H. rhamnoides* and *E. nigrum*, since it shades the surrounding vegetation more effectively and has a stronger developed root system (Isermann 2008a, b). Furthermore, *R. rugosa*-dominated stands contain a higher number of neophytes (Isermann 2008a). Not only is *R. rugosa* a threat to species richness through competition, but it also hybridizes with native roses, thus triggering their genetic assimilation (Mercure and Bruneau 2008). Vanderhoeven et al. (2005) discovered that *R. rugosa* also alters soil properties, namely by increasing concentrations of exchangeable essential nutrients beneath its canopy.

Native Japanese *R. rugosa* communities are more species rich and are probably analogous to European *H. rhamnoides* communities, possibly due to the broader environmental niche in its new range (Isermann 2008a). While adult plants effectively

shade the surrounding vegetation, *R. rugosa* seedlings seem to prefer small-scale disturbed sites (Kollmann et al. 2007), and are probably dependent on mycorrhiza (Gemma and Koske 1997).

*R. rugosa* is difficult to control either mechanically or with herbicides (Bruun 2006); however, releasing potential biocontrol agents may constitute a threat to cultivated roses. Burning does not seem to be a permanent management option either, as *R. rugosa* plants constantly resprout after fire (Tsuda et al. 1999).

### 12.9.4 *Unsuccessful Invasion or Missing data?* R. canina

Despite the fact that *R. canina* has a wider distribution in Eurasia and is more abundant than, e.g., *R. rubiginosa* (Meusel and Jäger 1965; Timmermann and Müller 1994), it is not as equally successful an invader. Notwithstanding, it has been introduced to North America, Australia, New Zealand, and South America (Parsons 2001; Amrine 2002; Weber 2003; Damascos and Bran 2006); however, we did not find evidence of any capacity to build up such dominant stands as the other three species. Studies on invasive *R. canina* are few and a comparison with the other invasive European rose species would be desirable.

### 12.9.5 *Causes for the Invasion Success:* R. rubiginosa

*R. rubiginosa* is currently the most successful invader of these four rose species, being invasive in North America, Australia, New Zealand, southern Africa, Chile, and Argentina (Kissel et al. 1987; Hatton 1989; Damascos and Gallopin 1992; Amrine 2002; Weber 2003). Invasive species most certainly pose a threat to the local biodiversity, yet they also provide an opportunity for the study of evolutionary and biogeographic processes on a global scale. A comparison between native European *R. rubiginosa* populations in Germany and Spain with invasive Argentinean populations is currently underway which will enhance the understanding of invasion processes (H. Zimmermann et al. unpublished data). It is uncertain when *R. rubiginosa* was introduced to Argentina, however, it is assumed to have been at least 100 years ago (Damascos et al. 2004). Spanish immigrants are often held responsible for bringing this species to the New World, however, preliminary genetic analysis points to a Central European origin (Zimmermann et al. 2010).

*R. rubiginosa* shrubs in Argentina outgrow their European ancestors in both number and size, and populations cover much larger areas than in Europe (Zimmermann et al. 2008, 2010). The invasion success of *R. rubiginosa* could be due to special characteristics of this species (invasiveness) or attributes of the new environment (invasibility). Much like the other invasive roses, *R. rubiginosa* spreads clonally, resprouts after cutting or fire, is able to produce apomictic seeds, and its spiny leaves and branches form an efficient defense against herbivores. In contrast to Germany and Spain, propagule pressure is high in Argentina, simply because bigger shrubs produce more rose hips. Moreover, seeds are not only dispersed by birds and small mammals, but also by horses and cattle (Damascos et al. 2005). Reciprocal transplantation experiments in Europe and Argentina will clarify whether Argentinean roses are extremely successful ecotypes due to rapid genetic changes (Blossey and Nötzbold 1995).

It seems unlikely that the release from natural herbivores or parasites could be an attribute of the new environment as *R. rubiginosa* is equally well defended against herbivory in the invasive and native ranges. In addition, there seems to be no differences in the spectra of further parasites and fungi (Havrylenko 1995). Preliminary analysis of abiotic soil parameters and climatic variance did not point to more favorable conditions in Argentina (H. Zimmermann unpublished data). Since European *R. rubiginosa* populations are always intermixed with other *Rosa* species, the absence of competing roses in the same habitat could be an advantage for *R. rubiginosa* in Argentina.

Our geographic approach highlights that land-use history is an important factor determining population sizes on a continental scale (Zimmermann et al. 2008). Within the old cultural landscape of Europe, suitable *R. rubiginosa* habitat is limited to a few protected areas, where it is cut in order to promote plant biodiversity. This implies that Argentinean populations might simply be relatively large, because European populations are managed. All invaded habitats in Argentina had experienced anthropogenic disturbance

as well, however, disturbance was a singular event which enabled the establishment of the species.

In southern Argentina, *R. rubiginosa* is already falsely accepted as a typical Patagonian plant. Its image can be found on postcards, and fruits of naturalized *R. rubiginosa* are harvested to fabricate teas, marmalade, and rose oil (Damascos et al. 2004). Therefore, it is especially important to raise awareness that this species is invasive and to offer alternative indigenous replacements.

**Acknowledgments** Research of the authors is funded by, amongst others, grants from DAAD, DFG, TTI Green Genetics, and the National Science Fund of the Ministry of Education and Science, Bulgaria.

## References

Achuthan C, Babu B, Padikkala J (2003) Antioxidant and hepatoprotective effects of *Rosa damascena*. Pharm Biol 41: 357–361

Agaoglu Y, Ergul A, Baydar N (2000) Molecular analyses of genetic diversity of oil rose (*Rosa damascena* Mill.) grown in Isparta (Turkey) region. Biotechnol Biotechnol Equip 14:16–18

Ahmadi N, Mibus H, Serek M (2008) Isolation of an ethylene-induced putative nucleotide laccase in miniature roses (*Rosa hybrida* L.). J Plant Growth Regul 27:320–330

Allum JF, Bringloe DH, Roberts AV (2007) Chromosome doubling in a *Rosa rugosa* Thunb. hybrid by exposure of in vitro nodes to oryzalin: the effects of node length, oryzalin concentration and exposure time. Plant Cell Rep 26: 1977–1984

Amrine JW (2002) Multiflora rose. In: Driesche RV, Lyon S, Blossey B, Hoodle M, Reardson R (eds) Biological control of invasive plants in the Eastern United States. USDA Forest Service Publication FHTET-2002-04, Washington DC

Amrine JW, Hindale DF, Stasny TA, Williams RL, Coffman CC (1988) Transmission of the rose rosette disease agent to *Rosa multiflora* by *Phyllocoptes fructiphilus* (Acari: Eriophyidae). Entomol News 99(5):239–252

Aridogan BC, Baydar H, Kaya S, Demirci M, Ozbasar D, Mumcu E (2002) Antimicrobial activity and chemical composition of some essential oils. Arch Pharm Res 25: 860–864

Auldridge ME, McCarty DR, Klee HJ (2006) Plant carotenoid cleavage oxygenases and their apocarotenoid products. Curr Opin Plant Biol 9:315–321

Babaei A, Tabaei-Aghdaei SR, Khosh-Khui M, Omidbaigi R, Naghavi MR, Esselink GD, Smulders MJM (2007) Microsatellite analysis of Damask rose (*Rosa damascena* Mill.) accessions from various regions in Iran reveals multiple genotypes. BMC Plant Biol 7:12

Basim E, Basim H (2003) Antibacterial activity of *Rosa damascena* essential oil. Fitoterapia 74:394–396

Basye R (1990) An amphidipioid of *Rosa banksiae* and *R. laevigata* induced by colchicine. Am Rose Ann 75:82–88

Baudino S (2003) Parfum de rose. Jardins de France Mai 2003:24–26

Baydar N, Baydar H, Debener T (2004) Analysis of genetic relationships among *Rosa damascena* plants grown in Turkey by using AFLP and microsatellite markers. J Biotechnol 111:263–267

Beales P, Duncan W, Fagan G, Grant W, Grapes K, Harkness P, Hughes K, Mattock J, Ruston D, Sutherland P, Williams T (1998) Botanica's roses: the encyclopedia of roses. Random House Australia, Milsons Point, NSW, Australia

Bean WJ (1951) Trees and shrubs – Hardy in the British Isles. John Murray, London, UK

Bergougnoux V, Caissard JC, Jullien F, Magnard JL, Scalliet G, Cock JM, Hugueney P, Baudino S (2007) Both the adaxial and abaxial epidermal layers of the rose petal emit volatile scent compounds. Planta 226:853–866

Biswas N, Gupta S, Das G, Kumar N, Mongre P, Haldar D, Beri S (2001) Evaluation of Ophthacare eye drops – a herbal formulation in the management of various ophthalmic disorders. Phytother Res 15:618–620

Blechert O, Debener T (2005) Morphological characterisation of the interaction between Diplocarpon rosae and various rose species. Plant Pathol 54:82–90

Blossey B, Nötzbold R (1995) Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. J Ecol 83:887–889

Boskabady M, Kiani S, Rakhshandah H (2006) Relaxant effects of *Rosa damascena* on guinea pig tracheal chains and its possible mechanism(s). J Ethnopharmacol 106:377–382

Brandes D, Weishaupt A, Grote S, Becher R, Griese D, Hartwig U, Schlender H, Wenzel K, Nitzsche J (2003) Die aktuelle Situation der Neophyten in Braunschweig – The actual situation of Neophytes in the City of Braunschweig (Germany). Braunschweiger Naturkundliche Schriften 6 (4):705–760 (in German)

Bruneau A, Starr JR, Joly S (2007) Phylogenetic relationships in the genus *Rosa*: new evidence from chloroplast DNA sequences and an appraisal of current knowledge. Syst Bot 32:366–378

Bruun HH (2005) *Rosa rugosa* Thunb. ex Murray. J Ecol 93: 441–470

Bruun HH (2006) Prospects for biocontrol of invasive *Rosa rugosa*. Biocontrol 51:141–181

Byrne DH, Crane YM (2003) Amphidiploidy. In: Roberts AV, Debener T, Gudin S (eds) Encyclopedia of rose science. Elsevier Academic Press, Oxford, UK, pp 11–15

Byrne DH, Black W, Ma Y, Pemberton HB (1996) The use of amphidiploidy in the development of blackspot resistant rose germplasm. Acta Hortic 424:269–272

Channelière S, Rivière S, Scalliet G, Szecsi J, Jullien F, Dolle C, Vergne P, Dumas C, Bendahmane M, Hugueney P, Cock JM (2002) Analysis of gene expression in rose petals using expressed sequence tags. FEBS Lett 515:35–38

Condliffe PC, Davey MR, Power JB, Koehorst-van Putten H, Visser PB (2003) An optimized protocol for rose transformation applicable to different cultivars. Acta Hortic 612:115–120

Crespel L, Gudin S (2003) Evidence for the production of unreduced gametes by tetraploid *Rosa hybrida* L. Euphytica 133:65–69

Crespel L, Meynet J (2003) Manipulation of ploidy level. In: Roberts AV, Debener T, Gudin S (eds) Encyclopedia of rose science. Elsevier Academic Press, Oxford, UK, pp 5–11

Crespel L, Chirollet M, Durel CE, Zhang D, Meynet J, Gudin S (2002a) Mapping of qualitative and quantitative phenotypic traits in *Rosa* using AFLP markers. Theor Appl Genet 105:1207–1214

Crespel L, Gudin S, Meynet J, Zhang D (2002b) AFLP-based estimation of 2n gametophytic heterozygosity in two parthenogenetically derived dihaploids of *Rosa hybrida* L. Theor Appl Genet 104:451–456

Crespel L, Ricci SC, Gudin S (2006) The production of 2n pollen in rose. Euphytica 151:155–164

Daels-Rakotoarison DA, Gressier B, Trotin F, Brunet C, Luyckx M, Dine T, Bailleul F, Cazin M, Cazin JC (2002) Effects of *Rosa canina* fruit extract on neutrophil respiratory burst. Phytother Res 16:157–161

Damascos MA, Bran D (2006) *Rosa canina* (Rosaceae) nueva cita para la flora de Argentina. Hickenia 3(63):285–288

Damascos M, Gallopin GG (1992) Ecologiá de un arbusto introducido (*Rosa rubiginosa* L. = *Rosa eglanteria* L.): riesgo de invasión y efectos en las comunidades vegetales de la región andino-patagónica de Argentina. Rev Chil Hist Nat 65:395–407

Damascos M, Bran D, Lopez C, Ayesa J, Umana F (2004) Invasiveness of *Rosa rubiginosa* in wild areas of Patagonia, Argentine. Southern temperate ecosystems & Biota: contributions towards a global synthesis. University of Cape Town, South Africa

Damascos MA, Ladio AH, Rovere AE, Ghermandi L (2005) Semillas de rosa mosqueta: dispersión y germinación en diferentes bosques nativos andino-patagónicos. Patagon For 11(4):2–6

Darbyshire SJ (2003) Inventory of Canadian agricultural weeds. Agriculture and Agri-Food Canada, Research Branch, Ottawa, Ontario, Canada

David N, Wang J, Shalit M, Guterman I, Bar E, Beuerle T, Menda N, Shafir S, Zamir D, Adam Z, Vainstein A, Weiss D, Pichersky E, Lewinsohn E (2002) O-methyltransferases involved in the biosynthesis of volatile phenolic derivatives in rose petals. Plant Physiol 129:1899–1907

De Cock K (2008) Genetic diversity of wild roses (*Rosa* spp.) in Europe, with an in-depth morphological study of Flemish populations. PhD Thesis, Ghent University, Belgium

De Cock K, Vander Mijnsbrugge K, Quataert P, Breyne P, Van Huylenbroeck J, Van Slycken J et al (2007) A morphological study of autochthonous roses (*Rosa*, Rosaceae) in Flanders. Acta Hortic 751:305312

De Cock K, Van Der Mijnsbrugge K, Breyne P, Van Bockstaele E, Van Slycken J (2008) Morphological and AFLP-based differentiation within the taxonomical complex section Caninae (subgenus *Rosa*). Ann Bot 102:685–697

de Jong J, Visser PB (2000) Vermindering van energieverbruik bij kasroos door toepassing van transgene onderstammen. Nov Rep 335123/6203, pp 1–14 (in Dutch)

De Vries DP (2003) Breeding – selection strategies for pot roses. In: Roberts AV, Debener T, Gudin S (eds) Encyclopedia of rose science. Elsevier, Academic, Oxford, UK, pp 41–48

De Vries DP, Dubois L (1996) Rose breeding: past, present, prospects. Acta Hortic 424:241–248

Debener T (1999) Genetic analysis of horticulturally important morphological and physiological characters in diploid roses. Gartenbauwissenschaft 64:14–20

Debener T, Mattiesch L (1999) Construction of a genetic linkage map for roses using RAPD and AFLP markers. Theor Appl Genet 99:891–899

Debener T, Mattiesch L, Vosman B (2001a) A molecular marker map for roses. Acta Hortic 547:283–287

Debener T, von Malek B, Mattiesch L, Kaufmann H (2001b) Genetic and molecular analysis of important characters in roses. Acta Hortic 547:45–49

Debener T, Malek BV, Schreiber M, Drewes-Alvarez R (2003) Marker assisted background selection for the introgression of black spot resistance into cultivated roses. Eur J Hortic Sci 68:245–252

Derks FHM, van Dijk AJ, Hänisch ten Cate CH, Florack DEA, Dubois LAM, de Vries DP (1995) Prolongation of vase life of cut roses via introduction of genes coding for antibacterial activity. Somatic embryogenesis and Agrobacterium-mediated transformation. Acta Hortic 405:205–209

Dewitte A, Leus L, Van Huylenbroeck J, Van Bockstaele E, Höfte M (2007) Characterization of reactions to powdery mildew (Podosphaera pannosa) in resistant and susceptible rose genotypes. J Phytopathol 155:264–272

Dobson HEM, Danielson EM, Van Wesep I (1999) Pollen odor chemicals as modulator of bumble bee foraging on *Rosa rugosa* Thunb. (Rosaceae). Plant Species Biol 14:153–166

Dohm A, Ludwig C, Schilling D, Debener T (2001) Transformation of roses with genes for antifungal proteins. Acta Hortic 547:27–33

Dohm A, Ludwig C, Schilling D, Debener T (2002) Transformation of roses with genes for antifungal proteins to reduce their susceptibility to fungal diseases. Acta Hortic 572:105–111

Drewes-Alvarez R (2003) Disease – black spot. In: Roberts AV, Debener T, Gudin S (eds) Encyclopedia of rose science. Elsevier, Academic, Oxford, UK, pp 148–153

Dubois A, Raymond O, Maene M, Baudino S, Langlade NB, Boltz V, Vergne P, Bendahmane M (2010) Tinkering with the C-function: a molecular frame for the selection of double flowers in cultivated roses. PLoS ONE 5:e9288

Dubra J, Olšauskas A (2002) The main problems of the protection of the sand coasts in Lithuania. In: Proceedings of international conference Littoral 2002, The changing coast, vol 3. EUROCOAST/EUCC, Porto, Portugal, pp 45–48

Dugo M, Satovic Z, Millan T, Cubero JI, Rubiales D, Cabrera A, Torres AM (2005) Genetic mapping of QTLs controlling horticultural traits in diploid roses. Theor Appl Genet 111:511–552

Eichholzer M, Lüthy J, Gutzwiller F, Stähelin HB (2001) The role of folate, antioxidant vitamins and other constituents in fruits and vegetables in the prevention of cardiovascular disease: the epidemiological evidence. Int J Vitam Nutr Res 71:5–17

El Mokadem H (2001) Haploïdisation de rosiers, comportement des dihaploïdes de *Rosa hybrida* L. et utilisation à des fins de sélection et d'analyses génétiques. Thèse de l'Univ de Toulouse, France, 206 p

El Mokadem H, Meynet J, Jacob Y, Gudin S (2000) Utilization of parthenogenetic diploid plants of *Rosa hybrida* L. in interspecific hybridizations. Acta Hortic 508:185–190

El Mokadem H, Crespel L, Meynet J, Gudin S (2002a) The occurrence of 2n-pollen and the origin of sexual polyploids in dihaploid roses (*Rosa hybrida* L.). Euphytica 125:169–177

El Mokadem H, Meynet J, Crespel L (2002b) The occurrence of 2n eggs in the dihaploids derived from *Rosa hybrida* L. Euphytica 124:327–332

Epstein AH, Hill JH (1999) Status of rose rosette disease as a biological control for multiflora rose. Plant Dis 83:92–101

Ercisli S (2007) Chemical composition of fruits in some rose (*Rose* spp.) species. Food Chem 104:1379–1384

Eugster HC (1985) Carotenoid structure, old and new problems. Pure Appl Chem 57:639–647

Firoozabady E, Moy Y, Courtney-Gutterson N, Robinson K (1994) Regeneration of transgenic rose (*Rosa hybrida*) plants from embryogenic tissue. Nat Biotechnol 12:609–613

Flament I, Debonneville C, Furrer A (1993) Volatile constituents of roses: characterization of cultivars based on the headspace analysis of living flower emissions. In: Teranishi R, Buttery RG, Sugisawa H (eds) Bioactive volatile compounds from plants. American Chemical Society, Washington, DC, USA, pp 269–281

Foucher F, Chevalier M, Corre C, Soufflet-Freslon V, Legeai F, Hibrand-Saint Oyant L (2008) New resources for studying the rose flowering process. Genome 51:827–837

Fukuchi-Mizutani M, Savin K, Cornish E, Tanaka Y, Ashikari T, Kusumi T, Murata N (1995) Senescence-induced expression of a homologue of delta 9 desaturase in rose petals. Plant Mol Biol 29:627–635

Fukuchi-Mizutani M, Ishiguro K, Nakayama T, Utsunomiya Y, Tanaka Y, Kusumi T, Ueda T (2000) Molecular and functional characterization of a rose lipoxygenase cDNA related to flower senescence. Plant Sci 160:129–137

Gao X, Björk L, Trajkovski V, Uggla M (2000) Evaluation of antioxidant activities of ethanol extracts in different test systems. J Sci Food Agric 80:2021–2027

Gemma JN, Koske RE (1997) Arbuscular mycorrhizae in sand dune plants of the north Atlantic Coast of the US: field and greenhouse inoculation and presence of mycorrhizae in planting stock. J Environ Manag 50:251–264

Grossi C, Jay M (2002) Chromosome studies of rose cultivars: application into selection process. Acta Bot Gallica 149: 405–413

Gudin S (2000) Rose: genetics and breeding. Plant Breed Rev 17:159–189

Gudin S (2001) Rose breeding technologies. Acta Hortic 547:23–26

Gudin S (2003) Breeding. In: Roberts AV, Debener T, Gudin S (eds) Encyclopedia of rose science. Elsevier, Academic, Oxford, UK, pp 25–30

Gunes E (2005) Turkey rose oil production and marketing: a review on problem and opportunities. J Appl Sci 5: 1871–1875

Gürbüz I, Üstün O, Yesilada E, Sezik E, Kutsal O (2003) Anti-ulcerogenic activity of some plants used as folk remedy in Turkey. J Ethnopharmacol 88:93–97

Guterman I, Shalit M, Menda N, Piestun D, Dafny-Yelin M, Shalev G, Bar E, Davydov O, Ovadis M, Emanuel M, Wang J, Adam Z, Pichersky E, Lewinsohn E, Zamir D, Vainstein A, Weiss D (2002) Rose scent: genomics approach to discovering novel floral fragrance-related genes. Plant Cell 14:2325–2338

Guterman I, Masci T, Chen XL, Negre F, Pichersky E, Dudareva N, Weiss D, Vainstein A (2006) Generation of phenylpropanoid pathway-derived volatiles in transgenic plants: rose alcohol acetyltransferase produces phenylethyl acetate and benzyl acetate in petunia flowers. Plant Mol Biol 60:555–563

Håkansson Å, Stene C, Mihaescu A, Molin G, Ahrné S, Thorlacius H, Jeppsson B (2006) Rose hip and *Lactobacillus plantarum* DSM 9843 reduce ischemia/reperfusion injury in the mouse colon. Dig Dis Sci 51:2094–2101

Halvorsen B-L, Holte K, Myhrstad MCW, Barikmo I, Hvattum E, Remberg S-F, Wold A-B, Haffner K, Baugerød H, Frost Andersen L, Jø M, Jacobs D-R Jr, Blomhoff R (2002) A systematic screening of total antioxidants in dietary plants. Am J Nutr Sci 132:461–471

Hartzler B (2003) Multiflora rose and rose rosette disease. http://www.weeds.iastate.edu/mgmt/2003/multiflorarose.shtml. Accessed 20 Oct 2008

Halliwell B (2006) Polyphenols: antioxidant treats for healthy living or covert toxins? J Sci Food Agricult 86:1992–1995

Hattendorf A, Debener T (2007) Molecular characterization of NBS-LRR-RGAs in the rose genome. Physiol Plant 129:775–786

Hattendorf A, Linde M, Mattiesch L, Debener T, Kaufmann H (2004) Genetic analysis of rose resistance and their localisation in the rose genome. Acta Hortic 651:123–130

Hatton TJ (1989) Spatial patterning of sweet briar (*Rosa rubiginosa*) by two vertebrate species. Aust J Ecol 14:199–205

Havrylenko M (1995) Erysiphaceous species from Nahuel Huapi National Park, Argentina. Part I. NZ J Bot 33: 389–400

Hibrand Saint Oyant L, Crespel L, Rajapakse S, Zhang L, Foucher F (2008) Genetic linkage maps of rose constructed with new microsatellite markers and locating QTL controlling flowering traits. Tree Genet Genomes 4:11–23

Hornero-Méndez D, Mínguez-Mosquera MI (2000) Carotenoid pigments in *Rosa mosqueta* hips, an alternative carotenoid source for foods. J Agric Chem 48:825–828

Hunter JC, Mattice JA (2002) The spread of woody exotics into the forests of a northeastern landscape, 1938–1999. J Torr Bot Soc 129:220–227

Hurst C (1941) Notes on the origin and evolution of our garden roses. J R Hortic Soc 66:77–289

Isermann M (2008a) Classification and habitat characteristics of plant communities invaded by the non-native *Rosa rugosa* Thunb. in NW Europe. Phytocoenologia 38:133–150

Isermann M (2008b) Effects of *Rosa rugosa* invasion in different coastal dune vegetation types. In: Tokarska-Guzik B, Brock JH, Brundu G, Child L, Daehler CC, Pysek P (eds) Plant invasions. Backhuys, Leiden, Netherlands

ISSG (2008) Global invasive species database. http://www.issg.org/database/species/ecology.asp?si=1390&fr=1&sts=sss&lang=EN. Accessed 25 Oct 2008

Iwata H, Kato T, Ohno S (2000) Triparental origin of Damask roses. Gene 259:53–59

Jäger AK, Petersen KN, Thamsen G, Christensen SB (2008) Isolation of linoleic and alpha-linolenic acids as COX-1 and -2 inhibitors in rose hip. Phytother Res 22:982–984

Jaillon O, Aury J-M, Noel B, Policriti A, Clepet C, Casagrande A et al (2007) The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. Nature 449:463–465

Jan CH, Byrne DH, Manhart J, Wilson H (1999) Rose germplasm analysis with RAPD markers. HortScience 34:341–345

Jesse LC, Moloney KA, Obrycki JJ (2006) Abundance of arthropods on the branch tips of the invasive plant, *Rosa multiflora* (Rosaceae). Weed Biol Manag 6:204–211

Jirong X, Yunhai X, Guolu L, Zaiquan C, Kaixue T, Xingqi H (2008) Identification of differentially expressed genes in fragrant rose Jinyindao with suppressive subtraction hybridization. Sci Hortic 116:318–323

Joichi A, Yomogida K, Awano K, Ueda Y (2005) Volatile components of tea-scented modern roses and ancient Chinese roses. Flav Fragr J 20:152–157

Joly S, Bruneau A (2007) Delimiting species boundaries in *Rosa* sect. *Cinnamomeae* (Rosaceae) in eastern North America. Syst Bot 32:819–836

Joly S, Starr JR, Lewis WH, Bruneau A (2006) Polyploid and hybrid evolution in roses east of the Rocky Mountains. Am J Bot 93:412–425

Jung S, Jesudurai C, Staton M, Du Z, Ficklin S, Cho I, Abbott A, Tomkins J, Main D (2004) GDR (Genome Database for Rosaceae): integrated web resources for Rosaceae genomics and genetics research. BMC Bioinformatics 5:130

Jung S, Staton M, Lee T, Blenda A, Svancara R, Abbott A, Main D (2008) GDR (Genome Database for Rosaceae): integrated web-database for Rosaceae genomics and genetics data. Nucleic Acids Res 36:D1034–D1040

Jürgens AH, Seitz B, Kowarik I (2007) Genetic differentiation of Rosa canina (L.) at regional and continental scales. Plant Syst Evol 269: 39–53

Katsumoto Y, Fukuchi-Mizutani M, Fukui Y, Brugliera F, Holton TA, Karan M, Nakamura N, Yonekura-Sakakibara K, Togami J, Pigeaire A, Tao GQ, Nehra NS, Lu CY, Dyson BK, Tsuda S, Ashikari T, Kusumi T, Mason JG, Tanaka Y (2007) Engineering of the rose flavonoid biosynthetic pathway successfully generated blue-hued flowers accumulating delphinidin. Plant Cell Physiol 48:1589–1600

Kaufmann H, Mattiesch L, Lörz H, Debener T (2003) Construction of a BAC library of *Rosa rugosa* Thunb. and assembly of a contig spanning Rdr1, a gene that confers resistance to blackspot. Mol Genet Genomics 268:666–674

Kermani MJ, Sarasan V, Roberts AV, Yokoya A, Wentworth J, Sieber VK (2003) Oryzalin-induced chromosome doubling in *Rosa* and its effects on plant morphology and pollen viability. Theor Appl Genet 107:1195–1200

Khosravi P, Kermani MJ, Nematzadeh GA, Bihamta MR, Yokoya K (2008) Role of mitotic inhibitors and genotype on chromosome doubling of *Rosa*. Euphytica 160:267–275

Kiani M, Zamania Z, Khalighia A, Fatahia R, Byrne D (2008) Wide genetic diversity of *Rosa damascena* Mill. germplasm in Iran as revealed by RAPD analysis. Sci Hortic 115:386

Kim CK, Chung JD, Park SH, Burrell AM, Kamo KK, Byrne DH (2004) *Agrobacterium tumefaciens*-mediated transformation of *Rosa hybrida* using the green fluorescent protein (GFP) gene. Plant Cell Tiss Org Cult 78:107–111

Kiritikar KR, Basu BD (1987) *Rhus semialata* Murr. In: Blatter E (ed) Indian medicinal plants. International Book Distributors, Dehra Dun, India, pp 646–647

Kissel RM, Wilson JB, Bannister P, Mark AF (1987) Water relations of some native and exotic shrubs of New Zealand. New Phytol 107:29–37

Kitahara K, Matsumoto S (2000) Rose MADS-box genes 'MASAKO C1 and D1' homologous to class C floral identity genes. Plant Sci 151:121–134

Kollmann J, Frederiksen L, Vestergaard P, Bruun HH (2007) Limiting factors for seedling emergence and establishment of the invasive non-native *Rosa rugosa* in a coastal dune system. Biol Invas 9:31–42

Koning-Boucoiran CFS, Dolstra O, van der Linden CG, van der Schoot J, Gitonga VW, Verlinden K, Maliepaard CA, Krens FA (2009) Specific mapping of disease resistance genes in tetraploid cut roses. Acta Hortic 836:137–142

Koopman WJM, Vosman B, Sabatino GJH, Visser D, Van Huylenbroeck J, De Riek J, De Cock K, Wissemann V, Ritz CM, Maes B, Werlemark G, Nybom H, Debener T, Linde M, Smulders MJM (2008) AFLP markers as a tool to reconstruct complex relationships in the genus *Rosa* (Rosaceae). Am J Bot 95:353–366

Kornman K, Rogus J, Roh-Schmidt H, Krempin D, Davies AJ, Grann K, Randolph RK (2007) Interleukin-1 genotype-selective inhibition of inflammatory mediators by a botanical: a nutrigenetics proof of concept. Nutrition 23:844–852

Kovacs S, Toth M, Facsar G (2004) Evaluation of fruit quality parameters of *Rosa* taxa from the Carpathian basin. Int J Hortic Sci 10:81–87

Kovarik A, Werlemark G, Leitch AR, Souckova-Skalicka K, Lim KY, Khaitová L, Koukalova B, Nybom H (2008) The asymmetric meiosis in pentaploid dogroses (*Rosa* sect. *Caninae*) is associated with a skewed distribution of rRNA gene families in the gametes. Heredity 101:359–367

Kovats ES (1987) Composition of essential oils. Bulgarian oil of rose (*Rosa damascena* Mill). J Chromatogr 406:185–222

Kvacek Z, Walther H (2004) Oligocene flora of Bechlejovice at Decin from the neovolcanic area of the Ceske stredohori Mountains, Czech Republic. Acta Musei Nationalis Pragae 60(1–2):9–60

Lavid N, Wang J, Shalit M, Guterman I, Bar E, Beuerle T, Menda N, Shafir S, Zamir D, Adam Z, Vainstein A, Weiss D, Pichersky E, Lewinsohn E (2002) O-Methyltransferases involved in the biosynthesis of volatile phenolic derivatives in rose petals. Plant Physiol 129:1899–1907

Leus L, Dewitte A, Van Huylenbroeck J, Vanhoutte N, Van Bockstaele E, Höfte M (2006) *Podosphaera pannosa* (syn. *Sphaerotheca pannosa*) on *Rosa* and *Prunus* spp.: characterization of pathotypes by differential plant reactions and ITS-sequences. J Phytopathol 154:23–28

Li X, Krasnyanski SF, Korban SS (2002) Optimization of the *uid*A gene transfer into somatic embryos of rose via *Agrobacterium tumefaciens*. Plant Physiol Biochem 40:453–459

Li X, Gasic K, Cammue B, Broekaert W, Korban SS (2003) Transgenic rose lines harboring an antimicrobial protein gene, *Ace-AMP1*, demonstrate enhanced resistance to powdery mildew (*Spaerotheca pannosa*). Planta 218:226–232

Lim KY, Werlemark G, Matyasek R, Bringloe JB, Sieber V, El Mokadem H, Meynet J, Hemming J, Leitch AR, Roberts AV (2005) Evolutionary implications of permanent odd

polyploidy in the stable sexual, pentaploid of *Rosa canina* L. Heredity 94:501–506

Linde M, Debener T (2003) Isolation and identification of eight races of powdery mildew or roses (Podosphaera pannosa (Wallr.: Fr.) de Bary) and the genetic analysis of the resistance gene Rpp 1. Theor Appl Genet 107:256–262

Linde M, Shishkoff N (2003) Disease – powdery mildews. In: Roberts AV, Debener T, Gudin S (eds) Encyclopedia of rose science. Elsevier, Academic, Oxford, UK, pp 158–165

Linde M, Mattiesch L, Debener T (2004) Rpp 1, a dominant gene providing race-specific resistance to rose powdery mildew (Podosphaera pannosa): molecular mapping SCAR development and confirmation of disease resistance data. Theor Appl Genet 109:1261–1266

Linde M, Hattendorf A, Kaufmann H, Debener T (2006) Powdery mildew resistance in roses: QTL mapping in different environments using selective genotyping. Theor Appl Genet 113:1081–1092

Loux MM, Underwood JF, Amrine JW, Chandran R (2005) Multiflora rose control. Ohio State Univ Bull 857:1–16

Ma Y, Byrne DH, Chen J (1997) Amphidiploid induction from diploid rose interspecific hybrids. HortScience 32:292–295

Ma N, Tan H, Liu X, Xue J, Li Y, Gao J (2006) Transcriptional regulation of ethylene receptor and CTR genes involved in ethylene-induced flower opening in cut rose (*Rosa hybrida*) cv. Samantha. J Exp Bot 57:2763–2773

Mahmood N (1996) The anti-HIV activity and mechanisms of action of pure compounds isolated from *Rosa damascena*. Biochem Biophys Res Commun 229:73–79

Marchant R, Power JB, Lucas JA, Davey MR (1998a) Biolistic transformation of rose (*Rosa hybrida* L.). Ann Bot 81:109–114

Marchant R, Davey MR, Lucas JA, Lamb CJ, Dixon RA, Power JB (1998b) Expression of a chitinase transgene in rose (*Rosa hybrida* L.) reduces development of blackspot disease (*Diplocarpon rosae* Wolf). Mol Breed 4:187–194

Martin M, Piola F, Chessel D, Jay M, Heizmann P (2001) The domestication process of the modern rose: genetic structure and allelic composition of the rose complex. Theor Appl Genet 102:398–404

Matsumoto S, Kouchi M, Yabuki J, Kusunoki M, Ueda Y, Fukui H (1998) Phylogenetic analyses of the genus *Rosa* using the *matK* sequence: molecular evidence for the narrow genetic background of modern roses. Sci Hortic 77:73–82

Matsumoto S, Kouchi M, Fukui H, Ueda Y (2000) Phylogenetic analyses of the subgenus *Eurosa* using the ITS nrDNA sequence. Acta Hortic 521:193–202

McNeill J, Barrie FR, Burdet HM, Demoulin V, Hawksworth DL, Marhold K, Nicolson DH, Prado J, Silva PC, Skog JE, Wiersema JH, Turland NJ (2006) Decisions concerning conservation of names in rose taxonomy: International Code of Botanical Nomenclature (Vienna Code) adopted by the 17th International Botanical Congress, Vienna, Austria, July 2005. Regnum Vegetabile 146:389

Mercure M, Bruneau A (2008) Hybridization between the escaped *Rosa rugosa* (Rosaceae) and native *R. blanda* in eastern North America. Am J Bot 95:597–607

Merriam RW (2003) The abundance, distribution and edge associations of six non-indigenous, harmful plants across North Carolina. J Torr Bot Soc 130(4):283–291

Mertens-Talcott SU, Bomser JA, Romero C, Talcott ST, Percival SS (2005) Ellagic acid potentiates the effect of quercitin on $p21^{waf1/ci1}$, p53, and MAP-kinases without affecting intracellular generation of reactive oxygene species in vitro. J Nutr 135:609–614

Meusel H, Jäger EJ (1965) Vergleichende Chorologie der zentraleuropäischen Flora. Gustav Fischer, Jena, DDR

Meynet J, Barrade R, Duclos A, Siadous R (1994) Dihaploid plants of roses (*Rosa x hybrida*) obtained by parthenogenesis induced using irradiated pollen and in vitro culture of immature seeds. Agronomie 2:169–175

Mikanagi Y, Yokoi M, Ueda Y, Saito N (1995) Flower flavonol and anthocyanin distribution in subgenus *Rosa*. Biochem Syst Ecol 23:183–200

Ming OC, Wen AS, Sinniah U, Xavier R, Subramaniam S (2007) Cysteine and acetosyringone are the two important parameters in *Agrobacterium*-mediated transformation of rose hybrid (*Rosa hybrida* L.) cv. Nikita. J Plant Sci 2:387–397

Mottley J, Yokoya J, Matthews K, Squirrel D, Wentworth JE (1996) Protoplast fusion and its potential role in de genetic improvement of roses. Acta Hortic 424:393–397

Müller R, Owen CA, Xue ZT, Welander M, Stummann BM (2002) Characterization of two CTR-like protein kinases in *Rosa hybrida* and their expression during flower senescence and in response to ethylene. J Exp Bot 53:1223–1225

Nikolov N, Dragostinov P, Tsousoulova A, Portarska F, Apostolova B, Nenov N (1977) Bulgarian rose oil and its quality. In: 7th International congress of essential oils, Kyoto, Japan, 7–11 Oct 1977, pp 159–163

Nikolov N, Dragostinov P, Tsoutsoulova T, Apostolova B, Portarska F (1978) Essence de rose bulgare et autres produits aromatiques de la rose oleifere de Kazanlik. Pharmachim 205:35–40

Nilsson Ö (1999) Wild roses in Norden: taxonomic discussion. Acta Bot Fennica 162:169–173

Ninomiya K, Matsuda H, Kubo M, Morikawa T, Nishida N, Yoshikawa M (2007) Potent anti-obese principle from *Rosa canina*; Structural requirements and mode of action of transtiliroside. Bioorg Med Chem Lett 27:3059–3064

Nybom H, Olsson Å, Werlemark G (1996) Morphometric variation in Nordic dogroses (*Rosa* sect. *Caninae*, Rosaceae). Symb Bot Ups 31(3):59–68

Nybom H, Esselink GD, Werlemark G, Vosman B (2004) Microsatellite DNA marker inheritance indicates preferential pairing between highly homologous genomes in polyploid and hemisexual dog-roses *Rosa* L. sect. *Caninae*. Heredity 92:139–150

Nybom H, Esselink GD, Werlemark G, Leus L, Vosman B (2006) Unique genomic configuration revealed by microsatellite DNA in polyploid dogroses. Rosa sect. Caninae. J Evol Biol 19:635–648

Ohba T, Miyawaki A, Tüxen R (1973) Pflanzengesellschaften der japanischen Dünen-Küsten. Vegetatio 26:1–143

Ohloff G (1994) Scent and fragrances. The fascination of odors and their chemical perspectives, Translated by Pickenhagen W, Lawrence B. Springer, Berlin, Germany

Olsson ÅME, Nybom H, Prentice HC (2000) Relationships between Nordic dogroses (*Rosa* L. sect. *Caninae*, Rosaceae) assessed by RAPDs and elliptic Fourier analysis of leaflet shape. Syst Bot 25:511–521

Olsson ME, Gustavsson K-E, Andersson S, Nilsson Å, Duan R-D (2004) Inhibition of cancer cell proliferation in vitro by fruit and berry extracts and correlations with antioxidant levels. J Agric Food Chem 52:7264–7271

Ozkan G, Sagdic O, Baydar N (2004) Antioxidant and antibacterial activities of *Rosa damascena* flower extracts. Food Sci Technol Int 10:277–281

Pan HC, Li JH, Wang XZ (2005) Involvement of ethylene and 1-aminocyclopropane-1-carboxylate synthase gene in regulation of programmed cell death during rose (*Rosa x hybrida*) flower development. Zhi Wu Sheng Li Yu Fen Zi Sheng Wu Xue Xue Bao 31:354–360

Parsons WT (2001) Noxious weeds of Australia. CSIRO, Collingwood, Australia

Pati PK, Sharma M, Ahuja PS (2008) Rose protoplast isolation and culture and heterokaryon selection by immobilization in extra thin alginate film. Protoplasma 233:165–171

Peng Y, Chen W, Moens M (2003) Resistance of *Rosa* species and cultivars to *Pratylenchus penetrans*. HortScience 38:560–564

Pirseyedi S, Mardi M, Davazdahemami S, Kermani M, Mohammadi S (2005) Analysis of the genetic diversity of 12 Iranian Damask rose (*Rosa damascena* Mill.) genotypes using amplified fragment length polymorphism markers. Iran J Biotechnol 3:225–230

Raev R (1984) Mutation breeding of Kazanlushka oil bearing rose (*Rosa damascena* Mill.). Rastenievudni Naouki 8: 92–98 (in Bulgarian)

Rajapakse S, Byrne DH, Zhang L, Anderson N, Arumuganathan K, Ballard RE (2001) Two genetic linkage maps of tetraploid roses. Theor Appl Genet 103:575–583

Reimann-Philipp R (1981) Cytogenetics and breeding in diploid roses from the triploid hybrid R. multiflora x garden cultivars. Ornamental Meeting on Rose Breeding (Ahrensburg) Eucarpia p. 27–29

Rehder A (1940) Manual of cultivated trees and shrubs, Hardy in North America, 2nd edn. Dioscorides, Portland, OR, USA

Reynders-Aloisi S, Bollereau P (1996) Characterisation of genetic diversity in genus *Rosa* by randomly amplified polymorphic DNA. Acta Hortic 424:253–259

Ritz CM, Schmuths H, Wissemann V (2005) Evolution by reticulation: European dogroses originated by multiple hybridization across the genus *Rosa*. J Hered 96:4–14

Roberts AV, Lloyd D, Short KC (1990) In vitro procedures for the induction of tetraploidy in a diploid rose. Euphytica 49:33–38

Roberts AV, Gladis T, Brumme H (2009) DNA amounts of roses (*Rosa* L.) and their use in attributing ploidy levels. Plant Cell Rep 28:61–71

Robertson DJ, Robertson MC, Tague T (1994) Colonization dynamics of 4 exotic plants in a northern Piedmont natural area. Bull Torr Bot Club 121:107–118

Rusanov K, Kovacheva N, Vosman B, Zhang L, Rajapakse S, Atanassov A, Atanassov I (2005a) Microsatellite analysis of *Rosa damascena Mill.* accessions reveals genetic similarity between genotypes used for rose oil production and old Damask rose varieties. Theor Appl Genet 111:804–809

Rusanov K, Kovacheva N, Atanassov A, Atanassov I (2005b) Microsatellite analysis of oil-bearing roses which do not belong to the species *Rosa damascena* Mill. Bulg J Agric Sci 11:1–9

Rusanov K, Kovacheva N, Atanassov A, Atanassov I (2005c) Lessons from the microsatellite characterization of a segregating population derived from seeds of open pollinated *Rosa damascena* Mill *f.* Trigintipetala plants. Biotechnol Biotechnol Equip 19:72–79

Scalliet G, Journot N, Jullien F, Baudino S, Magnard J-L, Channelière S, Vergne P, Dumas C, Bendahmane M, Cock JM, Hugueney P (2002) Biosynthesis of the major scent components 3,5-dimethoxytoluene and 1,3,5-trimethoxybenzene by novel rose O-methyltransferases. FEBS Lett 523:113–118

Scalliet G, Lionnet C, Le Bechec M, Dutron L, Magnard JL, Baudino S, Bergougnoux V, Jullien F, Chambrier P, Vergne P, Dumas C, Cock JM, Hugueney P (2006) Role of petal-specific orcinol O-methyltransferases in the evolution of rose scent. Plant Physiol 140:18–29

Scalliet G, Piola F, Douady CJ, Réty S, Raymond O, Baudino S, Bordji K, Bendahmane M, Dumas C, Cock MJ, Hugueney P (2008) Scent evolution in Chinese roses. Proc Natl Acad Sci USA 105:5927–5932

Scariot V, Akkak A, Botta R (2006) Characterization and genetic relationships of wild species and old garden roses based on microsatellite analysis. J Am Soc Hortic Sci 131:66–73

Schlätzer G (1974) Some experiences from attempts at establishing broadleaved woody plants in some Danish dunelands. Int J Biometeorol 18(2):159–167

Schouten HJ, Krens FA, Jacobsen E (2006) Cisgenic plants are similar to traditionally bred plants. EMBO Rep 7:750–753

Schum A, Hofmann K (2001) Use of isolated protoplasts in rose breeding. Acta Hortic 547:35–45

Schum A, Hofmann K, Felten R (2002) Fundamentals for integration of somatic hybridization in rose breeding. Acta Hortic 572:29–36

Semeniuk P, Arisumi T (1968) Colchicine induced tetraploid and cytochimeral roses. Bot Gaz 129:190–193

Shalit M, Guterman I, Volpin H, Bar E, Tamari T, Menda N, Adam Z, Zamir D, Vainstein A, Weiss D, Pichersky E, Lewinsohn E (2003) Volatile ester formation in roses. Identification of an acetyl-coenzyme A Geraniol/Citronellol acetyltransferase in developing rose petals. Plant Physiol 131:1868–1876

Souq F, Coutos-Thevenot P, Yean H, Delbard G, Maziere Y, Barbe JP, Boulay M (1996) Genetic transformation of roses, 2 examples: one on morphogenesis, the other on anthocyanin biosynthetic pathway. Acta Hortic 424:381–388

Spethmann W, Feuerhahn B (2003) Genetics – species crosses. In: Roberts AV, Debener T, Gudin S (eds) Encyclopedia of rose science. Elsevier, Academic, Oxford, UK, pp 299–312

Spiller M, Linde M, Hibrand-Saint Oyant L Tsai C-J, Byrne D, Smulders M, Foucher F, Debener T (2011) Towards a unified genetic map of diploid rose. Theor Appl Genet http://dx.doi.org/10.1007/s00122-010-1463-x

Squirrell J, Mandegaran Z, Yokoya K, Roberts AV, Mottley J (2005) Cell lines and plants obtained after protoplast fusions of *Rosa+Rosa, Rosa+Prunus* and *Rosa+Rubus*. Euphytica 146:223–231

Staikov V, Kalaijiev I (1980) Results from the hybridization of the oil bearing rose (*Rosa damascena Mill.*). Rastenievudni Naouki 5:21–33 (in Bulgarian)

Szentmihályi K, Vinkler P, Lakatos B, Illés V, Then M (2002) Rose hip (*Rosa canina* L.) oil obtained from waste hip seeds by different extraction methods. Bioresour Technol 82:195–201

Tabaei-Aghdaei SR, Babaei A, Khosh-Khui M, Jaimand K, Rezaee MB, Assareh MH, Naghavi MR (2007) Morphological and oil content variations amongst Damask rose (Rosa damascena Mill.) landraces from different regions of Iran. Scientia Horticulturae 113:44–48

Timmermann G, Müller T (1994) Wildrosen und Weißdorne Mitteleuropas–Landschaftsgerechte Sträucher und Bäume. Verlag des Schwäbischen Albvereins e.V, Stuttgart

Topalov V (1978) The Kazanlushka rose and the rose production in Bulgaria (in Bulgarian). Christo Danov, Plovdiv, Bulgaria

Tsuda S, Fujita H, Nishisaka K (1999) Resprouting behavior of *Rosa rugosa* Thunb. after an experimental burning in Koshimizu Nature Reserve, Hokkaido, Japan. Actinia: Bulletin of the Manazuru Marine Laboratory for Science Education, Faculty of Education and Human Sciences, Yokohama National University 12:113–121

van der Salm TPM, Hänisch ten Cate CH, Dons HJM (1996) Prospects for application of *rol* genes for crop improvement. Plant Mol Biol Rep 14:207–228

van der Salm TPM, van der Toorn CJG, Bouwer R, Hänisch ten Cate CH, Dons HJM (1997) Production of *ROL* gene transformed plants of *Rosa hybrida* L. and characterization of their rooting ability. Mol Breed 3:39–47

van der Salm TPM, Bouwer R, van Dijk AJ, Keizer LCP, Hänisch ten Cate CH, van der Plas LHW, Dons HJM (1998) Stimulation of scion bud release by *rol* gene transformed rootstocks of *Rosa hybrida* L. J Exp Bot 49:847–852

Van Huylenbroeck J, Eeckhaut T, Leus L, Werlemark G, De Riek J (2007) Introduction of wild germplasm in modern roses. Acta Hortic 751:285–290

Vanderhoeven S, Dassonville N, Meerts P (2005) Increased topsoil mineral nutrient concentrations under exotic invasive plants in Belgium. Plant Soil 275:169–179

Vecchia DI, Altieri A, Tavani A (2001) Vegetables, fruit, antioxidants and cancer: a review of Italian studies. Eur J Nutr 40:261–267

Velasco R, Zharkikh A, Troggio M, Cartwright DA, Cestaro A et al (2007) A high quality draft consensus sequence of the genome of a heterozygous grapevine variety. PLoS ONE 2:e1326

Vergne P, Maene M, Chauvet A, Debener T, Bendahmane M (2010) Versatile somatic embryogenesis systems and transformation methods for the diploid rose genotype *Rosa chinensis* cv Old Blush. Plant Cell Tiss Organ Cult 100:73–81

von Malek B, Weber WE, Debener T (2000) Identification of molecular markers linked to Rdr1, a gene conferring resistance to blackspot in roses. Theor Appl Genet 101:977–983

Wang D, Fan J, Ranu RS (2004a) Cloning and expression of 1-aminocyclopropane-1-carboxylate synthase cDNA from rose (*Rosa x hybrida*). Plant Cell Rep 22:422–429

Wang X, Jacob Y, Mastrantuono S, Bazzano J, Voisin R, Esmenjaud D (2004b) Spectrum and inheritance of resistance to the root-knot nematode *Meloidogyne hapla* in *Rosa multiflora* and *R. indica*. Plant Breed 123:79–83

Warholm O, Skaar S, Hedman E, Mølmen HM, Eik L (2003) The effects of a standardized herbal remedy made from a subtype of *Rosa canina* in patients with osteoarthritis: a double-blind, randomized, placebo-controlled clinical trial. Durr Ther Res 64:21–31

Weber E (2003) Invasive plant species of the world: a reference guide to environmental weeds. CABI, Cambridge, UK

Widrlechner MP (1981) History and utilization of *Rosa damascena*. Econ Bot 35:42–58

Winther K, Apel K, Thamsborg G (2005) A powder made from seeds and shells of a rose-hip subspecies (*Rosa canina*) reduces symptoms of knee and hip osteoarthritis: a randomized double-blind, placebo-controlled clinical trial. Scand J Rheumatol 34:302–308

Wisseman V, Ritz CM (2005) The genus *Rosa* (Rosoideae, Rosaceae) revisited: molecular analysis of nrITS-1 and *atp*B-*rbc*L intergenic spacer (IGS) versus conventional taxonomy. Bot J Linn Soc 147:275–290

Wissemann V (1999) Genetic constitution of *Rosa* Sect. *Caninae* (*R. canina*, *R. junzillii*) and Sect. Gallicanae (*R. gallica*). J Appl Bot 73:191–196

Wissemann V (2002) Molecular evidence for allopolyploid origin of the *Rosa canina*-complex (Rosaceae, Rosoideae). J Appl Bot 76:176–178

Wissemann V (2003) Conventional taxonomy (wild roses). In: Roberts AV, Debener T, Gudin S (eds) Encyclopedia of rose science. Elsevier, Academic, Oxford, UK, pp 111–117

Wu SQ, Ueda Y, He HY, Nishihara S, Matsumoto S (2000) Phylogenetic analysis of Japanese *Rosa* species using *matK* sequences. Breed Sci 50:275–281

Wylie AP (1954) The history of garden roses, part I. J R Hortic Soc Lond 79:555–571

Xue J, Li Y, Tan H, Yang F, Ma N, Gao J (2008) Expression of ethylene biosynthetic and receptor genes in rose floral tissues during ethylene-enhanced flower opening. J Exp Bot 59:2161–2169

Yan Z, Denneboom C, Hattendorf A, Dolstra O, Debener T, Stam P, Visser PB (2005) Construction of an integrated map of rose with AFLP, SSR, PK, RGA, RFLP, SCAR and morphological markers. Theor Appl Genet 110:766–777

Yan Z, Dolstra O, Prins T, Stam P, Visser P (2006) Assessment of partial resistance to powdery mildew (*Podosphaera pannosa*) in a tetraploid rose population using a spore-suspension inoculation method. Eur J Plant Pathol 114:301–308

Yasmin A, Debener T (2010) Transient gene expression in rose petals via *Agrobacterium* infiltration. Plant Cell Tiss Org Cult 102:245–250

Yates ED, Levia DF, Williams CL (2004) Recruitment of three non-native invasive plants into a fragmented forest in southern Illinois. For Ecol Manag 190:119–130

Yokoya K, Roberts AV, Mottley J, Lewis R, Brandham PE (2000) Nuclear DNA amounts in roses. Ann Bot 85:557–561

Zhang LH, Byrne DH, Ballard RE, Rajapakse S (2006) Microsatellite marker development in rose and its application in tetraploid mapping. J Am Soc Hortic Sci 131:380–387

Zimmermann H, Renison D, Damascos M, Welk E, Wesche K, Hensen I (2008) Density and growth of *Rosa rubiginosa* L.; invasive in Argentina and declining in Europe. Verhandlungen der Gesellschaft für Ökologie, Leipzig 38:383

Zimmermann H, Ritz CM, Hirsch H, Renison D, Wesche K, Hensen I (2010) Highly reduced genetic diversity of *Rosa rubiginosa* L. populations in the invasive range. Int J Plant Sci 171:435–446

Zlesak DC, Thill CA, Anderson ON (2005) Trifluralin-mediated polyploidization of *Rosa chinensis minima* (Sims) Voss seedlings. Euphytica 141:281–290