Phylogenetic Analysis of Japanese *Rosa* Species Using *matK* Sequences

Shuiqin Wu¹⁾, Yoshihiro Ueda^{*1)}, Haiyang He¹⁾, Sayako Nishihara¹⁾ and Shogo Matsumoto²⁾

¹⁾ Laboratory of Floricultural Science, Faculty of Horticulture, Chiba University, 648 Matsudo, Matsudo, Chiba 271-8510, Japan

²⁾ Department of Biology, Faculty of Education, Gifu University, Yanagido, Gifu, Gifu 501-1193, Japan

The *matK* DNA was sequenced in each of the 15 Japanese *Rosa* taxa, 17 other Asian taxa, and two American taxa to analyse their phylogenetic relationships. All the 14 tested taxa in the section *Synstylae*, associated with the section *Indicae*, formed one clade. It was confirmed that sections *Synstylae* and *Indicae*, with the common styles exerting from the hypanthium, are closely related to each other. Furthermore, *R. brunonii* and *R. moschata* in the section *Synstylae* formed an inner clade which is consistent with their common morphological traits. Two clones of *R. acicularis* formed one single clade which was separated from the remaining species of the section *Rosa*. This finding suggests that *R. acicularis* might be a distinctive species in the section *Rosa*. The remaining species of the section *Rosa* formed one clade which was further subdivided into two inner clades: *R. rugosa* and *R. marretii* formed one clade, while *R. acicularis* var. *nipponensis* and *R. willmottiae* formed another clade. The distinctive relationship with *R. acicularis* implies that diploid *R. acicularis* var. *nipponensis*, traditionally treated as one variety of polyploid *R. acicularis* by some authors, is a primitive taxon and that it should be reassigned to its original specific rank as *R. nipponensis*.

Key Words: *Rosa*, *matK* DNA, phylogeny, Japanese native species.

Introduction

The genus *Rosa* contains more than 150 species that are widely distributed throughout the Northern Hemisphere. The largest concentration of species is indigenous to the regions of Western Asia and China. About 15 taxa of the genus *Rosa* are distributed in Japan (Ueda 1996), and only 3 of these species have contributed to the development of the modern commercial rose, namely *R. multiflora*, *R. wichurana* and *R. rugosa*. The land area of Japan extends over nearly 21° of latitude from about 24°N to 45°N from the subtropical zone of the southern area to the frozen zone of the alpine summits in the northern part of Hokkaido. Therefore, we consider that there are many important genetic resources

among the Japanese indigenous species. For example, two taxa in the section *Synstylae*, *R. sambucina* with large white flowers and *R. multiflora* var. *adenochaeta* with pinkish white flowers, are both potentially useful for rose breeding. The use of another species in the section *Rosa*, *R. marretii*, may enable to introduce cold resistance genes into the modern rose.

In order to utilize such wild species for modern rose breeding, their crossing compatibility should be determined. Biosystematic studies of these species may provide rose breeders with such basic information, since phylogenetic relationships have been found to be strongly correlated with the corresponding crossability in some plants (Hawkes 1981, Kim and Jansen 1998). In fact, it is relatively easy to raise the first progeny in rose breeding using wild species. However, further hybrids may result in sterility if the parental wild species are not closely related. It is considered that a clarification of their phylogenetic relationship may provide useful information for future breeding experiments.

The wide morphological variation within species and the weak barriers to interspecific hybrids call in question the traditional classification of this genus based on morphological traits (e. g. Rehder 1940). In the recent decade, diversified molecular approaches have been used to resolve the taxonomic problems of the genus *Rosa* (Debener *et al.* 1996, Millan *et al.* 1996, Matsumoto and Fukui 1996, Matsumoto *et al.* 1997, Jan *et al.* 1999). Among these approaches, comparative sequencing of chloroplast DNA, as well as nuclear genes, has been found to be extremely useful (Matsumoto *et al.* 1998, Matsumoto *et al.* 2000).

The *matK* gene, located in the intron of *trnK*, encodes a maturase that acts as an essential splicing factor (Vogel *et al.* 1997). Due to its high nucleotide substitution rate and lower structural conservation, this gene has been employed for the reconstruction of phylogenetic relationships not only within families, but also within genera in higher plants (Johnson and Soltis 1995). In this study, we used *matK* sequence variation to examine the phylogenetic relationships of Japanese *Rosa* species, which had not been analyzed previously (Matsumoto *et al.* 1998), and to consider the implications for the classification of the genus *Rosa*.

Materials and Methods

Plant samples

The species investigated (Table 1) included all of the 15 taxa of the genus *Rosa* native to Japan. For comparison

Communicated by M. Shibata

Received March 22, 2000. Accepted June 26, 2000.

*Corresponding author (e-mail: yueda@midori.h.chiba-u.ac.jp)

Table 1. Taxa included in the phylogenetic study of the genus *Rosa* with *matK* sequences

Species	Geographical origin	Voucher and source ^{a)}	DDBJ accession number ^{b)}
Genus <i>Rosa</i>			
Subgenus <i>Hulthemia</i>			
<i>R. persica</i> Michx.	Xinjiang, China	98UGX-04	AB039289 ^{d)}
Subgenus <i>Rosa</i>			
Section <i>Pimpinellifoliae</i>			
<i>R. spinosissima</i> L.	Xinjiang, China	97UGX-16	AB039290
<i>R. platyacantha</i> Schrenk	Xinjiang, China	98UGX-09-03	AB039291
Section <i>Carolinae</i>			
<i>R. foliolosa</i> Nutt. ex Torr. et Gray	Southeastern America	KRN, Chiba	AB039292
Section <i>Rosa</i>			
<i>R. acicularis</i> Lindl. -1	Hokkaido, Japan	96UN-SA-03	AB039293
<i>R. acicularis</i> Lindl. -2	Northeastern China	87S-619	AB039294
<i>R. acicularis</i> var. <i>nipponensis</i> Koehne.	Gunma, Japan	96UN-OZ-11	AB039295
<i>R. rugosa</i> Thunb.	Aomori, Japan	85P-85	AB039296 ^{c)}
<i>R. marretii</i> Lév.	Nagano, Japan	96UN-NA-16	AB039297
<i>R. willmottiae</i> Hemsl.	Western China	KRN, Chiba	AB039298
Section <i>Synstylae</i>			
<i>R. luciae</i> Franch. et Rochebr.	Kagoshima, Japan	96UN-CH-04	AB039299
<i>R. luciae</i> var. <i>onoiei</i> Momiyama	Kagoshima, Japan	96UN-IS-04	AB039300
<i>R. luciae</i> var. <i>hakonensis</i> Franch. et Sav.	Kanagawa, Japan	96UN-HA-01	AB039301
<i>R. luciae</i> var. <i>fujisanensis</i> Makino	Kanagawa, Japan	96UN-HA-32	AB039302
<i>R. luciae</i> var. <i>paniculigera</i> Momiyama	Gifu, Japan	99UIG-01	AB039303
<i>R. multiflora</i> Thunb.	Kanagawa, Japan	96UN-HA-77	AB039304 ^{d)}
<i>R. multiflora</i> var. <i>adenochaeta</i> Makino	Fukuoka, Japan	96UN-HI-02	AB039305
<i>R. sambucina</i> Koidz.	Hiroshima, Japan	96UN-MI-05	AB039306
<i>R. wichurana</i> Crép.	Kagoshima, Japan	96UN-HA-11	AB039307 ^{d)}
<i>R. moschata</i> Herrm.	Western Asia	KRN, Chiba	AB039308 ^{d)}
<i>R. maximowicziana</i> Regel	Korea	95S-06	AB039309
<i>R. henryi</i> Bouleng.	Sichuang, China	97SR-03-1	AB039310
<i>R. tunquinensis</i> Crép.	Paksong, Laos	99UML-08	AB039311
<i>R. brunonii</i> Lindl.	Godawari, Nepal	92NN-01	AB039312
Section <i>Indicae</i>			
<i>R. chinensis</i> Jacq. var. <i>spontanea</i> (Rehd. et Wils.) Yü et Ku	Sichuang, China	89S-612	AB039313 ^{d)}
<i>R. gigantea</i> Collett ex Crép.	Yunnan, China	97UGY-012	AB039314 ^{d)}
Section <i>Banksianae</i>			
<i>R. banksiae</i> Ait. f. <i>lutescens</i> Voss.	West China	PBR, UK	AB039315
<i>R. banksiae</i> var. <i>normalis</i> Regel	Southwestern China	KRN, Chiba	AB039316 ^{c)}
<i>R. cymosa</i> Tratt.	Sichuang, China	89S-613	AB039317
Section <i>Laevigatae</i>			
<i>R. laevigata</i> Michx.	Fujian, China	96UWF-01	AB039318 ^{c)}
Section <i>Bracteatae</i>			
<i>R. bracteata</i> Wendl.	Okinawa, Japan	92S-269	AB039319 ^{c)}
Subgenus <i>Platyrrhodon</i>			
<i>R. roxburghii</i> Tratt. var. <i>hirtula</i> Rehd. et Wils.	Kanagawa, Japan	96UN-HA-06	AB039320
<i>R. roxburghii</i> Tratt. var. <i>normalis</i> Rehd. et Wils.	Southwestern China	KRN, Chiba	AB039321
Subgenus <i>Hesperhodos</i>			
<i>R. stellata</i> Woot. var. <i>mirifica</i> Cockerell	Northern America	FFCP, Gifu	AB039322 ^{c)}
Genus <i>Rubus</i>			
<i>Rubus trifidus</i> Thunb. ^{e)}	Chiba, Japan	Field of Chiba Uni.	AB039323

^{a)} Abbreviations used: PBR=Peter Beales Roses; FFCP=Flower Festival Commemorative Park; KRN=Keisei Rose Nursery.

^{b)} The nucleotide sequence data of the samples will appear in the DDBJ, EMBL and GenBank Nucleotide Sequence Databases with accession numbers AB039289 through AB039323.

^{c)} We confirmed that the nucleotide sequences of the samples were completely the same as those in previously examined samples (Matsumoto *et al.* 1998).

^{d)} We confirmed that the nucleotide sequences of the samples were slightly different from those of previously examined samples (Matsumoto *et al.* 1998) due to the use of subclones of PCR products. We then used our nucleotide sequences for analysis.

^{e)} outgroup

with the Japanese species, other 19 taxa (13 taxa native to China, one species from Korea, one from Nepal, one from Laos, one from Western Asia and two from North America) were also used. Each of these taxa is included in 4 subgenera (*Hulthemia*, *Rosa* (formerly *Eurosa*), *Platyrhodon*, *Hesperhodos*) and 8 sections of the subgenus *Rosa* (Rehder 1940). In this study, based on the assumption that the type species for the genus is *R. cinnamomea* L. (Rowley 1976, Nicolson 1992), we used the section name *Rosa* and not *Cinnamomeae* (*Cassiorhodon*). *Rubus trifidus* was selected as the outgroup, because *Rubus* is closely related to *Rosa* (Kalkman 1988, Eriksson *et al.* 1998). All the samples were grown in the experimental farm of the Faculty of Horticulture, Chiba University except for *R. stellata* var. *mirifica* which was collected from the Flower Festival Commemorative Park in Gifu Prefecture. *Rubus trifidus* was collected from the wild field in the experimental farm of the Faculty of Horticulture, Chiba University.

DNA extraction, PCR amplification, subcloning and sequencing

Total DNA was extracted from young leaf tissue by a modified CTAB method (Doyle and Doyle 1987). The portion of *trnK* DNA including the entire *matK* region was amplified by PCR. PCR amplification was performed in 25 µl reaction solutions containing 17.1 µl of sterile water, 2.5 µl 10×*Ex Taq* polymerase reaction buffer (Takara Shuzo Co. Ltd.), 2.0 µl 2.5 mM dNTPs in an equimolar ratio, 0.15 µl of *Ex Taq* DNA polymerase (5 units/µl) (Takara Shuzo Co. Ltd.), 0.625 µl of 20 µM “*trnK*-3914” and “*trnK*-2R” primers (Johnson and Soltis 1995) and 2 µl of template DNA (10 ng/µl). The solutions were first denatured for 2 min at 95°C, followed by 30 cycles of amplification which consisted of 1 min at 95°C (denaturation), 1 min at 50°C (annealing of primers), and 2 min at 72°C (polymerization). After amplification, the PCR products were ligated into a pGEM-T system vector and at least two clones was screened out and used for DNA sequence analysis.

The purified plasmid DNA of each clone was sequenced on an ABI PRISM 377 DNA sequencer (Perkin-Elmer) using dRhodamine Terminator Cycle Sequencing Kits (Perkin-Elmer). Six primers were used for sequencing: *matK*-AF; *matK*-WF; *matK*-VF; *matK*-UF; *matK*-QR; *matK*-1412R, which were the same as those used by Matsumoto *et al.* (1998). We also designed one primer, *matK*-854F (5'-CTGGCATCAAAAGATACTCC-3'), which starts at the position of 1617 relative to the *Nicotiana* sequence (Sugita *et al.* 1985). We used it for sequence analysis of the corresponding unresolved parts for some samples.

Sequence analysis

Boundaries of *matK* DNA were determined by comparison of the DNA sequences with published ones (Matsumoto *et al.* 1998). DNA sequences were aligned manually by sequential pairwise comparison with the DNASIS program. The alignment was easy due to the identical length in

the whole tested taxa except for *R. stellata* var. *mirifica* with a six-base insertion at the corresponding position 573-578. Therefore, six gaps were introduced at this corresponding position of the other DNA sequences in order to align all the sequenced data. The resulting matrix was then used for phylogenetic analyses with PAUP (Version 3.1.1) (Swofford 1993). A heuristic search was carried out using MULPARS and TBR (tree bisection-reconnection) branch-swapping for identifying the most parsimonious trees. Bootstrap values were calculated from a 100 replicate parsimony analysis. Another decay analysis was performed with PAUP to determine the relative support for various clades found in the parsimony analysis.

Results

A total of 1518 nucleotides were obtained after alignment. Seventy two base substitutions were observed in all the tested taxa (variant rate=4.7%) and among these substitutions, 17 base substitutions were parsimony-informative characters. All the aligned sequence data were used to search for the maximum parsimonious trees and a single most parsimonious tree with bootstrap values was obtained, as shown in Fig. 1, (tree length=154, consistency index (C. I.)=0.9416, retention index (R. I.)=0.8953).

All the 14 taxa in the section *Synstylae*, associated with two taxa in the section *Indicae*, formed one clade with a 70% bootstrap confidence value. In this clade, *R. brunonii* and *R. moschata* further formed an inner clade with a 57% bootstrap confidence value.

Two clones (1 and 2) of *R. acicularis* formed one clade with a 91% bootstrap confidence value, and this clade was separated from the other taxa of the section *Rosa*. The remaining taxa in the section *Rosa* formed one clade with a 52% bootstrap confidence value. This clade was further subdivided into two inner clades: *R. rugosa* and *R. marretii* formed one clade with a 86% bootstrap confidence value, while *R. acicularis* var. *nipponensis* and *R. willmottiae* formed another single clade with a 63% bootstrap confidence value. In the latter clade, *R. platyacantha* in the section *Pimpinellifoliae* was included. Furthermore, *R. foliolosa* in the section *Carolinae*, associated with the two inner clades, was included in the large clade of the section *Rosa* (Fig. 1).

Three taxa in the section *Banksianae* formed one clade with a 65% bootstrap confidence value. Each of the *Bracteatae* and *Laevigatae* sections, and subgenera *Hulthemia* and *Hesperhodos* failed to form clades with any of the species. Two species, *R. spinosissima* and *R. platyacantha* in the section *Pimpinellifoliae*, also did not form any clade, but *R. platyacantha* was included in the inner clade formed by *R. acicularis* var. *nipponensis* and *R. willmottiae* of the section *Rosa* as described above. Furthermore, another two taxa (*R. roxburghii* var. *normalis* and *R. roxburghii* var. *hirtula*) in the subgenus *Platyrhodon* also failed to form a clade.

Fig. 1. Most-parsimonious tree in 154 steps based on *matK* sequence data using the maximum parsimony method. C.I.=0.9416, R.I.=0.8953. Numbers above the clades indicate the bootstrap confidence values (%), Numbers below the clades indicate the number of character changes that are homoplastic, and those in brackets, the number of additional steps in the decay analysis in which the clade was no longer resolved in the strict consensus.

Discussion

All the tested taxa of the section *Synstylae* were included in one group, even though these taxa were collected over a wide area in Japan and other areas in Asia. These results were also supported by the analysis of the internal transcribed spacers (ITS) region of 18-26S nuclear ribosomal DNA (Wu *et al.* manuscript in preparation). We, therefore,

confirmed that this section, with the common adnate styles protruding from the calyx tube, is an evolved monophyletic group in the genus *Rosa* (Hutchinson 1973, Sporne 1977). In this section, *R. brunonii* is considered to be a closely related species to *R. moschata* (Yü and Ku 1985). Both species are distributed in the western part of Asia and share common morphological traits. As expected, these two species formed an inner clade in this study. However, *R.*

sambucina, which is native to Japan and is considered to be a closely related species to *R. moschata* (Kitamura 1979), was not included in this inner clade. Another four taxa (*R. luciae* var. *hakonensis*, *R. luciae* var. *fujisanensis*, *R. luciae* var. *onoei* and *R. luciae* var. *paniculigera*) in the section *Synsylae* are generally ranked as varieties of *R. luciae* based on the similarity of their morphological characteristics, though they have been treated as separate species by some authors (Momiyama 1989, Ohwi 1983). In this study, their phylogenetic relationships were also poorly identified. We assume that *matK* information is not suitable for elucidating such close relationships of taxa at the variety level.

Section *Indicae* was combined with the clade of section *Synstylae* in this study. The close relationship between them is consistent with their common styles that are exerting from the hypanthium. In the previous *matK* study (Matsumoto *et al.* 1998), similar results were obtained, though another two sections, *Caninae* and *Gallicanae*, were also included in the clade formed by sections *Synstylae* and *Indicae*. However, the latter two sections formed an inner clade prior to the former two ones except for *R. chinensis* var. *spontanea* in the section *Indicae*. *R. chinensis* var. *spontanea* shows a remarkable variation in the flower color even in a single individual (Ogisu 1996). Taking the peculiarity of this heterogeneous species in consideration, we suppose that these two data sets were consistent with each other. The isozyme data (Kim and Byrne 1996) and RAPD data (Jan *et al.* 1999) also supported the validity of our results.

The section *Rosa* formed one clade except for two clones (1 and 2) of *R. acicularis*. This clade was further subdivided into two inner clades while *R. foliolosa* in the section *Carolinae* was related to them. Furthermore, *R. platyacantha* in the section *Pimpinellifoliae* was included in one of the two inner clades (Fig. 1). It is likely that the section *Rosa* is polyphyletic. Matsumoto *et al.* (1998) showed that this section formed one clade except for *R. californica* 'Plena' while another taxon *R. spinosissima* var. *pimpinellifolia* in the section *Pimpinellifoliae* was included in this clade. Since the section *Rosa* consists of the largest number of species in the genus *Rosa*, the present study dealing only with several species is inadequate for the phylogenetic analysis of this section.

Two clones of *R. acicularis*, collected from the boreal area of China and Japan, formed one clade with a high bootstrap confidence value, and this clade was separated from the other taxa of the section *Rosa*. *R. acicularis* is likely to be a distinctive species in the section *Rosa*. However, *R. acicularis* var. *nipponensis*, traditionally treated as one variety of *R. acicularis* by some authors, was included in another clade formed by the remaining taxa of the section *Rosa*. This variety exhibits a very local distribution in the central area of mainland Japan in contrast to its mother species that is generally distributed in the arctic zone of the Northern Hemisphere. Furthermore, this variety is known to be diploid (Erlanson 1929, Flory 1950) compared with the mother spe-

cies that includes various hexaploids and octoploids (Lewis 1957). Rowley (1967) stated that the genus *Rosa* has evolved from an ancestral diploid core to regular polyploids. We assume that *R. acicularis* var. *nipponensis* is a primitive taxon and it should be reassigned to its original specific rank as *R. nipponensis* Crépin, as proposed by Hara (1952) (Lewis 1959). *R. acicularis* might have been derived from the diploid ancestor by polyploidization when it migrated to the arctic areas. Apparently, this distinctive species has evolved into a infraspecific complexities as a result of diversified distribution.

R. marretii, another species in this section, is morphologically closely related to *R. acicularis* and *R. acicularis* var. *nipponensis* by the common traits of glabrous branches among these taxa (Momiyama 1989). In contrast to the traditional concept, our data showed that *R. marretii* is more closely related to *R. rugosa*. *R. rugosa* could be distinguished from all the three taxa described above by its prickles and tomentose branches. However, several natural hybrid intermediates between *R. marretii* and *R. rugosa* have been found in the overlapping regions of their indigenous areas in Hokkaido. The presence of the intermediates suggests the existence of a potentially close relationship between *R. marretii* and *R. rugosa*.

As described above, *R. foliolosa* in the section *Carolinae*, associated with the section *Rosa*, formed one clade with some species of the section *Rosa*. Section *Carolinae* has been found to be closely related to section *Rosa* based on the similarity of their morphological traits (Lewis 1957). RAPD evidence (Jan *et al.* 1999) and *matK* data (Matsumoto *et al.* 1998) also support this close relationship.

Two species, *R. spinosissima* and *R. platyacantha*, in the section *Pimpinellifoliae* did not form any clade. *R. spinosissima* did not form any clade with any of the species, but *R. platyacantha* was included in the clade of section *Rosa*. This section consists of nine diploid species and three tetraploid species but it is widely distributed in Asia and Europe (Roberts 1977). We suggest that it may be one of the polyphyletic groups. Another recent study has also supported this assumption (Matsumoto unpublished data). In particular, the tetraploid *R. spinosissima* occurs from the Korean Peninsula to Western Europe and it also shows an overlapping range of morphological variation. In conjunction with this polymorphic phenomenon, *R. spinosissima* did not form any clade in the present study, while one of the varieties, *R. spinosissima* var. *pimpinellifolia* showed a close relationship with the section *Rosa* in the previous study (Matsumoto *et al.* 1998). Furthermore, the association of the variety with the section *Rosa* suggests that the section *Pimpinellifoliae* may be closely related to the section *Rosa*. Roberts (1977) has stated that two Asian diploid species in the section *Pimpinellifoliae*, *R. farreri* and *R. koreana*, exhibit an affinity to the Chinese *R. forrestiana* in the section *Rosa*. *R. platyacantha* in this study was also included in the clade of the section *Rosa*. In brief, the section *Pimpinellifoliae* is probably one of the polyphyletic groups. Alternatively, some spe-

cies should be included in the section *Rosa*.

Two other tested taxa (*R. roxburghii* var. *normalis* and *R. roxburghii* var. *hirtula*) in the subgenus *Platyrhodon* did not form any clade. *R. roxburghii* var. *normalis* is distributed in Southwestern China while *R. roxburghii* var. *hirtula* shows a limited distribution in the Honshu area of Japan. The separate distribution may have caused this divergence. However, the similarity in their morphological traits does not support this hypothesis. Other ITS sequence data also revealed a close relationship between them (Wu *et al.* manuscript in preparation). Since the *matK* DNA is inherited from the mother line, it is possible that the maternal DNA information partially revealed their relationship. However, additional studies should be carried out to resolve this discrepancy.

We reconsidered the whole Japanese species and also provided implications for the classification of the genus *Rosa* based on the *matK* sequences. Our results showed that the use of *matK* is suitable for clarifying the taxonomy of the genus *Rosa*. Nevertheless, additional genes with a higher mutation rate should be used for a comprehensive analysis of the systematic relationship of the genus *Rosa* and especially for gaining an insight into the relationships of such taxa at the variety level.

Acknowledgements

We thank Dr. Kodama of the Faculty of Horticulture, Chiba University for his technical advice. This research was supported by a Grant-in-Aid for Science Research from the Ministry of Education, Culture, Sports and Science of Japan (No. 09660018).

Literature Cited

- Debener, T., C. Bartels and L. Mattiesch (1996) RAPD analysis of genetic variation between a group of rose cultivars and selected wild rose species. *Mol. Breeding* 2: 321-327.
- Doyle, J.J. and J.L. Doyle (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bul.* 19: 11-15.
- Eriksson, T., M.J. Donoghue and M.S. Hibbs (1998) Phylogenetic analysis of *Potentilla* using DNA sequences of nuclear ribosomal internal transcribed spacers (ITS), and implications for the classification of *Rosoideae* (*Rosaceae*). *Pl. Syst. and Evol.* 211: 155-179.
- Erlanson, E.W. (1929) Cytological conditions and evidences for hybridity in North American wild roses. *Bot. Gaz.* 87: 443-506.
- Flory, W.S. (1950) Pollen condition in some species and hybrids of *Rosa* with a consideration of associated phylogenetic factors. *Virg. J. Sci.* 1: 11-59.
- Hara, H. (1952) Contributions to the study of variations in Japanese plants closely related to those of Europe or North America. Part 1. *J. Fac. Sci. Univ. Tokyo Sect. 3.* 6(2): 29-96.
- Hawkes, J.G. (1981) Biosystematic studies as an aid to breeding research and plant breeding. *Kulturpflanze* 29: 327-336.
- Hutchinson, J. (1973) *General Principles adopted for the classification of flowering plant in the present system.* In "The families of flowering plants, 3rd. ed", Oxford Univ. Press, London, 22-23.
- Jan, C.H., D.H. Byrne, J. Manhart and H. Wilson (1999) Rose germplasm analysis with RAPD markers. *HortScience* 34: 341-345.
- Johnson, L.A. and D.E. Soltis (1995) Phylogenetic inference in Saxifragaceae sensu stricto and *Gilia* (Polemoniaceae) using *matK* sequences. *Ann. Missouri Bot. Gard.* 82: 149-175.
- Kalkman, C. (1988) The phylogeny of the Rosaceae. *Bot. J. Linnean Society* 98: 37-59.
- Kim, K.-J. and R.K. Jansen (1998) A chloroplast DNA phylogeny of lilacs (*Syringa*, Oleaceae): Plastome groups show a strong correlation with crossing groups. *Am. J. Bot.* 85: 1338-1351.
- Kim, Y. and D.H. Byrne (1996) Interspecific hybrid verification of *Rosa* with isozymes. *HortScience* 31: 1207-1209.
- Kitamura, S. (1979) *Rosa*. In "Colored illustrations of woody plants of Japan. Vol. 2". Kitamura, S. and G. Murata, Hoikusha, Osaka, 49-59, pls. 82-83 (in Japanese).
- Lewis, W.H. (1957) An introduction to the genus *Rosa* with special reference to *R. acicularis*. *Virg. J. Sci.* 8: 197-202.
- Lewis, W.H. (1959) A monograph of the genus *Rosa* in North America. I. *R. acicularis*. *Brittonia* 11: 1-24.
- Matsumoto, S. and H. Fukui (1996) Identification of rose cultivars and clonal plants by random amplified polymorphic DNA. *Scientia Hort.* 67: 49-54.
- Matsumoto, S., H. Wakita and H. Fukui (1997) Molecular classification of wild roses using organelle DNA probes. *Scientia Hort.* 68: 191-196.
- Matsumoto, S., M. Kouchi, J. Yabuki, M. Kusunoki, Y. Ueda and H. Fukui (1998) Phylogenetic analyses of the genus *Rosa* using the *matK* sequence: molecular evidence for the narrow genetic background of modern roses. *Scientia Hort.* 77: 73-82.
- Matsumoto, S., M. Kouchi, H. Fukui and Y. Ueda (2000) Phylogenetic analyses of the subgenus *Eurosa* using the ITS nrDNA sequence. *Acta Horticulturae*, 521: 193-202.
- Millan, T., F. Osuna, S. Cobos, A.M. Torres and J.I. Cubero (1996) Using RAPDs to study phylogenetic relationships in *Rosa*. *Theor. Appl. Genet.* 92: 273-277.
- Momiyama, Y. (1989) *Rosa*. In "Wild flowers of Japan. Woody plants, Vol. 1" Satake, Y., H. Hara, S. Watari and T. Tominari (eds.), Heibonsha, Tokyo, 199-204, pls. 217-224 (in Japanese).
- Nicolson, D.H. (1992) Seventy-two proposals for the conservation of types of selected Linnaean generic names, the report of Subcommittee 3C on the lectotypification of Linnaean generic names. *Taxon* 41: 552-583.
- Ogisu, M. (1996) Some thoughts on the history of China roses. *The new plantsman* 3: 152-157.
- Ohwi, J. (1983) *Rosa*. In "New flora of Japan. Phanerogam", Ohwi, J. and M. Kitagawa, Shibundo, Tokyo, 860-864 (in Japanese).
- Rehder, A. (1940) *Rosa*. In "Manual of cultivated trees and shrubs hardy in North America, 2nd ed.", MacMillan, New York, 426-451.
- Roberts, A.V. (1977) Relationship between species in the genus *Rosa*, section *Pimpinellifoliae*. *Bot. J. Linnean Society* 74: 309-328.
- Rowley, G.D. (1967) Chromosome studies and evolution in *Rosa*. *Bull. Jard. Bot. Nat. Belg.* 34: 45-52.
- Rowley, G.D. (1976) Typification of the genus *Rosa* L. *Taxon* 25: 181.
- Sporne, K.R. (1977) Some problems associated with character correlation. *Pl. Syst. Evol. Suppl.* 1: 33-51.
- Sugita, M., K. Shinozaki and M. Sugiura (1985) Tobacco chloroplast tRNA^{Lys} (UUU) gene contains a 2.5-kilobasepair intron: an open reading frame and a conserved boundary sequence in the intron. *Proc. Natl. Acad. Sci. USA* 82: 3557-3561.
- Swofford, D.L. (1993) PAUP: Phylogenetic analysis using parsimony,

- version 3.1.1. Computer program distributed by the Illinois Natural History Survey, Champaign, Illinois.
- Ueda, Y. (1996) *Gensan To Saibaishi*. In "Nogyo Gijutsu Taikei Kakihen, Vol. 7. Bara", Sakamoto N. (eds), Nobunkyo. Tokyo, 257-265 (in Japanese).
- Vogel, J., T. Hübschmann, T. Börner and W. R. Hess (1997) Splicing and intron-internal RNA editing of *trnK-matK* transcripts in Barley plastids: support for *matK* as an essential splicing factor. *J. Mol. Biol.*, 270: 179-187.
- Yü, T.-T., and T.-C. Ku (1985) *Rosa brunonii*. In "Flora Republicae Popularis Sinicae, Tomus 37", Yü, T.-T. (ed.), Science Press, Beijing, 436-437 (in Chinese).