Classification of Flowering Quince Cultivars (genus *Chaenomeles*) using Random Amplified Polymorphic DNA Markers

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Flowering quince (genus *Chaenomeles*) comprises six species, i.e., *C. cardinalis* Carr., *C. cathayensis* (Hemsl.) Sch., *C. eburnea* Nakai, *C. lagenaria* Koidz., *C. speciosa* Nakai and *C. japonica* Lind., and is indigenous to China or Japan (Miyazawa and Wakisaka 1969, Asayama 1986). Many valuable cultivars of flowering quince were developed during the Taisho and early Showa Periods (1910~1940) (Nagao 1966). They are said to have been bred not only by interspecific or intraspecific crossing (Saito and Kaneko 1975), but also by spontaneous bud mutation (Nagao 1966). The lineage for the development and differentiation of cultivars, however, has remained unsolved in spite of the morphological and cytogenetical approaches. Breeding works in flowering quince have been performed for such traits as thornless, large flower, pure-yellow flower, unstanding, early trunk-growth, multibearing, and crown gall resistance (Nakamura 1976). Few cultivars possess these traits except for the thornless cultivar of 'Hinode' and the large flowering ones of 'Sekai-ichi' and 'Mona Lisa'.

It is assumed that Chinese quince (*Pseudocydonia sinensis* Schneid.) and quince (*Cydonia oblonga* Mill.) are closely related to flowering quince (Kubo 1962, Asayama 1986). On the other hand, Chinese quince has been described as *Chaenomeles sinensis* (Miyazawa and Wakisaka 1969). Weber (1964) proposed that these three plant groups belong to the same genus *Cydonia*. Both Chinese quince and quince possess useful traits for flowering quince breeding, but hybrids between these two species and flowering quince could not be developed in spite of much effort (Weber 1964).

Recently, a molecular biological technique based on a random amplified polymorphic DNA (RAPD) assay has been widely applied to the classification and identification of genotypes in arbor crops such as fruit trees (Koller *et al.* 1993, Luo *et al.* 1993) and flowering shrubs (Millan *et al.* 1996, Bartish *et al.* 1999).

In this study, we tried to clarify the genetic relationships among 31 cultivars of flowering quince, Chinese quince 'Oomi-karin' and an uncertain clone of quince by RAPD assay to obtain some information to crossbreeding of flowering quince.

Exploration of primers for RAPD assay in flowering quince

Young leaves developed after pruning were excised from the plants. Total DNA was extracted according to the method of Doyle and Doyle (1987) with a modification of four times rinsing with ethyl alcohol for the elution of impurities from the DNA pellet. DNA could be extracted successfully by this method, in a sufficient volume to serve as the template.

For the classification of flowering quince cultivars, the primers that gave reasonable numbers of strong amplification products using DNA from cv. 'Asahi-botan' as template, were selected from one hundred primers (kits OPA, OPE, OPR, OPT, OPAX, Operon Tech. Co., Alameda, USA) with ten base synthetic oligonucleotides. DNA amplification was performed using a thermal cycler (Program-tempucontrol system PC-700, Astec Co., Fukuoka, Japan). The thermal cycle used was 95°C for 3 min.; then 44 cycles of 94°C for 1 min., 42°C for 2 min and 72°C for 2 min; and finally 72°C for 5 min. RAPD samples were electrophoresed on 1.6% agarose gels at 50 V for 120 min, and then stained with ethidium bromide for 30 min following decolorization for 20 min. Gels were photographed with black and white film #667 (Polaroid, Cambridge, Mass, USA) under UV light. The amplification products per sample were replicated twice for analysis. Analysis was made with the constantly amplified bands. Of 100 primers surveyed, nine (OPE 08, OPR 01, OPR 14, OPT 03, OPT 09, OPT 11, OPT 17, OPT 18 and OPT 19) were finally selected for the classification of flowering quince cultivars. Six primers (OPE 08, OPR 14, OPT 03, OPT 17, OPT 18 and OPT 19) had a GC content of 60% and the remaining three had that of 70%. Shimada *et al.* (1994) reported that adequate amplification products were obtained with the primers with a high GC contents in mume (*Prunus mume* Sieb. et Zucc.). This has also been reported in apricot (*Prunus armeniaca* L.) (Takeda *et al.* 1998). Since the adequate GC content in the primers for RAPD assay in flowering quince seemed to be 60%~70%, these nine primers were employed.

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Fig. 1. Dendrogram from the data of polymorphic DNA bands among 31 cultivars of flowering quince, Chinese quince and quince.

Analysis of genetic relationships among flowering quince cultivars, Chinese quince and quince

Out of 99 RAPD amplification bands produced by the nine primers in 31 cultivars of flowering quince, Chinese quince and quince, 86 bands (87%) were ascertained to be polymorphic. All bands produced by both OPT 17 and OPT

19 were polymorphic in flowering quince cultivars, Chinese quince and quince. In Chinese quince, a specific band was observed with the corresponding four primers (OPR 01, OPR 14, OPT 17 and OPT 19). Moreover a specific band was detected for quince with six primers (OPE 08, OPR 01, OPT 03, OPT 09, OPT 11 and OPT 17) in addition to two

bands produced by OPR 14 primer. Nine bands were deleted in both Chinese quince and quince. For flowering quince cultivars, however, no specific band was recognized with any of the nine primers employed.

The similarity index (SI) among cultivars was calculated by the methods by Kresovich *et al.* (1992) and Mailer *et al.* (1994). A dendrogram was constructed based on the SI matrix data by applying the unweighted pair group method with arithmetic averages (UPGMA) cluster analysis using the PHYLIP ver. 3.572C (Felsenstein 1993).

The dendrogram constructed by SI is shown in Fig. 1. The dendrogram reveals two distinctive clusters: one comprises all cultivars of flowering quince and the other includes both Chinese quince and quince. Accordingly, the present study confirmed that Chinese quince and quince are closely related to each other and are genetically distinct from flowering quince.

Flowering quince cultivars also formed two major clusters which comprised three cultivars, 'Shiro-choju-bai', 'Choju-kan' and 'Hichi-henge', and the remaining 28 cultivars. The latter group of 28 cultivars was further subdivided into nine minor clusters. In the former, 'Choju-kan' resembled 'Shiro-choju-bai' in some morphological traits except for flower color. This cultivar might have been bred from the crossing of 'Beni-choju-bai' (original cultivar for 'Shiro-choju-bai') with 'Beni-botan' (Masuda 1983). Studying the morphological traits, some researchers proposed that 'Shiro-choju-bai' belonged to *C. japonica* (Miyazawa and Wakisaka 1969, Nakamura 1976, Masuda 1983, Asayama 1986). Although Masuda (1983) proposed that 'Hichi-henge' was bred through hybridization between 'Showa-nishiki' and 'Tsukasa-botan', three cultivars in the former group were classified as *C. japonica* type in the present study.

'Kumo-no-mine', 'Hi-aka-kanboke', 'Musashi' and 'Kokko' were included in the same cluster. Since 'Kumo-no-mine' morphologically resembles 'Hi-aka-kanboke', the results seemed to be reasonable. However, Nakamura (1976) indicated that 'Musashi' was derived from the crossing of 'Hi-no-mihata' with 'Hinode-nishiki'. In this study, 'Musashi' was included in the extremely remote cluster of the maternal parent 'Hi-no-mihata', although we did not investigate 'Hinode-nishiki'. Moreover, 'Kokko' was included in this cluster, although 'Kokko' exhibits peculiar traits such as crimson flower color, slender trunk, an irregular chromosome configuration at meiosis and male sterility.

As to the cluster of 'Hi-no-tsukasa', 'Hi-no-hakama' and 'Kagaribi', Miyazawa and Wakisaka (1969) proposed that 'Hi-no-hakama' was derived from 'Toyo-nishiki' (old cultivar) by bud mutation. In the present study, 'Hi-no-hakama' was more closely related to 'Kagaribi' (new cultivar) and 'Hi-no-tsukasa' than 'Toyo-nishiki'. On the other hand, Nagao (1966) reported that 'Takane-nishiki' and 'Nichi-getsu-sei' were bred through the spontaneous bud mutation of 'Toyo-nishiki'. The results of our RAPD assay are in agreement with these reports.

'Wata-boushi' was a hybrid progeny between 'Iwato-

kagura' and 'Showa-nishiki' (Nagao 1966). In this study, these 3 cultivars were included in the same cluster together with two cultivars of 'Iwai-nishiki' and 'Beni-komachi', confirming the relationship of 3 cultivars suggested in the previous study.

As shown in Fig. 1, the remaining 13 cultivars formed five distinctive clusters. In three clusters comprising 'Katsuranishiki' to 'Choju', 'Tsukasa-botan' to 'Hinode', and 'Taikonishiki' to 'Hakusen', respectively, three cultivars in the same cluster exhibited some distinctive traits. Thus, the cultivars which belonged to each of the three clusters were also suggested to be closely related by RAPD assay in the present study.

'Hi-no-mihata' closely resembled 'Maiko' in morphology. 'Asahi-botan' and 'Akane' were introduced from the same nursery and exhibited similar morphology and plant form. In this study, the former two cultivars formed one cluster, and the latter two formed another cluster. Accordingly, it was ascertained that cultivars in each cluster were closely related to each other.

Recently, Bartish *et al.* (1999) reported that 14 accessions belonging to four species (*C. cathayensis*, *C. speciosa*, *C. × superba* and *C. japonica*) formed four independent clusters by an RAPD assay. In more developed cultivars of flowering quince, cultivars in the same cluster might be closer in their lineage relationship.

In this study, both Chinese quince and quince were classified as species genetically distinct from the flowering quince, which explains the failure of hybrid production between these species and the flowering quince. Secondly, we propose one independent genetic group of *C. japonica* type and nine minor groups for 31 cultivars of flowering quince that have been chaotically classified by the conventional morphological and cytological works. In Japan, collection of flowering quince cultivars requires great efforts since it is a minor ornamental plant. We are now collecting the genetic resources for breeding of flowering quince in addition to the 31 specimens described in this study. In this context, the exploitation for native species in China would play a role in the development of new cultivars of Japanese flowering quince. Analysis for phylogenetic relationships among accessions available in the future should provide some valuable information for genetic and breeding works of flowering quince.

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