



Short communication

Citrus hybrid seedlings reduce initial time to flower when grafted onto shiikuwasha rootstock

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ABSTRACT

The long juvenile period of citrus seedlings before flowering is one of the major obstacles in citrus breeding. Under the citrus breeding program of National Institute of Fruit Tree Science in Japan, 299 citrus hybrid seedlings were grafted onto shiikuwasha (*Citrus depressa* HAYATA) and cultivated. Approximately one-third of the plants flowered two and a half years after grafting. The average length of the main stems of flowering plants was 308 cm, while that of non-flowering plants was 264 cm. Half of the plants having main stem length ≥ 300 cm formed flowers, while most of the plants having main stem length < 210 cm formed no flower. These results indicate that plant vigor influences the flowering and that grafting effectively accelerated flowering. The proportion of flowering plants varied among cross combinations, ranging from 71.4% to 8.0% among the 10 cross combinations used.

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1. Introduction

Woody plant breeding is time consuming and requires a number of years before plants can be evaluated. In the case of citrus, almost 20 years are required from crossing to cultivar release to the industry. This is primarily due to the extremely long period of juvenile period of citrus seedlings before flowering, which is one of the major obstacles for citrus breeders. Effective methods are desirable to shorten the juvenile period and to increase number of fruits for evaluation of the fruit characteristics.

Several attempts to shorten the juvenile period of woody plants have been reported. Zimmerman (1971) showed that growing crabapple seedlings in a greenhouse greatly reduced the juvenile period. Furr et al. (1947) demonstrated that ringing in summer induced flowering in 7-year-old citrus seedlings. On the other hand, Takahara et al. (1980) showed that the effect of ringing on flowering varied depended on the female parent of the seedlings. Grafting is one of the effective methods to shorten the juvenile

period. Citrus hybrid seedlings grafted onto satsuma mandarin (*Citrus unshiu*) interstocks or onto trifoliolate orange (*Poncirus trifoliata* L. Raf.) rootstocks formed flowers two to three years after grafting (Okudai et al., 1980). In Japan, trifoliolate orange is used as rootstock for the cultivation of most citrus cultivars because many citrus cultivars are compatible with trifoliolate orange and this species is tolerant to cold and resistant to tristeza virus disease. However, trifoliolate orange is not suitable for use as rootstock of less vigorous cultivars.

Vigor is a very important factor to induce flowering in fruit breeding (Zimmerman, 1972). A negative relationship between vigor and juvenile period has been reported in apple (Visser, 1970; Way, 1971) and pear (Zimmerman, 1977). Shiikuwasha (*C. depressa* HAYATA) is native to the southwestern areas of Japan and Taiwan, and instead of trifoliolate orange, it is considered to be one of the most promising rootstocks for cultivars such as 'Otani Iyokan' (*C. iyo* hort. ex Tanaka), which is characterized by a low tree vigor (Takahara et al., 1994).

In this study, citrus hybrid seedlings were grafted onto shiikuwasha rootstocks to accelerate flowering of the hybrid seedlings. Growth characteristics of the hybrid seedlings were examined. The use of grafting to accelerate progeny derived from citrus breeding programs was also discussed.

2. Materials and methods

This study was carried out in the breeding orchard and the greenhouse of the Kuchinotsu Citrus Research Station, National Institute of Fruit Tree Science (36° 33'N, 136° 21'E).

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Table 1
Cross combinations of citrus hybrid seedlings grafted onto shiikuwasha rootstock

No.	Cross combination		No. of seedlings
	Seed parent	Pollen parent	
1	Ariake	× ((Encore × Okitsu wase) × Youkou)	16
2	Clementine	× ((Encore × Okitsu wase) × Youkou)	25
3	(Encore × Okitsu wase)	× (Kiyomi × Otani iyokan)	31
4	(Kiyomi × Encore)	× (Kiyomi × Otani iyokan)	35
5	((Kiyomi × Encore) × Encore)	× (Kiyomi × Otani iyokan)	49
6	((Kiyomi × Encore) × Encore)	× (Nishinokaori × Otani iyokan)	36
7	(Kiyomi × Kuchinotsu No. 4)	× (Kiyomi × Encore) × Minneola)	24
8	Nankou	× ((Encore × Okitsu wase) × Youkou)	14
9	Nankou	× (Kiyomi × Otani iyokan)	28
10	Nankou	× (Nishinokaori × Otani iyokan)	41
Total			299

Ariake, *C. sinensis* L. Osbeck × Clementine; Clementine, *C. clementina* 'Clementine'; Encore, *C. nobilis* × *C. deliciosa*; Kiyomi, *C. unshiu* Marc. 'Miyagawa wase' × *C. sinensis* 'Trovia'; Kuchinotsu No. 4, *C. unshiu* Marc. 'Hayashi unshiu' × Clementine; Minneola, *C. paradisi* 'Duncan' × *C. tangerina* 'Dancy'; Nankou, *C. unshiu* Marc. 'Miho wase' × Clementine; Nishinokaori, Kiyomi × *C. sinensis* 'Trovia'; Okitsu wase, *C. unshiu* Marc. 'Okitsu wase'; Otani iyokan, *C. iyo hort. ex Tanaka*; Youkou, Kiyomi × *C. reticulata* Blanco.

Cross combinations and numbers of the citrus hybrid seedlings for the combinations used in this study are shown in Table 1. The crosses were made in the spring of 1995. Hybrid seeds were germinated in pots in the fall of 1995 and grown in the greenhouse for one year. Four or five buds were cut off from each of the hybrid seedlings and they were side-grafted onto a 3-year-old shiikuwasha (*C. depressa* HAYATA) seedling in the orchard in the fall of 1996. From the spring of 1997, one shoot per plant was allowed to grow while the other shoots were removed. The upper part of the rootstock from the grafted union of the left shoot was cut down. Plants were grown as a single stem by removing lateral branches until the spring of 1998. In April 1998, each plant was inclined by 30° from the vertical at a height of 180 cm and tied to a pole. Then, they were grown with lateral branches until the spring of 1999.

In April 1999, the length of the main stem, the circumference of rootstock, the circumference of scion, the number of lateral shoots, and the number of flowers were measured.

3. Results

Of the 301 citrus hybrid seedlings grafted, 299 grew successfully. Table 2 shows the growth characteristics of flowering and non-flowering plants raised by grafting citrus hybrid seedlings onto shiikuwasha rootstocks. The average length of the main stems of 299 plants was 279 cm, and approximately one-third of the plants grown formed flowers two and a half years after grafting. The maximum number of flowers per plant was approximately

2600 (data not shown) and the average number of flowers per flowering plant was approximately 200.

The proportion of flowering plants was low for plants whose main stems were shorter than 240 cm, while half of the plants whose main stems were longer than 300 cm formed flowers (Fig. 1A). The proportion of flowering plants was very low for plants having circumference of the scion less than 60 mm, while approximately 60% of the plants having circumference of the scion greater than 75 mm formed flowers (Fig. 1B). The relationship between number of lateral branches and proportion of flowering plants was also examined (Fig. 1C). Approximately 60% of the plants with more than 150 lateral branches formed flowers, while only 40% of the plants with less than 120 lateral branches formed flowers.

There are also strong evidences of the relationship of hybrid cross combination and the proportion of flowering (Table 1, Fig. 2). Among the 10 cross combinations, the proportion of flowering plants ranged from 71.4% (No. 5; ((Kiyomi × Encore) × Encore) × (Kiyomi × Otani iyokan)) to 8.0% (No. 2; Clementine × ((Encore × Okitsu wase) × Youkou)).

4. Discussion

Yoshida (1980) reported that citrus hybrid seedlings (not grafted onto rootstocks) began to form flowers seven years after germination. After 10 years, the proportion of hybrid seedlings that formed flowers was 57%. Compared to this study, grafting accelerated flowering of citrus hybrid seedlings and therefore, is an effective method to shorten the juvenile period. Our results show that plant vigor influences flowering. Carbohydrates levels have been suggested as a limiting factor for flower formation in citrus. For example, it has been reported that girdling and fruit removal in October increased flower production in 'Murcott' (Goldschmidt et al., 1985). More vigorous seedlings might form flowers earlier as a result of higher levels of carbohydrate.

Citrus hybrid seedlings grafted both onto satsuma mandarin interstocks and onto trifoliolate orange rootstocks formed flowers a few years after grafting (Okudai et al., 1980). In that study, flowering of hybrid seedlings grafted onto satsuma mandarin interstocks in the open air occurred earlier than that of hybrid seedlings grafted onto trifoliolate orange rootstocks in a greenhouse. Thus, in the breeding program conducted at the National Institute of Fruit Tree Science in Japan, the method of grafting onto satsuma mandarin interstocks has been used. That method accelerates breeding because flowering occurs early and 10–15 citrus hybrid seedlings can be grafted onto an interstock. One disadvantage, however, is that a large number of more than 10-year-old interstocks should be prepared for the cross breeding program every year. Meanwhile, it was reported that growing crabapple seedlings in a greenhouse greatly shortened the juvenile period (Zimmerman, 1971), but growing seedlings in a glasshouse cannot be carried out on a large scale because of space limitations (Okudai et al., 1980). Although it is difficult to compare the results of shiikuwasha rootstocks in this study with those of trifoliolate orange rootstocks in the study of Okudai et al. (1980) the growth and the

Table 2
Growth characteristics of flowering and non-flowering plants raised by grafting citrus hybrid seedlings onto shiikuwasha rootstocks

	No. of plants	Circumference of rootstock ^a (mm)	Circumference of main stem ^a (mm)	Length of main stem ^a (cm)	No. of lateral branches ^a	No. of flowers
Flowering	102	123.1 ± 19.0a	82.3 ± 16.4a	308.2 ± 41.1a	91.9 ± 54.4a	212
Non-flowering	197	109.6 ± 17.7b	61.6 ± 17.0b	264.4 ± 69.7b	55.2 ± 45.9b	–
Total	299	114.2 ± 19.2	68.7 ± 19.4	279.4 ± 64.8	72.2 ± 51.9	–

Different letters (a, b) within columns indicate significant difference at $P < 0.05$ by Tukey's test.

^a Data are means ± S.D. ($n = 102$ or 197 or 299).

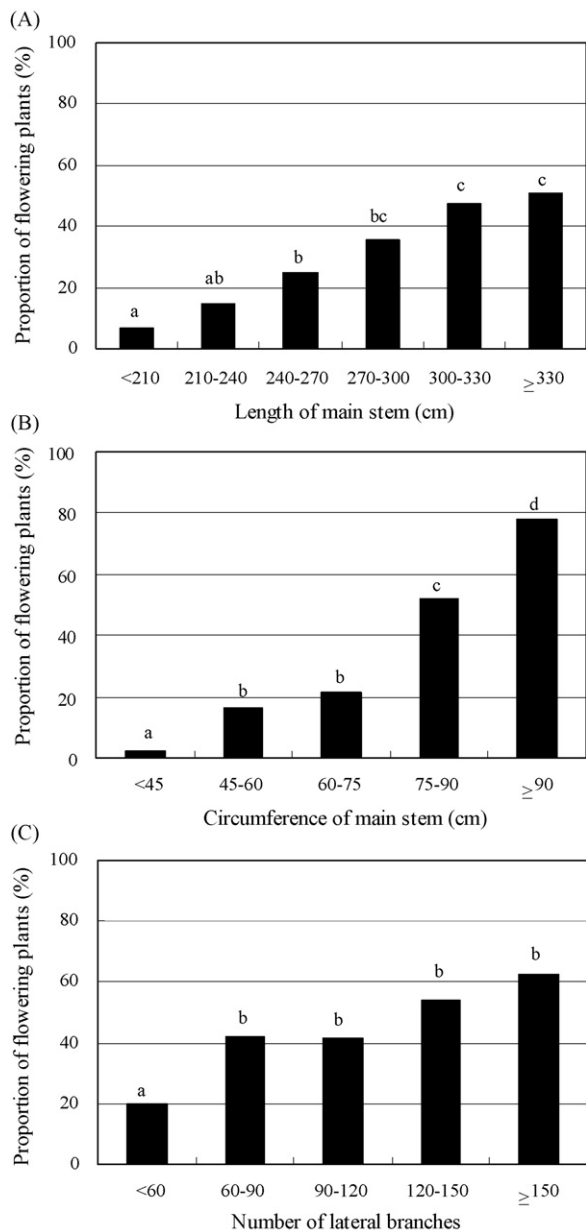


Fig. 1. Relationship between growth characteristics and proportion of flowering of plants raised by grafting citrus hybrid seedlings onto shiikuwasha rootstocks. (A) Relationship between main stem length and proportion of flowering. (B) Relationship between circumference of the main stem and proportion of flowering. (C) Relationship between number of lateral branches and proportion of flowering. Different letters within each graph frame indicate significant differences at $P < 0.05$ by Fisher's exact test.

proportion of flowering plants in the two studies are comparable. Thus, we can conclude the grafting of hybrid seedlings onto shiikuwasha rootstock is an effective method to induce rapid growth even in open air and reduce the juvenile period.

Our grafting method was tested in the breeding program and found to be applicable to various cross combinations. As shown in Table 1, crosses were made with various varieties and cultivars; thus, it would be difficult to analyze in detail the effect of cross combination on the proportion of flowering plants. Nevertheless, we found that the proportion of flowering plants of No. 5 was higher than that of No. 6, and that of No. 9 was higher than that of No. 10 (Fig. 2), indicating that the proportion of flowering plants derived from pollen parent 'Kiyomi' × 'Otani iyokan' was higher

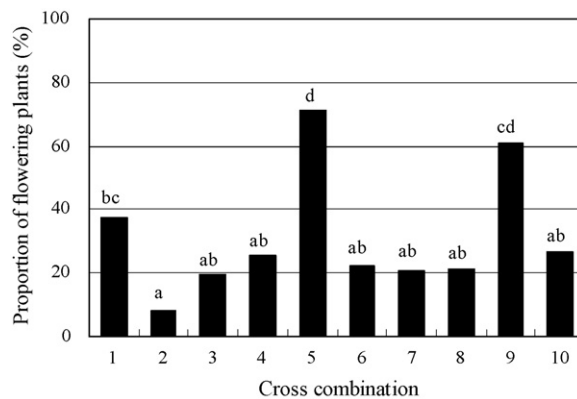


Fig. 2. Relationship between cross combination and proportion of flowering plants raised by grafting citrus hybrid seedlings onto shiikuwasha rootstocks. Cross combination numbers correspond to the numbers in Table 1. Different letters indicate significant differences at $P < 0.05$ among cross combinations by Fisher's exact test.

than that from the pollen parent 'Nishinokaori' × 'Otani iyokan'. It has been previously reported that seedlings of lemon, lime, and mandarin have shorter juvenile periods than seedlings of other genotypes, such as grapefruit and orange (Spiegel-Roy and Vardi, 1982; Snowball et al., 1994). As shown in Table 1, 'Nishinokaori' was derived from 'Kiyomi' (*C. unshiu* Marc. 'Miyagawa wase' × *Citrus sinensis* 'Trovita') × *C. sinensis* 'Trovita', which might have lowered the proportion of flowering seedlings of No. 6 and 10 lower. Together, the results demonstrate that it might take a longer time for citrus hybrid seedlings derived from varieties and/or cultivars with longer juvenile periods to form flowers, even if they are grown with our grafting method.

Of course, citrus seedlings may show early flowering if early flowering genotypes are used as parents. In our breeding program, seed and pollen parents are often selected depending on the fruit characteristics. Citrus breeders may use late flowering genotypes as parents. Thus, the method to shorten the juvenile period should be applicable to almost of all genotypes. In recent years, transformed plants have been produced to constitutively express the *Arabidopsis* *LEAFY* (*LFY*) or *APETALA1* (*API*) gene that promotes flower initiation in *Arabidopsis*. The expression of either of these genes is sufficient to promote precocious flowering in citrange (Peña et al., 2001). On the other hand, homologues of both genes were isolated from *C. sinensis* and characterized (Pillitteri et al., 2004). These results would contribute to the development of new methods to shorten the juvenile period of hybrid seedlings in the future. At this stage, however, these genes cannot be utilized for large-scale cross breeding programs. Consequently, grafting methods should be improved continuously to breed new cultivars with favorable characteristics by optimizing the information of rootstocks and cultivation techniques.

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