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Relationship between Seedlessness of Keraji (*Citrus keraji* hort. ex Tanaka) and Female Sterility and Self-incompatibility

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Summary

We characterized the mechanism of seedlessness of Keraji (*Citrus keraji* hort. ex Tanaka), cultivar grown in Kikaijima island, Kagoshima. This cultivar is a diploid (2n=18) that produces a medium amount of fertile pollen which readily fertilizes other citrus cultivars. It is highly parthenocarpic, setting 30-50% of unpollinated flowers. When Keraji flowers are hand-pollinated with 'Hassaku' pollen, these fruits produce only 2 to 3 seeds. However, 19-20% of these hand-pollinated flowers set seedless fruits, whereas 30-38% of open-pollinated flowers set parthenocarpically. Self-pollinated flowers resulted in 91-93% parthenocarpic fruits; the remaining 7-9% of the fruits had only one seed. Six days after pollination, the self-pollinated flowers had 1.4 pollen tubes at the base of the style, a figure that is much lower than that in cross-pollinated flowers with 'Hassaku' pollen (61 ± 7.4). These observation demonstrated that seedlessness of Keraji results from strong female sterility, self-incompatibility and parthenocarpy.

Key Words: citrus, incompatibility, Keraji, seedlessness, sterility.

Introduction

Keraji (*Citrus keraji* hort. ex Tanaka) which is cultivated in Kikaijima island in Kagoshima Prefecture has relatively small fruit with little or no seeds but excellent flavor. Seedlessness is a very desirable characteristic in citrus, so that several studies have been conducted to elucidate the mechanism of seedlessness (Iwamasa, 1966; Soost and Cameron, 1969; Yamamoto et al., 1995). Male and female sterility and self-incompatibility influence seedlessness in fruits derived from open pollination; triploid individuals produce seedless fruits.

However, there is no report about the mechanism of seedless fruit production of Keraji. This research was conducted to determine ploidy, male and female sterility/fertility, self-incompatibility/compatibility, and parthenocarpic ability of this cultivar.

Materials and Methods

All trees were grafted on trifoliate orange (*Poncirus trifoliata* Raf) and grown in the Toso orchard, Faculty of Agriculture, Kagoshima University.

In late April 1999, 100 flowers of 'Kawano natsudaidai' (*C. natsudaidai* Hayata) were hand-pollinated with pollen of Keraji. The flowers were emasculated and enclosed in bags to prevent open pollination. All hand- and 50 open-pollinated fruits were sampled at random in December 1999, and their weight and seed numbers recorded.

Pollination experiments, using Keraji as a seed parent, were conducted in late April 1999 and early May 2000. The three treatments were: pollinated with 'Hassaku' (*C. hassaku* hort. ex Tanaka) pollen, self-pollinated, and unpollinated. In both years, 100 flowers on each treatment were emasculated and enclosed in bags, but only 50 flowers were pollinated with pollen of 'Hassaku' in 1999. All treated fruits and 50 open-pollinated fruits were sampled at random in November 1999 and 2000, and their weight and seed numbers recorded. The seeds from 100 open-pollinated fruits of Keraji were also counted in November 1998.

Pollen tube growth in the pistils of self- and cross ('Hassaku')-pollinated Keraji flower was examined on May 2000. Six days after pollination, 10 flowers were collected and fixed in methanol - acetic acid (3:1, V/V). Squash preparation of pistils were stained with decolorized anilin blue (Martin, 1958) for fluorescence microscopy.

Keraji chromosomes from root tips of open-pollinated seedlings were stained with 2% Giemsa solution (Merck Co.) and observed as described by Yamamoto et al. (1999).

The pollen fertility and the number of embryos of Keraji were determined according to Ueno (1986) and Okudai et al. (1981), respectively.

Results and Discussion

The number of seeds per fruit and the percentage of seedless fruits for open-pollinated flowers of Keraji in

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1998 to 2000 were consistently 1.6-1.9, and 30.0-38.0 %, respectively (Table 1). Chromosome counts confirmed that Keraji was diploid (2n=18) (Fig. 1). Those results indicate that seedlessness of Keraji is caused by the sterility of reproductive organs and/or incompatibility at the diploid level. However, Keraji is not male sterile as it produces a medium yield of fertile pollen (Table 2); 'Kawano natsudaidai' fruits produced 29.5 seeds on an average after pollination with Keraji versus 24.2 seeds per fruit for fruits resulting from open-pollinated flowers (Table 3). Furthermore, fruits obtained from Keraji-pollinated flowers were larger than those from open-pollinated flowers (Table 3).

In 1999, Keraji fruits resulting from pollination with 'Hassaku' pollen contained 2.6 seeds per fruit (Table 4), whereas self-pollinated fruits contained only 0.1 seed per fruit; thus, 93.1% of these fruits were seedless. Although 30% of unpollinated flowers set, those fruits were relatively smaller than the pollinated ones. Similar results were obtained in 2000. As many as 61 pollen tubes were observed at the base of the style six days after cross pollination, while only 1.4 pollen tubes were present at the base of the styles in self-pollinated ones (Table 5).

Since Keraji and 'Hassaku' are cross-compatible (Table 5), the low seed number of Keraji crossed with Hassaku, demonstrate female sterility of Keraji. Because female sterility/fertility which is directly related to seediness (Yamamoto et al., 1995), female sterility of Keraji is almost as strong as those of Satsuma mandarin (*C. unshiu* Marc.) and Navel orange (*C. sinensis* (L.) Osb.). The latter cultivars are considered to be female

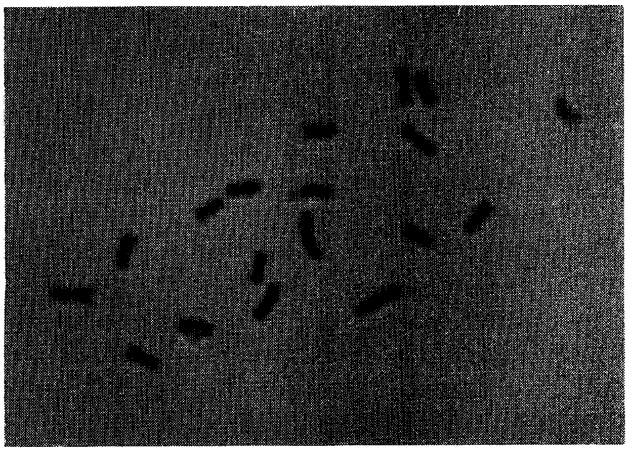

Fig. 1. Keraji chromosomes at metaphase plate (2*n*=18).

Table 1. Number of seeds per fruit and percentage of seedless fruits in open-pollinated Keraji fruits.

Year	Number of fruits observed	Number of seeds per fruit	Percentage of seedless fruits
1998	100	1.7 ± 0.19	32.0
1999	50	1.9 ± 0.28	30.0
2000	50	1.6 ± 0.29	38.0

Table 2. Pollen yield and fertility of Keraji.

Year	Pollen yield[z]	Pollen fertility[y] (%)
1999	Medium	73.8
2000	Medium	70.0

[z]A standard cultivar giving medium pollen yield is Miyauchi iyokan.

[y]Pollen fertility was determined by an acetocarmin method accrding to Ueno (1986).

Table 3. Number of seeds per fruit and fruit weight of 'Kawano natsudaidai' fruit pollinated with Keraji or open-pollinated (1999).

Pollen parent	Number of flowers pollinated	Number of fruits set	Number of seeds per fruit	Fruit weight (g)
Keraji	100	34	29.5 ± 1.06	412 ± 8.4
Open-pollinated	-	50	24.2 ± 0.61	377 ± 7.9

Table 4. Pollen source effects on number of seeds per fruit, percentage of seedless fruits and fruit weight of Keraji.

Pollen parent or treatment	Number of flowers pollinated	Number of fruits set	Number of seeds per fruit	Percentage of seedless fruits	Fruit weight (g)
1999					
Hassaku	50	39	2.6 ± 0.38	20.5	78 ± 4.2
Self-pollinated	100	29	0.1 ± 0.13	93.1	69 ± 2.1
Unpollinated	100	30	0	100	57 ± 2.5
Open-pollinated	-	50	1.9 ± 0.28	30.0	82 ± 1.5
2000					
Hassaku	100	73	2.2 ± 0.22	19.2	69 ± 1.5
Self-pollinated	100	45	0.1 ± 0.04	91.1	57 ± 1.7
Unpollinated	100	50	0	100	55 ± 1.7
Open-pollinated	-	50	1.6 ± 0.29	38.0	62 ± 1.7

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Table 5. Number of pollen tubes observed in the style of Keraji six days after pollination (2000).

Pollen parent or treatment	Number of pollen tubes		
	Top of the style	Middle of the style	Base of the style
Self-pollinated	29.4 ± 6.5	4.6 ± 4.6	1.4 ± 1.4
Hassaku	>100	>100	61.0 ± 7.4

sterile and usually produce seedless fruits (Nishiura and Iwasaki, 1963; Yamamoto et al., 1995).

The production of seedless fruits and inhibition of pollen tube growth after self-pollination demonstrated self-incompatibility in Keraji. Self-incompatibility impedes seed formation (Yamamoto et al., 1995); several cultivars grown in single plantings produce seedless fruit because of self-incompatibility (Soost, 1964; Iwamasa and Oba, 1980; Li, 1980). However, mixed cultivation of self-incompatible cultivars with male fertile ones yield seedy fruits unless they exhibit female sterility. In this study, Keraji trees, surrounded by various male fertile cultivars, formed mostly seedless fruits (Table 1). These results confirm that Keraji is female sterile and self-incompatible, and therefore produce seedless or fruits with few seeds even if mix-planted with male fertile cultivars.

Keraji usually sets parthenocarpically, e. g., the percentage of fruit set on unpollinated flowers was relatively high in both years. However, unpollinated fruits were smaller than pollinated ones. Since the average fruit size of Keraji is smaller than those of many other commercial cultivars, the reduction in size of seedless fruit may be a disadvantage in the market.

This study reveals that seedless Keraji results because it is inherently strong female sterile, self-incompatible, and it sets parthenocarpic fruits. Self-incompatibility in *Citrus* is ruled by a gametophytic S-gene (Soost, 1969) so that female sterility and parthenocarpy are heritable traits (Sykes, 1998; Vardi et al., 2000; Yamamoto et al., 2001). Thus, we think that Keraji has value as a parent in the breeding of new seedless cultivars. It is sometimes difficult to obtain many hybrids from sterile parents (Nishiura and Iwasaki, 1963). Keraji is not suitable as a seed parent because of its female sterility and high embryo count (15.9 ± 1.12) which probably impedes production of hybrids. However, it is a good pollen parent to conduct seedless breeding because it produces sufficient fertile pollen.

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Literature Cited

Iwamasa, M., 1966. Studies on the sterility in genus *Citrus* with special reference to the seedlessness. Bull. Hort. Res. Sta. B 6: 1-81.

Iwamasa, M. and Y. Oba. 1980. Seedlessness due to self-incompatibility in Egami-Buntan, a Japanese pummelo cultivar. Bull. Fac. Agr., Saga Univ. 49: 39-45 (In Japanese with English summary).

Li, S.-J. 1980. Self-incompatibility in 'Matou' wentan (*Citrus grandis* (L.) Osb.). HortScience 15: 298-300.

Martin, F. W. 1958. Staining and observing pollen tubes in the style by means of fluorescence. Stain Technol. 34: 125-128.

Nishiura, M. and T. Iwasaki. 1963. Studies on the citrus breeding I. Variation of seed formation in citrus crossing. Bull. Hort. Res. Sta. B 2: 1-13 (In Japanese with English summary).

Okudai, N., I. Oiyama and T. Takahara. 1981. Studies on the improvement of zygotic seedling yield of polyembryonic citrus. I. Difference in embryo number per seed and zygotic seedling yield among its varieties and strains. Bull. Fruit Tree Res. Stn. D 3: 9-21 (In Japanese with English summary).

Soost, R. K. 1964. Self-incompatibility in *Citrus grandis* (Linn.) Osbeck. Proc. Amer. Soc. Hort. Sci. 84: 137-140.

Soost, R. K. 1969. The incompatibility gene system in citrus. Proc. First Int. Citrus Symp. vol. 1: 189-190.

Soost, R. K. and J. W. Cameron. 1969. Tree and fruit characters of *Citrus* triploids from tetraploids by diploid crosses. Hilgardia 39: 569-579.

Sykes, S. R. 1998. The current status of conventional breeding of improved citrus scion varieties for Australia by CSIRO. p.63-67. In: M. Omura, T. Hayashi and N. S. Scott (eds.). Breeding and biotechnology for fruit trees. Proc. 2nd Japan-Australia International Workshop, NIFTS/CSIRO.

Ueno, I. 1986. Studies on the inheritance of citrus flower characteristics. I. Segregation of viable pollen production in hybrid seedlings. Bull. Fruit Tree Res. Stn. B 13: 1-9 (In Japanese with English summary).

Vardi, A., H. Neumann, A. Frydman-Shani, Y. Yaniv and P. Speigel-Roy. 2000. Tentative model on the inheritance of juvenility, self-incompatibility and parthenocarpy. Acta Hort. 535: 199-205.

Yamamoto, M., T. Haji, M. Yamaguchi, H. Yaegaki, T. Sanada, K. Kudo and N. Mase. 1999. Fluorescent banding pattern of peach [*Prunus persica* (L.) Batsch] chromosomes. J. Japan. Soc. Hort. Sci. 68: 471-475.

Yamamoto, M., R. Matsumoto and T. Kuniga. 2001. Inheritance of female sterility in *Citrus*. Breed. Res. 3: 49-51 (In Japanese).

Yamamoto, M., R. Matsumoto and Y. Yamada. 1995. Relationship between sterility and seedlessness in citrus. J. Japan. Soc. Hort. Sci. 64: 23-29.

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雌性不稔性および自家不和合性に起因するケラジ (*Citrus keraji* hort. ex Tanaka) の無核性

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摘　要

ケラジ (*Citrus keraji* hort. ex Tanaka) は，無核性で果実に特有の芳香を備えた鹿児島県喜界島の特産カンキツである．本研究ではその果実の無核性機構の解明を行なった．

1999年には，自然受粉果の含核数は1.9個で，無核果率は30.0%であった．他家受粉区でも含核数は2.6個と少なく，自家受粉区では含核数は0.1個で無核果率は93.1%にもなった．花粉遮断区の果実は小さかったが，30%が結実した．本種は二倍体 (2n=18) であり，花粉稔性は73.8%で，その量も多かった．2000年も1999年とほぼ同様の結果が得られた．また，受粉6日後において他家受粉では61.0本の花粉管が花柱の基部まで達していたのに対して，自家受粉ではわずかに1.4本の花粉管が花柱基部に達していたにすぎなかった．以上から，ケラジの示す無核・少核性は，本種が雌性不稔性，自家不和合性および単為結果性を備えていることに起因することが明らかになった．

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