

## Self- and Cross-incompatibility of Various *Citrus* Accessions

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Self-incompatibility and compatibility of 65 citrus accessions were determined by pollen tube growth in the style. Lemon was self-compatible whereas the six pummelos and seven out of 11 its relatives were self-incompatible. Furthermore, among sour oranges and their relatives, two out of six accessions were, likewise, self-incompatible as were one out of five sweet orange and their relatives. Every yuzu and its relatives, except hyuga-natsu, were self-compatible. Of the mandarin and its relatives, 14 out of 28 accessions were self-incompatible, whereas calamondin, a kumquat relative, and Hedzuka daidai of diverse origin were self-compatible. Thirty-one out of 65 accessions used in this study were self-incompatible. Of the self-incompatible accessions, clementine and ‘Ariake’, a hybrid of navel orange and clementine, were cross-incompatible. Thus, the incompatible genotypes of those two accessions were considered to be identical.

**Key Words:** citrus, cross-incompatibility, pollen fertility, pollen tube, self-incompatibility.

### Introduction

Self-incompatibility and parthenocarpy in *Citrus* are very important traits for fruit production because they result in seedless fruit (Iwamasa and Oba, 1980; Li, 1980; Yamamoto and Tominaga, 2002; Yamamoto et al., 1995). Without parthenocarpy, however, cross pollination is required to achieve stable fruit production (Hearn, 1969; Krezdorn and Robinson, 1958; Mustard et al., 1956; Reece and Register, 1961; Soost, 1956). Thus, much research has been conducted to determine self-incompatibility of major cultivars and many accessions, such as pummelos. These studies have uncovered cross-incompatibility among several artificial hybrids (Hearn, 1969; Iwamasa and Oba, 1980; Krezdorn and Robinson, 1958; Li, 1980; Miwa, 1951; Mustard et al., 1956; Nagai and Tanikawa, 1928; Ngo, 2001; Nishiura and Iwasaki, 1963; Soost, 1956, 1964; Yamamoto and Tominaga, 2002).

Soost (1969) reported gametophytic incompatibility systems in *Citrus*. He determined the incompatibility (S) allele of some self-incompatible cultivars. Moreover, he discovered the existence of one self-incompatible allele among self-compatible *C. paradisi*. Vardi et al. (2000) and Ngo (2001) reported the S genotypes of several important accessions that are both self-incompatible and compatible.

Although various studies of self- and cross-incompatibility have been conducted, determination of incompatibility was limited. Thus, there are still unresolved accessions and cross combinations, so that,

in this study, we examined self-incompatibility of various accessions and cross-incompatibility of several cross combinations that resulted in new self- and cross-incompatibility progenies. In addition, we proposed the S genotype of a few accessions.

### Materials and Methods

*Citrus* accessions were grafted on trifoliolate orange (*Poncirus trifoliata*) and preserved in the Toso orchard of the Faculty of Agriculture, Kagoshima University. All pollination experiments were conducted during the flowering season (late April to early May) in 2000 to 2004.

Self- and cross-incompatibility/compatibility were based on by pollen tube growth in the style. The hand-pollinated flowers that were emasculated and enclosed in bags to prevent open pollination were collected 6 to 8 days later and fixed in methanol–acetic acid (3:1, v/v) and stored at –20°C until use. Squash preparations of pistils were stained with decolorized anilin blue (Martin, 1958) and examined by fluorescence microscopy. Sixty-five accessions were used to determine self-incompatibility and compatibility (Table 1). To determine cross-incompatibility and compatibility of closely related self-incompatible accessions, eight cross pollinations were conducted (Table 2). The number of pollen tubes present in the upper, middle, and basal portions of the style was counted and their growth behavior was observed to determine incompatibility or compatibility.

The pollen fertility of 65 accessions was determined according to Ueno (1986) and their yield was categorized into five grades: 1, poor; 2, slightly poor; 3, medium; 4, slightly rich; and 5, rich. Example of accessions with poor, medium, and rich yield of pollen were ‘Encore’

**Table 1.** Pollen yeild and fertility and self-incompatibility/compatibility of *Citrus* accessions used in this study.

Classi- fica- tion number <sup>z</sup>	Accession	Latin name Cross combination	Pollen			Self incompatibility/compatibility							
			Year	Yield <sup>y</sup>	Fertility (%)	Year	No. of days after pollination	No. of flowers observed	No. of pollen tubes <sup>x</sup>			SI or SC <sup>w</sup>	Result of previous report <sup>v</sup>
									Top of the style	Middle of the style	Base of the style		
Lemon													
36	Allen eureka	<i>Citrus limon</i> (L.) Burm. f.	2003	3	54.5	2004	6	3	46.3	15.7	10.3	SC	
Pummelo													
56	Anseikan	<i>C. maxima</i> (Burm.) Merr.	2003	5	93.4	2001	6	4	17.3	1.7	0.8	SI	SI(11) <sup>v</sup>
56	Banokan	<i>C. maxima</i> (Burm.) Merr.	2003	3	89.1	2001	6	4	28.8	1.3	0.0	SI	
56	Benimadoka	<i>C. maxima</i> (Burm.) Merr.	2003	5	97.0	2003	6	3	67.7<	6.3	4.3	SI	SI(10)
56	Chandler	<i>C. maxima</i> (Burm.) Merr.	2003	5	97.2	2003	6	4	75.5<	16.3	0.0	SI	SI(11)
56	Hayasaki	<i>C. maxima</i> (Burm.) Merr.	2003	5	93.0	2003	7	4	32.0	20.5	0.5	SI	SI(9)
56	Suisyo buntan	<i>C. maxima</i> (Burm.) Merr.	2002	5	90.4	2002	8	3	37.0	6.7	2.3	SI	SI(11)
Pummelo relatives													
62	Marsh	<i>C. paradisi</i> Macfad.	2002	2	12.0	2002	8	5	54.2<	41.2<	22.2	SC	
74	Hassaku	<i>C. hassaku</i> hort. ex Tanaka	2001	5	95.9	2000	6	5	27.4	2.2	0.0	SI	SI(5)
77	Naruto	<i>C. medioglobosa</i> hort. ex Tanaka	2003	3	83.7	2001	6	8	100<	66.9<	25.0<	SC	
78	Kawano natsudaikai	<i>C. natsudaikai</i> Hayata	2002	5	80.0	2002	7	4	100<	71.5<	31.0	SC	SC(11)
79	Kinkoji	<i>C. obovoidea</i> hort. ex I. Takah.	2003	3	97.4	2001	6	4	67.0<	2.3	1.0	SI	
80	Tosa buntan	<i>C. ootachibana</i> hort. ex Yu. Tanaka	2003	5	91.1	2003	6	3	3.0	0.0	0.0	SI	SI(8)
81	Hyokan	<i>C. ampullacea</i> hort. ex Tanaka	2002	5	77.4	2002	7	5	23.0	1.8	1.2	SI	SI(11)
83	Yamabuki	<i>C. yamabuki</i> hort. ex Yu. Tanaka	2002	4	88.3	2002	8	4	23.5	0.8	0.8	SI	SI(1)
84	Sanbokan	<i>C. sulcata</i> hort. ex I. Takah.	2002	4	84.3	2001	6	4	61.3<	0.3	0.0	SI	SC(11)
						2002	7	3	13.0	0.0	0.0	SI	
						2003	6	4	18.0	2.3	0.0	SI	
—	Kawachi bankan	<i>C. kawachiensis</i> hort. ex Yu. Tanaka	2003	5	93.6	2003	6	4	26.0	1.3	0.0	SI	SI(7)
—	Southern yellow	<i>C. maxima</i> × <i>C. kinokuni</i>	2003	3	77.2	2003	6	4	100<	84.8<	38.8	SC	
Sour orange and its relatives													
93	Kabusu	<i>C. aurantium</i> L.	2003	4	79.9	2003	6	5	90.0	57.8	10.4	SC	
93	Furi daidai	<i>C. aurantium</i> L.	2003	4	74.6	2004	6	3	80.3<	26.0	7.7	SC	
94	Chinotto	<i>C. myrtifolia</i> Rafin.	2003	3	46.5	2003	6	3	91.0<	90.7<	50.0<	SC	
95	Rokugatsumikan	<i>C. rokugatsu</i> hort. ex Yu. Tanaka	2002	2	96.3	2002	7	4	11.5	0.8	0.3	SI	
99	Kikudaikai	<i>C. canaliculata</i> hort. ex Yu. Tanaka	2003	5	87.8	2003	6	4	7.5	4.0	0.0	SI	
—	Shiikuu <sup>h</sup>	<i>C. spp.</i>	2003	3	73.7	2004	6	3	100<	80.0<	64.0	SC	
Sweet orange and its relatives													
100	Hamlin	<i>C. sinensis</i> (L.) Osbeck	2002	3	85.3	2003	6	8	54.2	24.3	11.7	SC	
103	Tarumizu Igo	<i>C. tankan</i> Hayata	2003	1	36.9	2003	6	4	56.0<	24.3	13.0	SC	
105	Miyauchi iyokan	<i>C. iyo</i> hort. ex Tanaka	2002	3	86.0	2002	7	5	100<	100<	23.3	SC	SC(11)
—	Ariake	<i>C. sinensis</i> × <i>C. clementina</i>	2003	2	82.5	2001	6	8	10.4	1.3	0.0	SI	
						2003	6	8	18.9	0.4	0.0	SI	
—	Murcott	parentage unknown, probably tangor	2003	1	84.5	2003	6	4	37.5<	37.0<	31.5<	SC	
Yuzu and its relatives													
107	Hyuga-natsu	<i>C. tamurana</i> hort. ex Tanaka	2003	5	90.5	2003	6	4	18.3	0.0	0.0	SI	SI(2)
110	Kawabata mikan	<i>C. aurea</i> hort. ex Tanaka	2002	4	82.0	2001	6	6	100<	50.7<	30.0	SC	
						2002	7	5	80.8<	46.2<	33.5	SC	
113	Yuzu	<i>C. junos</i> Siebold. ex Tanaka	2004	3	93.0	2004	6	3	100<	100<	100<	SC	
114	Hanayu	<i>C. hanaju</i> Siebold ex Shirai	2002	3	86.7	2002	7	4	100<	51.0	24.3	SC	SC(11)
115	Sudachi	<i>C. sudachi</i> hort. ex Shirai	2002	3	49.0	2002	7	5	87.4<	72.0<	21.4	SC	
121	Kabosu	<i>C. sphaerocarpa</i> hort. ex Tanaka	2002	3	90.7	2002	7	5	100<	100<	39.4	SC	SC(11)
Mandarin and its relatives													
123	Kunenbo	<i>C. nobilis</i> Lour.	2002	4	85.9	2001	6	4	34.0	0.5	0.3	SI	SI(1)
123	Tokunin <sup>i</sup>	<i>C. nobilis</i> Lour.	2003	3	90.8	2003	6	4	3.3	0.0	0.0	SI	
126	Keraji	<i>C. keraji</i> hort. ex Tanaka	2001	3	77.9	2001	6	4	95.5<	0.8	0.5	SI	SI(12)

(Continued next page)

Table 1. Continued.

Classi- fica- tion number <sup>z</sup>	Accession	Latin name Cross combination	Pollen			Self incompatibility/compatibility							
			Year	Yield <sup>y</sup>	Fertility (%)	Year	No. of days after pollination	No. of flowers observed	No. of pollen tubes <sup>x</sup>			SI or SC <sup>w</sup>	Result of previous report <sup>v</sup>
									Top of the style	Middle of the style	Base of the style		
126	Kabuchi	<i>C. keraji</i> hort. ex Tanaka	2002	2	89.0	2002	7	5	15.3	1.0	0.2	SI	SI(11)
126	Natsukinin <sup>t</sup>	<i>C. keraji</i> hort. ex Tanaka	2003	3	94.9	2003	6	4	29.0	1.0	0.0	SI	
130	Yoshida ponkan	<i>C. reticulata</i> Blanco	2002	1	95.2	2002	8	5	86.0<	56.6<	28.2	SC	
131	Mediterranean	<i>C. deliciosa</i> Ten.	2002	1	52.6	2002	7	5	100<	89.2<	52.0<	SC	
132	Usukawa <sup>u</sup>	<i>C. genshokan</i> hort. ex Tanaka	2002	1	96.0	2002	7	3	100<	68.3<	11.3	SC	
133	Dancy	<i>C. tangerina</i> hort. ex Tanaka	2002	2	96.6	2002	7	4	100<	100<	100<	SC	SI(11)
134	Clementine	<i>C. clementina</i> hort. ex Tanaka	2003	3	89.4	2003	7	4	27.8	4.3	0.7	SI	SI(4)
143	Tachibana	<i>C. tachibana</i> (Makino) Tanaka	2002	3	73.9	2002	7	5	100<	89.2<	52.0<	SC	SC(11)
145	Kinokuni	<i>C. kinokuni</i> hort. ex Tanaka	2002	2	95.4	2002	7	5	100<	39.2<	6.2	SC	
148	Sunki	<i>C. sunki</i> (Hayata) hort. ex Tanaka	2002	2	98.6	2002	8	3	100<	100<	84.0	SC	
149	Cleopatra	<i>C. reshni</i> hort. ex Tanaka	2002	3	96.9	2002	8	5	100<	100<	100<	SC	
153	Shiikuwasha	<i>C. depressa</i> Hayata	2002	3	87.7	2002	8	5	10.0	3.4	0.0	SI	SC(11)
						2003	6	4	11.5	0.0	0.0	SI	
153	Shiikunin (A) <sup>t</sup>	<i>C. depressa</i> Hayata	2003	2	97.1	2003	7	4	86.0<	76.0<	65.8<	SC	
153	Shiikunin (K) <sup>t</sup>	<i>C. depressa</i> Hayata	2003	3	95.3	2003	6	4	27.3	1.7	0.0	SI	
154	Koji	<i>C. leiocarpa</i> hort. ex Tanaka	2002	2	91.5	2001	6	3	39.3	4.3	0.3	SI	SC(11)
						2003	6	4	46.3	13.8	1.5	SI	
—	Kimikan	<i>C. flaviculpus</i> hort. ex Tanaka	2003	4	58.5	2001	6	7	6.3	1.4	1.0	SI	
—	Kozu <sup>s</sup>	<i>C. spp.</i>	2003	3	95.2	2003	6	2	100<	100<	100<	SC	
—	Encore	<i>C. nobilis</i> × <i>C. deliciosa</i>	2004	1	92.8	2004	6	5	100<	100<	100<	SC	
—	Fortune	<i>C. clementina</i> × <i>C. tangerina</i>	2002	2	87.6	2002	7	5	14.8	1.4	0.6	SI	
—	Hareyaka	Encore × <i>C. reticulata</i>	2003	1	88.9	2003	6	4	38.3<	32.8<	28.5<	SC	
—	Nova	<i>C. clementina</i> × Orlando <sup>r</sup>	2002	1	75.1	2002	7	5	15.6	0.0	0.0	SI	SI(6)
—	Page	Minneola × <i>C. clementina</i>	2003	3	86.0	2003	6	4	38.5	13.8	1.8	SI	SI(6)
—	Youkou	Kiyomi <sup>q</sup> × <i>C. reticulata</i>	2003	1	89.4	2003	6	4	41.0	21.3	9.3	SC	
—	Amakusa	KO14 <sup>p</sup> × Page	2003	2	84.3	2003	6	4	95.3<	72.3	26.5	SC	
—	Minneola	<i>C. paradisi</i> × <i>C. tangerina</i>	2003	3	86.4	2003	6	4	13.5	2.3	0.0	SI	SI(3)
Kumquat relatives													
159	Calamondin	<i>C. madurensis</i> Lour.	2003	3	77.4	2003	6	4	49.3	36.5	20.5	SC	
Miscellaneous													
—	Hedzuka daidai	<i>C. spp.</i>	2002	5	53.3	2002	7	5	84.0<	40.2	13.2	SC	

<sup>z</sup> Classified by Tyozaburo Tanaka's system (Tanaka, 1969, 1977).

<sup>y</sup> 1: poor, 2: slightly poor, 3: medium, 4: slightly rich, 5: rich.

<sup>x</sup> When numbers of observed pollen tubes were more than 100, we recorded it '100<'. In this case, we could not calculate exact mean number. We calculated mean number '100<' as 100, and add '<'.  
<sup>w</sup> SI: self-incompatibility, SC: self-compatibility.

<sup>v</sup> Number indicates source reference. 1: Nagai and Tanikawa (1928), 2: Miwa (1951), 3: Mustard (1956), 4: Soost (1956), 5: Nishiura and Iwasaki (1963), 6: Hearn (1969), 7: Mishima and Inaba (1971), 8: Yamada (1988), 9: Okudai et al. (1991), 10: Yamada et al. (1993), 11: Ngo (2001), 12: Yamamoto and Tominaga (2002).

<sup>u</sup> Local accession which was collected in Kikai-jima island.

<sup>t</sup> Local accession which was collected in Tokuno-shima island.

<sup>s</sup> Local accession which was collected in Yaku-shima island.

<sup>r</sup> *C. paradisi* × *C. tangerina*.

<sup>q</sup> *C. unshiu* × *C. sinensis*.

<sup>p</sup> Kiyomi × *C. unshiu*.

(*C. nobilis* × *C. deliciosa*), 'Miyachi iyokan' (*C. iyo*) and hassaku (*C. hassaku*), respectively. *Citrus* accessions were basically classified according to Tanaka's system (Tanaka, 1969, 1977).

## Results and Discussion

### Pollen yield and fertility

Pollen yield and fertility of all accessions were rated

before their self-incompatibility status was determined. The reason for poor pollen tube growth in the style for pollen sterile accessions could be attributed to male sterility or self-incompatibility (Vithanage, 1986).

In many accessions, pollen yield was rated as 3 (medium) to 5 (rich) and fertility was in excess of 80%. The pollen fertility of 'Marsh' was the lowest at 12.0%. Although some accessions produced poor yield of pollen,

**Table 2.** Cross-incompatibility/compatibility of related accessions of *Citrus*.

Cross Combination	Year	No. of days after pollination	No. of flowers observed	No. of pollen tubes <sup>z</sup>			CI or CC <sup>y</sup>	Result of previous report <sup>x</sup>
				Top of the style	Middle of the style	Base of the style		
Ariake <sup>w</sup> × Clementine	2002	8	5	7.6	1.2	0.0	CI	
	2003	6	4	33.8	15.8	0.3	CI	
Clementine × Ariake <sup>w</sup>	2002	8	5	6.8	3.0	0.2	CI	
	2003	8	8	24.7	2.7	0.0	CI	
Clementine × Page <sup>v</sup>	2003	7	4	90.0<	60.8<	42.8<	CC	
Minneola × Page <sup>v</sup>	2003	6	4	100<	100<	100<	CC	
Nova <sup>u</sup> × Minneola <sup>t</sup>	2002	7	5	100<	73.4	37.8	CC	
Nova <sup>u</sup> × Clementine	2002	8	5	70.6<	67.4<	54.6<	CC	
Nova <sup>u</sup> × Page <sup>v</sup>	2003	6	4	100<	71.3	31.5	CC	CC <sup>x</sup>
Page <sup>v</sup> × Clementine	2003	6	4	100<	88.8<	27.5	CC	
Hayasaki <sup>t</sup> × Benimadoka <sup>t</sup>	2003	6	4	100<	100<	100<	CC	
Beniamanatsu × Ariake	2003	6	4	100<	83.3<	46.7	CC	
Beniamanatsu × Clementine	2003	6	4	100<	100<	100<	CC	

<sup>z</sup> When numbers of observed pollen tubes were more than 100, we recorded it '100<'. In this case, we could not calculate exact mean number. We calculated mean number '100<' as 100, and add '<'.  
<sup>y</sup> CI: cross-incompatibility, CC: cross-compatibility.

<sup>x</sup> Reported by Hearn (1969).

<sup>w</sup> Seike navel × clementine.

<sup>v</sup> Minneola × clementine.

<sup>u</sup> clementine × Orlando.

<sup>t</sup> Matou buntan × Hirado buntan.

pollen fertility was high except in 'Tarumizu 1 go' (36.9%) and mediterranean (52.6%) (Table 1). When flowers of 'Kawano natsudaikai' were pollinated with pollen of 'Tarumizu 1 go', the mean length of pollen tubes in the pistil were 71.3, 50.3, and 22.5 in the upper, middle, and basal part of the style respectively. This indicates that 'Tarumizu 1 go' produces sufficient viable pollen. Although pollen fertility of 'Marsh' was lower than that of 'Tarumizu 1 go', pollen yield of the former was a 2 compared to 1 in the later; so that the difference in the total amount of viable pollen grains produced slight. Thus, we considered that these cultivars produce enough viable pollen to determine self-incompatibility or compatibility, although the total amount of viable pollen grains of those three accessions was categorized as poor to slightly poor.

#### Self-incompatibility/compatibility

Pollen tube growth was strongly inhibited in the accessions known to be self-incompatible (Table 1), such as hyuga-natsu (Miwa, 1951) and hassaku (Nishiura and Iwasaki, 1963). Moreover, those pollen tubes were abnormally twisted and filled with heavy and irregular callose (data not shown). However, in self-compatible 'Kawano natsudaikai', many normal pollen tubes penetrated into the style (Table 1). These results agree with those of Ngo et al. (2001). Thus, both the number of pollen tubes and the pollen tube behavior in the styles were used as indices for judging incompatibility or compatibility in this study.

The results of self-incompatibility or compatibility testing of 65 *Citrus* accessions (Table 1) revealed that lemon was self-compatible, whereas the six tested pummelos and seven out of 11 relatives were self-incompatible. Among the sour orange and its relatives, two out of six accessions were self-incompatible. Within the sweet orange and its relatives, one out of five accessions was self-incompatible. But every yuzu and its relatives, except hyuga-natsu, were self-compatible. Fourteen out of 28 accessions of mandarin and its relatives were self-incompatible. Both calamondin, a kumquat relative, and Hedzuka daidai of miscellaneous were self-compatible. Therefore, 31 out of 65 accessions examined were self-incompatible.

Ngo (2001) revealed that self-incompatibility reaction between pollen tubes and style is classified according to degree of self-incompatibility of each portion of the style. Some self-incompatible cultivars such as 'Hirado buntan' showed moderately self-incompatible in top of the style and highly self-incompatible in base of the style. In our result with 'Benimadoka', 67.7<, 6.3 and 4.3 pollen tubes were observed in the top, middle and base of the style, respectively. The self-incompatibility reaction of 'Benimadoka' is similar to 'Hirado buntan': moreover, their pollen tubes exhibited abnormal behavior, typical of self-incompatible accessions, as we and Ngo et al. (2001) observed in hyuga-natsu and hassaku. Thus, 'Benimadoka' was judged as self-incompatible, whereas, Furi daidai, kinokuni and 'Youkou' were classified as to self-compatible even

**Table 3.** Relationship between presumed *S*-genotype of Seike navel (*C. sinensis*), clementine and Ariake.

Cross combination	Hypothesis	Genotype of parents <sup>y</sup>	Presumed genotype of Ariake (SI) <sup>z</sup>
Seike navel (SC) <sup>z</sup> × Clementine (SI) <sup>z</sup>	1	$S_c S_x \times S_y S_z$	$S_c S_y, S_c S_z, S_x S_y, S_x S_z$
	2	$S_c S_x \times S_x S_y$	$S_c S_y, S_x S_y$

<sup>z</sup> SC: self-compatibility, SI: self-incompatibility.

<sup>y</sup>  $S_c$ : compatible allele;  $S_x$ ,  $S_y$ , and  $S_z$ : incompatible alleles.

<sup>x</sup> Since these genotypes are self-compatible, they are not presumed genotype of self-incompatible 'Ariake'.

though less than ten pollen tubes were observed in the base of the style. Normal pollen tubes penetrated into the style in these accessions. The number of viable pollen grains and their speed of growth seem to affect how many reach the base of the style.

To our knowledge, this study is the first to report self-incompatibility in 13 out of 31 accessions; the status of the remaining 18 accessions were previously reported (Table 1). Our results generally agree with those previously reported, the exception is that of shiikuwasha (*C. depressa*) which Ngo (2001) found to be self-compatible, whereas our accessions were found to be both self-incompatible and compatible. Accessions of *C. depressa* exhibit high genetic variability (Yamamoto et al., 1998), so that self-incompatible and compatible accessions probably exist. The wide variations in the morphology of koji (Iwamasa, 1976) may be attributed to the use of different accessions. Similarly, our results with both dancy and sanboku disagreed with those of Ngo (2001) although typical strains were experimented. 'Amakusa' has been considered to be self-incompatible because it produces viable pollen and nearly seedless fruit (Matsumoto et al., 1999), but we found it to be self-compatible.

Ngo (2001) found that all 71 pummelo accessions examined were self-incompatible as were six accessions that we examined. These results indicate that all pummelos are self-incompatible. Since pummelo (*C. maxima*) is a basic *Citrus* species and the ancestor of various citrus accessions (Yamamoto et al., 1993), it may be a major source of incompatible allele.

Mandarin is also considered to be a basic *Citrus* species (Yamamoto et al., 1993), but some accessions exhibit genetic traits of the pummelo. For example, kunenbo seems to be a tangor (hybrid of sweet orange and mandarin) that carries genes originating from pummelo, a probable ancestor of several mandarins, such as keraji and kabuchi (Ngo, 2001). Although incompatible alleles of these accessions may originate in pummelo, mandarins that originated in China (Mediterranean, kinokuni and sunki) and India ('Yoshida ponkan', dancy and cleopatra) are considered to be true mandarins that are self-compatible. Two out of three accessions of *C. depressa* that originated in Nansei (SouthWest) islands of Japan are self-incompatible but carry no morphological traits of pummelo, sweet orange or kunenbo. The origin of this species is unclear, but it

may be a hybrid of tachibana, a Japanese mandarin, and Chinese mandarin (Yamamoto et al., 1998). Further studies are needed to resolve whether incompatible alleles of *C. depressa* is derivative of Japanese mandarin or pummelo.

#### Cross-incompatibility/compatibility

Pollen tube growth in the styles of 'Ariake' × clementine and the reciprocal cross combination, clementine × 'Ariake' was inhibited, although both accessions could produce viable pollen (Table 2), so that those two accessions are cross-incompatible of identical genotype. On the other hand, cross-incompatibility was not found in the other seven cross combinations (Table 2).

Self-incompatible hybrids with known pedigree are very useful for estimating the *S* genotype; Soost (1969) reported the *S* genotype of several hybrids and their parents. This study revealed that clementine and its progeny 'Ariake', a hybrid from 'Seike navel' (*C. sinensis*) × clementine (Yamada et al., 1995), have the same *S* alleles. Determining self-incompatibility or compatibility of 'Seike navel' is impossible because it is male sterile and all members of *C. sinensis* are self-compatible. Our results also show that 'Hamlin' belonging to the same species is, likewise, self-compatible. Hirai and Kajiura (1987) also revealed low genetic variability among *C. sinensis*.

We propose the *S* genotypes of 'Seike navel', clementine and 'Ariake' (Table 3). 'Seike navel' has one *S* allele because its progeny 'Ariake' is self-incompatible. When 'Seike navel' and clementine have the same *S* allele, 'Ariake' and clementine are cross-incompatible.

The present study uncovered an incompatible allele in three accessions whose origin we plan to trace by hybridizing more accessions of known parentage.

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## カンキツにおける自家および交雑不和合性

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カンキツにおける自家不和合性は結実不良の原因となることもあるが、単為結果性が備わった場合には無核果の生産につながる重要な形質である。そのため、本研究では主としてわが国原産のカンキツ類 65 種・品種（以下、品種と略）を供試して、その自家不和合性について解明するとともに、血縁関係のある自家不和合性品種間の交雑不和合性についても検定した。なお、不和合性は花柱内の花粉管伸長によって検定した。レモンは自家不和合性であった。ブンタンでは 6 品種すべて、ブンタン類縁種では 11 品種中 7 品種、ダイダイおよびその類縁種では 6 品種中 2 品種、スイートオレンジおよびその類縁

種では 5 品種中 ‘ありあげ’ のみの 1 品種、ユズおよびその類縁種では 5 品種中ヒュウガナツのみの 1 品種、マンダリンおよびその類縁種では 28 品種中 14 品種が自家不和合性であった。キンカン類縁のシキキツおよび分類上の位置が不詳の辺塚ダイダイは自家不和合性であった。すなわち、本研究で供試したカンキツ全 65 品種中 31 品種が自家不和合性であった。交雑不和合性はクレメンティンとその後代である ‘ありあげ’ との正逆交雑のみ認められ、両者の不和合性に関する遺伝子型が一致していると推定できた。