J. Japan. Soc. Hort. Sci. 78 (4): 402–409. 2009.
Available online at www.jstage.jst.go.jp/browse/jjshs1

Characteristics of Fruiting and Pollen Tube Growth of Apple Autotetraploid Cultivars Showing Self-compatibility

Yoshiteru Adachi[1], Sadao Komori[1]*, Yoshimasa Hoshikawa[1], Norimitsu Tanaka[1], Kazuyuki Abe[2], Hideo Bessho[2], Manabu Watanabe[3] and Akira Suzuki[1]

[1]*Faculty of Agriculture, Iwate University, Morioka 020-8550, Japan*
[2]*Apple Research Station, National Institute of Fruit Tree Science, Shimokuriyagawa, Morioka 020-0123, Japan*
[3]*Field Science Center, Faculty of Agriculture, Iwate University, Morioka 020-8550, Japan*

Apples (*Malus*×*domestica* Borkh.) have gametophytic self-incompatibility (SI). Generally, the polyploidy breaks down gametophytic SI; however, there is little data on apples. In this study, we collected basic data on the self-compatibility (SC) mechanism of apple autotetraploid cultivars and polyploid mutants of original diploid cultivars. The autotetraploids showed self-fertility, and their pollen was compatible with the pistils of their original diploid cultivars, which share two *S*-alleles. However, the original diploid cultivars' pollen was incompatible with the pistils of autotetraploid cultivars. Although the pollen of autotetraploid cultivars induced SC, the fruit set percentage and seed number were lower when autotetraploid cultivars were selfed or used as pollen parents in the pistils of the original diploid cultivars than in compatible diploid cross-combinations. The pollen tubes of autotetraploid cultivars grew significantly further than those of the selfed diploid cultivars, but grew significantly slower than in compatible diploid cross-combinations. These results show that the pollen of apple autotetraploid cultivars induced SC but maintained partial incompatibility.

Key Words: apple, pollen tube, polyploidy, self-incompatibility, tetraploid.

Introduction

Apples (Rosaceae) show gametophytic self-incompatibility (SI), and the fruit set after self-pollination in most apple cultivars is less than 10% (Komori et al., 1999). *S*-RNase-based gametophytic SI has been found in the Solanaceae, Rosaceae, and Plantaginaceae, and is controlled by a single, multi-allelic locus, the *S*-locus, which contains stylar-*S* and pollen-*S* genes. The stylar-*S* gene encodes a ribonuclease (S-RNase) (McClure et al., 1989; Sassa et al., 1992), which is expressed in the pistil and specifically degrades incompatible pollen RNA (Kao and McCubbin, 1996). The pollen-*S* gene encodes an F-box gene named *S*-locus F-box (*SLF*) in *Antirrhinum* (Lai et al., 2002), *PmSLF* in *Prunus mume* (Entani et al., 2003), *PiSLF* in *Petunia inflata* (Sijacic et al., 2004), *S*-haplotype-specific F-box gene (*SFB*) in *Prunus dulcis* (Ushijima et al., 2003), *PaSFB* in *Prunus avium* (Yamane et al., 2003), and *PcSFB* in *Prunus cerasus* (Ikeda et al., 2004). Since the well-documented function of F-box protein is substrate recognition as a component of SCF complex, a kind of E3 ubiquitin ligase, it has been hypothesized that SLF/SFB distinguishes self and non-self *S*-RNase in the pollen tube, ubiquitinates nonself *S*-RNase for degradation by the 26S proteasome, and allows the compatible pollen tube to fertilize (Qiao et al., 2004; Ushijima et al., 2003, 2004); however, it is unclear how gametophytic SI is triggered by the interaction between SLF/SFB and *S*-RNase. Recently, the *S* locus F-box brothers (*SFBB*) were derived as polymorphic genes in the Maloideae: apple and Japanese pear (Sassa et al., 2007); however, whether only one, some or none of the *SFBB*s in a haplotype are involved in pollen *S* specificity is unknown. In the Solanaceae and Rosaceae, *S*-RNase-based gametophytic SI is broken down by polyploidization (Crane and Lewis, 1942; Entani et al., 1999a, 1999b; Hauck et al., 2002, 2006; Lewis and Modlibowska, 1942; de Nettancourt et al., 1974; Pandey, 1968; Sato and Kitayama, 1992; Stout and Chandler, 1942; Ueda and Akimoto, 2001). In the Solanaceae, this phenomenon is called 'competitive interaction' (Golz et al., 1999, 2001), in which pollen grains containing two copies of the same

Received; November 11, 2008. Accepted; April 3, 2009.
* Corresponding author (E-mail: komoris@iwate-u.ac.jp).

pollen-*S* allele (homoallelic pollen) are arrested if the cognate *S*-RNase is present in the style, while pollen grains containing two different pollen-*S* alleles (heteroallelic pollen) are compatible and recognized as nonself *S*-RNase.

In the Rosaceae, tetraploid sour cherry (*Prunus cerasus*) and hexaploid common plum (*Prunus domestica*) have self-compatible (SC) cultivars; however, tetraploid sour cherry has both SI and SC types (Hauck et al., 2002), because SC is induced by the accumulation of nonfunctional mutations in pollen-*S* or stylar-*S* genes (Hauck et al., 2006). Thus, the SC mechanism of polyploidy differs between Solanaceae by 'competitive interaction' and *Prunus* by the mutation of *S*-genes. In apples, the tetraploid cultivar 'Doud Golden Delicious' (sport of 'Golden Delicious') also shows SC (Sato and Kitayama, 1992), but the mechanism is unknown. In this study, we verified the features of the SC of apple autotetraploid cultivars and polyploid mutants of original diploid cultivars by crossing and pollen tube growth tests.

Materials and Methods

Pollination test

Pollination tests were performed three times in early May from 2003 to 2007 using trees of autotetraploid cultivars (Table 1) growing at the Apple Research Station, National Institute of Fruit Tree Science (NIFTS), National Agriculture and Food Research Organization (NARO), Morioka, Japan. We compared autotetraploid cultivars and original diploid cultivars; for example, 'Tensei' (4X) is the autotetraploid cultivar of 'Fuji' (2X).

Buds were collected before the balloon stage. Anthers were also collected, dried for 1–2 days at 20°C, and stored at −80°C until use. Pollen germination was investigated *in vitro* within 3 weeks after pollination: pollen was incubated at 25°C for 24 h on germination medium (17% sucrose, 1% Bacto agar). Pollination was performed using balloon stage flowers. All flowers were emasculated (except self-pollinated flowers), pollinated, and bagged. At 3 weeks after pollination, fruit set was counted. Fruits were harvested at the end of August, and the number of seeds per fruit was counted. Compatibility and incompatibility were discriminated according to the number of seeds per fruit and the fruit set percentage, as shown in Table 2 (Komori et al., 1999). *S*-allele genotypes are quoted from Matsumoto et al. (2002); the autotetraploid cultivar is shown as a putative *S*-allele genotype.

Pollen tube growth test

1. Pollen tube growth test in vitro (on germination medium)

Pollen was incubated for 12 h at 15, 20, or 25°C under humid conditions on germination medium (17% sucrose, 1% Bacto agar). The pollen tube was observed by light microscope. Pollen tube length was measured after 3, 6, 9, and 12 h from incubation of 20 randomly selected

Table 1. Characteristics of 10 apple cultivars used in this study.

Cultivar	Ploidy[z]	*S*-allele genotype[y]	Parentage (♀ × ♂)[z]	References (page)[z]
'Golden Delicious'	2X	S_2S_3	'Grimes Golden' × ?	365
'Doud Golden Delicious'	4X	$S_2S_2S_3S_3$	sport of 'Golden Delicious'	255
'Fuji'	2X	S_1S_9	'Ralls Janet' × 'Delicious'	336–337
'Tensei'	4X	$S_1S_1S_9S_9$	sport of 'Fuji'	—
'Jonathan'	2X	S_7S_9	'Esopus Spitzenburg' × ?	478
'Welday Jonathan'	4X	$S_7S_7S_9S_9$	sport of 'Jonathan'	1077
'Spartan'	2X	S_9S_{10}	'McIntosh' × 'Yellow Newton Pippin'	962
'Sweden Spartan'	4X	$S_9S_9S_{10}S_{10}$	sport of 'Spartan'	1000
'Sweden Alpha 68A'	4X	unknown	unknown	1000
'Hokuto'	3X	$S_1S_7S_9$	'Fuji' × 'Rero 11'[x]	—

[z] Yoshida (1986), 'Tensei' ploidy quoted from Fukushima Tenkoen Co., Ltd. (http://www.fukuten.com/, December 25, 2007).
[y] Matsumoto et al. (2002); the autotetraploid cultivar is shown as a putative *S*-allele genotype.
[x] Kitahara et al. (2005)

Table 2. Criteria of number of seeds per fruit and fruit set percentage (Komori et al., 1999).

Parameter	Incompatible	Mixed region	Compatible
① Fruit set (%)	<20	≥20–<30	≥30
② Number of seeds per fruit	<1.2	≥1.2–<3.0	≥3.0

More than 3.0 seeds per fruit indicated compatibility, and fewer than 1.2 indicated incompatibility. More than 30% fruit set indicated compatibility, and less than 20% indicated incompatibility. Intermediate values indicated mixed compatibility and incompatibility. It is better to perform reciprocal crosses or to examine both parameters over more than 2 years (1) when the fruit set percentage and number of seeds per fruit are opposed and (2) when the value of one parameter shows either compatibility or incompatibility and that of the other parameter lies in the mixed region.

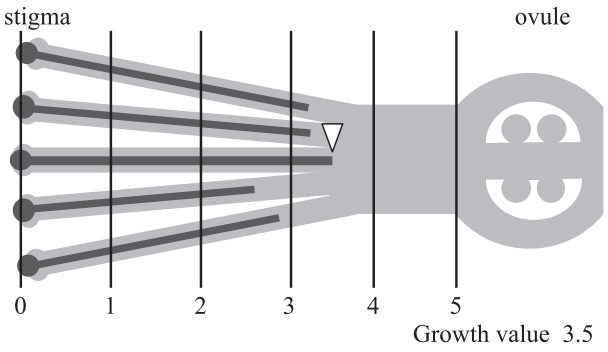

Fig. 1. Evaluation method of maximum growth point of pollen tube in the style of pistils. Pollen tube growth was scored as the maximum growth point (▽), and assessed as the mean of three styles per cross-combination on a scale of 0 (no growth from stigma) to 5 (growth to junction with ovary) at 24, 48, and 96 h after pollination.

pollen grains by image analysis software Win ROOF Version 5.0 (Mitani Co., Fukui, Japan). Statistical analysis was carried out on the pollen tube after 12 hours by Student's *t*-test.

2. Pollen tube growth test in vivo (in style)

In 2004, flowers were pollinated as above, collected for observation of pollen tube growth 24, 48, and 96 h after pollination, and fixed in FAA (5% formaldehyde, 5% acetic acid, 45% ethanol) for more than 24 h. The styles were hydrolyzed with softening solution (55% 1 N HCl, 45% acetic acid) at 60°C for 12 h, and then rinsed with distilled water. The samples were stained with 0.1% aniline blue for 10 h, and observed by fluorescence microscope. Pollen tube growth was scored as the maximum growth point, and assessed as the mean of three styles per cross-combination on a scale of 0 (no growth from stigma) to 5 (growth to junction with ovary) (Fig. 1). Statistical analysis was performed on the pollen tube after 96 h by Fisher's LSD.

Results

Pollination test

1. Selfing

The number of seeds per fruit and the fruit set percentage of autotetraploid cultivars 'Doud Golden Delicious' (sport of 'Golden Delicious'), 'Tensei' (sport of 'Fuji'), and 'Welday Jonathan' (sport of 'Jonathan') were judged to indicate SC in all 3 years (Table 3). 'Sweden Spartan' (sport of 'Spartan') was judged to be SC in 2 years (test could not be performed in the 3rd year). 'Sweden Alpha 68A' (origin unknown) was judged to be SC.

Among the diploid cultivars, 'Golden Delicious' fruited in all 3 years (Table 3), the number of seeds per fruit and the fruit set percentage indicated mixed SC/SI or SI, and the fruits could not be maintained to harvest; therefore, 'Golden Delicious' was judged as SI. 'Spartan' and 'Fuji' did not fruit in any year. 'Jonathan' and 'Hokuto' were also judged as SI.

2. Crossing autotetraploid cultivars and their original diploid cultivars

In the crosses of diploid cultivars' pollen with the pistils of autotetraploid cultivars, all combinations were judged as incompatible (Table 4).

On the other hand, all crosses of autotetraploid pollen with original diploid pistils showed compatibility (Table 4). 'Fuji' × 'Tensei' and 'Jonathan' × 'Welday Jonathan' were judged as compatible in all 3 years. 'Golden Delicious' × 'Doud Golden Delicious' was judged as compatible in 2 years. 'Spartan' × 'Sweden Spartan' and 'Spartan' × 'Hunter Spartan' were judged as compatible except in the fruit set of the former in 2004.

Pollen tube growth test

1. Pollen tube growth in vitro

Pollen tube length on germination medium is shown in Figure 2. Pollen tube length incubated at 20°C was most extended after 12 h. There was no significant difference in the pollen tube length between 'Doud Golden Delicious' and its original diploid cultivar 'Golden Delicious' at any incubation temperature. A significant difference was observed in the pollen tube length between 'Tensei' and its original diploid cultivar 'Fuji' at 25°C and between 'Welday Jonathan' and its original diploid cultivar 'Jonathan' at 15 and 25°C; 'Tensei' and 'Welday Jonathan' pollen tubes were significantly further extended than 'Fuji' and 'Jonathan' (Student's *t*-test, $P < 0.05$).

2-1. Pollen tube growth in self-pollination

The pollen tubes of the selfed autotetraploid cultivars were significantly longer than those of diploid and triploid cultivars (Fig. 3), but their growth was slower than the control ('Fuji' × 'Golden Delicious', a compatible diploid cross-combination). The pollen tubes of the control and selfed autotetraploid cultivars continued to grow with a pointed tip (Fig. 4); however, those of selfed diploid and triploid cultivars had a swollen tip at 48 and 96 h after pollination, and these prevented fertilization (Fig. 5).

2-2. Pollen tube growth in reciprocal crosses between autotetraploids and diploids

Pollen tube growth in reciprocal crosses between the autotetraploid 'Doud Golden Delicious' and its original diploid cultivar 'Golden Delicious' is shown in Figure 6. 'Fuji' pollen was used as a control with 'Golden Delicious'. The growth of 'Golden Delicious' pollen tubes in pistils of 'Doud Golden Delicious' and 'Golden Delicious' was significantly slower than the control (Fig. 6). On the other hand, the growth of 'Doud Golden Delicious' pollen tubes in pistils of itself and 'Golden Delicious' was significantly longer than that of 'Golden Delicious' pollen, but significantly slower than that of control 'Fuji' pollen (Fig. 6).

We also investigated the pollen tube growth of selfed 'Tensei' and 'Welday Jonathan', and of cross-pollinated 'Tensei' and 'Fuji', and 'Welday Jonathan' and

Table 3. Fruit set and seed number per fruit in the self-pollination of apple autotetraploid cultivars showing self-compatibility and their original diploid cultivar.

Cultivar	Ploidy[z]	*S*-allele genotype[y]	Year	Pollen germination (%)	Fruit set (%)	No. of fruit harvest	No. of seed/fruit
'Doud Golden Delicous'	4X	$S_2S_2S_3S_3$	03	19.4	30.0	1	7.0
			04	12.5	78.1	18	4.1
			05	21.4	46.7	6	5.5
			07	32.3	56.0	14	3.9
'Golden Delicious'	2X	S_2S_3	03	82.6	10.0	0	0
			04	22.4	10.0	0	0
			05	42.5	25.0	0	0
'Sweden Spartan'	4X	$S_9S_9S_{10}S_{10}$	04	28.3	71.4	10	4.2
			07	38.5	30.0	8	3.6
'Spartan'	2X	S_9S_{10}	03	54.3	0	0	0
			04	60.9	0	0	0
			05	38.2	0	0	0
'Sweden Alpha 68A'	4X	unknown	04	35.7	44.4	3	5.3
'Tensei'	4X	$S_1S_1S_9S_9$	04	52.1	89.7	31	4.6
			05	13.7	92.9	9	6.6
			06	24.8	62.5	24	4.6
			07	44.1	88.2	42	4.7
'Fuji'	2X	S_1S_9	04	67.3	0	0	0
			05	22.0	0	0	0
			06	36.5	0	0	0
			07	82.7	0	0	0
'Welday Jonathan'	4X	$S_7S_7S_9S_9$	03	29.1	75.0	7	4.1
			04	46.0	66.7	5	4.2
			05	39.5	73.3	9	3.2
'Jonathan'	2X	S_7S_9	03	43.2	10.0	0	0
			05	45.0	0	0	0
			06	84.3	0	0	0
'Hokuto'	3X	$S_1S_7S_9$	04	30.2	0	0	0
			05	25.7	6.7	1	3.0
			06	45.8	0	0	0

[z] Yoshida (1986), 'Tensei' ploidy quoted from Fukushima Tenkoen Co., Ltd. (http://www.fukuten.com/, December 25, 2007).
[y] Matsumoto et al. (2002); the autotetraploid cultivar is shown as a putative *S*-allele genotype.

'Jonathan'. The results are similar to those of 'Doud Golden Delicious' and 'Golden Delicious' (Figs. 7 and 8).

Discussion

The autotetraploid cultivars 'Doud Golden Delicious', 'Sweden Spartan', 'Sweden Alpha 68A', 'Tensei', and 'Welday Jonathan' were SC (Table 3). In addition, autotetraploid pollen was compatible with the pistils of their original diploid cultivars (Table 4). On the other hand, diploid cultivar pollen was rejected by the pistils of autotetraploid cultivars, which shared two *S*-alleles (Table 4); therefore, the SI in autotetraploid pistils was fully functional, so the cause of SC in autotetraploid cultivars was the pollen. However, the fruit set percentage and seed number were lower when autotetraploids were selfed and used as the pollen parent in crossing with their original diploid cultivars than in compatible diploid cross-combinations (Tables 3 and 4). Thus, overcoming incompatibility by using the pollen of autotetraploid cultivars was imperfect, and the cultivars seemed to maintain partial incompatibility.

In pollen tube growth on germination medium (Fig. 2), no significant differences were observed in the ability of pollen tube growth between autotetraploid pollen and their original diploid cultivar pollen, and autotetraploid pollen was fully normal; however, the pollen tubes of autotetraploid cultivars grew significantly further than those of selfed diploid and triploid cultivars, but grew significantly slower than compatible diploid cross-combinations (Fig. 3). This result was consistent with the pollination test. Pollen tube growth, fruit set percentage, and seed number when using autotetraploid cultivar pollen were lower than in compatible diploid cross-combinations; therefore, the autotetraploid cultivars were SC but maintained partial incompatibility. Most pollen tubes did not reach the ovary within 96 h, yet seeds were obtained. Thus, the pollen tubes of

Table 4. Fruit set and seed number per fruit in the reciprocal crossing of apple autotetraploid cultivars and their original diploid cultivar.

♀	Ploidy[z]	♂	Ploidy[z]	Year	Pollen germination (%)	Fruit set (%)	No. of fruit harvest	No. of seed/fruit
'Doud Golden Delicious'	4X	'Golden Delicious'	2X	03	82.6	0	0	0
				04	22.4	18.8	2	0.5
				05	42.5	7.1	0	0
'Golden Delicious'	2X	'Doud Golden Delicious'	4X	05	21.4	50.0	6	5.5
				06	44.0	73.1	37	5.2
'Sweden Spartan'	4X	'Spartan'	2X	03	54.3	0	0	0
				04	60.9	0	0	0
'Spartan'	2X	'Sweden Spartan'	4X	04	28.3	13.3	4	5.8
				05	10.6	58.3	4	5.8
		'Hunter Spartan'	4X	05	13.3	86.7	11	4.6
'Tensei'	4X	'Fuji'	2X	04	67.3	4.3	1	2.0
				05	22.0	0	0	0
				06	36.5	0	0	0
'Fuji'	2X	'Tensei'	4X	04	52.1	50.0	10	4.4
				05	13.7	93.3	12	6.4
				06	24.8	64.0	62	4.9
'Welday Jonathan'	4X	'Jonathan'	2X	03	43.2	6.7	0	0
				04	64.2	0	0	0
				07	84.6	0	0	0
'Jonathan'	2X	'Welday Jonathan'	4X	04	46.0	56.7	17	4.1
				05	39.5	86.7	5	5.8
				06	29.2	54.3	45	4.3
'Fuji'	2X	'Golden Delicious'	2X	05	42.5	100	12	8.3
				06	89.7	100	13	10.8
				07	83.7	93.3	20	10.1

[z] Yoshida (1986), 'Tensei' ploidy quoted from Fukushima Tenkoen Co., Ltd. (http://www.fukuten.com/, December 25, 2007).

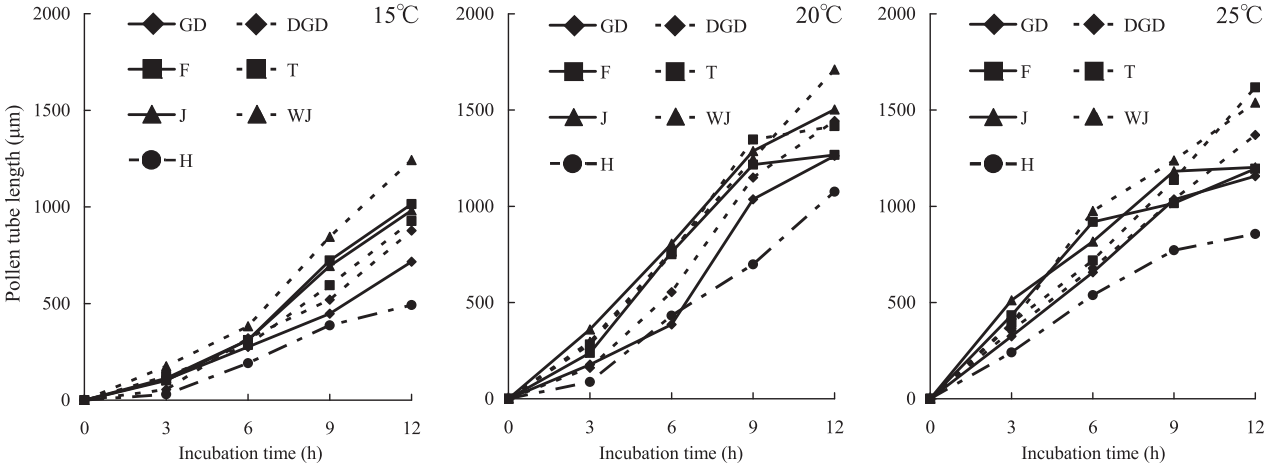

Fig. 2. Pollen tube growth on pollen germination media at each temperature. GD, 'Golden Delicious' (2X); DGD, 'Doud Golden Delicious' (4X); F, 'Fuji' (2X); T, 'Tensei' (4X); J, 'Jonathan' (2X); WJ, 'Welday Jonathan' (4X); H, 'Hokuto' (3X).

compatible diploid cross-combinations and autotetraploids must have continued to grow for more than 96 h after pollination and fertilized the ovules.

Okuse (1972) reported that the self-fertility of 'Megumi' might be related to strong pollen tube growth. The pollen tube growth of autotetraploid cultivars is larger than or equal to that of their original diploid cultivars (Fig. 2), yet its pollen tube growth was slower than that of the compatible diploids (Fig. 3); therefore, the SC mechanism of autotetraploid cultivars differs from that of 'Megumi'.

In apple crossing tests, the diploid cultivar pollen produced little or no fruit by either self-pollination or autotetraploid progeny, which shared two *S*-alleles; therefore, the *S-RNase* gene and *S*-RNase of autotetraploids functioned normally. However, the pollen tubes

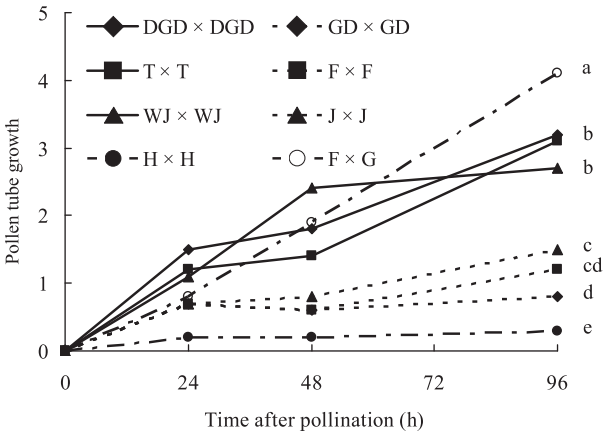

Fig. 3. Pollen tube growth in the self-pollination pistil. Statistical analysis was conducted 96 h after pollination. Means followed by different letters are significantly different at $P < 0.01$ by Fisher's LSD test. DGD, 'Doud Golden Delicious' (4X); GD, 'Golden Delicious' (2X); T, 'Tensei' (4X); F, 'Fuji' (2X); WJ, 'Welday Jonathan' (4X); J, 'Jonathan' (2X); H, 'Hokuto' (3X).

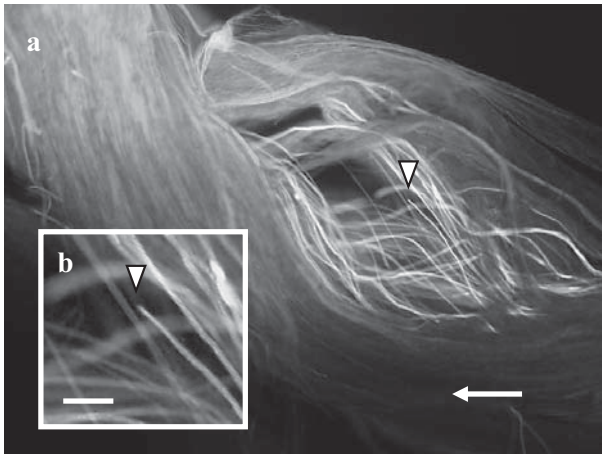

Fig. 4. Pollen tube growth in the middle style more than 96 h from pollination ('Fuji' × 'Golden Delicious'). a. Arrow head indicates the direction of pollen tube growth. Triangle head indicates the growth position of pollen tube. Arrow bar = 0.1 mm. b. Scaled up a. Triangle head indicates the growth position of the pollen tube. Pollen tube tip was sharpened. Bar = 0.02 mm.

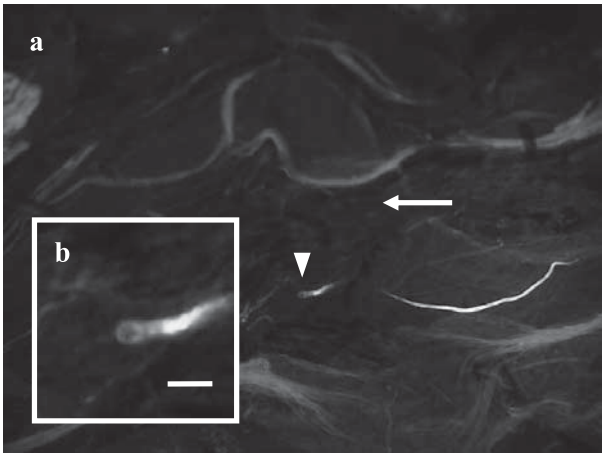

Fig. 5. Pollen tube growth at the stigma surface position more than 96 h from pollination ('Hokuto' × 'Fuji'). a. Arrow head indicates the direction of pollen tube growth. Triangle head indicates the stop position of pollen tube. Arrow bar = 0.1 mm. b. Scaled up a. Pollen tube tip was swollen. Bar = 0.02 mm.

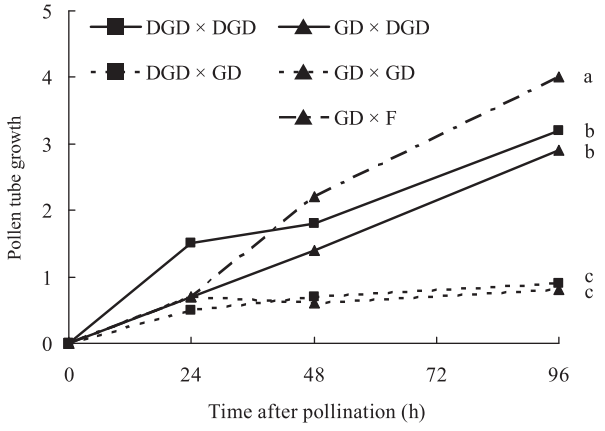

Fig. 6. Pollen tube growth of 'Doud Golden Delicious' and 'Golden Delicious' pollen in self- and cross-pollination pistils. Statistical analysis was conducted 96 h after pollination. Means followed by different letters are significantly different at $P < 0.01$ by Fisher's LSD test. DGD, 'Doud Golden Delicious' (4X); GD, 'Golden Delicious' (2X); F, 'Fuji' (2X).

of autotetraploids fertilized their own styles and those of their original diploid cultivars. Thus, the apple autotetraploid cultivar pollen could not be exactly recognized as self-pollen in the pistils of autotetraploid and diploid cultivars which shared two *S*-alleles, but the heteroallelic diploid pollen tube could be recognized as partially self-pollen by *S*-RNase and slightly inhibited pollen tube RNA synthesis.

A diploid cultivar ($2n$) produces haploid gametes (n). Autotetraploid cultivars ($4n$) produce diploid gametes ($2n$). The diploid gametes are either homoallelic or heteroallelic at the *S*-locus. Homoallelic diploid pollen might be arrested in a style containing its cognate *S*-RNase, because the pollen produces only one pollen-*S* protein. On the other hand, heteroallelic diploid pollen might be compatible in the same style—that is, its cognate *S*-RNase cannot recognize the pollen—because the pollen produces two kinds of pollen-*S* proteins: 'competitive interaction' (Golz, 1999, 2001). In the Solanaceae and Rosaceae *Prunus*, F-box protein is encoded by pollen *S*-genes and distinguishes between cognate *S*-RNase and nonself *S*-RNase (Qiao et al., 2004; Ushijima et al., 2003, 2004). In the Maloideae, the *SFBB* gene has been derived as pollen-*S* gene, which encodes an F-box protein (Sassa et al., 2007), and its molecular mechanism remains unknown. Consequently, there is no model to explain how pollen-*S* (SLF, SFB, and SFBB) and stylar-*S* (*S*-RNase) interact in the heteroallelic pollen tube—'competitive interaction'.

Recently, Hua et al. (2008) proposed a new biochemical model to explain 'competitive interaction'.

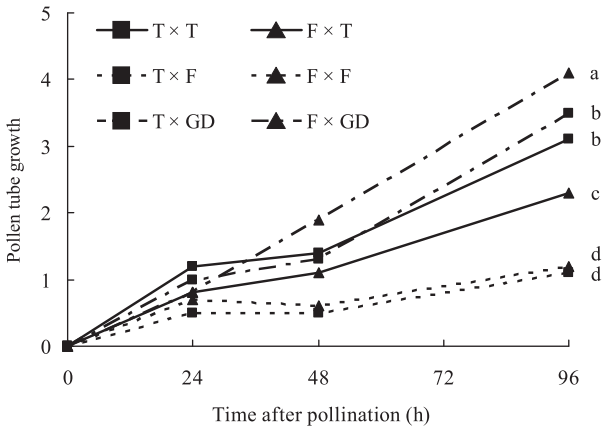

Fig. 7. Pollen tube growth of 'Tensei' and 'Fuji' pollen in self- and cross-pollination pistils. Statistical analysis was conducted 96 h after pollination. Means followed by different letters are significantly different at $P < 0.01$ by Fisher's LSD test. T, 'Tensei' (4X); F, 'Fuji' (2X); GD, 'Golden Delicious' (2X).

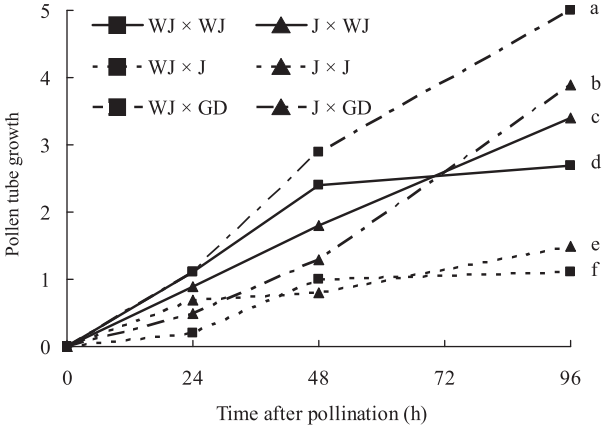

Fig. 8. Pollen tube growth of 'Welday Jonathan' and 'Jonathan' pollen in self- and cross-pollination pistils. Statistical analysis was conducted 96 h after pollination. Means followed by different letters are significantly different at $P < 0.01$ by Fisher's LSD test. WJ, 'Welday Jonathan' (4X); J, 'Jonathan' (2X); GD, 'Golden Delicious' (2X).

This model is based on the results of various *in-vitro* assays (Hua and Kao, 2006, 2008; Hua et al., 2007), and a modified protein-degradation model that the targeted *S*-RNase protein is degraded by the ubiquitin-26S proteasome pathway in the pollen tube. Thus, in incompatible pollination, since the interaction between $PiSLF_1$ and its self-*S*-RNase, S_1-RNase, in an S_1 pollen tube would be weaker than the favorable interaction between $PiSLF_1$ and non-self *S*-RNase (other *S*-RNases but S_1-RNase), the $PiSLF_1$-S_1-RNase complex would not be stable, and then most S_1-RNase molecules would exist in the free form and degrade pollen RNA, resulting in the growth inhibition of the S_1 pollen tube. On the other hand, in the case of compatible pollination, since the interaction between $PiSLF_1$ and non-self *S*-RNase (other *S*-RNases but S_1-RNase) in an S_1 pollen tube would interact more strongly than the interaction between $PiSLF_1$ and its self-*S*-RNase, S_1-RNase, this strong interaction would result in the formation of a stable $PiSLF_1$-non-self *S*-RNase complex, and then the non-self S-RNase would be ubiquitinated to target it for degradation and the growth of the S_1 pollen tube would not be inhibited. Additionally, in the case of 'competitive interaction', such as pollination of an S_1S_2 pistil by S_1S_2 heteroallelic pollen, two different PiSLFs, $PiSLF_1$ and $PiSLF_2$, and their respective self *S*-RNases, S_1-RNase and S_2-RNase, are present in the cytoplasm of the same pollen tube. $PiSLF_1$ and $PiSLF_2$ would preferentially interact with their respective non-self *S*-RNases to form stable $PiSLF_1$-S_2-RNase and $PiSLF_2$-S_1-RNase complexes, as in the case of non-self compatible pollination. $PiSLF_1$ or $PiSLF_2$ might also interact with their respective self *S*-RNases; however, these interactions would not result in stable complexes. Since the dissociated S_1-RNase and S_2-RNase would again preferentially interact with their non-self PiSLFs, all S_1-RNase and S_2-RNase would be in stable complexes with $PiSLF_2$ and $PiSLF_1$, respectively. As a result, all S_1-RNase and S_2-RNase would be ubiquitinated and degraded; consequently, S_1S_2 heteroallelic pollen would be fully compatible.

However, our results show that the SC of apple autotetraploid cultivars is incomplete and autotetraploid cultivar pollen must be recognized by its cognate *S*-RNase. Thus, this model could not prove the SC mechanism of apple autotetraploid cultivars, and these results suggest that the SC mechanism of apple autotetraploid cultivars might differ from that of the Solanaceae or Rosaceae *Prunus*. Further analysis of the *S*-genotypes of seedlings of autotetraploid cultivars are required to assess the effects of pollen *S*-genotypes on fertilization. It is necessary to investigate whether SC is induced by heteroallelic pollen, and to clarify the SC mechanism in apple autotetraploid cultivars.

Literature Cited

Crane, M. B. and D. Lewis. 1942. Genetical studies in pears. III. Incompatibility and sterility. J. Genet. 43: 31–42.

Entani, T., M. Iwano, H, Shiba, F. S. Che, A. Isogai and S. Takayama. 2003. Comparative analysis of the self-incompatibility (*S*) locus region of *Prunus mume*: identification of a pollen-expressed F-box gene with allelic diversity. Genes Cells 8: 203–213.

Entani, T., M. Iwano, H. Shiba, S. Takayama, K. Fukui and A. Isogai. 1999a. Centromeric localization of an S-RNase gene in *Petunia hybrida* Vilm. Theor. Appl. Genet. 99: 391–397.

Entani, T., S. Takayama, M. Iwano, H. Shiba, F.-S. Che and A. Isogai. 1999b. Relationship between polyploidy and pollen self-incompatibility phenotype in *Petunia hybrida* Vilm. Biosci. Biotechnol. Biochem. 63: 1882–1888.

Golz, J. F., H.-Y. Oh, V. Su, M. Kusaba and E. Newbigin. 2001. Genetic analysis of Nicotiana pollen-part mutants is consistent with the presence of an S-ribonuclease inhibitor at the S locus. PNAS 98: 15372–15376.

Golz, J. F., V. Su, A. E. Clarke and E. Newbigin. 1999. A molecular description of mutations affecting the pollen component of

the *Nicotiana alata S* locus. Genetics 152: 1123–1135.

Hauck, N. R., H. Yamane, R. Tao and A. F. Iezzoni. 2002. Self-compatibility and incompatibility in tetraploid sour cherry (*Prunus cerasus* L.). Sex. Plant Reprod. 15: 39–46.

Hauck, N. R., H. Yamane, R. Tao and A. F. Iezzoni. 2006. Accumulation of nonfunctional *S*-haplotypes results in the breakdown of gametophytic self-incompatibility in tetraploid Prunus. Genetics 172: 1191–1198.

Hua, Z. H., A. Fields and T-H. Kao. 2008. Biochemical models for S-RNase-based self-incompatibility. Mol. Plant. 1: 575–585.

Hua, Z. H. and T-H. Kao. 2006. Identification and characterization of components of a putative Petunia S-Locus F-Box-containing E3 ligase complex involved in S-RNase-based self-incompatibility. Plant Cell 18: 2531–2553.

Hua, Z. H. and T-H. Kao. 2008. Identification of major lysine residues of S_3-RNase of *Petunia inflata* involved in ubiquitin-26S proteasome-mediated degradation *in vitro*. Plant J. 54: 1094–1104.

Hua, Z. H., X. Y. Meng and T-H. Kao. 2007. Comparison of *Petunia inflata S*-locus F-box protein (Pi SLF) with Pi SLF-like proteins reveals its unique function in S-RNase-based self-incompatibility. Plant Cell 19: 3593–3609.

Ikeda, K., B. Igic, K. Ushijima, H. Yamane, N. R. Hauck, R. Nakano, H. Sassa, A. F. Iezzoni, J. R. Kohn and R. Tao. 2004. Primary structural features of the *S* haplotype-specific F-box protein, SFB, in *Prunus*. Sex. Plant Reprod. 16: 235–243.

Kao, T. H. and A. McCubbin. 1996. How flowering plants discriminate between self and non-self pollen to prevent inbreeding. Proc. Natl. Acad. Sci. USA 93: 12059–12065.

Kitahara, K., S. Matsumoto, T. Yamamoto, J. Soejima, T. Kimura, H. Komatsu and K. Abe. 2005. Molecular characterization of apple cultivars in Japan by *S*-RNase analysis and SSR markers. J. Amer. Soc. Hort. Sci. 130: 885–892.

Komori, S., J. Soejima, Y. Ito, H. Bessho, K. Abe and N. Kotoda. 1999. Discrimination of cross incompatibility by number of seeds per fruit and fruit set percentage in apples. Bull. Natl. Inst. Fruit Tree Sci. 33: 97–112.

Lai, Z., W. S. Ma, B. Han, L. Z. Liang, Y. S. Zhang, G. F. Hong and Y. B. Xue. 2002. An F-box gene linked to the self-incompatibility (s) locus of *Antirrhinum* is expressed specifically in pollen and tapetum. Plant Mol. Biol. 50: 29–42.

Lewis, D. and I. Modlibowska. 1942. Genetical studies in pears. IV. Pollen-tube growth and incompatibility. J. Genet. 43: 211–222.

Matsumoto, S. 2002. Ringo no S-idenshigata to jikafuwagouseiseiritsukatei. p. 14–20. (In Japanese). Kagakukenkyuhi-hojyokinkenkyuseikahoukokusyo.

McClure, B. A., V. Haring, P. R. Ebert, M. A. Anderson, R. J. Simpson, F. Sakiyama and A. E. Clarke. 1989. Style self-incompatibility gene products of *Nicotiana alata* are ribonucleases. Nature 342: 955–957.

de Nettancourt, F. Saccardo, U. Laneri and E. Capaccio. 1974. Self-compatibility in a spontaneous tetraploid of *Lycopersicon peruvianum* Mill. p. 77–84. In: Polyploidy and induced mutations in plant breeding. International Atomic Energy Agency, Vienna.

Pandey, K. K. 1968. Colchicine induced changes in self-incompatibility behavior of *Nicotiana*. Genetica 39: 257–271.

Qiao, H., F. Wang, L. Zhao, J. L. Zhou, Z. Lai, Y. S. Zhang, T. P. Robbins and Y. B. Xue. 2004. The F-box protein AhSLF-S_2 controls the pollen function of S-RNase-based self-incompatibility. Plant Cell 16: 2307–2322.

Sassa, H., H. Hirano and H. Ikehashi. 1992. Self-incompatibility-related RNases in styles of Japanese pear (*Pyrus serotina* Rehd). Plant Cell Physiol. 33: 811–814.

Sassa, H., H. Kakui, M. Miyamoto, Y. Suzuki, T. Hanada, K. Ushijima, M. Kusaba, H. Hirano and T. Koba. 2007. *S Locus F-Box Brothers*: Multiple and pollen-specific F-box genes with S haplotype-specific polymorphisms in apple and Japanese pear. Genetics 175: 1869–1881.

Sato, K. and H. Kitayama. 1992. Ringo jikaketsujitsuhinsyu no ketsujitsutokusei. J. Japan. Soc. Hort. Sci. 61 (Suppl. 2): 720 (In Japanese).

Sijacic, P., X. Wang, A. L. Skirpan, Y. Wang, P. E. Dowd, A. G. McCubbin, S. Huang and T. H. Kao. 2004. Identification of the pollen determinant of S-RNase-mediated self-incompatibility. Nature 429: 302–305.

Stout, A. B. and C. Chandler. 1942. Heredity transmission of induced tetraploidy and compatibility in fertilization. Science 96: 257.

Ueda, Y. and S. Akimoto. 2001. Cross- and self-compatibility in various species of the genus *Rosa*. J. Hort. Sci. Biotechnol. 76: 392–395.

Ushijima, K., H. Sassa, A. M. Dandekar, T. M. Gardziel, R. Tao and H. Hirano. 2003. Structural and transcriptional analysis of the self-incompatibility locus of almond: Identification of a pollen-expressed F-box gene with haplotype-specific polymorphism. Plant Cell 15: 771–781.

Ushijima, K., H. Yamane, A. Watari, E. Kakehi, K. Ikeda, N. R. Hauck, A. F. Iezzoni and R. Tao. 2004. The *S* haplotype-specific F-box protein gene, *SFB*, is defective in self-compatible haplotypes of *Prunus avium* and *P. mume*. Plant J. 39: 573–586.

Yamane, H., K. Ikeda, K. Ushijima, H. Sassa and R. Tao. 2003. A pollen-expressed gene for a novel protein with an F-box motif that is very tightly linked to a gene for S-RNase in two species of cherry, *Prunus cerasus* and *P. avium*. Plant Cell Physiol. 44: 764-769.

Yoshida, Y. 1986. Encyclopedia of Apple Cultivars. p. 255–1077. (In Japanese). JA Zennou Nagano, Nagano.