Crossability of Self-compatible Mutant 'Osa-Nijisseiki' (*Pyrus serotina* Rehd.)¹

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Summary

Crossability of self-compatible mutant 'Osa-Nijisseiki' was investigated in the field and *in vitro* by crossing with the other cultivars possessing various S-alleles (S₁S₄, S₂S₄, S₄S₅, S₅S₇).

'Osa-Nijisseiki' crossed successfully with all cultivars tested when used as female, whereas it did not cross well with cultivars having S₂S₄ genotype when used as male. The mechanism of cross-incompatibility of 'Osa-Nijisseiki' is elicited when S₂S₄-alleles are present in female tissue. However, the degree of incompatibility of 'Nijisseiki' (S₂S₄) was different from that of 'Kikusui' (S₂S₄) when crossed with 'Osa-Nijisseiki': the former combination was completely incompatible but the latter resulted in some fruit setting which persisted on a tree until harvest time. This shows that the S-gene in pollen of 'Osa-Nijisseiki' acts differently on the 2 cultivars, even though they have the same S₂S₄ genotype.

By using the excised-stylar system, crossing tests were also carried out. Pollen tubes with various S-alleles penetrated 'Osa-Nijisseiki' styles well, but pollen tubes of 'Osa-Nijisseiki' were completely arrested in excised styles of 'Nijisseiki' and partially arrested in styles of 'Kikusui'. Pollen tubes of selfed 'Osa-Nijisseiki' penetrated relatively well to the cut ends of its excised styles. Fluorescent microscopic observations showed that the growth ratio of pollen tubes in selfed style of 'Osa-Nijisseiki' was inferior to that in a crossed style.

These facts suggest that the style of 'Osa-Nijisseiki' loses its ability to arrest self pollen tubes but that the crossing barrier of 'Nijisseiki' and 'Kikusui' with 'Osa-Nijisseiki' is present in their styles. However, it seems that 'Osa-Nijisseiki' still possesses the properties of weak self-incompatibility.

Introduction

'Osa-Nijisseiki' is the only self-compatible cultivar of Japanese pear in Japan. This pear is a mutant from 'Nijisseiki' which was found in Tottori Prefecture. Furuta *et al.* (1) compared properties of this pear such as flowering time, number of flowers in a cluster, and sugar and acid content in mature fruit with those of 'Nijisseiki' and concluded that these 2 cultivars were almost the same

except for self-compatibility. They also studied the crossability of this pear with 'Nijisseiki' and 'Chōjūrō' and found that cross-incompatibility was elicited in the combination, 'Nijisseiki' × 'Osa-Nijisseiki'.

Machida *et al.* (6) investigated 'Osa-Nijisseiki' from a genetic standpoint and suggested that the compatibility was transferred to the progeny.

However, the characteristics of 'Osa-Nijisseiki' with respect to self- or cross-compatibility are not well understood now. In the present experiments, therefore, the characteristics of crossability and self-compatibility of this pear were studied by using a excised-

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stylar system and fluorescent microscopic method, together with field experiments.

Materials and methods

Plant materials

The self-compatible mutant of Japanese pear (*Pyrus serotina* Rehd. *culta* Rehd.) 'Osa-Nijisseiki', top-grafted on 'Nijisseiki' and grown in clay pots in the orchard of Chiba University, was employed. Adult trees of 'Kikusui' (S_2S_4), 'Nijisseiki' (S_2S_4), 'Yakumo' (S_1S_4), 'Suisei' (S_1S_4), 'Kōsui' (S_4S_5), 'Shinsui' (S_4S_5), 'Shinseiki' ($S_4S_5?$), and 'Kumoi' (?) grown in the same orchard were also used (4, 5, 7). 'Okusankichi' (S_5S_7) was kindly provided by Mr. Tanaka of Ichikawa city.

Anthers of each cultivar were collected immediately before flower opening, dehiscent in a desiccator and stored under dry conditions in a refrigerator (4°C) until used.

Field tests of crossability

Flowers of 'Osa-Nijisseiki' emasculated one day before anthesis were pollinated with pollen having various S-alleles. The pollinated flowers were covered with paraffin paper bags. On the other hand, 'Osa-Nijisseiki' pollen

was supplied to the emasculated flowers of each cultivar described above and they were covered with bags. Fruit set was recorded 3 weeks after pollination.

Pollen tube growth in excised styles

Pistils with petals, filaments and calyxes removed were pollinated and the styles were cut with a clean razor blade at the calyx end. They were then carefully placed on an 1% agar medium containing 10% sucrose and 100 mg/l H_3BO_3 in a Petri dish. After culturing 2 days, pollen tubes protruding from cut ends of styles were stained with 0.5% cotton blue solution and observed under a light microscope. The detailed procedures were described in a previous report (2).

Fluorescent microscopic observation of pollen tubes in pistils

Selfed flowers of 'Osa-Nijisseiki' and 'Nijisseiki', and the flowers of 'Nijisseiki' crossed with 'Chōjūrō', were sampled every 12 hours after pollination. Then the pistils were fixed with F. A. A. (formalin : acetic acid : 80% ethanol = 5 : 5 : 90), dehydrated with ethanol-butanol series, embedded in paraffin and cut into 10 μ m sections. The sections, with paraffin removed through xylene-ethanol se-

Table 1. Fruit set in the combinations between 'Osa-Nijisseiki' and cultivars having various S-alleles.

Combination		S-allele	No. of flowers pollinated	No. of fruit set	% of fruit set
♀	♂				
Osa-Nijisseiki	× Osa-Nijisseiki	—	28	25	89.3
〃	× Kikusui	S_2S_4	24	24	100
〃	× Nijisseiki	S_2S_4	30	29	96.7
〃	× Yakumo	S_1S_4	26	24	92.3
〃	× Suisei	S_1S_4	10	10	100
〃	× Kōsui	S_4S_5	23	23	100
〃	× Shinsui	S_4S_5	30	29	96.7
〃	× Shinseiki	(S_4S_5)	34	34	100
〃	× Okusankichi	S_5S_7	33	33	100
〃	× Kumoi	—	34	27	79.4
Kikusui	× Osa-Nijisseiki		29	11	37.9
Nijisseiki	× 〃		28	3	10.7
Yakumo	× 〃		24	24	100
Suisei	× 〃		36	36	100
Kōsui	× 〃		30	29	96.7
Shinsui	× 〃		28	27	96.4
Shinseiki	× 〃		29	29	100
Okusankichi	× 〃		—	—	—
Kumoi	× 〃		32	31	96.9

— : S-allele is not defined, () : S-allele is not defined well.

ries, were stained for 2 hours with 0.1% aniline blue dye dissolved in 0.1 N K_3PO_4 and observed under a fluorescent microscope to determine the number of pollen tubes present at each site in a pistil.

Twenty-five styles and 5 ovaries were employed for the observations and the mean number of pollen tubes in each tissue is shown in the Table 2 and 3.

Results and discussion

Field tests of crossability

When flowers of 'Osa-Nijisseiki' were pollinated with the pollen of various cultivars, they set fruit with all cultivars tested (Table 1). 'Osa-Nijisseiki' pollen also functioned well with other cultivars except for 'Nijisseiki' and 'Kikusui'. The failure of the combination, 'Nijisseiki' $\times$ 'Osa-Nijisseiki', agreed with data from Furuta *et al.* (1). Since 'Nijisseiki' and 'Kikusui' have the same S genotype (S_2S_4), the cross-incompatibility mechanism of 'Osa-Nijisseiki' should be elicited when S_2S_4 -alleles are present in the female tissue. Crossing tests over 3 years from 1982 to 1984, however, showed higher percentages of fruit set in the combination

Fig. 1. Fruit (upper) and seeds (lower) obtained by 'Kikusui' $\times$ 'Osa-Nijisseiki'.

of 'Kikusui' with 'Osa-Nijisseiki' than in that of 'Nijisseiki' with 'Osa-Nijisseiki'. The former combination produced a few fruit containing several fully developed seeds and they persisted on a tree until harvest time (Fig. 1). In the latter combination, even

Table 2. Pollen tube emergence from the excised styles in the combinations between 'Osa-Nijisseiki' and cultivars having various S-alleles.

Combination		No. of styles pollinated	No. of styles with tubes	% of styles with tubes
♀	♂			
Osa-Nijisseiki	$\times$ Osa-Nijisseiki	35	16	46
♂	$\times$ Kikusui	33	22	67
♂	$\times$ Nijisseiki	35	16	46
♂	$\times$ Yakumo	34	29	85
♂	$\times$ Suisei	36	27	75
♂	$\times$ Kōsui	32	31	97
♂	$\times$ Shinsui	31	27	87
♂	$\times$ Shinseiki	32	25	78
♂	$\times$ Okusankichi	30	22	73
♂	$\times$ Kumoi	33	10	30
Kikusui	$\times$ Osa-Nijisseiki	36	5	14
Nijisseiki	$\times$ ♂	36	0	0
Yakumo	$\times$ ♂	34	26	76
Suisei	$\times$ ♂	34	31	91
Kōsui	$\times$ ♂	30	13	43
Shinsui	$\times$ ♂	35	16	46
Shinseiki	$\times$ ♂	32	8	25
Okusankichi	$\times$ ♂	32	20	63
Kumoi	$\times$ ♂	30	9	30

when the combination caused some fruit setting, all fruit abscised during the early stages of fruit development.

Pollen tube growth in excised styles

When 'Osa-Nijisseiki' was used as female, percentages of styles which had pollen tubes emerging from cut ends were relatively high in all cases tested except for 3 combinations using 'Osa-Nijisseiki', 'Kumoi' and 'Nijisseiki' as male parents, respectively (Table 2). However, selfed styles of 'Osa-Nijisseiki' also showed well protruded pollen tubes, even though the percentage was lower than the other combinations. From this result, 'Osa-Nijisseiki' should be a stylar mutant. This idea fits the assumption of Machida *et al.* (6) who proposed the S genotype of 'Osa-Nijisseiki' as $S_2^{sm}S_4$ or $S_2S_4^{sm}$ (sm indicates stylar-part mutant). They also succeeded in producing self-compatible progenies by crossing 'Osa-Nijisseiki' with other commercial cultivars of Japanese pear.

The relatively lower percentage of tube protrusion from selfed 'Osa-Nijisseiki' styles may suggest that this pear still possesses the weak characteristics of self-incompatibility. From this viewpoint, the growth rate of pollen tubes in a selfed style of 'Osa-Nijisseiki' was compared in detail with that in selfed and crossed styles of 'Nijisseiki' in the next experiments.

When 'Osa-Nijisseiki' was used as male,

overall levels of pollen tube protrusion were lower. In the combination, 'Nijisseiki' × 'Osa-Nijisseiki', no styles with protruded tubes were observed. This agreed with the result from field experiments. The percentage of styles with protruded tubes in 'Kikusui', which has the same S genotype as 'Nijisseiki', was higher than in 'Nijisseiki'. This suggests that the styles of these cultivars react differently to 'Osa-Nijisseiki' pollen.

Fluorescent microscopic observation of pollen tubes in pistils

Pollen tubes in the pistils of selfed 'Osa-Nijisseiki', selfed 'Nijisseiki', and 'Nijisseiki' crossed with 'Chōjūrō' were observed under the fluorescent microscope in order to know the detailed growth pattern of pollen tubes among 3 combinations. In the styles of crossed 'Nijisseiki', pollen tubes reached the basal part of a style 48 hours after pollination and many then entered the ovary (Table 3). Pollen tube growth in styles of selfed 'Nijisseiki' was similar to that after crossing, up to 36 hours after pollination, but then many tubes ceased to grow at the basal part of the styles. These growth patterns corresponded well with those in selfed 'Chōjūrō' reported previously (3). On the other hand, in the styles of selfed 'Osa-Nijisseiki' pollen tubes grew similarly to those in the styles of selfed 'Nijisseiki' till 72 hours

Table 3. Pollen tube growth in the styles of selfed 'Osa-Nijisseiki' and 'Nijisseiki', and 'Nijisseiki' crossed with 'Chōjūrō'.

Hours after pollination	Distance (mm) from the stigmatic surface											
	Osa-Nijisseiki (Self)				Nijisseiki (Self)				Nijisseiki (Cross)			
	2	4	6	B	2	4	6	B	2	4	6	B
12	0	0	0	0	0	0	0	0	0	0	0	0
24	14	0	0	0	+	0	0	0	0	0	0	0
36	15	10	7	2	+	21	2	0	+	+	8	0
48	+	14	8	0	16	12	7	0	+	+	17	2
60	+	17	3	2	+	+	0	0	+	+	+	+
72	4	3	2	0	+	+	5	0	+	+	+	+
84	15	8	5	2	20	11	4	0	+	+	+	+
96	+	17	13	13	+	+	+	5*				
108	+	+	+	+	+	+	16	2	+	+	+	+

Each value is the mean number of pollen tubes per style.

+ : 30 or more pollen tubes were detected, * : many pollen tubes were detected in only one style,

B : basal part of style.

Fig. 2. Horizontal section of an ovary of 'Osa-Nijisseiki' 96 hours after self-pollination. The section was cut at the zone 1mm from the calyx end. Note the basal parts of styles. Arrows indicate pollen tubes.

Fig. 3. Horizontal section of an ovary of 'Osa-Nijisseiki' 120 hours after self-pollination. The section was cut at the zone 2mm from the calyx end. Note the basal or middle parts of ovules. Arrows indicate pollen tubes.

after pollination, after which they grew gradually and entered into ovaries at about 96 hours (Fig. 2).

Growth patterns of pollen tubes in ovaries of 3 combinations are shown in Table 4. As the middle or basal part of ovules was present at the zone 2 mm (Fig. 3), pollen tubes present at this region indicate that fertilization had already occurred, or would occur soon. In the crossed flowers of 'Nijisseiki', fertilization occurred about 72 hours after pollination, whereas in the selfed ones of 'Osa-Nijisseiki' fertilization occurred at about 108 hours. In the selfed flowers of 'Nijisseiki', no pollen tubes were observed at the basal part of an ovary at 160 hours after pollination.

Among 3 combinations, the growth ratio of pollen tubes in the styles was calculated at 60 and 108 hours after pollination. The ratio was shown as follows; Number of pollen tubes presented at 6mm from the stigmatic surface or at basal part of a style/Number of pollen tubes presented at 2 mm from the stigmatic surface (Fig. 4). At 60 hours, growth ratio in selfed 'Osa-Nijisseiki' was similar to that in selfed 'Nijisseiki'. However, at 108 hours the ratio in selfed 'Osa-Nijisseiki' almost agreed with that in crossed 'Nijisseiki'. It is probable that 'Osa-Nijisseiki' shows a self-incompatible property at early stages of tube elongation, and at the later stages it shows self-compatibility and then fertilization occurs normally.

Table 4. Pollen tube growth in the ovaries of selfed 'Osa-Nijisseiki' and 'Nijisseiki', and 'Nijisseiki' crossed with 'Chōjūrō'.

Hours after pollination	Distance (mm) from the calyx end											
	Osa-Nijisseiki (Self)				Nijisseiki (Self)				Nijisseiki (Cross)			
	0	1	2	3	0	1	2	3	0	1	2	3
60									8	8	0	0
72									11	7	5	0
84	1	0	0	0					15	5	2	0
96	+	7	0	0					+	13	3	0
108	21	11	3	0	0	0	0	0				
120	11	12	2	0								
132	10	7	3	0								

Each value is the mean number of pollen tubes per style (0, 1mm) or locule (2, 3mm). + : 30 or more pollen tubes were detected. Zone 0 or 1mm indicates basal part of styles (styles embedded into upper part of ovary); 2 or 3mm indicates middle or basal part of ovules.

Fig. 4. Growth ratio of pollen tubes in the styles of selfed 'Osa-Nijisseiki' and 'Nijisseiki', and 'Nijisseiki' crossed with 'Chōjūrō' at 60 and 108 hours after pollinations. A : No. of tubes present at 6 mm from the stigmatic surface/No. of tubes present at 2 mm from the stigmatic surface, B : No. of tubes present at basal part of the style/No. of tubes present at 2 mm from the stigmatic surface.
 ● : 'Osa-Nijisseiki' (Self), △ : 'Nijisseiki' (Self), ○ : 'Nijisseiki' (cross).

The conclusion of this paper is that the characteristics of 'Osa-Nijisseiki' with reference to self- or cross-compatibility are rather complex. Therefore, the properties of this

pear could not be fully explained by conventional S-genes for Japanese pears, as proposed by the previous researchers.

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ニホンナシの自家和合性突然変異体‘おさ二十世紀’の交配和合性

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摘 要

ニホンナシの自家和合性突然変異体である‘おさ二十世紀’と、さまざまな不和合性のS因子を有する品種との交配和合性について、ほ場および *in vitro* で試験を行なった。

1. ‘おさ二十世紀’を雌とした時は、試験された全ての品種と和合性を示した。逆に雄として用いた時は、 S_2S_4 因子をもつ品種と和合性を示さなかった。しかし、同じ S_2S_4 因子をもつ‘菊水’と‘二十世紀’とでは‘おさ二十世紀’に対する不和合性の程度が異なった。前者は若干の着果が見られたが、後者の場合は全く着果が認められなかった。

2. 切り取った花柱のシステムを用いて‘おさ二十世紀’の和合性を調べたところ、‘おさ二十世紀’は自家受粉でも花柱切断面から花粉管を突出した。しかし‘二

十世紀’の花柱では、‘おさ二十世紀’の花粉管生長は完全に抑制され、‘菊水’の花柱内でもかなり抑制された。これらの実験結果より、‘おさ二十世紀’は花柱が突然変異を起したために自家和合性になったものと考えられた。

3. けい光顕微鏡下で‘おさ二十世紀’の自家受粉、‘二十世紀’の自家受粉および‘二十世紀’と‘長十郎’との他家受粉花内の花粉管生長率及び生長速度を調査した結果、‘おさ二十世紀’の自家受粉区では‘二十世紀’の他家受粉区より生長速度が劣った。また、2で行なった花柱切断面からの花粉管出現率も、‘おさ二十世紀’の自家受粉区は他の区より劣る傾向を示しており、‘おさ二十世紀’は自家不和合的な性質も有しているものと考えられた。