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# Inheritance of Male Sterility in Japanese Apricot (*Prunus mume*)

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*Abstract.* Pollen fertility and inheritance patterns of male sterility were analyzed in various cultivars and selections of Japanese apricot (*Prunus mume* Sieb. et Zucc.). Male sterility segregated differently in two types of crosses. In pairings of male-sterile and male-fertile parents, progenies were either all male-fertile, all male-sterile, or mixed. Crossing two male-fertile plants resulted in offspring that were either all male-fertile or mixed. Male sterility in Japanese apricot appears to be of the gene-cytoplasmic type. The genotypes of 10 cultivars and three selections are determined.

Japanese apricot originated in southwest China and presently is one of the main fruit trees in Japan, where it has been cultivated from ancient times. Many cultivars are self-unfruitful; some are also male-sterile (Yaegaki et al., 2002a, 2002b), and fruit set can be adversely affected by meteorological conditions during the flowering period (February and March in Japan). Knowledge of the mode of inheritance of male sterility in Japanese apricot would be helpful in breeding self-fruitful cultivars, which will help to stabilize fruit production. However, the mechanisms of male sterility in Japanese apricot are presently not well established. Therefore, we analyzed pollen fertility and inheritance patterns in various cultivars, selections, and their progeny in order to determine the mode of inheritance of male sterility in Japanese apricot.

#### **Materials and Methods**

Two hundred sixty-six seedlings (≈4-6 years old) from 22 crosses of 14 cultivars and four selections of Japanese apricot, grown at the National Institute of Fruit Tree Science, Tsukuba, Japan, were examined (Tables 1 and 2). Pollen collected during the flowering periods from 1997 to 2002 was stored at -4  $^{\circ}$ C under desiccated conditions for  $\approx 1$  month. Pollen samples were incubated for ≈12 h at 20 °C on 1% agar plates with a 10% sucrose solution, and germination rates were determined on 200 pollen grains in each sample. Pollen grains were considered germinated when pollen tube growth was longer than the diameter of the pollen grain. In male-sterile genotypes, pollen grains were small, shriveled, and did not germinate.

### **Results and Discussion**

The number of seedlings showing male sterility (MS) or male fertility (MF) in each cross is presented in Table 2. Male sterility in all seedlings was observed in seven out of 12 crosses of the type male-sterile  $\times$  male-fertile (MS  $\times$  MF). In two MS  $\times$  MF crosses, all seedlings were male-fertile. Progenies of the three remaining MS  $\times$  MF crosses segregated into male-sterile and male-fertile seedlings. In three out of 10 crosses of two male-fertile parental plants (MF  $\times$  MF), progenies consisted of both male-fertile and male-sterile seedlings. In the remaining seven MF  $\times$  MF crosses, all seedlings were male-fertile.

Taken together, male sterility segregated differently in the two types of crosses. In MS × MF crosses, the progenies were all male-fertile, all male-sterile, or mixed. Crossing two malefertile plants (MF × MF) resulted in progenies that were either all male-fertile or mixed.

Male sterility can be caused by two distinct mechanisms. It may be controlled by genes in the nucleus (genic male sterility), or by cyto-

plasmic genes interacting with nuclear genes (gene-cytoplasmic male sterility)(Kaul, 1988). Male sterility in apricot (P. armeniaca L.) and peach [P. persica (L.) Batsch.] is controlled by a single recessive gene in the nucleus (Burgos and Ledbetter, 1994; Hesse, 1975). However, our results indicate that the inheritance pattern of male sterility in Japanese apricot is genecytoplasmic. All 21 seedlings obtained from crossing 'Shirokaga' and 'Nankou' (i.e., MS × MF), were male-sterile. In contrast, recovery of fertility was observed in seedlings obtained from the crossing 'Shirokaga' (MS) with 'Orihime' or 'Gessekai' (both MF). The 15 seedlings obtained from 'Nankou' × 'Juurou' (both MF) were all male-fertile. All 21 seedlings from the cross between 'Kagajizou' (MS) and 'Ume Tsukuba No. 4' (MF) were male-sterile, whereas all four seedlings from self-pollinated 'Ume Tsukuba No. 4' were male-fertile. Thus, it appears that male sterility in Japanese apricot is of the gene-cytoplasmic type.

Gene-cytoplasmic male sterility is common in higher plants (Kaul, 1988), and has been reported in the commercial fruit trees Citrus (Yamamoto et al., 1997) and olive (Besnard et al., 2000). Two genetic factors were responsible for gene-cytoplasmic male sterility (Kaul, 1988): "S," the cytoplasmic genome inducing male sterility, and "Rf," the nuclear genes that suppress the action of the cytoplasmic genome and promote normal pollen production. Male sterility is thought to be induced by the genotype (S)rfrf, i.e., a cytoplasmic genome favoring male sterility (S) together with homozygosity in the recessive nuclear gene rf. In the presence of the dominant nuclear fertility restoring gene (Rf) and/or the normal cytoplasmic genome (N), male fertility is recovered and normal pollen is produced.

The estimated genotypes of the gene-cytoplasmic male sterility model of cultivars and selections observed in this study are shown in Table 3. The genotypes of male-sterile cultivars, such as 'Shirokaga', 'Gyokuei', and 'Kagajizou', are presumed to be (S)rfrf.

Table 1. Male fertility, self-fruitfulness, and parentage of Japanese apricot cultivars and selections used in this study.

Cultivars/	Male	Self-			
selections	fertility <sup>z</sup>	fruitfulnessy	Parentage		
Shirokaga	MS	SU	Unknown		
Kagajizou	MS	SU	Shirokaga × Jizouume		
Gyokuei	MS	SU	Unknown		
Nankou	MF	SU	Unknown		
Gessekai	MF	SU	Jyousyuushiro × Ohshuku		
Baigou	MF	SU	Unknown		
Kairyouuchidaume	MF	SU	Unknown		
Gecchibai	MF	SU	Unknown		
Juurou	MF	SU	Unknown		
Ume Tsukuba No. 7 <sup>x</sup>	MF	SU	Gessekai × Baigou		
Orihime	MF	SF	Unknown		
Ryuukyoukoume	MF	SF	Unknown		
Jizouume	MF	SF	Unknown		
Hachirou	MF	SF	Jizouume open pollinated		
Inazumi	MF	SF	Unknown		
Ume Tsukuba No. 4 <sup>x</sup>	MF	SF	Jizouume open pollinated		
Ume Tsukuba No. 9 <sup>x</sup>	MF	SF	Orihime self-pollination		
MM-38-16 <sup>x</sup>	MF	SF	Youseiume self-pollination		

 $^{z}MS = male-sterile; MF = male-fertile.$ 

 $^{y}SU =$  self-unfruitful; SF = self-fruitfulness.

SO = sch-uninumun, Sr = sch-inumumess.

<sup>x</sup>Selection is bred at the National Institute of Fruit Tree Science, Tsukuba, Japan.

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Table 2. Segregation of male-sterile/fertile seedlings from crosses of Japanese apricot.

		No. of	Male fertility	Expected			Expected		
Cross combination		seedlings	MS : MF	ratio	$\chi^2$	Р	ratio	$\chi^2$	Р
MS × MF									
Shirokaga	× Nankou	21	21:0	1:0					
-	× Ryuukyoukoume	21	21:0	1:0					
	× Inazumi	5	5:0	1:0					
	× Jizouume	2	2:0	1:0					
	× Orihime	6	0:6	0:1					
	× Gessekai	3	0:3	1:1	3.000	0.08			
Kagajizou	× Kairyouuchidaume	25	25:0	1:0					
	× Ume Tsukuba No. 4	21	21:0	1:0					
	× MM-38-16	15	15:0	1:0					
	× Gecchibai	20	10:10	1:1	0.000	1.00			
	× Ume Tsukuba No. 7	10	3:7	1:1	1.600	0.21			
Gyokuei	× Gessekai	24	11:13	1:1	0.166	0.68	1:3	1.000	0.32
$MF \times MF$									
Nankou	× Juurou	15	0:15	0:1					
	× Orihime	4	0:4	0:1					
Gessekai	× Baigou	8	2:6	1:3	0.000	1.00			
	× Nankou	2	1:1	1:1	0.000	1.00			
Baigou	× Hachirou	19	7:12	1:1	1.315	0.25	1:3	1.420	0.23
-	× Orihime	8	0:8	0:1					
Hachirou	× Ume Tsukuba No. 9	20	0:20	0:1					
Ume Tsukuba No. 4	× Juurou	5	0:5	0:1					
Ume Tsukuba No. 4	Self pollination	4	0:4	0:1					
Orihime	Self pollination	8	0:8	0:1					

Table 3. Cytoplasmic and nuclear genotype of Japanese apricot cultivars and selections used in this study.

Cytoplasmic and	
nuclear gene type	Cultivars/selections
(S)rfrf	Shirokaga, Kagajizou, Gyokuei
(S)Rfrf	Gessekai, Baigou, Ume Tsukuba No. 7
(N)rfrf	Nankou, Kairyouuchidaume, Ryuukyoukoume, Inazumi, Hachirou <sup>z</sup> ,
	Ume Tsukuba No. 4, MM-38-16
(N)rf-	Jizouume
(–)RfRf	Orihime, Ume Tsukuba No. 9
(–)Rfrf	Gecchibai
Unknown	Juurou

In summary, inheritance patterns and the genotypes of male sterility were determined in several cultivars and selections of Japanese apricot. It is concluded that male sterility is of the nuclear-cytoplasmic type in this species. These investigations are continuing in order to produce new self-fruitful cultivars of Japanese apricot.

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<sup>z</sup>According to chi-square probability, 'Hachirou' is more likely to be (N)rfrf than (N)Rfrf.

The nuclear genes in the male-fertile cultivars and selections 'Nankou', 'Ryuukyoukoume', 'Kairyouuchidaume', 'Inazumi', 'Ume Tsukuba No. 4', and 'MM-38-16' probably are rfrf, since these cultivars and selections produced only male-sterile seedlings when crossed with the male-sterile cultivars and selections. Since they are male-fertile, they do not seem to possess the cytoplasmic male sterility factor, and can therefore be denoted (N)rfrf.

'Gessekai' and 'Baigou' carry the male sterility factor in the cytoplasm and are heterozygous in their fertility restoring genes denoted (S)Rfrf, because both male-sterile and fertile seedlings were produced in crosses with 'Gessekai' and 'Baigou' as female and/ or male parents. The genetic constitution of 'Ume Tsukuba No. 7', a selection derived from 'Gessekai'× 'Baigou', is also (S)Rfrf, because the progenies obtained from crossing 'Ume Tsukuba No. 7' with the male-sterile 'Kagajizou' segregated into male-sterile and fertile plants. Progenies of 'Shirokaga'× 'Orihime' and 'Baigou' × 'Orihime' were all male-fertile. Thus, 'Orihime' must be homozygous in the fertility restoring gene (i.e., RfRf), but its cytoplasmic factor is unknown. If so, 'Ume Tsukuba No. 9', which is derived from selfpollination of 'Orihime', must also carry RfRf. The inheritance pattern of 'Gecchibai' indicates the presence of heterozygous fertility restoring genes (Rfrf), because progenies derived from 'Gecchibai' pollen segregated into male-sterile and fertile seedlings. The cytoplasmic factor of 'Gecchibai' is unknown. The genotype of 'Jizouume', the female parent of 'Ume Tsukuba No. 4', must include the normal cytoplasmic factor (N) and at least one recessive nuclear gene. The progenies of the cross between pollen of 'Hachirou', which originated from the natural pollination of 'Jizouume', and 'Baigou' as female parent, segregated into seven malesterile seedlings and 12 male-fertile ones. This is closer to a ratio of 1:1 than 1:3 (Table 2), and we infer that the genotype of 'Hachirou' is more likely to be (N)rfrf than (N)Rfrf.