Selection of a Suitable Pollinizer for ‘Summer Fantasia’ Plum

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Abstract. Being self-incompatible, most Asian plums should be planted with a compatible pollinizer to produce fruits. Therefore, the selection of an adequate pollinizer is essential when new plum cultivars are released. To select a suitable pollinizer for ‘Summer Fantasia’ plum, the *S* genotype, cross-compatibility, pollen viability, and flowering time of five candidate cultivars were evaluated. Using polymerase chain reaction (PCR) analysis, the *S* genotype of ‘Summer Fantasia’ was determined as *S^aS^c*, which was expected to be compatible with other *S* genotypes. To test cross-compatibility, the trees were covered with caging net to prevent unintended pollination, and pistils were hand pollinated without emasculation. Fruit set percentage was calculated 10 weeks after pollination. The fruit set percentage resulting from the cross between ‘Summer Fantasia’ and ‘Taiyo’ (*S^bS^c*) was 13.8%, whereas that resulting from other combinations was less than 5.0%. Pollen germination percentage was investigated to monitor pollen viability; it varied yearly among cultivars, although ‘Formosa’ (10.6%) and ‘Taiyo’ (13.8%) showed the highest pollen germination percentages among the cultivars. When averaged over three years and two locations, ‘Summer Fantasia’ bloomed 2–3 days after ‘Akihime’, ‘Formosa’, ‘Oishiwase’, and ‘Purple Queen’. Blooming period of ‘Summer Fantasia’ and ‘Taiyo’ overlapped almost entirely. Overall, the results indicated that ‘Taiyo’ was the most suitable pollinizer for ‘Summer Fantasia’.

Most Asian plum (*Prunus salicina* Lindl.) cultivars have a self-incompatible mechanism that prevents self-fertilization (Okie and Hancock, 2008). In fact, most rosaceous fruit tree species, including *P. salicina*, exhibit gametophytic self-incompatibility, which is genetically controlled by at least two loci encoding the pollen and pistil allelic determinants (Kao and Tsukamoto, 2004; Tao and Iezzoni, 2010). In this mechanism, when the *S*-allele expressed in the pollen is identical to either of the two *S*-alleles expressed in the style, pollen tube growth is arrested in the style. By contrast, when the *S*-allele of the haploid pollen grain is different from the two *S*-alleles expressed in the style tissue, the pollen tube can grow through the style and fertilize the ovule (Guerra et al., 2009). Because of this mechanism, diploid plums often exhibit fruit set problems, and therefore, it is necessary to interplant suitable pollinizers in plum orchards. These pollinizers should flower with commercial cultivars and have abundant, cross-compatible, and active pollen (Crassweller et al., 1980; Kang et al., 2002). Among temperate fruit trees, the most actively studied pollinizers are those of apple cultivars (Bessho et al., 2009; Broothaerts et al., 2004; Halász et al., 2011).

Although studies on plum pollinizers are scarce, similar screening procedures for suitable pollinizers can be adapted from those used for apple cultivars.

Several experimental methods can be used to screen suitable pollinizers. Traditionally, controlled pollination under field conditions has been used for evaluating cross-compatibility. Pollen is collected from unopened blossoms just before the flowers open. Anthers are collected by rubbing the flowers through a sieve and then slightly dried overnight at room temperature or using extra heat; the pollen is easily shed from the dried anthers and applied to the stigmas. To prevent stigmas from contacting other pollen grains before cross-pollination, flowers are generally emasculated or covered with caging net. Although this method might be affected by environmental and agrotechnical factors, it is a critical process in determining suitable pollinizers. The microscopic observation of pollen tube growth in flowers is also used to determine cross-compatibility (Beppu et al., 2003).

The PCR-based *S*-*RNase* allele typing has been used widely to determine self-incompatibility among groups. This method allows identification of different *S*-alleles based on their characteristic amplicon size through PCR amplification (Beppu et al., 2002). This PCR method has been used to determine *S*-haplotypes in several rosaceous fruit tree cultivars such as almond (Tamura et al., 2000), apple (Janssens et al., 1995), pear (Ishimizu et al., 1999), and sweet cherry (Tao et al., 1999). Furthermore, the *S*-*RNase*

gene-specific primer set designed for sweet cherry can be used to determine *S*-haplotypes in apricot (Halász et al., 2005; Vilanova et al., 2005), mume (Tao et al., 2000; Yaegaki et al., 2001), and plum (Beppu et al., 2002; Jun et al., 2007) because the *S*-*RNase* genes of *Prunus* species are highly conserved. This approach is useful to identify cross-incompatibility groups as it can be carried out at any time of the year, not relying on flower availability. Also, it can be easily applied to determine *S* genotypes in new plum cultivars. ‘Soldam’ was the first *S*-genotyped Asian plum cultivar (Yamane et al., 1999), and the *S* genotypes of various other Asian plum cultivars were later determined by PCR (Beppu et al., 2002, 2003; Guerra et al., 2009; Halász et al., 2007; Jun et al., 2007; Sapir et al., 2008b; Zhang et al., 2007). To date, 42 *S*-*RNase* alleles and 15 *SFB* alleles have been identified (Guerra and Rodrigo, 2015).

Not every plum cultivar is a good source of pollen (Okie and Weinberger, 1996). Pollen viability or germination percentage varies among plum cultivars (Calić et al., 2013). Many studies on pollen viability and germination in mume (Du et al., 2006), peach (Hedhly et al., 2005; Sharafi, 2011), plum (Nikolić and Milatović, 2010), and sweet cherry (Hedhly et al., 2004; Koyuncu and Tosun, 2005) have indicated that pollen viability, germination, and tube growth significantly vary according to the species or cultivar. Similarly, various plum cultivars have different pollen vitality, and some plum cultivars produce infertile pollen or pollen that germinates poorly (Flory and Tomes, 1943; Jun et al., 2010; Okie and Weinberger, 1996). Therefore, evaluating pollen viability among cultivars is an important process in the selection of a suitable pollinizer.

In addition, the bloom periods of the pollinizer and main cultivar should overlap to enhance fertilization. Because cultivars do not bloom at the same time, monitoring the flowering period is an essential process when selecting a suitable pollinizer. Thus, the pollinizer should be selected by considering not only its genetic compatibility but also its blooming period (Sapir et al., 2008b).

To select suitable pollinizer for ‘Summer Fantasia’ plum, five plum cultivars were evaluated as pollinizer candidates. To investigate the genetic backgrounds of self-incompatibility, *S*-*RNase* alleles were monitored using the PCR method and primers designed from the conserved regions of rosaceous *S*-*RNase* (Beppu et al., 2002). In addition, cross-pollination and pollen germination tests were conducted, and flowering period was monitored to test the compatibility under field conditions.

Materials and Methods

Plant materials. Five plum cultivars were used in this study as candidate pollinizers for ‘Summer Fantasia’. Trees were grown at the experimental orchard of the National Institute of Horticultural and Herbal Science (NIHHS),

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Rural Development Administration, Republic of Korea. The experiments were conducted in Suwon (37°2'N, 127°0'E; Gyeonggi) in 2014–15 growing season and in Wanju (35°8'N, 127°0'E; Jeonbuk) in 2016. (Because of the relocation of the institution, the experimental field of the NIHHS was changed from Suwon to Wanju during 2013–15.) The experimental trees at Suwon were 7-years old in 2014, and those at Wanju were 4-years old in 2016. The trees were planted with 4 × 5 m spacing, and pruning, fertilization, and pest management were performed following the standard guidelines for plum cultivation (Chung et al., 2013).

DNA extraction. Young leaves were pulverized in liquid nitrogen using TissueLyser II (Qiagen, Hilden, Germany), and genomic DNA was then extracted with a DNeasy Plant Mini Kit (Qiagen, Germany) according to manufacturer's instructions. The quality of the extracted DNA was screened on a 0.8% agarose gel, and its quantification was performed using a spectrophotometer (NanoDrop 1000; Thermo Scientific, Wilmington, DE).

PCR amplification. The PCR protocol consisted of 35 cycles at 94 °C for 1 min, 56 °C for 1 min, and 72 °C for 90 s, which were preceded by an initial denaturation step at 94 °C for 3 min, followed by a final extension step at 72 °C for 7 min. The PCR mixture (25 µL) contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 200 µM each dNTP, 400 nM each primer, 20 ng template DNA, and 1 U Taq polymerase (Takara Shuzo Co., Kyoto, Japan). Two primer sets, Pru-C2 (Tao et al., 1999) and PCE-R (Yamane et al., 2001), and Pru-C2 and Pru-C6R (Vilanova et al., 2003), were used (Table 1). Pru-C2 and PCE-R were designed from C2 and C3 conserved regions of rosaceous *S-RNase* of sweet cherry, respectively (Tao et al., 1999; Yamane et al., 2001). The PCR products were subjected to electrophoresis on a 1.5% agarose gel, and DNA bands were visualized by ethidium bromide staining.

Evaluation of cross-compatibility under field conditions. Cross-compatibility was evaluated in 2014 and 2016. The trees were covered with caging net to prevent unintended pollination by insects, and pistils were hand pollinated without emasculation when flowers were in full bloom. More than 300 flowers were pollinated per replication, and pollination was replicated three times for each cross combination. Pollinated flowers were counted and fruit set was evaluated 10 weeks after pollination.

Pollen germination. Germination of the pollen used in hand pollination was determined in a solution containing 10% sucrose, 0.1% boric acid, and 1% agar. Pollen grains were dusted on agar mounts on glass slides and germinated in a controlled incubator for 8 h at 25 °C. Pollen grains were observed under an optical microscope (BX60; Olympus, Tokyo, Japan) and considered germinated when pollen tube length was greater than or equal to pollen diameter. Pollen germination percentage was calculated by

dividing the number of germinated pollen grains by the total number of pollen grains. Each glass slide was considered one replicate, and three replicates were evaluated for each cultivar. In each replicate, more than 30 pollen grains were observed.

Flowering time. The flowering times of plum cultivars were monitored from 2014 to 2016. The beginning of flowering and full bloom were recorded when 10% and 80% of flowers, respectively, were open. Petal falls were recorded when 10% of flower petals had fallen.

Statistical analysis. Data were evaluated statistically by analysis of variance followed by Duncan's multiple range test at the 1% level of significance using R (version 3.3.1) (R Core Team, 2014).

Results and Discussion

Variation in the *S-RNase* gene. Gene fragments were amplified in each cultivar using the primer sets Pru-C2 and PCE-R and Pru-C2 and Pru-C6R (Figs. 1 and 2). The primers Pru-C2 and PCE-R amplify the *S^a*, *S^b*,

S^c, *S^d*, and *S^e*-alleles with approximate length of 460, 1540, 1170, 1290, and 520 bp, respectively (Jun et al., 2007). Using this primer set, we obtained 460- and 1170-bp fragments from 'Summer Fantasia' (Fig. 1), which corresponded to the *S^a* and *S^c*-alleles, being consistent with previous results (Jun et al., 2015). The results obtained for the other five plum cultivars, for which *S* genotypes were already known, were identical to those of previous studies (Beppu et al., 2003; Jun et al., 2007).

The fragment lengths of *S^a*, *S^b*, *S^c*, *S^d*, and *S^e*-alleles detected using the Pru-C2 and Pru-C6R primer set are ≈400, 1500, 850, 1200, and 450 bp, respectively (Jun et al., 2007). The six plum cultivars, including 'Summer Fantasia', examined in the present study showed consistent banding patterns to those obtained by the Pru-C2 and PCE-R primer set (Fig. 2). The band for *S^c-RNase* was not observed in 'Summer Fantasia' using Pru-C2 and Pru-C6R, but it was amplified with the Pru-C2 and PCE-R primer set (Fig. 1).

Combining the PCR results for the two primer sets, the *S* genotype of 'Summer

Table 1. Sequence of primers used to amplify the *S-RNase* genes in plum.

Name	Sequence (5'→3')	Reference
Pru-C2 (forward)	CTT TGG CCA AGT AAT TAT TCA AAC C	Tao et al. (1999)
PCE-R (reverse)	TGT TTG TTC CAT TCG CYT TCC C	Yamane et al. (2001)
Pru-C6R (reverse)	CAT TGC CAC TTT CCA CGT C	Vilanova et al. (2003)

Fig. 1. Results of the polymerase chain reaction analysis of plum cultivars using the *S-RNase* gene-specific primer pair Pru-C2 and PCE-R. M, molecular size marker; 1, 'Akihime' (*S^b*); 2, 'Formosa' (*S^bS^d*); 3, 'Oishiwase' (*S^dS^c*); 4, 'Purple Queen' (*S^bS^c*); 5, 'Summer Fantasia' (*S^cS^c*); 6, 'Taiyo' (*S^bS^c*).

Fig. 2. Results of the polymerase chain reaction analysis of plum cultivars using the *S-RNase* gene-specific primer pair Pru-C2 and Pru-C6R. M, molecular size marker; 1, 'Akihime' (*S^b*); 2, 'Formosa' (*S^bS^d*); 3, 'Oishiwase' (*S^dS^c*); 4, 'Purple Queen' (*S^bS^c*); 5, 'Summer Fantasia' (*S^cS^c*); 6, 'Taiyo' (*S^bS^c*).

Fantasia' was determined as S^cS^c . Because 'Summer Fantasia' originated from a cross between 'Soldam' (S^cS^b , female parent) and 'Taiyo' (S^bS^c , male parent) (Jun et al., 2015), the S genotype determined for 'Summer Fantasia' (S^cS^c) is acceptable. This S genotype indicates that 'Summer Fantasia' needs a pollinizer with a different S genotype to ensure fruit set.

In plum cultivars, the S^c -allele is related to self-compatibility, and cultivars with the S^c -allele, such as 'Santa Rosa' and 'Beauty', show self-compatibility (Beppu et al., 2005). In sweet cherry, the self-compatible $S^{c'}$ -haplotype is attributed to a mutation (Ikeda et al., 2004), whereas in plum, no mutation is identified in the open reading frame of S^c -*RNase*, the mRNA quantity is very low, and the S^c -*RNase* protein is not detected (Watari et al., 2007). Thus, decreased levels of transcription, translation, or both of S^c -*RNase* seem to induce self-compatibility in plum.

In contrast, some self-fertile cultivars without the S^c -allele were reported (Beppu et al., 2012; Guerra et al., 2009). Interestingly, these self-fertile cultivars have the S^b -allele in common; Guerra et al. (2009) assumed that such cultivars might have a self-compatible S -*RNase* allele different from S^b but of similar size. Although self-compatible cultivars without the S^c -allele are not common, additional factors seem to affect self-compatibility in plum cultivars. Therefore, self-pollination tests were conducted to confirm the self-incompatibility of 'Summer Fantasia' (see section *Cross-compatibility*).

Plum cultivars are divided into various groups according to their S genotypes. However, the S^cS^c type is rare. Jun et al. (2007) reported that only 'White Queen' is the S^cS^c type, and more than half of plum cultivars belong to S^cS^b , S^bS^c , or S^cS^d groups. Among the 26 plum-incompatibility groups, only three cultivars were reported in the S^cS^c (XIV) group so far (Guerra and Rodrigo, 2015). Therefore, considering the S genotype, it is not difficult to select a candidate pollinizer whose S genotype is completely different from that of 'Summer Fantasia'. In the present study, the S genotype of all cultivars differed from that of 'Summer Fantasia' in at least one allele. Although 'Oishiwase' (S^cS^d), 'Purple Queen' (S^bS^c), and 'Taiyo' (S^bS^c) shared the S^c -allele with 'Summer Fantasia', they can be regarded as candidate pollinizers.

Cross-compatibility. The cross-compatibility of 'Summer Fantasia' with five plum cultivars was evaluated in 2014 and 2016 (Table 2). In addition, the self-pollination test of 'Summer Fantasia' was conducted in 2016 to confirm its self-incompatibility under field conditions. The cross-compatibility test found no combination in which fruit set was 0%, except for the self-pollination in 2016. Together with the S -genotype results, these compatibility tests confirmed that 'Summer Fantasia' is self-infertile and should be planted with a suitable pollinizer to ensure fruit set.

Although the percentage of fruit set was generally low (Table 2), it seems that the cross-compatibility among cultivars is attributable to their different S genotypes. When 'Summer Fantasia' (S^cS^c) was pollinated with 'Akihime' (S^bS^b), 'Formosa' (S^bS^d), 'Oishiwase' (S^cS^d), and 'Purple Queen' (S^bS^c), the fruit set was less than 5%. However, when 'Summer Fantasia' was pollinated with 'Taiyo', the average fruit set was 13.8%, which was the highest fruit set among the studied combinations.

In gametophytic self-incompatibility, cross-compatibility is divided into full compatibility and semicompatibility (Sapir et al., 2008a). In full compatibility, two cultivars differ in both S -alleles, whereas in semicompatibility, they share one of their S -alleles. Full compatibility has been reported as superior to semicompatibility for fruit set in plum cultivars (Sapir et al., 2008a). Other studies revealed that fully compatible pollinizers have better fruit set than semicompatible ones in other rosaceous fruit tree species, including apple (Goldway et al., 1999; Schneider et al., 2001) and pear (Goldway et al., 2005; Zisovich et al., 2005). The lower fruit set in semicompatible cultivars could be due to the rejection of half of the pollen. However, fruit set is not only affected by the genetic backgrounds, but also by a specific combination of cultivars and environmental conditions. For example, semicompatible pollinizer cultivars in Rosaceae, such as apple (Schneider et al., 2001), pear (Zisovich et al., 2005), and plum (Sapir et al., 2008a), showed a fruit set as high as that of the fully compatible cultivars. In the present study, fruit set with 'Taiyo' (S^bS^c) (13.84%), which is semicompatible with 'Summer Fantasia' (S^cS^c), was higher than that of 'Akihime' (S^bS^b) (2.56%) and 'Formosa' (S^bS^d) (3.24%), both of which are fully compatible with 'Summer Fantasia'. Although half of the semicompatible pollen grains were rejected in the pistil, enough pollen was provided by hand pollination that could overcome the deficiency of semicompatibility. However, the excessive amount of pollen does not always bring about high fruit set. For example, walnut (*Juglans regia* L.), which belongs to Juglandaceae, showed pistillate flower abscission in the presence of excessive pollen load (McGranahan et al., 1994). Therefore, other factors (such as pollen conditions) might influence fruit set in addition to the compatibility type. Therefore, pollen germination percentage was also evaluated (see section *Pollen germination*).

In artificial pollination, the fruit set of *P. salicina* generally ranged from 0% to 15% (Guerra et al., 2010; Nyéki and Szabó, 1996). In the present study, the fruit set using hand pollination was 1% to 16% (Table 2). According to Nyéki and Szabó (1997), combinations producing fruit set values between 0% and 1% are incompatible, between 1.1% and 5% are partially incompatible, and above 5% are compatible. Following this classification, 'Akihime', 'Formosa', 'Oishiwase', and 'Purple Queen' were partially incompatible, and 'Taiyo' was compatible with 'Summer Fantasia'.

Usually, fruit set of the Asian plum (5% to 14%) is lower than that of other *Prunus* species such as almond (30% to 40%), apricot (20% to 33%), peach (30% to 32%), sour cherry (25% to 50%), and sweet cherry (18% to 25%) (Guerra and Rodrigo, 2015). The fruit set dropped to even lower values when flowers were emasculated because of ovule degeneration caused by the emasculation (Guerra et al., 2010). Therefore, when parentage is designed, cross-pollination inside caging nets without emasculation is preferred in breeding systems to enhance fruit set.

However, caging nets also lower fruit set as revealed by Sapir et al. (2008a) who reported increased fruit set when caging nets are not used. These authors hypothesized that this was due to caging nets rubbing young fruitlets, maintaining high temperature inside the nets, and providing 30% of shade. Although the caging net is a preferred method when parentage is intended for breeding or study, the fruit set percentage could be increased without the caging net in commercial production.

Pollen germination. Pollen germination percentages were significantly different among the cultivars (Table 3), which was consistent with previous studies (Čalić et al., 2013; Du et al., 2006). Pollen germination percentage also varied among the years: it ranged between 4.99% and 18.74% in 2014 and between 0.88% and 14.12% in 2016. In particular, the pollen germination of 'Purple Queen' significantly decreased from 17.10% in 2014 to 1.64% in 2016. This variation seems to be due to the complicated interactions between environmental and genetic factors. In this two-year research, 'Formosa', 'Oishiwase', and 'Taiyo' showed higher and more stable pollen germination percentages

Table 2. Fruit set obtained from the controlled cross- and self-pollination of 'Summer Fantasia' in 2014 and 2016.

Pollen cultivar	Fruit set (%) ^z		
	2014	2016	Avg
Akihime	1.88 b	3.23 b	2.56
Formosa	2.80 b	3.68 b	3.24
Oishiwase	2.08 b	1.36 b	1.72
Purple Queen	4.11 b	4.82 b	4.47
Summer Fantasia	—	0.00 c	0.00
Taiyo	11.68 a	15.99 a	13.84

^zLetters across columns indicate statistical differences according to Duncan's multiple range test at $P \leq 0.01$.

Table 3. Pollen germination percentage of plum cultivars in 2014 and 2016.

Cultivar	Pollen germination (%) ^z		
	2014	2016	Avg
Akihime	4.99 b	0.88 c	2.94
Formosa	7.03 ab	14.12 a	10.58
Oishiwase	9.89 ab	9.74 ab	9.82
Purple Queen	17.10 ab	1.64 c	9.37
Summer Fantasia	—	11.39 ab	11.39
Taiyo	18.74 a	8.85 b	13.80

^zLetters across columns indicate statistical differences according to Duncan's multiple range test at $P \leq 0.01$.

compared with other cultivars. Therefore, these three cultivars were considered suitable pollinizers in terms of pollen viability.

Flowering time. The flowering times of the six plum cultivars were monitored during 2014–15 in Suwon and in 2016 in Wanju; the average flowering time across these 3 years is shown in Table 4. The average full bloom date for ‘Summer Fantasia’ was 10 April. In most plum cultivars, flowering began 3–4 d before the full bloom and flower abscission occurred 4–5 d after the full bloom. The flowering period, from the beginning of flowering to flower abscission, was similar among cultivars and lasted 8–10 d.

The average full bloom date of ‘Akihime’, ‘Formosa’, ‘Oishiwase’, and ‘Purple Queen’ was 7–8 Apr., which is 2–3 d earlier than that of ‘Summer Fantasia’ and ‘Taiyo’. In addition, the late flowering of ‘Summer Fantasia’ and ‘Taiyo’ was repeated in the 3 years. To be suitable, the flowering season of the pollinizer should be similar to that of the main cultivar (Crassweller et al., 1980). However, pollinizers with slightly earlier flowering than the main cultivar are also suitable. Among the investigated cultivars, the flowering time of ‘Taiyo’ was synchronized with that of ‘Summer Fantasia’. Therefore, in terms of flowering time, ‘Taiyo’ was the most suitable pollinizer among the ones investigated.

Another interesting aspect is the different bloom period of cultivars according to their locations. As mentioned above, the flowering time estimated at Suwon in 2014–15 and at Wanju in 2016 indicated that ‘Summer Fantasia’ was in full bloom 5 d later compared with ‘Akihime’, which showed the earliest full bloom. However, in 2016, the full bloom date of ‘Summer Fantasia’ was similar to that of ‘Akihime’, indicating that the span of full bloom among the cultivars was wider in Suwon than in Wanju. Flowering time, a

phenological characteristic, is readily influenced by environmental conditions and may change every year, especially due to temperature. Many reports have mentioned the different length of blooming periods according to locations and temperature. LaRue and Norton (1989) reported that winter with enough chilling often results in a relatively short blooming period and in a good overlap of pollinizer cultivars. By contrast, in warmer regions or after mild winters, the time span between the full blooms of early and late flowering cultivars was longer than that in cooler climates (Hartmann and Neumüller, 2009). Certain combinations of cultivars may not cross-pollinate effectively because their bloom periods do not coincide (LaRue and Norton, 1989). Therefore, when selecting a suitable pollinizer, the bloom period should be carefully monitored, particularly in warm places where it can be extended. In the present study, the six plum cultivars bloomed almost simultaneously in 2016 but not in 2014–15. The average temperature (recorded over 30 years) in Wanju (13.3 °C), which is located in Southern Korea, is similar or slightly higher than that in Suwon (12.0 °C) (Korea Meteorological Administration, 2011). However, the average temperatures during 20–25 and 10–15 d before the full bloom were lower in Wanju than in Suwon (Table 5), and this seems to have affected the bloom period. The mean temperature 20–25 d before full bloom was 9.1–9.5 °C at Suwon in 2014–15 and 4.7 °C at Wanju in 2016. Similarly, the mean temperature 10–15 d before full bloom was 12.1–14.2 °C at Suwon in 2014–15 and 7.2 °C in 2016. Considering the short bloom period (2–3 d) of cultivars in 2016 (Table 4), the lower temperatures before full bloom seem to be related with the short bloom period.

The chilling requirement of most Asian plums is 500–800 h, although some low-chill

plum cultivars have been reported whose chilling requirement is less than 450 h (Okie and Hancock, 2008). In the present study, because the flower and leaf buds were normally germinated, the chilling hours were sufficient for plum cultivars to break the rest period of buds in Suwon and Wanju. Moreover, the chilling hours (below 7 °C) in Suwon and Wanju are around 2300 and 2000 hr, respectively. Therefore, the winter chill of Suwon and Wanju was sufficient to break bud dormancy of the plum cultivars. Although chilling requirement is one of the major factors that influence flowering time, other factors such as heat requirement also affect the flowering time (Okie and Blackburn, 2011). In the present study, the different length of the bloom period seems to result from different temperatures before flowering.

Conclusion. Although there are many self-compatible cultivars in the European plum (*P. domestica*), most cultivars of the Asian plum (*P. salicina*) are self-sterile or have a very low rate of self-fertility (Alderman and Angelo, 1932; Waugh, 1896). Therefore, most Asian plums should be cross-pollinated for a good fruit set. In the present study, the *S* genotype, cross-compatibility, pollen germination, and flowering period of *P. salicina* cultivars were evaluated to select an adequate pollinizer. Although these characteristics are essential criteria, others such as disease and insect resistance and fruit quality should also be evaluated when selecting pollinizers. For apple cultivars, for example, flower morphology including flower color was also considered because the interchange between cultivars was greatest when flowers had the same color (Crassweller et al., 1980).

In the present study, five cultivars were evaluated to select a suitable pollinizer for ‘Summer Fantasia’ plum. Because the *S* genotypes of all evaluated cultivars differed from that of ‘Summer Fantasia’, they were all

Table 4. Average flowering period of the six plum cultivars evaluated in this study during 2014–15 in Suwon and in 2016 in Wanju, Republic of Korea. The span of flowering time is colored from the beginning of flowering to flower abscission along the 3 years.

Cultivar	Flower period (April)															Full bloom		
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	2014	2015	2016
Akihime						● ^z										7 Apr.	10 Apr.	4 Apr.
Formosa						●										8 Apr.	10 Apr.	4 Apr.
Oishiwase							●									9 Apr.	11 Apr.	4 Apr.
Purple Queen						●										8 Apr.	10 Apr.	5 Apr.
Summer Fantasia									●							12 Apr.	15 Apr.	4 Apr.
Taiyo									●							12 Apr.	14 Apr.	6 Apr.

^zAverage full bloom date.

Table 5. Yearly full bloom date and average daily temperatures before full bloom in Suwon and Wanju, Republic of Korea.

Yr	Location	Full bloom	Avg daily temp (°C) ^z	
			20–25 d before full bloom	10–15 d before full bloom
2014	Suwon	9 Apr.	9.5 a	14.2 a
2015	Suwon	12 Apr.	9.1 a	12.1 a
2016	Wanju	5 Apr.	4.7 b	7.2 b

^zLetters across columns indicate statistical differences according to Duncan’s multiple range test at $P \leq 0.01$.

possible pollinizers based on their genetic background. However, other factors such as cross-compatibility, pollen germination, and flowering time should also be considered when selecting a suitable pollinizer. ‘Taiyo’ showed the highest fruit set and, despite the yearly variation in pollen germination, the highest pollen germination percentage among the cultivars. The flowering period of ‘Taiyo’ was synchronized with that of ‘Summer Fantasia’. Taken together, ‘Taiyo’ is the most suitable pollinizer for ‘Summer Fantasia’ among the evaluated cultivars.

However, before encouraging growers to use potential pollinizers, their bloom periods and compatibility characteristics must be evaluated. In the present study, we selected ‘Taiyo’, which blooms simultaneously with ‘Summer Fantasia’ in Suwon and Wanju. However, further studies are required in other climatic areas where the bloom periods might be much more extended. For example, several selected apple pollinizers in England were not suitable in the United States because their flowering times were not synchronized with that of the main cultivar (Kang et al., 2002). Therefore, local adaptability, especially regarding flowering time, should be monitored when selecting suitable pollinizers. To expand the range of choices in pollinizer cultivars, which has a high economic importance, additional plum cultivars should be evaluated.

Literature Cited

- Alderman, W.H. and E. Angelo. 1932. Self and cross sterility in plum hybrids. *Proc. Amer. Soc. Hort. Sci.* 29:118–121.
- Beppu, K., N. Komatsu, H. Yamane, H. Yaegaki, M. Yamaguchi, R. Tao, and I. Kataoka. 2005. *S*⁺-haplotype confers self-compatibility in Japanese plum (*Prunus salicina* Lindl.). *J. Hort. Sci. Biotechnol.* 80:760–764.
- Beppu, K., M. Kumai, H. Yamane, R. Tao, and I. Kataoka. 2012. Molecular and genetic analysis of the *S*-haplotype of the self-compatible Japanese plum (*Prunus salicina* Lindl.) ‘Methley’. *J. Hort. Sci. Biotechnol.* 87:493–498.
- Beppu, K., Y. Takemoto, H. Yamane, H. Yaegaki, M. Yamaguchi, I. Kataoka, and R. Tao. 2003. Determination of *S*-haplotypes of Japanese plum (*Prunus salicina* Lindl.) cultivars by PCR and cross-pollination tests. *J. Hort. Sci. Biotechnol.* 78:315–318.
- Beppu, K., H. Yamane, H. Yaegaki, M. Yamaguchi, I. Kataoka, and R. Tao. 2002. Diversity of *S*-RNase genes and *S*-haplotypes in Japanese plum (*Prunus salicina* Lindl.). *J. Hort. Sci. Biotechnol.* 77:658–664.
- Bessho, H., M. Wada, K. Kudo, Y. Inomata, H. Iwanami, K. Abe, J. Soejima, S. Komori, and A. Suzuki. 2009. Selection of crabapple pollinizers for ‘Fuji’ and ‘Tsugaru’ apple. *J. Amer. Pomol. Soc.* 63:2–13.
- Broothaerts, W., I. Van Nerum, and J. Keulemans. 2004. Update on and review of the incompatibility (*S*-) genotypes of apple cultivars. *HortScience* 39:943–947.
- Ćalić, D., N. Devrnja, I. Kostić, and M. Kostić. 2013. Pollen morphology, viability, and germination of *Prunus domestica* cv. Požegača. *Sci. Hort.* 155:118–122.
- Chung, K.H., S.K. Yoon, E.Y. Nam, I.K. Yun, J.M. Park, S.C. Lee, and D.S. Chung. 2013. The guide of agricultural technique, Plum and mume (in Korean). Rural Development Administration, Suwon, Republic of Korea.
- Crassweller, R.M., D.C. Ferree, and L.P. Nichols. 1980. Flowering crab apples as potential pollinizers for commercial apple cultivars. *J. Amer. Soc. Hort. Sci.* 105:475–477.
- Du, Y.H., S.L. Zhang, X.T. Jiang, and J. Wu. 2006. Characteristics of pollen germination and pollen tube growth of *Prunus mume* in vitro. *Xibei Zhiwu Xuebao* 26:1846–1852.
- Flory, W.S. and M.L. Tomes. 1943. Studies of plum pollen, its appearance and germination. *J. Agr. Res.* 67:337–342.
- Goldway, M., O. Shai, H. Yehuda, A. Matityahu, and R.A. Stern. 1999. ‘Jonathan’ apple is a lower-potency pollenizer of ‘Topred’ than ‘Golden Delicious’ due to partial *S*-allele incompatibility. *J. Hort. Sci. Biotechnol.* 74:381–385.
- Goldway, M., R.A. Stern, A.H. Zisovich, and S. Shafir. 2005. Application of *S*-RNase allele molecular analysis as a means for elucidating low yields in the pear orchard. *Acta Hort.* 671:137–142.
- Guerra, M.E. and J. Rodrigo. 2015. Japanese plum pollination: A review. *Sci. Hort.* 197:674–686.
- Guerra, M.E., J. Rodrigo, M. López-Corrales, and A. Wüsch. 2009. *S*-RNase genotyping and incompatibility group assignment by PCR and pollination experiments in Japanese plum. *Plant Breed.* 128:304–311.
- Guerra, M.E., A. Wüsch, M. López-Corrales, and J. Rodrigo. 2010. Flower emasculation as the cause for lack of fruit set in Japanese plum crosses. *J. Amer. Soc. Hort. Sci.* 135:556–562.
- Halász, J., A. Hegedűs, Z. György, É. Pállinger, and M. Tóth. 2011. *S*-genotyping of old apple cultivars from the Carpathian basin: Methodological, breeding and evolutionary aspects. *Tree Genet. Genomes* 7:1135–1145.
- Halász, J., A. Hegedűs, R. Hermán, É. Stefanovits-Bányai, and A. Pedryc. 2005. New self-incompatibility alleles in apricot (*Prunus armeniaca* L.) revealed by stylar ribonuclease assay and *S*-PCR analysis. *Euphytica* 145:57–66.
- Halász, J., A. Hegedűs, Z. Szabó, J. Nyéki, and A. Pedryc. 2007. DNA-based *S*-genotyping of Japanese plum and pluot cultivars to clarify incompatibility relationships. *HortScience* 42:46–50.
- Hartmann, W. and M. Neumüller. 2009. Plum breeding, p. 161–231. In: S.M. Jain and P.M. Priyadarshan (eds.). *Breeding plantation tree crops: Temperate species*. Springer, New York.
- Hedhly, A., J.I. Hormaza, and M. Herrero. 2004. Effect of temperature on pollen tube kinetics and dynamics in sweet cherry, *Prunus avium* (Rosaceae). *Amer. J. Bot.* 91:558–564.
- Hedhly, A., J.I. Hormaza, and M. Herrero. 2005. The effect of temperature on pollen germination, pollen tube growth, and stigmatic receptivity in peach. *Plant Biol.* 7:476–483.
- Ikeda, K., A. Watari, K. Ushijima, H. Yamane, N.R. Hauck, A.F. Iezzoni, and R. Tao. 2004. Molecular markers for the self-compatible *S*⁺-haplotype, a pollen-part mutation in sweet cherry (*Prunus avium* L.). *J. Amer. Soc. Hort. Sci.* 129:724–728.
- Ishimizu, T., K. Inoue, M. Shimonaka, T. Saito, O. Terai, and S. Norioka. 1999. PCR-based method for identifying the *S*-genotypes of Japanese pear cultivars. *Theor. Appl. Genet.* 98:961–967.
- Janssens, G.A., I.J. Goderis, W.F. Broekaert, and W. Broothaerts. 1995. A molecular method for *S*-allele identification in apple based on allele-specific PCR. *Theor. Appl. Genet.* 91:691–698.
- Jun, J.H., J.H. Kwon, and K.H. Chung. 2007. Identification of self-incompatibility genotypes in Japanese plum (*Prunus salicina* Lindl.) by polymerase chain reaction and cross-pollination test. *Hort. Environ. Biotechnol.* 48:228–234.
- Jun, J.H., J.H. Kwon, K.R. Do, I.K. Yun, K.B. Ma, and J.B. Kim. 2010. Analysis of ploidy and *S*-alleles of ‘Kiyo’ a Japanese plum. *Kor. J. Hort. Sci. Technol.* 28:98–102.
- Jun, J.H., E.Y. Nam, K.H. Chung, S.K. Yun, J.H. Kwon, I.K. Yun, Y.B. Kwack, S.J. Kim, and K.H. Cho. 2015. ‘Summer Fantasia’ Japanese plum. *HortScience* 50:932–933.
- Kang, I.K., G.J. Lee, M.J. Kim, S.I. Kwon, P.Y. Peak, and D.G. Choi. 2002. Selection of crabapple as pollinizers for major apple cultivars in apple orchard. *Kor. J. Hort. Sci. Technol.* 20:330–334.
- Kao, T. and T. Tsukamoto. 2004. The molecular and genetic bases of *S*-RNase-based self-incompatibility. *Plant Cell* 16(Suppl.):72–83.
- Korea Meteorological Administration. 2011. Climatological normals of Korea. Korea Meteorological Administration. Seoul, Republic of Korea.
- Koyuncu, F. and F. Tosun. 2005. Evaluation of pollen viability and germinating capacity of some sweet cherry cultivars grown in Isparta, Turkey. *Acta Hort.* 795:71–74.
- LaRue, J.H. and M.V. Norton. 1989. Japanese plum pollination, p. 49–55. In: J.H. LaRue and R.S. Johnson (eds). *Peaches, plums, and nectarines: Growing and handling for fresh market*. Univ. of California, Oakland, CA.
- McGranahan, G.H., D.G. Voyiatzis, P.B. Catlin, and V.S. Polito. 1994. High pollen loads can cause pistillate flower abscission in walnut. *J. Amer. Soc. Hort. Sci.* 119:505–509.
- Nikolić, D. and D. Milatović. 2010. Examining self-compatibility in plum (*Prunus domestica* L.) by fluorescence microscopy. *Genetika* 42:387–396.
- Nyéki, J. and Z. Szabó. 1996. Fruit set of plum cultivars under Hungarian ecological conditions. *Acta Hort.* 423:185–192.
- Nyéki, J. and Z. Szabó. 1997. Cross-incompatibility in stone fruits. *Acta Hort.* 437:213–218.
- Okie, W.R. and B. Blackburn. 2011. Increasing chilling reduces heat requirement for floral budbreak in peach. *HortScience* 46:245–252.
- Okie, W.R. and J.F. Hancock. 2008. Plums, p. 337–357. In: J.F. Hancock (ed.). *Temperate fruit crop breeding: Germplasm to genomics*. Springer, Netherlands.
- Okie, W.R. and H. Weinberger. 1996. Plums, p. 559–607. In: J. Janick and J.N. Moore (eds.). *Fruit breeding. Vol. I. Tree and tropical fruits*. Wiley, New York, NY.
- R Core Team. 2014. R: A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. <<http://www.R-project.org/>>.
- Sapir, G., R.A. Stern, S. Shafir, and M. Goldway. 2008a. Full compatibility is superior to semi-compatibility for fruit set in Japanese plum (*Prunus salicina* Lindl.) cultivars. *Sci. Hort.* 116:394–398.
- Sapir, G., R.A. Stern, S. Shafir, and M. Goldway. 2008b. *S*-RNase based *S*-genotyping of Japanese plum (*Prunus salicina* Lindl.) and its implication on the assortment of cultivar-couples in the orchard. *Sci. Hort.* 118:8–13.
- Schneider, D., R.A. Stern, D. Eisikowitch, and M. Goldway. 2001. Analysis of *S*-alleles by PCR for determination of compatibility in the ‘Red Delicious’ apple orchard. *J. Hort. Sci. Biotechnol.* 76:596–600.

- Sharafi, Y. 2011. An investigation on the pollen germination and tube growth in some *Prunus persica* genotypes and cultivars. *Afr. J. Microbiol. Res.* 5:2003–2007.
- Tamura, M., K. Ushijima, H. Sassa, H. Hirano, R. Tao, T.M. Gradziel, and A.M. Dandekar. 2000. Identification of self-incompatibility genotypes of almond by allele-specific PCR analysis. *Theor. Appl. Genet.* 101:344–349.
- Tao, R., T. Habu, H. Yamane, A. Sugiura, and K. Iwamoto. 2000. Molecular markers for self-compatibility in Japanese apricot (*Prunus mume*). *HortScience* 35:1121–1123.
- Tao, R. and A.F. Iezzoni. 2010. The S-RNase-based gametophytic self-incompatibility system in *Prunus* exhibits distinct genetic and molecular features. *Sci. Hort.* 124:423–433.
- Tao, R., H. Yamane, A. Sugiura, H. Murayama, H. Sassa, and H. Mori. 1999. Molecular typing of S-alleles through identification, characterization and cDNA cloning for S-RNases in sweet cherry. *J. Amer. Soc. Hort. Sci.* 124:224–233.
- Vilanova, S., C. Romero, D. Abernathy, A.G. Abbott, L. Burgos, G. Llacer, and M.L. Badenes. 2003. Construction and application of a bacterial artificial chromosome (BAC) library of *Prunus armeniaca* L. for the identification of clones linked to the self-incompatibility locus. *Mol. Genet. Genomics* 269:685–691.
- Vilanova, S., C. Romero, G. Llacer, M.L. Badenes, and L. Burgos. 2005. Identification of self-(in) compatibility alleles in apricot by PCR and sequence analysis. *J. Amer. Soc. Hort. Sci.* 130:893–898.
- Watari, A., T. Hanada, H. Yamane, T. Esumi, R. Tao, H. Yaegaki, M. Yamaguchi, K. Beppu, and I. Kataoka. 2007. A low transcriptional level of S^c-RNase in the S^c-haplotype confers self-compatibility in Japanese plum. *J. Amer. Soc. Hort. Sci.* 132:396–406.
- Waugh, F.A. 1896. Problems in plum pollination. *Annu. Rpt. Vermont. Agr. Expt. Sta.* 10:87–98.
- Yaegaki, H., T. Shimada, T. Moriguchi, H. Hayama, T. Haji, and M. Yamaguchi. 2001. Molecular characterization of S-RNase genes and S-genotypes in the Japanese apricot (*Prunus mume* Sieb. et Zucc.). *Sex. Plant Reprod.* 13:251–257.
- Yamane, H., R. Tao, and A. Sugiura. 1999. Identification and cDNA cloning for S-RNases in self-incompatible Japanese plum (*Prunus salicina* Lindl. cv. Sordum). *Plant Biotechnol.* 16:389–396.
- Yamane, H., R. Tao, A. Sugiura, N.R. Hauck, and A.F. Iezzoni. 2001. Identification and characterization of S-RNases in tetraploid sour cherry (*Prunus cerasus*). *J. Amer. Soc. Hort. Sci.* 126:661–667.
- Zhang, S.L., S.X. Huang, H. Kitashiba, and T. Nishio. 2007. Identification of S-haplotype-specific F-box gene in Japanese plum (*Prunus salicina* Lindl.). *Sex. Plant Reprod.* 20:1–8.
- Zisovich, A., R.A. Stern, S. Shafir, and M. Goldway. 2005. Fertilisation efficiency of semi- and fully-compatible European pear (*Pyrus communis* L.) cultivars. *J. Hort. Sci. Biotechnol.* 80:143–146.