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RESEARCH ARTICLE

Maojun ZHANG, Baojiang LI, Qiang WANG, Lihua DING, Xingkai YAN, Guojie XING

Study on the mating compatibility of part pear varieties and wild types of *Pyrus ussuriensis*

Abstract To understand the mating compatibility of *Pyrus ussuriensis* Maxim., we studied the fertility of pollen and conducted a hand-pollination trial in the field on some pear varieties and wild types. The results showed that about 53% of varieties among 32 tested genotypes were male sterile. Not only did the pollen vitalities in normal varieties show distinct differences, but pollen vitalities from flower forcing in a glasshouse were found to be lower than those from natural flowering in the field, which had no apparent effect on fruit setting of tested varieties. Most of the tested genotypes such as Nanguoli, Pingxiangli, and Hanxiangli showed self-incompatibility (SI). Honghuagaili could bear fruit after hand pollination, but there were abnormal seeds in its fruits. So we suggested it was a recessive SI that happened during embryo development. Longxiangli has the capacity of self-compatibility (SC) to some extent, its fruit setting rate of inflorescence could reach 23.3%. Manual self-pollination during bud flowering could improve the fruit setting rate of part tested genotypes with SI, but had no effect on the fruit setting rate 3 days after flowering. Mating between female parents with the variety selected from F_1 generation showed that the majority of their combinations were compatible. There was one-way SC when Nanguoli was crossed with Hanhongli, while no fruits could be found after Hanhongli was crossed with Nanguoli. It may be related to the S-genotype or haplotype of Nanguoli. In addition, mating between the varieties derived from bud mutation with the female parent appeared incompatible. We concluded that *P. ussuriensis* Maxim. is similar to other grown pear systems with the characteristics of SI, the fruit setting rate of self pollination in some varieties and wild types can be improved by artificial self-pollination during bud flowering, and fruit cannot be developed through pollination between the varieties from bud mutation and the female parent.

Keywords *Pyrus ussuriensis* Maxim., self-incompatibility (SI), mating compatibility, self-pollination

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Maojun ZHANG, Baojiang LI (✉)
Horticulture College, Shenyang Agricultural University, Shenyang 110161, China
E-mail: libaojiang@hotmail.com

Maojun ZHANG, Qiang WANG, Lihua DING, Xingkai YAN, Guojie XING
Pomology Institute, Jilin Academy of Agricultural Sciences, Gongzhuling 136100, China

1 Introduction

Mating compatibility between individuals is a genetic trait that develops in flowering plants during a long period of evolution. One of them is the self-incompatibility (SI) system, which plays an important role in maintaining the hereditary variation of plants and widely exists in nature. It is estimated that more than 75 families (Lewis, 1979), 3000 species in flowering plants and half of angiosperms, have the characteristics of self-incompatibility (SI). SI is the inherited ability of a flower to reject its own pollen and promote out-crossing. It has a significant effect on plant breeding and cultivation, so researches are focused on relevant molecular biology and plant reproductive biology.

Pears are important deciduous fruit trees cultivated in temperate zones. There have been a lot of reports worldwide (Ishimizu et al., 1999: Norioka et al., 2001; Zuccherelli et al., 2002; Xu and Zhang, 2003; Xu et al., 2004) on the search for SI in *P. Prifolia* Nakai, *P. communis* and *P. bretschnider* in genetic or molecular mechanisms and determining S-genotype, but there are few studies dealing with *P. ussuriensis* Maxim.. Tan et al. (2005) and Wuyun et al. (2005) found 7 and 3 new *S* genes that controlled the pear SI in *P. prifolia* Nakai and *P. bretschnider*, respectively, by PCR-RELF detection and DNA sequence analysis of genome from Baili and Shali varieties grown in China. In this paper, we performed a systemic study on mating compatibility with varieties and wild types of *P. ussuriensis* by surveying their pollen fertility from different flower forcing methods and fruit setting rate from controlled hand pollination at different growth stages (flower bud, flowering and post flowering), and crossing reciprocally between bud mutation and female parent, female parent and the variety selected from F_1 generation. All the different varieties had similar phenotypes. The objective of our experiment was to provide a theoretical basis for genetic improvement, a highly efficient cultivation system, and information on SI of *P. ussuriensis*.

2 Materials and methods

2.1 Test materials

All of the experimental varieties and wild types came from the Gongzhuling Coldland Fruit Nursery of the National Fruit Germplasm Resources in China and the Pear Experiment Garden of the Pomology Institute at Jilin Academy of Agricultural and Sciences. There was a total of 32 genotypes including Nanguoli, Dananguoli, Pingxiangli, Xiehuatianli, Hanxiangli, Hanhongli, Honghuagaili, Changjianbali, Shanli1 (wild type), Shanli2, and so on. All the tested trees were over 10 years old.

2.2 Pollen collection

We cut off over 20 strong branches with flower buds from tested genotypes in mid April in the Gongzhuling district of Jilin Province. After tagging, they were put into water added with 10% sucrose and 0.1% borax for flowering in the greenhouse. Before flowering, we collected the anthers, dried them under 20–25°C, then removed the anther walls and reserved them in the vial for the following pollination experiment.

2.3 Investigation of pollen fertility and viability

The anther performance in different tested genotypes was surveyed and recorded during the normal flowering stage in the field in 2005 and 2006. The fertility and viability of the same variety pollen from greenhouse flower forcing and field had also been identified using the I-KI stain method (He et al., 2005) and a microscope. The pollen germination was recorded, and the materials without pollen or normal pollen were eliminated.

2.4 Manual self-pollination experiment

The manual self pollination was done in the period of bud-flower (two days before flowering), flowering, and late flowering (3 days after flowering) by selecting 3 trees for each variety, 10 flower buds per tree and 3 suitable flowers per inflorescence for pollination experiments, with the other flowers in the same inflorescence discarded. Before pollination, the anthers of the flowers that were left were cut off, thereafter hand pollinated, and finally the inflorescence was bagged and tagged. The bags were removed 7 days later to investigate the fruit setting rate, which was reinvestigated in early June, early July, and before harvest. After harvest, the seed number and seed development status in the experimental fruits were investigated. The fruit setting was recorded after each investigation.

2.5 Reciprocal crossing between female parent and F_1 generation

Test materials were Pingxiangli selected from Pingguoli natural seedlings, Hanxiang and Hanhong, which were the offspring from a combination of Daxiangshuili × Pingxiangli and Nanguoli × Jinsuli. To understand mating compatibility of the female parent with the F_1 generation, we performed the reciprocal crossing between Pingxiangli and Pingguoli, Hanxiangli and Pingxiangli, Hanhongli and Nanguoli. The experimental method and procedure were similar to the self pollination in flowering described above.

2.6 Reciprocal crossing between different varieties with a similar phenotype

Jianbazili, Ruanbazili and Changbazili, as well as Honghuagaili, Huagaili and Donghuagaili, have a similar phenotype in fruit trait and belong to the same variety group. To know mating compatibility within the same variety group, we independently did the reciprocal crossing between both of them. The experimental method and procedure were similar to the self pollination in flowering discussed above.

2.7 Reciprocal crossing between bud mutation and female parent

Nanguoli is a typical good variety of *P. ussuriensis*. Youhongli and Dananguoli were selected from the Nanguoli mutation. To understand mating compatibility between bud mutation and female parent, we performed the reciprocal crossing between them. The experimental method and procedure were similar to the self pollination in flowering as above.

3 Results and analysis

3.1 Pollen fertility of varieties from *P. ussuriensis*

Investigation in the field and indoor appraisal of anther or pollen quality showed that 17 varieties among the tested genotypes (Matihuangli, Tangli, Shangyali, Mali, Qingpicuili, Ruanbazili, Jianbazili, Hongtangli, Suanliguoli, Donghuagaili, Huagaili, Hululi, Daxiangshuili, Xiaoxiangshuili, Balixiangli, Seli, and Zaobaili) had anthers withered without opening, and no pollen was found in them by microscope. They were considered as male sterile and accounted for 53.1% of all the tested materials. Peach, persimmon and other fruit trees are also found with male sterility, but they do not have as high a percentage as *P. ussuriensis* in their cultivars. The fact that the male sterility rate in cultivar is higher than that of the wild population among *P. ussuriensis* is difficult to explain. This would need further research.

Figure 1 shows that pollen qualities of the same variety from normal flowering in the field and flowers in the greenhouse, identified by the I-KI stain method, were obviously different. The pollen viability from normal flowering in the field was significantly higher than that from the forced flowering in the greenhouse. This indicates that forced flowering in a greenhouse could promote early flowering and produce fertile pollen. However, it had a greater effect on pollen quality, which varied among varieties. Actually, flowers in the greenhouse had only 3.47% effect on the pollen germination in Honghuagaili from greenhouse (lower than that in the field), but had 21.6% effect on the pollen germination in Hanhong from greenhouse (lower than that in the field). Pollen viability was distinct among the tested varieties, and pollen germination of Honghuagai was the highest at 44.72% in the field and 41.25% in the greenhouse, with the lowest pollen germination of wild type at 12.88% in the field and 5.24% in the greenhouse. Although the pollen viability from forced flowering in the greenhouse was relatively low, we thought the pollen quantity of pear tree was so abundant that it could meet the need of fertilization. The method of forced flowering in the greenhouse for pollen can still be applied in breeding and SI research.

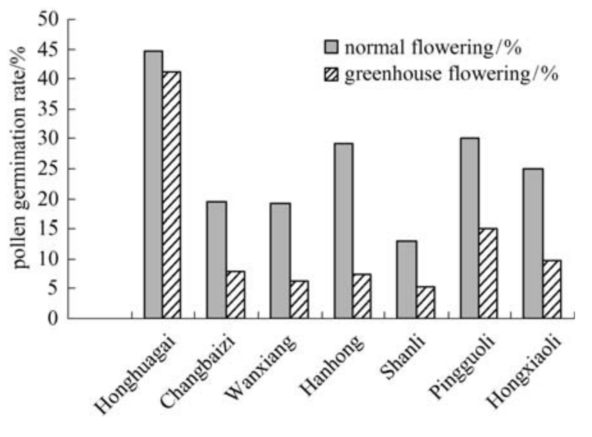

Fig. 1 Comparison of pollen fertility among different varieties

3.2 Self compatibility in *P. ussuriensis*

Fruit setting rates of 32 tested genotypes through controlled manual self pollination in blossoming are shown in Table 1. Results indicated that flowers self-pollinated from all of the tested varieties and wild types developed very well 7 days after bag removing (the first investigation) and were different from the flowers poorly pollinated in nature with yellow flower stalk or drop, which may be caused by bagging, so the results here could not represent the mating compatibility of these materials. Results on May 30 (the second investigation) showed that except for Honghuagaili and Longxiangli, most of the varieties dropped fruits without fruit setting. Among all the experimental materials of wild types, Nanguoli, Youhongli, Pingxiangli, Hanxiangli, Changbazili, Wanxiangli, Xiehuatianli8, Hanhongli, etc. had normal pollen, however their pollen infertility was attributed to SI, while the other materials, such as Matihuangli, Daxiangshuili, Xiaoxiangshuili, Huagaili, etc. were of male sterility and their pollen infertility could not be related with SI, indicating that *P. ussuriensis* had no capability of parthenocarpy. For Honghuagaili and Longxiangli, the fruit setting rates (the third investigation) on July 5 were 10% and 15%, respectively, similar to those on May 30, but remarkably lower than natural crossing at over 60%, even 100% from good compatibility.

3.3 Self fertility pollinated in different flowering phase

Table 2 shows the fruit setting rate of the tested varieties and wild types with male fertility through self pollination in different flowering phases. The results from the investigation before harvest showed that these materials (such as Shanli 3, Shanli 4, Shanli 5, Nanguoli, Youhongli, Pingxiangli, Hanxiangli, Changbazili, Wanxiangli, Xiehuatianli and Hanhongli), had infertility in the three flowering stages, which was regarded as full SI. The other species had some fertility in self pollinated flowers, but the fruit setting rate was remarkably lower than that of crossing in nature and different in the three phases. Wild types (Shanli 1 and Shanli 2) set self-pollinated fruits only in the bud flower stage, at a fruit setting rate of 1.11%. Cultivar Honghuagai, however, could set self-pollinated fruits not only in bud flower but also in flowering at a fruit setting rate of 2.33% and 10%, respectively. Cultivar Longxiangli could produce self-pollinated fruits in all the three stages at the fruit setting rate of 5.5%, 15.7% and 8.3% in bud flower, flowering and post flowering, respectively, with the flowering stage having the highest rate.

Table 3 shows the development of fruits and seeds from self pollination. It was shown that 27 fruits of cultivar Longxiang from self pollination in three phases of flowering developed normally, with some perfect seeds in its fruits. It is deduced that cultivar Longxiangli had a certain capability of self-compatibility (SC). Wild types of Sanli 1 and Shanli 2, were of SI, but both had two normal fruits only through pollination in bud flower stage, indicating that SI of variety sometimes can be changed by hand pollination in the bud flower stage. Cultivar Honghuagai had normal seeds

Table 1 Fruit-bearing status of self-pollinated *P. ussuriensis* in different time

cultivar	fruit setting rate per inflorescence/%			fruit setting number per inflorescence			fruit setting rate per flower/%		
	a	b	c	a	b	c	a	b	c
Shanli1	100	0	0	100	0	0	100	0	0
Honghuagaili	100	26.7	26.7	100	0.30	0.30	100	10	10
Longxiangli	100	23.3	23.3	100	0.47	0.44	100	15.67	14.67
others	100	0	0	100	0	0	100	0	0

Note: a, b and c represent the first investigation time (seven days after pollination), the second investigation time (May 30) and the third investigation time (July 5), respectively; Values in the table are the means of 30 numbers, and other test materials are Shanli2, Shanli3, Shanli4, Shanli5, Nanguoli, Youhong, Dananguo, Pingxiang, Hanxiang, Changbazi, Hanhong, Xiehuatian, Wanxiang, Matihuang, Tangli, Sanyali, Mali, Qingpicui, Ruanbazi, Jianbazi, Hongtangli, Suanliguo, Donghuagai, Huagai, Hululi, Balixiang, Seli, Zaobail, Fuxiang.

Table 2 Fruit-bearing status of pollinated *P. ussuriensis* in different flowering phase

cultivar	fruit setting rate per inflorescence/%			fruit set number per inflorescence			fruit setting rate per flower/%		
	BF	F	PF	BF	F	PF	BF	F	PF
Shanli1	3.33	0	0	0.03	0	0	1.11	0	0
Shanli2	10	0	0	0.13	0	0	4.44	0	0
Shanli3	0	0	0	0	0	0	0	0	0
Shanli4	0	0	0	0	0	0	0	0	0
Shanli5	0	0	0	0	0	0	0	0	0
Nanguoli	0	0	0	0	0	0	0	0	0
Youhong	0	0	0	0	0	0	0	0	0
Pingxiang	0	0	0	0	0	0	0	0	0
Hanxiang	0	0	0	0	0	0	0	0	0
Changbazi	0	0	0	0	0	0	0	0	0
Honghuagai	6.67	26.7	0	0.07	0.3	0	2.33	10	0
Wanxiang	0	0	0	0	0	0	0	0	0
Longxiang	13.3	23.3	30	0.17	0.47	0.53	5.57	15.67	17.7
Xiehuatian	0	0	0	0	0	0	0	0	0
Hanhong	0	0	0	0	0	0	0		0

Note: BF, F, and PF represent bud flower, flowering and post flowering, respectively; Values in the table are the means from 30 numbers.

through crossing in nature, but had abnormal seeds from self pollinated fruits in this experiment, with small fruit size and seeds all withered without viability, except one self fruit having 5 full seeds. Therefore we consider this variety as SI, whose discrimination and rejection stage is not in the pistil but in the post embryo.

3.4 Mating compatibility between female parent and F_1 generation

Table 4 shows the results of intercrossing compatibility from 3 pairs of parent-offspring combinations. Except for the combination of Nanguoli with Hanhong, two pairs had more high fruit setting rate of flower and inflorescence, especially the combination of Pingxiangli with Pingguoli, with the fruit setting rate of experimental inflorescence and flowering through reciprocal crossing reaching up to 80% and 50%, respectively. The combination of Nanguoli with Hanhongli was different from the above. When Hanhong was the male parent, Nanguoli had a very good fruit setting rate of flower with 93.3%. However, when Nanguoli was the male parent, fruit setting rate was zero. According to the genetic mechanism of SI, no fruits were produced in this experiment, as if Hanhong and Nanguoli

Table 3 Seeds development of fruits from self pollination in *P.ussuriensis*

cultivar	pollination time	total fruit bearing number	mean seed per fruit	normal seeds per fruit	fruit number with normal seed	mean single fruit weight/g
Honghuagai	BF	2	9.5	2.5	1	35
Honghuagai	F	9	8.6	0	0	13.6
Longxiang	BF	4	10	2.8	4	49.8
Longxiang	F	13	9.8	5.4	13	37.7
Longxiang	PF	10	9	4.8	10	56.1
Shanli 1	BF	1	10	4	1	–
Shanli 2	BF	1	6	6	1	–

Note: BF, F, and PF represent bud flower, flowering and post flowering, respectively; Fruit number with normal seed means the defined seed number per fruit > 7; The mean single fruit weight of Shanli 1 and Shanli 2 are omitted because of rot.

Table 4 Fruit-bearing status of crossing between parent and offspring varieties

mating combination	fruit setting rate per inflorescence/%	fruit setting number per inflorescence	fruit setting rate per flower/%
Hanxiangli × Pingxiangli	46.7	0.53	17.7
Pingxiangli × Hanxiangli	86.7	1.73	42.3
Pingxiangli × Pingguoli	100	2.27	75.7
Pingguoli × Pingxiangli	80	1.53	51
Nanguoli × Hanhongli	93.3	1.33	44.3
Hanhongli × Nanguoli	0	0	0

Note: The investigation time is July 5; Values in the table are the means from 30 numbers.

should have the same S-genotype, but it was deemed impossible and would need further research.

3.5 Mating compatibility between different varieties with similar phenotypes

Table 5 provides the reciprocal crossing compatibility results from the Jianbali and Huagaili groups. It shows that the fruit setting was different in different years, but all of the combinations in the same group could produce fruits, with the fruit setting rate per inflorescence reaching up to 80% in 2005. Though they were of similar phenotype in fruit appearance, the facts indicated that Changjianba, Jianba and Ruanbazi had different S-genotype. So did Huagai, Honghuagai and Donghuagai. They can be grown in the same garden for mutual pollination.

3.6 Mating compatibility between bud mutation and female parent

Cultivars of Dananguo and Youhong were selected from the individuals of bud mutations from Nanguoli. The results from reciprocal crossing between them showed the fruit setting rate was zero, suggesting incompatibility. So we deduced that the mutation did not affect the structure and function of their *S* gene and they still had the uniform S-genotype. And they cannot be planted in the same orchard as mutual pollination trees, which is similar to that of apple mutation varieties.

4 Discussion

4.1 Genetic mechanism of *P. ussuriensis* SI

SI of flowering plants was divided into Sporophytic Self-incompatibility (SSI) and Gametophytic Self-incompatibility (GSI). Crossing compatibility between individuals with SSI depends on both of their S-genotypes. When the same *S* gene is there, they are incompatible, and crossing between parents and their offspring usually produce no fruit. GSI is different from SSI. SI occurs when individuals with the same S-genotype are mated, and crossing between female parent with their offspring can normally produce fruit. In our experiment, cultivar Hanxiang was a cross hybrid from Pingxiangli. Pingxiangli was selected from Pingguoli natural seedlings. Since the mutual crossing among the combinations showed good fruit setting rates, it is confirmed that the incompatibility in self pollination of *P. ussuriensis* should be attributed to GSI.

4.2 S-genotype of Nanguoli

Nanguoli is an excellent variety in *P. ussuriensis.* Hanhongli was selected from crossing offspring of Nanguoli with Jinsu. In our experiment, one-way compatibility was found in the crossing between Nanguoli and Hanhong, wherein a combination of Nanguoli × Hanhong produced fruit. On the other hand, no fruit was produced in Hanhong × Nanguoli. There are two explanations for this: One of them is that the Nanguoli S-genotype consists of a pair of the same S-allele; the other is that one of the *S* genes in Nanguoli, which differs with that of Hanhong, could not produce a normal or functional male gamete. According to the GSI genetic mechanism, mating between male and female gametes with the same *S* gene is incompatible and cannot produce a homozygous individual with the same *S* gene. The first explanation is difficult to be accepted, and the second explanation needs to be further confirmed by molecular biology and pollination tests.

Table 5 Fruit-bearing status between different varieties in the same group

mating combination	fruit setting rate per inflorescence/%		fruit setting number per inflorescence		fruit setting rate per flower/%	
	2005	2006	2005	2006	2005	2006
Ruanbazi × Changbazi	80	13.3	1.4	0.2	28.6	6.7
Jianbazi × Changbazi	90	93.3	2.6	1.73	54.2	57.8
Donghuagai × Honghuagai	80	93.3	1.2	0.49	24.5	16.3
Huagai × Honghuagai	80	73.3	2.8	1.33	56	44.4

Note: 2005 and 2006 represent investigation year; The investigation time is July 5; Values in the table are the means from 15 numbers.

4.3 SI of Honghuagai

Based on the previous research, the pear SI mechanism is similar to that in Solanaceae plants. SRNase in the style has the capability to specifically recognize pollen, degrade RNA in a pollen tube with the same S-genotype, inhibit the pollen tube growth in the style, and finally fail to fertilize (Zhang and Hiratsuka, 2000; Ishimizu et al., 1996). In this experiment, most of the tested varieties and wild types of *P. ussuriensis* displayed their SI before the self-pollinated flowers began to develop, and all of the flowers dropped. It is clear that SI occurred in the process of pollination before a zygote was formed. Flowers of cultivar Honghuagai self-pollinated in flowering could produce fruits, which did not drop before harvest, but the fruits were small in size and had no normal seeds. So we deduced that cultivar Honghuagai also had SI, but SI occurred in the early development of the zygote than in the pistil.

5 Conclusions

There was a high percentage of male sterility cultivars with withered anther or without pollen in *P. ussuriensis*.

The pollen fertilities were different in cultivars and wild types of *P. ussuriensis*. Pollen vitality from forced flowering in the greenhouse was lower than that from natural flowering, but it did not affect the fruit setting of tested genotypes.

Most of the varieties of *P. ussuriensis* showed the capability of SI, but no parthenocarpy. Fruit setting of flowers from self pollination in different flowering stages were not remarkably affected and although a few cultivars with SI can be changed in the bud flower stage by self pollination, only a few seeds from its fruits were available.

Crossing reciprocally between a female parent and its offspring, as well as varieties with similar phenotype in fruit appearance, had a normal product and did not show the SI. So they can be grown in the same orchard for mutual pollination or utilized as crossing parents in pear breeding programs.

A mutated individual with S-genotype of was similar to its parent. Crossing between them was of SI. So they cannot be grown in the same orchard for mutual pollination or utilized as crossing parents in pear breeding programs.

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