Survey on Intra-Specific Crossing and F₁ Seedling Cultivation in Seven Combinations of Chinese Cherry

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The Chinese cherry (*Prunus pseudocerasus*/Cerasus *pseudocerasus*), which is native to China, is an economically important tetraploid fruiting cherry species. Its industry has been greatly limited due to some general disadvantages in the fruits such as small fruit size, high acid content, and short shelf-life. As such, it is urgent to carry out cross breeding and genetic improvement of this species. Here, seven cross combinations were designed by selecting five genotype landraces of the Chinese cherry and one semi-wild resource as cross parents. The fruit-set percentage, germination rate of the hybrid seeds, and growth status of the F₁ seedlings varied among different parental genotypes and cross combinations. Three or four S-genotypes were detected in six tetraploid parents, with a maximum of five different alleles between two parents. Both the pollen vigor of the male parent and the differential S-genotype between parents may contribute to the variation in fruit-set percentages, ranging from 0 to 28.55%. Significant differences in the F₀ fruit traits were observed among different combinations, indicating potential metaxenia of the Chinese cherry. Appropriate pretreatments, including the removal of endocarps, soaking with 150 mg/L GA₃ for 24 h, and chilling stratification for 7–10 days, could significantly increase the germination rate of the hybrid seeds. According to the overall performance, using (semi)-wild resources as one of the parents should be taken into consideration more in Chinese cherry breeding programs. On the basis of these findings, we further constructed a flow chart for successful intra-specific crossing and efficient cultivation of robust F₁ seedlings. This study will provide important references for the selection of cross parents, establishment of a feasible breeding program, and cultivation of robust F₁ progenies of the Chinese cherry.

Key Words: Chinese cherry, intra-specific crossing, parental genotypes, S-genotype, metaxenia.

Introduction

The Chinese cherry (*Prunus pseudocerasus*/Cerasus *pseudocerasus*) originates in China, and is an economically important cultivated tetraploid fruiting cherry

species (Yü, 1979; Wang et al., 2018). It is widely distributed across the Longmenshan Fault Zones, Qinling Mountains, North and East Plain, and Yunnan-Guizhou Plateau in China (Chen et al., 2016, 2020). Recently, cherry cultivation has been developing rapidly in China and has increasingly contributed to poverty alleviation and rural revitalization.

The cultivation history of the Chinese cherry dates back to 3000–4000 years ago (Liu and Liu, 1993; Dong and Liu, 2008). Through longtime domestication and artificial selection, a large number of landraces with significant phenotypic changes in fruit size, color, shape, and flavor have been produced and maintained by local farmers. These landraces contain greater genetic diversity than elite cultivars and represent an intermediate stage in domestication between wild and elite

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cultivars (Miller and Gross, 2011; Nikiforova et al., 2013), which often play crucial roles in breeding programs (Li et al., 2013). Over the past decade, we carried out a field investigation and comprehensive evaluation in around 1000 cherry resources in China, finding that the Chinese cherry has many advantages such as self-compatibility, early maturation, full-flavor, wide adaptability, and intensive disease/pest resistance (Huang et al., 2013; Chen et al., 2016, 2020). However, there are some general disadvantages in the current cultivated Chinese cherry including small fruit size, high acid content, and short shelf-life (Chen et al., 2016). Therefore, the urgent objective is to cultivate new varieties with early-ripening, large fruit size, sweet flavor, and better storability in the current Chinese cherry cross breeding program.

As is well known, it is difficult for breeders to obtain adequate hybrid seeds due to there being only one seed per fruit in stone fruit trees. Embryo abortion and arrest during the development of seeds from many crosses often cause loss of germination or a high death rate of young seedlings (Liu et al., 2004; Wu et al., 2021). After-ripening and chilling treatment are also essential for stone fruit seeds to break dormancy (Taiz et al., 2020). The low germination rate has severely hindered the cherry breeding process as sufficient hybrid seedlings could not be supplied for selection and breeding. Furthermore, adverse weather conditions in South China, such as frequent rainfall, late spring coldness, and hail from February to April, have become unavoi-

able obstacles to the cross-breeding process and hybrid seedling cultivation of the Chinese cherry. Based on our cross-breeding program over the past decade (Du et al., 2015), this study aimed (i) to evaluate crossing performance among different genotype parents and combinations, (ii) to illustrate the potential reasons for the different fruit-set percentages among cross combinations, and (iii) to construct a flow chart for successful intra-specific crossing and efficient cultivation of robust F_1 seedlings in South China.

Materials and Methods

Plant materials

In this study, five landraces and one semi-wild resource with obvious character differences, such as fruit size, color, shape, and maturation date, flower color, and pest/disease resistance, were selected as cross parents in 2017 (Table 1; Fig. 1). They were planted in the Teaching and Scientific Research Base of Sichuan Agricultural University (Chengdu Campus), China. In total, seven cross combinations were constructed using six genotype parents (Table 2).

Intra-specific crossing and fruit-set investigation

Pollen grains were obtained from the anthers of the bell-shaped flower buds and incubated at 25–27°C to accelerate pollen grain maturation. The number of pollen grains per anther was investigated using the method described by De Vries (1974). Specifically, the anther was deposited in a vial with 0.5% acetocarmine

Table 1. The locality and main economic characters of six parents in the Chinese cherry (2016).

Code	Full name	Locality	Blooming period	Mature period	Character	Ploidy level (2n)
‘HF’	‘Hongfei’	Xinjin, Sichuan	2/24–3/2	4/20–4/25	Large fruit size (4.5–5.5 g), full-flavored (14.5–16.2% TSS, 17.74 mg/100 mL vitamin C, 0.51 g/100 mL titratable acid), heart shape, purple-red peel color; susceptible to shot hole disease.	4x
‘NZH’	‘Nanzaohong’	Xinjin, Sichuan	2/23–3/1	4/10–4/12	Early-matured, medium fruit size (3.8–4.5 g), light flavor (11.5–13.5% TSS, 0.75 g/100 mL titratable acid), kidney shape, orange-red peel color; susceptible to gummosis disease.	4x
‘HC’	‘Huangcao’	Miyi, Sichuan	2/24–3/3	4/24–4/26	Small fruit size (1.8–2.7 g), sweet flavor (13.7–15.6% TSS, 0.62 g/100 mL titratable acid), kidney shape, red peel color.	4x
‘PZB’	‘Pengzhoubai’	Pengzhou, Sichuan	2/25–3/4	5/2–5/5	Late-matured, medium fruit size (3.5–4.5 g), sweet-sour flavor (13.5–15.8% TSS, 0.66 g/100 mL titratable acid), kidney shape, yellow peel color, short fruit stalk.	4x
‘PJHH’	‘Pujiang Honghua’	Pujiang, Sichuan	2/24–3/2	4/20–4/23	Semi-wild, intensive adaptability and disease/pest resistance, very small fruit size (1.5–2.5 g), flat round shape, red peel color, sour-sweet flavor (11.5–13.2% TSS, 0.79 g/100 mL titratable acid).	4x
‘HZZ’	‘Heizhenzhu’	Ba’nan, Chongqing	2/27–3/7	4/27–5/1	Late-matured, small fruit size (2.7–3.5 g), near round shape, purple-red peel color, sweet flavor (12.5–14.5% TSS, 17.85 mg/100 mL vitamin C, 0.80 g/100 mL titratable acid); approved by the provincial government in China.	4x

Fig. 1. Main characters of fruits (1) and flower buds (2) of cross parents in the Chinese cherry. A: ‘HF’ (‘Hongfei’); B: ‘NZH’ (‘Nanzaohong’); C: ‘HC’ (‘Huangcao’); D: ‘PJHH’ (‘Pujiang Honghua’); E: ‘PZB’ (‘Pengzhouhai’); F: ‘HZZ’ (‘Heizhenzhu’).

Table 2. The number of pollen grains and pollen germination rate of the male parent.

Male parent	‘HF’	‘NZH’	‘HC’	‘PZB’	‘PJHH’	‘HZZ’
Number of pollen grains per anther	4729a	3596c	4196b	2264d	4396b	5062a
Pollen germination rate	55.94b	59.84b	15.90e	22.42d	69.76a	49.38c

Values followed by different lowercase letters within the same row indicate a significant difference at the 0.05 level.

and, with a spatula, the anther was crushed against the vial wall. With a dropper, 10 samples of this suspension were pipetted onto a Fuchs-Rosenthal haemocytometer, and the number of pollen grains in these 10 samples was determined. Then, multiplication of the anther total per determination with $0.5/0.032 = 15.625$ resulted in an estimation of the real number of pollen grains per anther. For estimation of the pollen germination rate, pollen grains were cultured in 20% sucrose and observed with the method of differential staining (Chu et al., 1995). The matured and aborted pollen grains were stained purple red and green under a microscope, respectively.

Artificial pollination was conducted according to the method of emasculation with perianth-pollination-bagging (Chen et al., 1989). The petals and filaments were excised from the bell-shaped flower buds and the isolated stigmas were bagged to prevent open pollination. After two or three days, the stigmas were pollinated with brushes, and then bagged at once. At least 4,000 flowers were pollinated for each cross combination. The pollinated flowers were de-bagged two weeks later. The bagged self- and open pollinated flowers were used as controls. For the three types of pollination, each flower branch was considered a replicate. The fruit-set percentage was calculated at two weeks and

five weeks after pollination, and at the mature stage (eight to nine weeks). The F_0 mature fruits were harvested to analyze the fruit traits.

PCR amplification of genomic *S-RNases*

The *S-RNase* genotypes were also amplified to explain the different fruit-set percentages. The total genomic DNA was extracted from silica-gel dried leaf tissues using a modified cetyltrimethylammonium bromide (CTAB) method (Zhou, 2005). PCR to identify the *S*-alleles of the six cross parents analyzed was performed using forward primer Pru-C2 with reverse primers Pa-C3R and Pa-C5R (Supplementary Table 1) (Tao et al., 1999; Gu et al., 2010). Two and three specific primer sets were designed according to the alignment of known sequences and followed Gu et al. (2010) (Supplementary Table 1). PCR amplification was performed in a PTC-200 thermal cycler (Bio-rad Laboratories, Inc., USA). A volume of 25 μ L reaction mixture contained 20 ng of genomic DNA, 12.5 μ L of $2 \times$ EasyTaq PCR SuperMix (TransGen Biotech, Beijing), and 1 μ L of each primer (10 μ mol/L). The cycling program began with an initial pre-denaturation at 94°C for 3 min, followed by 35 cycles at 94°C for 30 s, 48–60°C for 30 s, 72°C for 20–60 s, and with a final extension at 72°C for 5 min. The PCR products were analyzed in

1.5% agarose gel and visualized by ethidium bromide staining under UV light.

Evaluation of the F_0 fruit traits

Data for the fruit traits were recorded for thirty fruits from five cross combinations, except for 'HF' × 'HC' and 'HF' × 'PZB'. The fruit longitudinal and transverse diameter and fruit stalk length were determined with a caliper. The total soluble solid content was measured using a refractometer (pocket PAL-1; ATAGO Co., Ltd., Japan). The contents of soluble sugar, titratable acid, and vitamin C were estimated based on Hou's method (Hou, 2015).

Hybrid seed pretreatment and seedling cultivation

The seeds from 'HF' × 'PJHH' were used to explore the effects of pretreatments on the germination rate. Hybrid seeds were pre-treated as follows: (i) whole seeds were under chilling stratification for 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 days; (ii) seeds without endocarp were under chilling stratification for 5, 10, 15, 20, 25, 30, 35, and 40 days; (iii) seeds without endocarp were soaked with 50, 100, 150, and 200 mg/L GA_3 for 24 h; (iv) seeds without endocarp were soaked with 150 mg/L of GA_3 under chilling stratification for 4, 7, 10, 13, 16, 19, 22, 25, and 28 days. Thirty seeds were used for each treatment with three biological replicates. The germination rates were calculated for each treatment.

The compound fertilizer 'Baolifeng' (N:P:K = 19:19:19) was used to strengthen the seedlings at 2, 4, 6, and 8 g per plant along with water as a control. For formula fertilizer, three factors and five levels were designed by the orthogonal method to cultivate strong seedlings. The five levels (g per plant) were 0, 1, 2, 3, 4 for both N and P, and 0, 0.5, 1, 1.5, 2 for K. N, P, and K corresponds to carbamide (46% N), superphosphate (12% P_2O_5), and potassium sulfate (52% K_2SO_4), respectively. The 3- and 4-month-old seedlings of 'HF' × 'PJHH' were irrigated two times. The height and stem diameter were measured with a caliper when they were 5 months old. The chlorophyll contents of the leaves were determined and calculated according to Arnon (1949). The photosynthetic rate (P_n), transpiration rate (T_r), stomatal conductance (G_s), and intercellular CO_2 concentration (C_i) were measured using a LI-6400 portable photosynthesis system (Li-Cor, Inc., USA).

Data analysis

The fruit-set percentage, nine fruit traits, germination rate of the hybrid embryos, and number of hybrid seedlings were recorded and calculated. The data were analyzed using one-way analysis of variance (ANOVA) and Duncan's multiple-range tests. A two-side P -value of 0.05 was considered a statistically significant difference. All statistical analyses were performed using IBM SPSS Statistics (IBM Corp., USA).

Results

Pollen vigor and fruit-set of the seven cross combinations

'HZZ' had the largest number of pollen grains per anther (5062), followed by 'HF' (4729), and the smallest was observed in 'PZB' (2264) (Table 2). 'PJHH' had the highest pollen germination rate of 69.76%, which was significantly higher than that of the other male parents (Table 2). 'PZB' and 'HC' exhibited the lowest values, being only 22.42% and 15.90%, respectively (Table 2).

A total of 41,486 flowers were artificially pollinated among the seven cross combinations. At two weeks after pollination, 'NZH' × 'HF', 'HF' × 'NZH', 'HF' × 'PJHH', and 'PZB' × 'HF' all showed relatively high fruit-set percentages ranging from 70.82 to 91.42%, which is slightly higher than 'HF' and 'NZH' by self-pollination and open pollination, but lower than that of 'PZB' (Table 3). 'HZZ' × 'PZB' exhibited the lowest fruit-set of 34.98%, and this value was also much lower than 'HZZ' by self-pollination and open pollination. At five weeks after pollination, different degrees of a sharp decrease in fruit-set percentages were observed in all combinations, and even decreased to 0 in 'HZZ' × 'PZB' (Table 3). At the mature stage, the fruit-set of 'HF' × 'PJHH' decreased to 28.55%, followed by 'PZB' × 'HF' (18.88%), and a reciprocal crossing between 'HF' and 'NZH' (13.70%, 13.93%). These values were all higher than those of the self-pollinated female parents (4.28–8.73%), but lower than those of the open pollinated female parents (44.25% for 'HF' and 34.68% for 'NZH'), except for 'PZB' (5.26%) (Table 3). Fruit-sets of only 0.29% and 0.34% were detected in 'HF' × 'HC' and 'HF' × 'PZB', respectively, which were much lower than those of the other cross combinations (Table 3). Finally, six cross combinations were successful in obtaining mature fruits, while 'HZZ' × 'PZB' was unsuccessful.

S-genotypes of six cross parents

The amplification products of four S -alleles were successfully generated from genomic DNA using forward primer Pru-C2 and reverse primer Pa-C3R or Pa-C5R. Four amplification bands were observed in 'NZH'; three specific bands present in 'HC' and 'PZB'; two bands in 'HF', 'PJHH', and 'HZZ'. The electrophoresis results indicated that the sizes of the specific amplification bands varied from 400 to 1500 bp amplified with primers Pru-C2F and Pa-C3R or Pa-C5R (Fig. 2A, B). Further sequence analysis and a Blast search in GenBank confirmed that all the amplified fragments contained four different S -alleles. They corresponded to *PpsS-RNase-1*, *PpsS-RNase-2*, *PpsS-RNase-4*, and *PpsS-RNase-9*. However, the bands of *PpsS-RNase-4* and *-9* in 'NZH' and *PpsS-RNase-4* in 'PZB' were much weaker than those in other cultivars.

Table 3. Fruit-set (%) of the Chinese cherry after cross, self-, and open pollination (2017).

Female parent	Male parent	No. of pollinated flowers	Fruit-set percentage (%)			No. of fruits
			2 weeks after pollination	5 weeks after pollination	Mature stage	
‘HF’ ^z	‘HC’ ^x	4547	66.95c	0.75e	0.29e	13
	‘PJHH’	11061	74.86bc	33.36b	28.55b	3137
	‘NZH’	4935	70.82c	14.25cd	13.70c	613
	‘PZB’	5339	85.23b	0.43e	0.34e	11
	Self-pollination	3565	66.52c	10.98d	8.19d	292
	Open pollination	4027	70.65c	47.90a	44.25a	1782
‘NZH’ ^z	‘HF’	7138	81.14b	22.65c	13.93c	994
	Self-pollination	4218	68.12c	17.39cd	8.73d	368
	Open pollination	3985	71.13c	51.45a	34.68b	1382
‘PZB’ ^y	‘HF’	4466	91.42a	24.92c	18.88c	686
	Self-pollination	3256	96.03a	11.11d	4.28d	139
	Open pollination	4205	91.88a	10.66d	5.26d	221
‘HZZ’ ^y	‘PZB’	4000	34.98d	0e	0de	0
	Self-pollination	3853	80.18b	15.26cd	10.35c	399
	Open pollination	4015	61.29c	49.18a	32.15b	1291

^z under rain shelter; ^y under an open field condition; ^x the blossing branches were from Miyi, Panzhihua City, about 500 km away from Chengdu City. In both cultivation environments (under a rain shelter and open field condition), different cultivars were not in proximity and there was no possibility of natural cross-pollination. Values followed by different lowercase letters within the same column indicate a significant difference at the 0.05 level.

Fig. 2. PCR products amplified from the genomic regions of six Chinese cherry germplasms. A: PCR with primer set Pru-C2/Pa-C3R for *PpsS-RNases*; B: PCR with primer set Pru-C2/Pa-C5R for *PpsS-RNases*; C–H: PCR with primer set for allele-specific primer set Pps-S3F/Pps-C2R for *PpsS-RNase-3* (C), Pps-S5F/Pps-S5R for *PpsS-RNase-5* (D), Pps-S6F/Pps-S6R for *PpsS-RNase-6* (E), Pps-S7F/Pps-C2R for *PpsS-RNase-7* (F) and Pps-S3F/Pps-S8R for *PpsS-RNase-8* (G). Lanes 1–6 corresponded to ‘HF’, ‘NZH’, ‘HC’, ‘PZB’, ‘PJHH’, and ‘HZZ’, respectively.

DNA gel extraction was unsuccessful from the PCR products, so *PpsS-RNase-4*, *-9*, and *PpsS-RNase-4* were excluded for ‘NZH’ and ‘PZB’.

PCR amplifications were also performed with five allelic specific primer sets in six cross parents. Amplification of the genomic DNA showed that *PpsS-RNase-3* was only found in ‘PZB’, ‘PJHH’, and ‘HZZ’ (Fig. 2C), *PpsS-RNase-6* was only found in ‘NZH’ and ‘PJHH’ (Fig. 2E), and *PpsS-RNase-8* was found in ‘HF’, ‘NZH’, and ‘HC’ (Fig. 2G). *PpsS-RNase-5* and *-7* were not detected in any of the cultivars (Fig. 2D, F). Therefore, the components of the *S*-genotypes were preliminary determined by consensus and specific PCR amplification, i.e., ‘HF’ and ‘HC’ were $S_1S_2S_8$; ‘PZB’ was $S_2S_3S_9$; ‘HZZ’ was $S_1S_3S_9$; ‘NZH’ was $S_1S_2S_6S_8$; and ‘PJHH’ was $S_1S_3S_4S_6$ (Table 4).

F₀ hybrid fruit traits of the cross combinations

The ripening stage of the F_0 hybrid fruits was slightly earlier than that of the self-pollinated and open pollinated female fruits. The F_0 fruits had the same color and shape as those of the female fruits by open pollination. In general, cross-pollination had no significant effect on the F_0 fruit traits compared with the female fruits from self- and open pollination such as ‘NZH’ and ‘PZB’ (Supplementary Table 2). When using ‘HF’ as the female parent, different pollen parents generated significant differences in the fruit size, transverse diameter, and fruit shape index of the F_0 hybrid fruits. The F_0 fruits of ‘HF’ × ‘PJHH’ showed the largest size and fruit weight, being 20.75 mm × 19.94 mm and 4.50 g, respectively, followed by the F_0 fruits of ‘HF’ × ‘PZB’. The internal F_0 traits, including total soluble solid,

titratable acid, TSS-acid ratio, and vitamin C content, were also affected by the pollen parent (Supplementary Table 2). The highest TSS content (16.60%) was observed in the F_0 fruits pollinated by ‘NZH’, followed by ‘HC’ (14.61%), and the lowest (13.36%) was observed in the fruits pollinated by ‘PJHH’. The F_0 fruits pollinated by ‘PZB’ had the highest titratable acid (0.67 g/100 mL), which is significantly higher than that of the fruits pollinated by the other pollen parents. The highest TSS-acid ratio (29.39) and vitamin C content (18.57 mg/100 mL) was detected in the F_0 fruits pollinated by ‘HC’ and ‘PJHH’, respectively (Supplementary Table 2). These results show that the pollen parent may affect the F_0 fruit traits of the Chinese cherry.

Hybrid seedling cultivation

In total, we obtained 5,454 hybrid seeds for six cross combinations, of which 4,682 (85.85%) were plump and 772 had grains empty of embryos (Table 5). In comparison, when seeds without an endocarp were treated with 150 mg/L of GA₃ under chilling stratification for a week, the germination rate increased to 78.67%, which is significantly higher than the other pretreatments (Supplementary Fig. 1). Based on the optimal pretreatment, a total of 3,371 seeds were germinated, accounting for 72.00%. Specifically, seeds of ‘HF’ × ‘PJHH’ had the highest germination rate of 78.66%, followed by reciprocal crossing of ‘HF’ and ‘NZH’ (72.42% and 68.20%, respectively), and the lowest rate of 37.26% was observed in ‘PZB’ × ‘HF’ (Table 5).

Appropriate compound and formula fertilizers could improve the growth status of hybrid seedlings by

Table 4. Distribution of nine *S*-genotypes in different Chinese cherry germplasms.

<i>S</i> -genotype	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9
‘HF’	+	+	–	–	–	–	–	+	–
‘NZH’	+	+	–	–	–	+	–	+	–
‘HC’	+	+	–	–	–	–	–	+	–
‘PZB’	–	+	+	–	–	–	–	–	+
‘PJHH’	+	–	+	+	–	+	–	–	–
‘HZZ’	+	–	+	–	–	–	–	–	+

+: *S*-genotype was found in the corresponding germplasm. –: *S*-genotype was not found in the corresponding germplasm.

Table 5. Numbers of seeds, germinated seeds, and seedlings among different cross combinations of the Chinese cherry.

Female parent	Male parent	No. of fruits (%)	No. of seeds (%)	No. of germinated seeds (%)	No. of young seedlings (%) in 2017	No. of 3-year-old progenies (%) in 2020
‘HF’	‘HC’	13	11 (84.62)	7 (63.64)	1 (9.09)	1 (9.09)
‘HF’	‘PJHH’	3137	2910 (92.76)	2289 (78.66)	1181 (40.58)	1163 (39.97)
‘HF’	‘NZH’	613	515 (84.01)	373 (72.42)	200 (38.83)	183 (35.53)
‘HF’	‘PZB’	11	10 (90.91)	6 (60.00)	1 (10.00)	0
‘NZH’	‘HF’	994	761 (76.56)	519 (68.20)	262 (34.43)	255 (33.51)
‘PZB’	‘HF’	686	475 (69.24)	177 (37.26)	2 (0.42)	2 (0.42)
Total		5454	4682 (85.85)	3371 (61.81)	1647 (30.20)	1421 (26.05)

increasing the growth indexes and increasing the photosynthetic capacity. Taking seedlings from 'HF' × 'PJHH' as an example, 4 g of compound fertilizer per plant or formula fertilizer of $N_2P_2K_3$ (N:P:K = 1:1:1) could significantly improve the growth of young seedlings (Supplementary Table 3). Finally, we obtained 1,647 seedlings with a ratio of 35.18%, including 1,181 for 'HF' × 'PJHH', 200 for 'HF' × 'NZH', 262 for 'NZH' × 'HF' (Table 5). Only three seedlings survived among the hybrids of reciprocal crossing between 'PZB' and 'HF'. 'HF' × 'PJHH' exhibited the highest seedling rate of 40.58%, followed by 'HF' × 'NZH' (38.83%) and 'NZH' × 'HF' (34.43%), which are significantly higher than those of 'PZB' × 'HF' and 'HF' × 'PZB' (Table 5). The survival rate of the 3-year-old progenies of 'HF' × 'PJHH' reached 98.48%, which is higher than those of 'NZH' × 'HF' (97.33%) and 'HF' × 'NZH' (91.50%) (Table 5).

Discussion

Factors affecting the fruit-set percentage

The relatively high fruit-set percentages at two weeks after pollination suggested compatibility among Chinese cherry germplasms. Then, they decreased sharply due to the physiological drop. At the mature stage, the fruit-set percentages varied among different cross combinations. Much lower values were observed in 'HF' × 'HC', 'HF' × 'PZB', and 'HZZ' × 'PZB' than the other cross combinations, which might be due to the low pollen vigor of the male parent. Moreover, the *S*-genotypes might contribute to the different fruit-set percentages of the Chinese cherry. It has been reported that full compatibility is superior to semi-compatibility for the fruit-set in *Prunus* species (Sapir et al., 2008). Based on our results, semi-compatibility was confirmed between these cross parents in the Chinese cherry. There were more differential *S*-genotypes between parents and higher fruit-set percentages were observed (Table 3). Five different *S*-genotypes were observed between 'HF' and 'PJHH', accounting for the highest fruit-set percentages, followed by four different *S*-genotypes between 'HF' and 'PZB', and reciprocal crossing between 'HF' and 'NZH' with one different *S*-genotype. While the lowest fruit-percentage was detected between 'HF' and 'HC' with the same *S*-genotypes.

Effect of pollen parents on the F_0 fruit quality

Metaxenia is a common phenomenon whereby the pollen genotype directly influences the fruit shape, maturation period, size, color, flavor, and substances content in the current year and results in variations in these traits (Yang et al., 2020), which has been reported in various fruit trees, including *Citrus* (Jahromi et al., 2019), *Pyrus* (Bisi et al., 2021), and *Carya* (Huang et al., 2020). Here, in the Chinese cherry, the F_0 fruits had the same color and shape as the female parent fruits with open pollination, which is consistent with those in

the sweet cherry (Wu et al., 2019). The source of the pollen grain affected the fruit quality, such as the fruit size, TSS, and acid content, indicating potential metaxenia in the Chinese cherry. The effect of pollen parents on the F_1 fruit quality needs to be further explored.

Effect of parental genotype on crossing

The appropriate parents will facilitate the exploitation of maximum genetic variability and production of superior recombinant genotypes (Bertan et al., 2007). Here, the germination rate and growth status of hybrid seedlings are greatly affected by the parental genotype and cross combinations, as reported in *Citrus* (Moriguchi et al., 1996) and the sweet cherry (Wu et al., 2019). In the Chinese cherry, the germinate rate and F_1 survival rate of landrace × (semi)-wild resource was significantly higher than those between landraces. Chen et al. (2013) suggested that the significant gene flows and pronounced seed dispersal abilities of the wild Chinese cherry could help retain some genotypes that favored reproduction during the long time needed for natural selection. In contrast, the landraces experienced strong artificial selection and domestication, inevitably leading to the loss of genotypes and genetic differentiation (Smykal et al., 2018), resulting in the embryo abortion of F_0 fruits between landraces. The latest field investigation found that hybrids of 'HF' × 'PJHH' exhibited robust growth and were taller and stronger than other combinations (data not shown). The semi-wild resource, 'PJHH', reveals intensive pest/disease resistance and barren tolerance (Cao et al., 2018), and also shows a relatively distant genetic relationship to the five landraces, while they are closely related to each other (Liu, 2020). As a consequence, heterosis apparently appeared in the F_1 progenies from 'HF' × 'PJHH'. Therefore, using (semi)-wild resources as one of the parents should be taken into consideration more in future breeding programs of the Chinese cherry.

In addition, bad weather (e.g., frequent rainfall, late spring coldness, and hail) in South China has also caused the poor development or embryo abortion of hybrid fruits and the death of young seedlings. This has been confirmed by our previous crossing under an open field condition (Du et al., 2015). Consequently, it is necessary to carry out crossing of the Chinese cherry under a rain shelter in South China, which could protect the flowers and fruits from cold damage. Overall, we summarize a flow chart for successful intra-specific crossing and efficient cultivation of robust F_1 seedlings for the Chinese cherry (Fig. 3).

Conclusion

Seven cross combinations were constructed using five genotype landraces and one semi-wild resource as cross parents in the Chinese cherry. The fruit-set percentage and germination rate of the hybrid seeds as well

Fig. 3. A flow chart for successful intra-specific crossing and efficient cultivation of robust seedlings of the Chinese cherry under a rain shelter.

as the growth status of the F_1 progenies were greatly affected by the parental genotype and cross combinations. Three or four S -genotypes were detected in six tetraploid parents, with a maximum of five different alleles between two parents. Both the pollen vigor of the male parent and differential S -genotypes between cross parents might contribute to the different fruit-set percentages. The F_0 fruit traits were also affected by the pollen parents, indicating potential metaxenia in the Chinese cherry. Chilling stratification, GA_3 treatment, and removing the endocarp could help break seed dormancy and effectively improve the germination rate and decrease the germination period. Using (semi)-wild resources with excellent characters, such as ‘PJHH’, as one of the parents could effectively promote the cross efficiency and improve the survival rate of the F_1 progenies. It is also necessary to conduct cross breeding of cherries under a safeguard, such as a rain shelter, to protect unmaturing F_0 hybrid fruits from cold damage. Finally, a flow chart for successful intra-specific crossing and efficient cultivation of robust F_1 seedlings is summarized for the Chinese cherry grown in South China.

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