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Determination of S-geontypes and Self-Fertility of Sweet Cherry in Summerland Advanced Selections

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Abstract

Self-incompatibility (S-) groups were determined using PCR based S-allele type analysis and self-fertility was confirmed by pollinataion trials of sweet cherry advanced selections from the Agriculture and Agri-Food Canada breeding program in Summerland, BC, Canada. Four S-allele genotypes (S1S3, S1S4, S3S4 and S2S4) were detected (PCR method can not differentiate S4 and A4'-pollen part mutant) among twenty-one selections. Ten selections were found to be self-fertile, four selections were likely self-fertile where S4' is presumed.

Introduction

Most sweet cherry (*Prunus avium* L.) cultivars are self-incompatible, and the majority are also incompatible with other cultivars in the same incompatibility group. Fruit set requires additional trees from a different pollination group in the same orchard. The production of sweet cherry and choice of cultivars have been strongly affected by compatibility and coincidence of flower times to ensure adequate pollination and fertilization. Many studies have been conducted world-wide to determine the cross-compatibility of sweet cherry cultivars (2, 5, 7, 10, 12). Development of self- fertile cherries has led to more efficiently pollinated orchards, more consistent cropping, and the possibility of single-cultivar orchards.

Self-incompatibility is controlled by a single genetic locus (S-locus) with many alleles (3). Rejection of pollen occurs when the single S-allele present in the haploid pollen grain matches either of the S-alleles present in the diploid tissues of the pistil (4). A self-fertile S-allele, S4', was initially derived from the John Innes seedling 2420 which is an offspring of 'Emperor Francis' (S3S4) and X-rayed pollen of 'Napoleon' (S3S4) (9). This "pollen part" mutation has been the basis of self-fertility in a number of new cultivars including 'Stella', 'Lapins' and 'Sweetheart', which produce universally accepted S4' pollen but still has normal function of S4 allele in the style tissue (9). It is unclear what changes have occurred to produce self-fertility but the mutations are closely linked to the S-locus.

Fifteen self-incompatibility alleles have been identified in sweet cherry to date and 9 genomic S-gene sequences (13) and 5 cDNA sequences from styles of sweet cherry (11) have been reported. The conserved region and specific sequences have been used to develop primers to amplify S-allele fragments using PCR (11,13). The S-allele genotypes of a large number of cherry cultivars have been determined (1, 6, 13,). In recent years, several new selections of sweet cherry have been developed by the Pacific Agri-Food Research Centre (PARC) breeding program in Summerland. These selections combine self-fertility with high yields, early onset of bearing, extended ripening season, high fruit quality and/or cracking resistance. These advanced selections are available for wider testing and some are being named as new cultivars. The purpose of the current study was to determine S-genotypes and presence of self-fertility for these advanced selections. The S-alleles were determined via PCR, and self-fertility was tested by controlled pollinaton experiments.

Materials and methods

Plant materials

Leaves of 21 different advanced sweet cherry selections were collected from the

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field at the Pacific Agri-Food Research Centre, Summerland, BC, Canada, flash frozen in liquid N_2 and stored at -70°C until use.

Genomic DNA isolation and purification

Genomic DNA was isolated as follows. Approximately 1 gram of frozen tissue was ground with mortar and pestle with liquid N_2, transferred to a centrifuge tube containing 6.5 ml of extraction buffer (100 mM Tris, pH 8.0; 50mM EDTA; 500 mM NaCl; 1.25% SDS (w/v); 2% polyvinylpyrrolidone-40 (PVP) (w/v); 0.38% $NaHSO_3$ (w/v)) and incubated at 65°C for 1 hour. After incubation, 2.5 ml 5 M KOAc was added and the mixture was placed on ice for 20 min. The homogenate was centrifuged at 5000 x g for 20 min at 4°C and the supernatant was transferred to a clean tube containing 4.5 ml of cold (-20 °C) isopropanol. Genomic DNA was precipitated at -20°C for 1 to 2 hours. The DNA was then either pelleted in a centrifuge or if visible collected with a pipet and transferred to a microfuge tube containing 1 ml of purifying buffer (70% EtOH, 0.3M NaOAc). After centrifugation, the DNA pellet was washed with 70% ethanol, spun again and air dried. The DNA was dissolved in 300 μl double distilled H_2O. One unit RNase-A was added to the solution to digest contaminating RNA at 37°C for 20 min. DNA was further purified once using a phenol-chloroform extraction method and ethanol precipitation.

PCR primers and amplification conditions

Four primers from previous research (13) were used to amplify three different sized genomic DNA fragments of S-RNase. SI-19, SI-20, SI-31 and SI-32. PCR reactions were performed in a Robo-Cycler 9600 (Stratagene, CA) with 5 min at 94°C for denaturing; 35 cycles of 45 s at 94°C, 45 s at 58°C, 90 s at 72°C for primer pair SI 31/32; or 35 cycles of 60 s at 94°C, 60 s at 58°C and 120 s at 72°C for primer pairs SI 19/20, SI 31/20, respectively; and 10 min at 72°C for final extension. The PCR products were analyzed in agarose gels in 1 X TAE buffer and visualized by staining with ethidium bromide.

Self-fertility testing of advanced selections

Mature fruiting trees from twenty-one selections (tree ages varied) were also investigated for their self-fertility. Four branches (range of flower numbers for each branch were given in Table 2, in general 82 to 613 flowers) from each selection were labeled for 1) open pollination, 2) bagging with no hand pollination, 3) emasculation, then pollination with self pollen, 4) emasculation, then pollination with 'Lapins' pollen (control, S4' is universal donor). Pollen was collected from flowers at the "balloon" stage by extracting anthers before they dehisced, drying overnight to release pollen and stored at 4°C until use. The pollen of 'Lapins' was a bulk collection and used for all the crosses. Emasculation and pollination were done at the "balloon" stage, any flowers that were open or that were not used were removed. Cloth bags were placed on branches that were bagged before any flowers were open and remained on until after petal fall. Flower buds were counted before bagging or artificial pollination and fruit numbers were counted a few weeks after pollination to determine the percentage of fruit set.

Results and Discussion

The PCR-detected S-alleles of the 21 selections fall mainly into the groups of S1S3, S1S4 and S3S4, and two selections, 'SPC 106' and 'SPC 136' belong to the S2S4 group (Table 1). The presence of self-fertility in these selections was determined by bagging branches and pollination tests in 1999 and 2000 (Table 2). The results are generally consistent between PCR and pollination test and with their known parents. According to the PCR results, there were 16 selections containing an S4 allele and 5 selections without an S4 allele. Results of pollination experiments indicated that ten out of the sixteen selections containing S4 were self-fertile, four were likely self-fertile, and one was self-incompatible, one was likely not self-fertile. While four out of the five selections lacking S4 were self-incompatible and one was ambiguous.

Table 1. S-alleles identified by PCR and parents of advanced sweet cherry selections from the Pacific Agri-Food Centre at Summerland.

Selection	S-alleles	Seed parent	Pollen parent
11W-27-07	S1 S3	Star (S3S4)	2S-41-27 (unknown)
13N-06-49	S1 S3	Lapins (S1S4')	2N-39-05 (S1S4' or S3S4')
13N-07-19	S1 S3	Lapins (S1S4')	2N-39-05 (S1S4' or S3S4')
13S -49-24	S1 S3	Compact Lambert[Z] (S3S4)	Lapins (S1S4')
SPC 088[Y]	S1 S3	Van (S1S3)	Sunburst (S3S4')
13N-07-39	S1 S4	2N-63-20 (S1S3 or S1S4)	Stella (S3S4')
13N-07-70	S1 S4	2N-63-20 (S1S3 or S1S4)	Stella (S3S4')
13S-18-15	S1 S4	2C-61-18 (S1S3 or S1S4)	2D-28-30 (S?S4')
SPC 105	S1 S4	4C-09-22 (S1S4)	2N-55-16 (S3S4' or S4S4')
SPC 108	S1 S4	2N-41-09(unknown)	2N-37-14 (S1S4' or S3S4')
SPC 232	S1 S4	Lapins (S1S4')	unknown
SPC 106	S2 S4	OSC #6 (unknown)	2S-28-39 (S1S4' or S3S4')
SPC 136	S2 S4	2S-36-36 (unknown)	Summit (S1S2)
13S-21-01	S3 S4	Sweetheart (S3S4')	unknown
SPC 086	S3 S4	Van (S1S3)	Sunburst (S3S4')
SPC 103	S3 S4	Sweetheart (S3S4')	unknown
SPC 104	S3 S4	Star (S3S4)	2S-41-27 (S?S4')
SPC 107	S3 S4	2N-63-20 (S1S3 or S1S4)	Stella (S3S4')
SPC 207	S3 S4	Stella (S3S4')	2S-84-10 (unknown)
SPC 243	S3 S4	Sweetheart (S3S4')	unknown
Staccato	S3 S4	Sweetheart (S3S4')	unknown

[Z]Compact Lambert is thought to be S3S4 because Lambert is S3S4.
[Y]PCR result is in conflict with recorded parental S alleles.

No molecular marker is available yet to distinguish between the S4 and S4' (in which S4' pollen is compatible with S4 style, but style still reject normal S4 pollen) phenotypes. The gene sequences for the S4-RNase from S4 and S4' are identical as well as showing the same expression patterns (Zhou et al., in preparation). The self-fertility of most selections could be predicted by combining PCR results and the known S-alleles of their parents based on the assumption that the S4' is tightly linked to the S4-RNase gene. For instance, three selections, 'SPC 243', 'SPC 103' and 'Staccato' are all offspring of 'Sweetheart' (S3S4') open pollination and all contain the S4 allele as demonstrated by PCR. It can be predicted that all three selections are self-fertile because their S4 allele must either come from the seed parent 'Sweetheart' which would make it S4' or from another S4' pollen donor (only S4' pollen could grow in S4 style). For selection of 'SPC 107', 'SPC 108', 'SPC 136' 'SPC 207', no prediction can be made based on the PCR result and known parentage information. Pollination tests demonstrated that 'SPC 107', 'SPC108' and 'SPC 207' probably contain S4' whereas 'SPC 136' should have a normal S4 allele (Table 2 and 3).

Selections '13S-21-01','13N-07-70', 'SPC 086' and 'SPC 232' have low fruit set in self cross test (Table 2). As mentioned earlier, these selections should carry the S4' allele according to PCR and the S-allele of known parents. '13S-21-01' developed fruit on the bagged limb in 2000 but the self crossing rate was low at 3.1% in 2000 and 0% in 1999 (Table 2). The PCR data consistently demonstrates the presence of the S4 allele in 13S-21-01 which suggests that '13S-21-01' should be self-fertile. The factors that affect their fertil-

Table 2. Fruit set (%) of branch units (open pollinated, bagged, emasculated and pollinated with own pollen or emasculated and pollinated with 'Lapins' pollen) of sweet cherry selections and cultivars from the Pacific Agri-Food Research Centre breeding program.

Selections	Pollination treatment				Range of flower # used
	Open pollinated	Bagged	Selfed	Lapins	
		1998			
11W-27-07	11	-	0	-	120 to 473
13N-06-49	36	-	1	-	213 to 613
		1999			
11W-27-07	15	-	0	1	196 to 586
13N-06-49	8	-	8	50	165 to 439
13S-21-01	14	-	0	12	155 to 376
		2000			
13N-07-19	2	0	0	0	148 to 294
13N-07-39	36	27	14	16	118 to 288
13N-07-70	20	55	0	3	180 to 413
13S-18-15	59	26	7	34	123 to 256
13S-21-01	15	14	3	1	129 to 230
13S-49-24	17	0	21	34	161 to 228
SPC 086	3	4	0	14	156 to 267
SPC 088	1	0	0	3	153 to 410
SPC 103	42	38	8	38	121 to 178
SPC 104	34	19	37	21	178 to 295
SPC 105	30	0	0	1	91 to 385
SPC 106	10	32	18	25	168 to 303
SPC 107	44	13	16	28	94 to 138
SPC 108	88	69	38	23	91 to 215
SPC 136	60	0	3	16	124 to 195
SPC 207	56	46	15	27	82 to 185
SPC 232	30	27	2	9	86 to 179
SPC 243	73	30	26	36	131 to 353
Staccato	59	56	16	8	168 to 225
		2001			
SPC 088	2	0	-	-	210 to 214
SPC 105	19	0	0	0	208 to 224

ization efficiency are unclear. In selection 13N-07-70, about 55% fruit set occurred on bagged limbs, whereas no fruit set occurred on flowers pollinated with self pollen. Pollination with 'Lapins' also resulted in very poor fruit set (3%) (Table 2). In previous work, fruit set occurred on bagged branches (data not shown) and the natural fruit set appears to follow the pattern of other self-fertile selections, (i.e. heavy fruit set, clustering of fruit and good fruit load in years when pollination conditions are poor and traditional self-incompatible cultivars have poor fruit set). It could be self-fertile with the S4 allele coming from the pollen parent 'Stella' and being detected in PCR reaction. Combining PCR result and cross test result, four selections ('13S-21-01', '13N-07-70', 'SPC 086' and 'SPC 232') are likely self-fertile. Failure to set fruit with self and 'Lapins' pollen could have resulted from poor pollen viability or style damage during emasculation and pollination. Further work needs to be done to clarify this situation. While for selection 'SPC 105', two

Table 3. Fertility assessment based on PCR test of S alleles and pollination test for advanced sweet cherry selections from the Pacific Agri-Food Research Centre breeding program.

Selection	Fertility Assessment	Reason for fertility assessment[z]			Comment
		PCR	Test-crosses	Pedigree	
11W-27-07	not self-fertile	√	√	?	No S4 allele; also multiple year no fruit set for selfed.
13N-06-49	not self-fertile	√	ambiguous	?	No S4 allele; one year low fruit set for selfed.
13N-07-19	not self-fertile	√	√	?	No S4 allele; no fruit set for selfed or bagged, have low fruit set in general.
13N-07-39	self-fertile	√	√	?	S4 allele; likely S4'; high fruit set in bag and selfed.
13N-07-70	likely self-fertile	√	ambiguous	√	S4 allele; likely S4'; high fruit set in bag but no fruit set from self; 'Lapins' cross also low, emasculating may damage flowers.
13S-18-15	self-fertile	√	√	√	S4 allele; likely S4'; good fruit set in test crosses.
13S-21-01	likely self-fertile	√	ambiguous	?	S4 allele; likely S4'; low set in self cross.
13S-49-24	unknown	?	ambiguous	?	No S4 allele; no fruit set in bagged but fruit set from selfing.
SPC 086	likely self-fertile	√	ambiguous	√	S4 allele; likely S4'; low to no fruit set in test cross.
SPC 088	not self-fertile	√	√	?	No S4 allele; very low fruit sete in test cross.
SPC 103	self-fertile	√	√	?	S4 allele; likely S4'; good fruit set in test cross.
SPC 104	self-fertile	√	√	?	S4 allele; likely S4'; good fruit set in test cross.
SPC 105	not self-fertile	?	√	?	S4 allele; no fruit in bag or selfed cross.
SPC 106	self-fertile	√	√	√	S4 Allele; likely S4'; good fruit set in test cross.
SPC 107	self-fertile	√	√	√	S4 allele; likely S4'; good fruit set in test cross.
SPC 108	self-fertile	√	√	√	S4 allele; likely S4'; good fruit set in test cross.
SPC 136	likely not self-fertile	√	√	?	S4 allele; low fruit set in bag or selfed cross.
SPC 207	self-fertile	√	√	?	S4 allele; likely S4'; good fruit set in test cross.
SPC 232	likely self-fertile	√	ambiguous	?	S4 allele; likely S4'; good fruit set in bag, however low in selfed cross.
SPC 243	self-fertile	√	√	?	S4 allele; likely S4'; good fruit set in test cross.
Staccato	self-fertile	√	√	?	S4 allele; likely S4'; good fruit set in test cross.

[z]√ represent result support assessment, ? not clear.

years cross test with no fruit set in both selfed and bagged branches (Table 2), suggesting it is not self-fertile.

'13S-49-24' was determined to be S1S3. It does not have the S4' gene and should be self-incompatible. However, pollinating with its own pollen resulted in 21% fruit set which was higher than natural pollination, whereas the bagged branches set no fruit. The S3 allele likely came from 'Compact Lambert' which we have occasionally been able to pollinate with its own pollen and obtain seedlings. 'Compact Lambert' is a mutant that was produced by irradiation of dormant scions of 'Lambert' with X-rays (8). 'Compact Lambert' may have a different mechanism of self-fertility, or its self-incompatibility may not be fully developed in early flower stages (e.g. balloon stage). This trait may have been transmitted to '13S-49-24'.

PCR reaction suggested that 'SPC 88' had the alleles S1 and S3, which was confirmed by the fact that bagged limbs and self-crosses produced no fruit. This selection does not have a history of low fruit set. According to the S-alleles of the parents, it should be self-fertile with either S1S4' or S3S4'. Multiple PCR tests using several primer pairs have consistently failed to detect the S4' allele in this selection suggesting that presumed parentage for this selection be wrong. This result is consistent with our AFLP fingerprint data (14)

The S-alleles of some parents of tested selections have also been deduced. For instance, the S-genotype for '13N-06-49' was determined to be S1S3, which fits the observation that it is not self-fertile. It was previously uncertain whether the pollen parent of this selection, '2N-39-05' (from 'Van' x 'Stella'), was S1S4' or S3S4'. Through the genotyping of '13N-06-49', it was indirectly determined that '2N-39-05' must have the alleles S3S4' because the S3 allele of '13N-06-49' would have to have come from this pollen and the S1 from 'Lapins', the seed parent. PCR results for '13N-07-19' also confirm that '2N-39-05' contains S3S4'.

In conclusion, 10 selections 'SPC 103', 'SPC 104', 'SPC 106', 'SPC 107', 'SPC 108', 'SPC 207', 'SPC 243', '13N-07-39', '13S-18-15', and 'Staccato' are self-fertile. Five selections 'SPC 088' 'SPC 105', '11W -27-07', '13N-06-49', '13N-07-19' are not self-fertile. Selections 'SPC 086' and SPC 232' '13N-07-70', '13S-21-01', are likely self-fertile while selection 'SPC 136' is likely not self-fertile. The self-fertility and S-allele genotypes of '13S-49-24' need to be tested further.

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Peaches for Subtropical South Florida

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Abstract

Low-chill peach [*Prunus persica* (L.) Batsch] genotypes with commercial quality have been developed and are adapted to the subtropical conditions in south Florida. The yellow flesh cultivars recommended are 'Flordaprince', 'TropicBeauty' and "UFGold'. Additionally, 'Flordaglo' and 'TropicSnow' are white flesh cultivars with high sugar content and noticeably sweet taste. These cultivars require from 150 to 200 chill units, ripen from mid-April through mid-May, and have fruit size greater than five cm diameter (85 to 140g). Trees are vigorous, upright to spreading depending on the cultivar, and produce fruit within two years from field planting. Fruit have high exterior red blush with yellow or cream background for yellow and white flesh cultivars, respectively. Fruit quality is good and these peach cultivars are suitable for commercial shipment, local markets and commercial u-pick, and are also suited for the home garden and landscape. Commercial peach plantings have been established in south Florida by growers that produce and market other subtropical and tropical tree fruit crops.

The low-chill stone fruit breeding program at the University of Florida in Gainesville, has developed peach cultivars adapted to the subtropical climatic conditions of central and south Florida. Yellow flesh cultivars, 'Flordaprince', 'TropicBeauty', (9, 18) and white-flesh cultivars 'Flordaglo' and 'TropicSnow' (8, 15), are low-chill cultivars with melting flesh currently recommended for limited commercial plantings and home gardens of central and south Florida (7, 10, 22). Additionally, "UFGold' (16), a non-melting, yellow-flesh cultivar, was recently released and is expected to be the first of several new cultivars with extended shelf life following harvest.

Growing high quality peaches [*Prunus persica* (L.) Batsch] with good flavor and fruit size, and low-chilling requirement (less that 250 chill units) in central and south Florida is appealing to homeowners, landscapers, and commercial fruit growers. Peach production has caught the interest of commercial producers of tropical and sub-tropical fruit crops that have ready markets established. The addition of a deciduous fruit crop is appealing since tropical fruits are subject to occasional cold damage. Peaches with the above mentioned characteristics would command high prices in commercial u-pick and local markets because the fruit ripens in south Florida with commercial blueberries,

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