

Other self-compatible sweet cherries varieties from Spain

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Introduction

Sweet cherry (*Prunus avium* L.) is self-incompatible. Self-compatibility is a main breeding objective in sweet cherry, but few sources of self-compatibility have been described in the species. One of these comes from the local Spanish variety 'Cristobalina' (Wünsch & Hormaza, 2004). In this work additional local sweet cherry varieties from Spain were studied to investigate self-compatibility. For this purpose self- and cross-pollinations followed by microscopic observation of pollen tubes was carried out in the laboratory and self-pollination fruit set was assayed in the field. Self-compatibility was further characterized by analysing molecular markers of self-incompatibility in self-progenies. Results of this study indicate that at least two additional varieties are self-compatible (Cachi and Wünsch, 2014).



Figure 1. Microscopic observation of sweet cherry pollen tube growth. A. pollen grains germinated in the stigma. B. Pollen tubes in the style. C. Pollen tubes in the ovary.

	Talegal Ahim (S_6S_{22})	Pico Colorado (S_6S_{22})	Son Miro (S_6S_{16})
Talegal Ahim (S_6S_{22})	+	-	
Pico Colorado (S_6S_{22})	+	-	
Son Miro (S_6S_{16})			+

Table 1. S-genotype, self-, and cross-compatibility of local sweet cherry cultivars determined by microscopic observation of pollen tube growth and by fruit set assay. +: compatible crossing, -: incompatible crossing.

Crossing experiments

Self-compatibility was studied in one variety from the same region than 'Cristobalina' ('Talegal Ahín') and another from the Balearics Island ('Son Miró'). Self- and cross-pollinations followed by microscopic observation of pollen tubes was carried out in the laboratory (Figure 1). Self-pollination was carried out in the field to evaluate fruit set. After self-pollination, pollen tubes of 'Talegal Ahín' and 'Son Miró' grew through the style to the ovary, indicating self-compatibility. Fruit set in the field was 13% for 'Talegal Ahín' and 43% for 'Son Miró', confirming they are self-compatible. Reciprocal crosses with a cultivar with the same S-genotype revealed that in 'Talegal Ahim' self-compatibility is due to a pollen part mutation (Table 1).

Genetic analysis

To further characterize the cause of self-compatibility of these two varieties, the progenies that resulted from self-pollination were analyzed for the S-locus (LG6) and for the type of self-compatibility identified in 'Cristobalina', Sc (LG3). DNA was extracted from the pit of the fruits and *S-RNase* (S-locus) and *EMPaS02* (linked to Sc), were analyzed by PCR and capillary electrophoresis in the two families.

Talegal Ahín

Genetic analysis (Table 2) revealed that the S locus segregated in a Mendelian fashion in 'Talegal Ahin' self-pollination. On the other side, *EMPaS02* segregation was distorted. *EMPaS02* segregation fit the model described for 'Cristobalina', in which *EMPaS02* is linked to self-compatibility sweet cherry GL3 (Cachi and Wünsch, 2011). Taking into account that self-compatibility in this variety is also caused by loss of pollen function, these results confirm that self-compatibility of 'Talegal Ahín' may be the same as described in 'Cristobalina'.

Son Miró

In this family the segregation of both markers was distorted (Table 3) and did not fit neither Mendelian segregation nor the model described for 'Cristobalina'. These results indicate that in 'Son Miró' self-compatibility is not linked to the S locus and is not of the type described for 'Cristobalina'. Interestingly, an heterozygous excess was observed in both loci. This segregation can be caused by a post-fertilization selection against homozygous embryos. Additional studies are needed to clarify the causes of self-compatibility in this cultivar.

Table 2. **Talegal Ahín:** Observed and expected *S-RNase* and *EMPaS02* segregations in self-progeny.

Loci (genotype)	Nr.	Observed segregation	Expected segregation	χ^2	Expected segregation (<i>EMPaS02</i> linked to Sc)	χ^2
<i>S-RNase</i> (S_6S_{22})	86	12:50:24 $S_6S_6: S_6S_{22}: S_{22}S_{22}$	1:2:1 $S_6S_6: S_6S_{22}: S_{22}S_{22}$	5.62 ns	-	-
<i>EMPaS02</i> (142/144)	86	36:49:1 142/142:142/144:144/144	1:2:1 142/142:142/144:144/144	30.16***	42:43:1 142/142:142/144:144/144	1.70 ns

Table 3. **Son Miró:** Observed and expected *S-RNase* and *EMPaS02* segregations in self-progeny.

Loci (genotype)	Nr.	Observed segregation	Expected segregation	χ^2	Expected segregation (<i>EMPaS02</i> linked to Sc)	χ^2
<i>S-RNase</i> (S_6S_{16})	95	0:92:3 $S_6S_6: S_6S_{16}: S_{16}S_{16}$	1:1 $S_6S_6: S_6S_{16}: S_{16}S_{16}$	83.38***	-	-
			1:2:1 $S_6S_6: S_6S_{16}: S_{16}S_{16}$	83.57***	-	-
<i>EMPaS02</i> (129/142)	95	0:87:8 129/129:129/142:142/142	1:2:1 129/129:129/142:142/142	67.04***	1.5:47.5:46.0 129/129:129/142:142/142	65.74***



Figure 3. Fruits of 'Cristobalina', 'Talegal Ahín' and 'Son Miró'.

- References:**
 Cachi AM, Wünsch A. 2011. Characterization and mapping of non-S gametophytic self-compatibility in sweet cherry (*Prunus avium* L.). J Exp Bot 62:1847-1856
 Cachi AM, Wünsch A. 2014. Characterization of self-compatibility in sweet cherry varieties by crossing experiments and molecular genetic analysis. Tree Genet Genomes 10(5): 1205-1212
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Conclusions

In 'Talegal Ahín' the source of self-compatibility appears to be the same as in 'Cristobalina'. The common origin of this character may make sense since both varieties are native to the same geographical area, 'Sierra de Espadán' in Castellón. Also, both share the S_6 -haplotype, which may indicate that they are genetically related. However, in 'Son Miró' self-compatibility appears to have a different cause and has not been described previously.

Preliminary data from the morphological characterization of these varieties also indicates that they differ from 'Cristobalina'. 'Cristobalina' is very early while these are mid-season varieties. Besides 'Talegal Ahín' produces larger and more heart-shaped fruits than 'Cristobalina' (Figure 3). 'Talegal Ahín' and 'Son Miró' are alternative sources of self-compatibility for cherry breeding.