Identification of a microsatellite marker linked to self-compatibility in `Cristobalina´ sweet cherry

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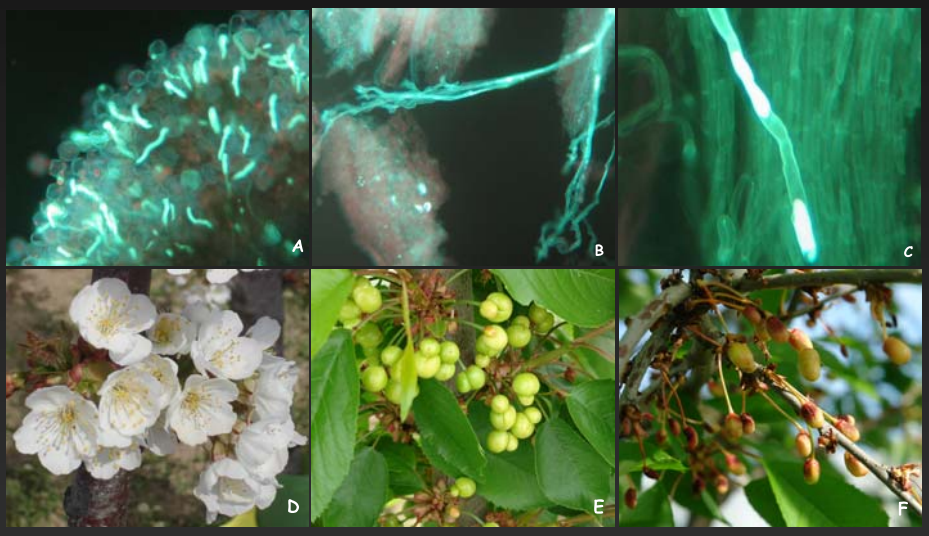
Introduction

Self-compatibility is a main breeding objective in self-incompatible species like sweet cherry (*Prunus avium* L.). In *Prunus*, gametophytic self-incompatibility is controlled by two linked genes at the *S* locus, *S-RNase* and *SBF*, that determine the specificity of the style and pollen respectively. Sweet cherry cultivars have a narrow genetic basis and it is of interest to identify and characterize new self-compatible sources that can be used in breeding. `Cristobalina´ is a local Spanish self-compatible cultivar in which self-compatibility is caused by breakdown of pollen function (Wünsch & Hormaza 2004). Previous studies with this cultivar also showed that no differences at the *S*-locus can be correlated with self-compatibility, suggesting that pollen modifier gene(s), not linked to the *S*-locus, may be the cause of self-compatibility (Wünsch et al. 2010). In this work a population derived from `Cristobalina' was phenotyped for self-(in)compatibility and markers linked to the trait were searched by Bulked Segregant Analysis (Michelmore et al. 1991). The markers identified will be use to develop markers that allow early selection of this trait in breeding populations.

Figure 1. Self-(in)compatibility phenotyping by microscopic observations of pollen tube growth (**A, B, C**) and by estimation of fruit set (**D, E, F**) . **A:** Pollen germination in the stigma. **B:** Pollen tubes in the ovary. **C:** Pollen tube growth arrested in the style. **D:** Self-pollinated flowers. **E:** Developing fruits of a self-compatible tree. **F:** Undeveloped fruits in a self-incompatible tree.



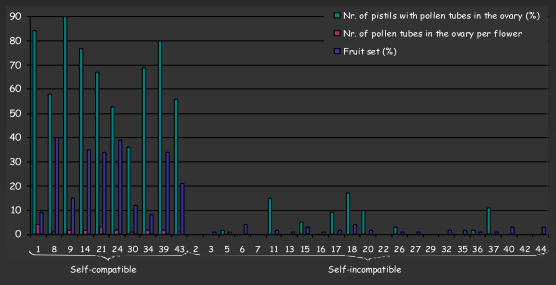
Microscopic observation of pollen tube growth

Determination of self-(in)compatibility in the `Brooks´ ($S_1S_9$, self-incompatible) x `Cristobalina´ (S_2S_6, self-compatible) population was carried out by microscopic observation of pollen tube growth and by estimation of fruit set after self-pollination.

For microscopic observations, flowers from each tree were self-pollinated in the laboratory and pollen tube growth was followed from the stigma to the ovary, along the style (Fig. 1. A, B, C). Results were expressed as the percentage of flowers with pollen tubes in the ovary in each genotype, and the number of pollen tubes reaching the ovary per flower in each tree (Fig. 2).

The percentage of flowers with pollen tubes in the ovary ranged from 0 to 90% and the mean number of pollen tubes in the ovary per flower ranged from 0 to 4 (Fig. 2). Trees with values of zero for both sets of data are expected to be self-incompatible whereas trees with high scored values in both sets of data are expected to be self-compatible (Fig. 2). Some trees, however, showed intermediate values for the percentage of flowers with pollen tubes reaching the ovary (i.e. 11, 18, 20, 37) and did not have a clear phenotype using this assay (Fig. 2).

Figure 2. Self-(in)compatibility phenotyping of `Brooks´ x `Cristobalina´ trees by microscopic observations of pollen tube growth and fruit set after self-pollination.

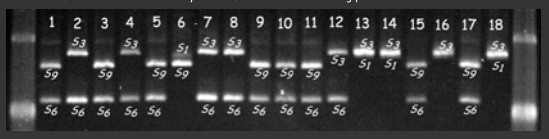


Fruit set

For the fruit set assay, trees were covered with an insect-proof mesh to prevent bees from visiting the trees and self-pollination of the flowers of each tree was done every day during flowering season. Fruit counts of each tree were carried out after last day of self-pollination until harvest date (Fig. 1. D, E, F). Fruit set was recorded as the percentage of fruits from the total number of self-pollinated flowers (Fig. 2). To estimate pollen cross-contamination in the final fruit set, fruit embryos were analysed for *S*-allele inheritance by PCR *S-RNase* typing. Embryo DNA from each fruit was analysed using the primers PruC2-PruC4R (Tao et al. 1999) and PCR products were detected by agarose gel electrophoresis (Figure 3).

Fruit set in the population ranged from 0 to 40% (Fig. 2). All the trees with a fruit set higher than 8% had at least 36% of the flowers with pollen tubes in the ovary and 1 pollen tube in the ovary per flower (Fig. 2). Additionally pollen cross-contamination in these trees was very low (maximum of 2%). These trees have been considered self-compatible (Fig. 2). Meanwhile trees with fruit set from 0 to 4% had a lower percentage of flowers with pollen tubes in the ovary but had no pollen tubes in the ovary per flower. Pollen cross-contamination in these trees was high (86 to 100%, Fig. 3) and thus self-pollination fruit set in these trees ranged from 0 to 2% and they have been considered self-incompatible (Fig. 2).

Figure 3. *S*-RNase PCR amplification of 18 embryos from the self-pollination of 2 `Brooks´ x `Cristobalina´ trees whose fruit set was 4 %. All embryos came from cross-contaminating pollen.



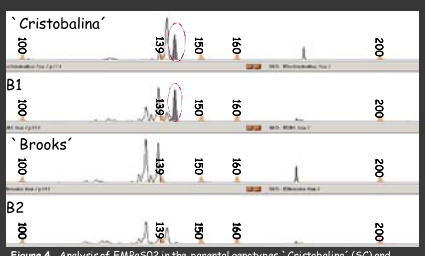


Figure 4. Analysis of EMPaS02 in the parental genotypes `Cristobalina´ (SC) and `Brooks´ (SI) and in two DNA bulks B1 (SC) and B2 (SI)

Bulked Segregant Analysis

A BSA approach was used to identify markers linked to self-compatibility in this population. For this purpose 88 *Prunus* SSR loci were initially analysed for polymorphism in the parental genotypes ('Brooks', 'Cristobalina') and those SSRs that resulted polymorphic, were analyzed in two DNA bulks made up of 8 self-compatible and 8 self-incompatible trees. SSR polymorphisms conserved in the parental genotypes and in the DNA bulks were analyzed in each genotype of the bulks. When the polymorphism was conserved in these genotypes the marker was analyzed for co-segregation in all the population.

Initial screening of the SSR markers revealed 54 SSRs (61%) that were polymorphic in the parental genotypes and 4 of these (4%) revealed polymorphisms that were conserved in the parental genotypes and in the DNA bulks. The analysis of these 4 markers in the population revealed that for 2 SSRs an allele co-segregated with self-(in)compatibility in most of the self-compatible trees. For microsatellite EMPaS02 (Vaughan & Russell 2004) an allele of 142 basepairs (Fig. 4) was found in the 10 self-compatible trees and was absent in 20 of the 21 self-incompatible genotypes. The genetic distance between self-compatibility and EMPaS02 was estimated to be 3.2 cM with a LOD of 6.19.

Conclusion

Self-compatibility phenotyping in a small F1 population that descends from `Cristobalina´ was accurately determined by using two complementing different methods, microscopic observations of pollen tube growth of self-pollinations in the laboratory and fruit set recording after self-pollination in the field. These results were used to construct two DNA bulks that were screened with SSR markers. This BSA approach was successful for the identification of an SSR marker linked to self-compatibility. SSR locus EMPaS02 is significantly linked to self-compatibility in 'Cristobalina'. The identification of this marker will allow the genetic mapping of this trait in cherry and will be use to develop efficient markers that allow early selection of this trait in breeding populations.

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