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 Prsloc (S allele) and Prloc1 (SI alleles) 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 & Hamouz 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 genes 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-incompatibility and markers linked to the trait were described by bulked segregant analysis (Michelmore et al., 1991). The markers identified will be used to develop markers that allow early selection of self compatible sweet cherry breeding populations.

Microscopic observation of pollen tube growth

Determination of self-incompatibility in the ‘Brooks’ (L53, S-self incompatible) × ‘Cristobalina’ (L53, S-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 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 6 (Fig. 2). Tress with values of 0 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. 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).

In order to further characterize the relationship between self-incompatibility and fruit set in this population, a bulked segregant analysis (BSA) was carried out on 50 plants representing the parental lines and the intermediate self-compatible form (Wünsch et al., 2010). The DNA was extracted from the genomic DNA of each bulk and was used for the analysis of SSR markers. A total of 21 self-incompatible trees and 20 self-compatible trees were examined.

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 in 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 field, fruit set in 10 fruit clusters were observed for all allelic combinations of the PzA and PzB using 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 6% had at least 36% of the flowers with pollen tubes in the ovary and 1 pollen tube in the ovary per flower (Fig. 2). In addition, 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 6% 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).

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-incompatible lines and fruit set records after self-pollination in the field. These traits were used to construct two DNA bulks that were screened with SSR markers. This BSA approach was successful for the identification of a SSR marker linked to self-compatibility. SSRs from EMPr002 are 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 used to develop efficient markers that allow early selection of this trait in breeding populations.

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