GENETIC RELATIONSHIPS AND POLLINATION STUDIES IN SWEET CHERRY
(Prunus avium L)

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ABSTRACT

Methods were developed for isozyme analysis of sweet cherry (Prunus avium) and the best results were achieved using cellulose acetate as the matrix for electrophoretic separation of isozymes.

Isozyme analysis was carried out on protein extracts from cherry leaves which facilitated the unique identification of 70 cultivars from a collection of 78. Cultivars were compared individually using the number of isozyme differences as a measure of genetic distance. Cultivars were also grouped into country of origin and allele frequencies were used to determine genetic distance between groups. The average number of isozyme differences ranged from 5.97 ± 1.73 for St Margaret to 3.37 ± 1.54 for Merton Glory. This indicates that the sweet cherry cultivars studied were closely related, differing by no more than 2 isozyme genotypes. Allele frequencies of geographically distinct groups produced dissimilarity indexes in the range of D = 0.41 to 0.54. This means that cultivars developed in different countries have diverged from the last common ancestor to a similar degree. There was only one statistically significant divergence for FDP which had a higher allelic frequency in the Swiss and Australian groups. Selection pressure associated with climate or breeding programmes was thought to be responsible for this result. Overall, cultivars from the U.S.A. showed the greatest divergence and this is probably because Bing dominates in the pedigree of those cultivars. The closest genetic distances were between the Australian and Canadian cultivar groups.

The examination of progeny from controlled hybridisations allowed genetic analysis of data to be carried out and the inheritance patterns of isozymes determined. Linkage between the isozymes and the self-incompatibility locus was also estimated. Glutamate oxaloacetate transaminase (GOT) was tightly linked to the S-locus (r = 0) and this linkage was reflected in the segregation ratios for GOT. An unexpected result occurred when Stella was selfed, in that all of the progeny showed the bc genotype and there was no segregation for either the bb or cc homozygous genotypes. Stella
is heterozygous for self-fertility and carries the mutant self-fertile allele \( S'_1 \). Progeny were expected to segregate as self-fertile heterozygotes \( S'_1S'_1 \) and self-fertile homozygotes \( S'_1S'_1 \). The segregation of the closely linked GOT isozyme showed that this was not occurring and only \( S'_1S'_1 \) progeny had been produced. Fluorescent microscopy was used to observe pollen tube growth in hand pollinated cherry styles. After Stella was self-pollinated, inhibition of the \( S'_1 \) pollen tubes was observed in the third style. Those pollen tubes growing to the base of the style \( (S'_1) \) were observed to penetrate the micropyle. Knowing that the self-fertile mutation was ‘pollen reaction lost’ and that the current model for gametophytic self-incompatibility based on work in *Nicotiana* depended on the pollen tube cell wall structure and uptake of S-RNases, it was concluded that \( S'_1 \) pollen tubes were recognised at fertilisation when they discharged their contents into the ovule. Because there are two ovules in the sweet cherry ovary either an incompatible reaction \( (S'_1, ovule) \) or compatible \( (S_1, ovule) \) mating would occur. Hence only \( S'_1S'_1 \) embryos were formed. The GOT isozyme acted as a marker for the \( S - \) alleles with all progeny showing the bc heterozygous genotype.

Isozymes were also used to determine gene flow in cherry orchards and to determine pollen donors of selected cultivars. Regarding the breeding behaviour of Stella, over a three year period 71% of embryos harvested from Stella trees at Lenswood Horticultural Centre were a result of outcrossing and 29% were generated by selfing. Sixty per cent of the outcrossing occurred with the nearest neighbouring trees flowering at the same time as Stella, in this study that was Venus, but all other sweet cherry cultivars are compatible with Stella and will cross pollinate if flowering times overlap. Interaction between honeybees (Apis mellifera) and orchard design was thought to be having the greatest effect on gene flow in cherry orchards. A range of commercial recommendations for pollination in cherry orchards was derived from this work.