Sexual Compatibility and Construction of Molecular Linkage Maps in Olives (*Olea europaea* L.)

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Abstract

The olive, *Olea europaea* L., is one of the oldest agricultural tree crops. The genus *Olea* consists of more than 38 different species, with only *Olea europaea* L. being cultivated. The olive tree is a perennial evergreen and its flowers show varying levels of self-incompatibility. It is widely planted in regions with Mediterranean climate and is economically important both for oil and table use.

Olive trees were introduced into Australia in the early 1800s, but in spite of many efforts made by the government and the new colonists, a viable commercial industry did not develop for a variety of reasons. In recent decades, however, the potential profits to be made from olives has stimulated a current investment boom. To be competitive, the Australian industry will need to concentrate on yield and quality by planting cultivar combinations with appropriate pollinations, and by developing cultivars that are well adapted to local environmental conditions. This objective will be achieved in part by determining the compatibility relationships between and among the common commercial cultivars, and by the use of molecular markers for quality traits that can be used in plant improvement. The construction of a genetic linkage map would be an important approach to aid marker-assisted selection. These topics were the focus of the research described in this thesis.

Self- and cross-compatibility was investigated in 1999 and 2000 using five common commercial cultivars. The results showed that Frantoio was cross-compatible, as either a male or female parent, with each of the other cultivars, but showed a high degree of self-incompatibility. Manzanillo, Kalamata, Pendolino, and Picual were cross-incompatible, and except for Manzanillo, were self-incompatible. It is concluded that Frantoio is a good general polleniser for the other cultivars investigated. Pollen tube growth decreased in discrete steps from stigma to upper style, and from upper style to lower style, with the result that only one, or rarely two, pollen tubes penetrated ovules. Self-incompatibility in olive is probably gametophytic, but more research is needed to clarify this. The sex ratio of flowers, pollen viability, and male sterility were also examined in the study. The results showed that complete flowers were predominant in Frantoio, Manzanillo, and Pendolino, but Kalamata and Picual had mainly male flowers. Frantoio had the highest pollen viability, Kalamata and Picual were intermediate, and Manzanillo and Pendolino the lowest. Male sterility was found in the cultivar SA Verdane during most of the flowering period of 2000 but it was male fertile in 1999.

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Fruit development continued about 5 months after pollination. The initial percent fruit set varied from 79.3% for SA Verdale x Manzanillo to 3.4% for Kalamata x Frantoio. Fruit abscission was evident as early as one month after pollination, and by two months, the highest fruit set of any cross was only 10.1%. Between two and five months after pollination, fruits retained declined to between 1.8% and 5.3%.

While fruit number was an important outcome from controlled pollinations, seed germination was needed for mapping studies. However, olive seed germination is slow and germination percentage is low. Therefore, germination conditions were studied and a germination percentage of 94% was obtained through optimised treatment of the seeds. The results showed that the best combination is a storage period of ten months at room temperature followed by four weeks at 4°C on ½ MS solidified medium; and then growth on the same medium at 20°C.

Molecular linkage maps are a powerful approach for the selection of quality traits in plant improvement programs. However, limited information is available for the linkage groups of olive. Therefore, the construction of a molecular linkage map was an objective of this project. A mapping population of 104 progeny was generated from a cross between the cultivars Frantoio and Kalamata, and molecular linkage maps were constructed based on a combination of RAPD, SCAR, and microsatellite markers using the pseudo-testcross strategy. The hybridity of the mapping population was confirmed by genetic similarity using 300 RAPD markers, nonmetric multidimensional scaling using 300 RAPD markers, and genotype matching based on 9 SSR markers. 194 molecular markers were used for map construction and 152 were mapped in the linkage groups.

Separate maps were produced using markers that were heterozygous in each of the parents and these were integrated to produce a map for the species using Joinmap v. 2.0. Twenty-three linkage groups were obtained for Kalamata, 27 for Frantoio, and 15 for the integrated map. The sizes of the genomic DNAs were estimated to be 2614 cM and 3427 cM for Frantoio and Kalamata respectively, and therefore the genomic size of the species was estimated around 3000 cM. The linkage groups for Frantoio cover 798 cM of the genome with 92 loci, and the average distance between loci is 12.3 cM. The linkage groups for Kalamata cover 759 cM of the genome with 89 loci, and the average distance between loci is 11.5 cM. The linkage groups for the integrated map cover 879 cM of the genome with 101 loci, and the average distance is 10.2 cM. It is estimated that 1333 cM of the olive genome was mapped with 152 markers in the present study, and this would account for about 45% of the genome.

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One SCAR marker, G07-700, which is linked to olive peacock disease resistance, was mapped in linkage group 1 of the Frantoio map, and linkage group 2 of the integrated map. When the mapping population pass through their juvenile phase and adopt their adult characters, the morphological markers will be added.

This study has contributed significantly to the information available for olive improvement in Australia, and the renaissance of the olive industry. However, further studies are needed to determine the sexual compatibility of other cultivars under Australian conditions, and to increase the number of molecular markers on the genetic maps.