THE DEVELOPMENT OF A GENETIC LINKAGE MAP FOR ALMOND BASED ON MOLECULAR AND AGRONOMIC MARKERS

A THESIS SUBMITTED BY

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Thesis Summary

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Almond, *Prunus dulcis*, is a tree nut crop that originated in central Asia and is now grown commercially worldwide. Within Australia there exists huge potential gain from optimisation of almond cultivars better suited to Australian conditions. This is the ultimate goal of the Australian Almond Breeding Program, which was established in 1997 at the University of Adelaide. As part of this breeding program a unique hybrid population was developed from a cross between the American self-incompatible cultivar 'Nonpareil' (NP) and European self-compatible cultivar 'Lauranne' (LA). The F_1 population derived from this cross is the focus of this study, the population consisted of 181 individuals, of which 93 were selected for use in the mapping study.

Investigation of a number of DNA extraction techniques was performed in order to optimise DNA extraction quality and integrity from almond leaves for future applications in molecular work.

To determine if the purported F_1 hybrids were true hybrids, derived from a cross between the cultivars NP and LA, both DNA fingerprinting with cluster analysis and *S*-

allele identification was performed, and the majority of F_1 putative hybrids clustered between the two parents when analysed using the simple matching coefficient and UPGMA. The genetic similarity between individuals comprising the mapping population ranged from 70% to 93% while the parents were 72% similar in comparison to each other. This indicated high genetic variability available for studying heritabilities and for production of a genetic map. Analysing the *S*-allele complement of all the F_1 hybrids was also performed to offer a more robust method for hybrid determination, since individuals in a breeding population with aberrant *S*-allele inheritance can be considered non-related. The inheritance of the self-fertility gene is important in breeding programs, since the majority of almond cultivars are self-incompatible, tracking the inheritance of this allele in breeding programs is therefore highly desirable.

A detailed morphological study was performed on the whole population over three growing seasons, 2001, 2002, and 2003. In 2001 tree characters such as disease prevalence, bare branches, close internodes, level of upright branches, leaf size and colour were measured. For all the seasons a number of other traits were also measured including: yield, bloom time, self-compatibility, percentage of double kernels, shell hardness, kernel weight, shape, taste, pubescence, and colour. The heritability, genetic variance, segregation and raw correlations between traits were calculated and used to establish a mode of inheritance for these traits. Rainfall and temperature maximum, minimum and monthly averages were collected and used to compare trends in the collected morphological data with these climatic data.

A preliminary investigation was undertaken to determine if the cellular structure of the kernel testa epidermis was responsible for the pubescent versus smooth mouthfeel of the F_1 hybrids. Light and scanning electron microscopy identified the presence of

cellular protuberances arising from the epidermis as a potential cause of the pubescent mouthfeel in almonds. Bulked segregant analysis using inter-simple sequence repeat (ISSR) primers identified a potential marker linked to the pubescent trait which was converted to a sequence characterised amplified region (SCAR), which was also used to screen twelve almond cultivars for this trait.

In addition to the use of BSA for the development of markers linked to traits of interest, the development of genetic linkage maps has the potential to greatly enhance current and future breeding programs by MAS. This study produced a genetic linkage map for this population, constructed using random amplified polymorphic DNA (RAPD), ISSR, and simple sequence repeats (SSR), with the mapping program Joinmap 3.0. Two parental maps were constructed, which coalesced into seven linkage groups for the female parent and eight linkage groups for the male parent, corresponding to the chromosome number of eight for almond. The marker density was 9.4 cM/marker for NP and 9.6 cM/marker for LA, covering 65% for the female and male parental maps in comparison to the highly saturated peach x almond map produced by the European *Prunus* Mapping Program (EPMP). Fourteen markers segregating in both parents were used to produce an integrated parental map for this cross, which coalesced into six linkage groups with a marker density of 11.6 cM/marker. The presence of anchor loci common to the EPMP map allowed homologous linkage groups to be established between the two populations.

This study has contributed to the understanding of key morphological traits important in almond breeding programs. The expression and influence of biotic factors on the expression of these traits was also investigated. Understanding factors

responsible for kernel taste is also an important objective and this study has contributed to this knowledge. The development of a genetic linkage map will serve as a permanent and practical resource for almond breeders in Australia, and contribute important data to the EPMP. This has significant benefit for *Prunus* breeders worldwide, and further enhances knowledge on an economically important nut crop.