

Genetic Analysis of Reproductive and Nut Traits in
Almond [*Prunus dulcis* (Mill.) D.A. Webb]

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the degree of the Doctor of Philosophy

By

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ABSTRACT

Almond is a perennial tree crop with a gametophytic self-incompatibility (SI) system. The SI system of almond is controlled by a multi-allelic locus, *S*, which is about 70,000 bp long. A nearly complete sequence for the entire *S* locus sequence has been available only for the *S*₇ haplotype. In this research, next-generation sequencing technology was implemented to sequence the entire *S* locus simultaneously from 15 haplotypes. The results confirmed the accuracy of available *S*₇ haplotype sequence, generated the entire *S* locus sequences for the *S*₁, *S*₇ and *S*₈ haplotypes and generated partial *S* locus sequences for 11 other haplotypes (*S*₃, *S*₅, *S*₆, *S*₉, *S*₁₃, *S*₁₄, *S*₁₉, *S*₂₂, *S*₂₃, *S*₂₅ and *S*₂₇). Comparisons among haplotype sequences revealed higher polymorphism in the region where the *S-RNase* and *SFB* genes are located and considerable differences in the number and locations of long terminal repeat retrotransposons.

There are about 50 known *S* alleles, of which one confers self-fertility. For some of these, complete or partial *S-RNase* and *SFB* sequences are available. Here, more complete sequences were generated for several alleles of the *S-RNase* gene (*S*₃, *S*₆, *S*₉, *S*₁₃, *S*₁₉, *S*₂₂ and *S*₂₅) and the *SFB* gene (*S*₉, *S*₂₃ and *S*₂₇).

In almond breeding, SI limits the parental combinations that can be used for crossing. Detection of *S* alleles prior to crossing would be beneficial. Until now, molecular detection of the *S* alleles has relied on detection of length polymorphisms in the *S-RNase* gene. Here, single nucleotide polymorphisms (SNPs) in the *S-RNase* and *SFB* genes were used in designing assays to distinguish among *S* alleles.

This thesis also reports on the construction of linkage maps for Nonpareil and Lauranne based on genotyping-by-sequencing (GBS) and on the design of uniplex assays for detection of SNPs

detected by GBS. These assays were applied to additional Nonpareil × Lauranne progeny and to progeny from three other Nonpareil crosses (Nonpareil × Constantí, Nonpareil × Tarraco and Nonpareil × Vairo). Data from all four populations were used to generate a composite map for Nonpareil. Comparisons of marker positions detected for Nonpareil and Lauranne with positions in the peach genome confirmed high collinearity between the almond and peach genomes.

Quantitative trait loci analysis detected 23 genomic regions as affecting nut and/or kernel traits in Nonpareil × Lauranne. Nine and 14 QTLs were detected for Nonpareil and Lauranne, respectively. Of the kernel and nut traits mapped here, shell weight, kernel shape, tocopherol concentration, fatty acid concentration and oleic/linoleic ratio were mapped for the first time in almond. For shell hardness and oleic/linoleic ratio, markers were identified that could be useful for marker-assisted selection. Some of the QTLs related to fatty acid and tocopherol concentration were closely located to the genes that are known to be involved in the synthesis of fatty acids and/or tocopherols. Some of the sequence information generated here may be useful for designing primers to amplify these genes (or components of these genes) for resequencing from multiple almond genotypes.

THESIS DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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LIST OF ABBREVIATIONS

AH	: amygdalin hydrolase
ADGH	: amygdalin diglucosidase
BAM	: binary alignment/map format
Bp	: base pair
BWA	: Burrows Wheeler Alignment
C	: conserved region
Ca ²⁺	: calcium ion
CDS	: coding sequences
CIG	: cross incompatibility group
CIGs	: cross incompatibility groups
CO ₂	: carbon dioxide
cv.	: cultivar
CYP	: cytochrome P450 monooxygenase
DdRAD	: double digest restriction site associated DNA
DMGGBQ	: 2,3-dimethyl -5-geranylgeranyl-1,4-benzoquinone
DMPBQ	: 2,3-dimethyl -5-phytyl-1,4-benzoquinone
DNA	: deoxyribonucleic Acid
EMBL	: European Molecular Biology Laboratory
F ₁	: filial 1 generation
FA	: fatty acid
G	: gram
Gb	: gigabit
GBS	: genotyping-by- sequencing
GC	: gas chromatography

GDR	: Genome database for Rosaceae
GT	: glucosyltransferase
GSTs	: glutathione S-transferases
H	: hydrogen
HGA	: homogentisic acid
HPLC	: high performance liquid chromatography
HPPD	: <i>p</i> -hydroxyphenylpyruvic acid dioxygenase
RHV	: hypervariable region
HV	: variable region
IGV	: integrative genomics viewer
IN	: integrase
ISSR	: inter simple sequence repeat
ISW	: in-shell weight
KASP™	: competitive allele-specific primer
Kb	: kilo base
KS	: kernel size
L	: linoleic acid
LDL	: low density lipoprotein
LG	: linkage group
LINEs	: long interspersed nuclear elements
LOD	: likelihood of odds
LTRs	: long terminal repeats
Mb	: mega bases
MDL	: mandelonitrile
Me	: methyl
MGGBQ	: 2-methyl-6-geranylgeranylplastoquinol
MIRA	: Mimicking Intelligent Read Assembler

MITEs	: miniature inverted-repeat transposable elements
MPBQ	: 2-methyl-6-phytylplastoquinol
MPBQ MT	: 2-methyl-6-phytylplastoquinol methyltransferase
NAM	: nested association mapping
NCBI	: National Centre for Biotechnology Information
NGS	: next- generation sequencing
O	: oleic acid
ORF	: open reading frame
PCR	: polymerase chain reaction
PDP	: phytyl diphosphate
PH	: prunasin hydrolase
PPM	: pollen part mutation
PR	: protease
QTL	: quantitative trait locus
QTLs	: quantitative trait loci
R	: retrotransposons
RAD	: restriction site associated DNA
RAPD	: randomly amplified polymorphic DNA
Res	: restriction enzymes
RFLP	: restriction fragment length polymorphism
RH	: RNase H
RT	: reverse transcriptase
SAM	: sequence alignment/map format
SAM	: S-adenosyl methionine
S locus	: self-incompatibility locus
SCAR	: sequence characterised amplified region
Sf	: self-fertility

SFB	: S haplotype-specific F-box
<i>SFB</i>	: S haplotype-specific F-box gene
SH	: shell hardness
SI	: self-incompatibility
SINEs	: short interspersed nuclear elements
SLF	: S locus F-box
<i>SLF</i>	: S locus F-box gene
SNP	: single nucleotide polymorphism
SNPs	: single nucleotide polymorphisms
SPM	: stylar part mutation
S-RNASE	: stylar-RNase
<i>S-RNASE</i>	: stylar-RNase gene
SSR	: simple sequence repeat
SW	: shell weight
TE	: transposable element
TIR	: terminal inverted repeats
TMT	: tocopherol methyltransferase
VCF	: variant call format
<i>VITE</i>	: genes for vitamin E biosynthesis

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