Self-incompatibility of Olive

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Abstract

The olive (*Olea europaea* L.) is one of the most ancient fruit trees and has been cultivated for its oil in the Mediterranean area for thousands of years. Today, the consumption of olive oil and table olives is increasing both in traditional producing countries and the entire world. Most olive cultivars are self-incompatible and do not produce a commercial yield after self-pollination. In this thesis, inflorescence architecture and sexual compatibility relationships of some olive cultivars, and gene expression in olive pistils during flowering were studied.

To study the inflorescence architecture of olive, 45 inflorescences in each of the cultivars Manzanillo, Mission, and Frantoio were checked every morning from flower opening to petal fall. The flower position on the inflorescence had a highly significant effect on the opening day in all cultivars. Terminal flowers and the flowers located on the primary branches opened earlier than flowers located on the secondary branches. Flower position also had a highly significant effect on gender in Manzanillo and Mission. In Manzanillo, the secondary branches had fewer perfect flowers than the primary branches. In Mission, the secondary branches had no perfect flowers at all. In Manzanillo, perfect flowers had significantly longer petal persistence than staminate flowers. To study flower competition within the inflorescence, the distal halves, on which the flowers tend to be perfect, of 120 inflorescences in three trees of Manzanillo were removed about one month before full bloom. This resulted in a highly significant increase in the percentage of perfect flowers on the proximal halves. The effects of shoot orientation and inflorescence location on inflorescence characteristics in the cultivars Frantoio, Kalamata, and Koroneiki were also studied. For each cultivar, inflorescence characteristics in three sections of shoots (top, middle, and base) and four sides of the three selected trees (north, south, east, and west) were recorded. The statistical analysis showed that basal inflorescences were shorter and with fewer flowers but with the same percentage of perfect flowers. Shoot orientation did not have any influence on these characteristics in any of the cultivars.

Sexual compatibility was assessed using two methods. In the first method, controlled crossings were performed in the cultivars Frantoio, Koroneiki, and Kalamata. The pistils were harvested one week after hand pollination and stained with 0.1% aniline blue. The styles and ovules were separated, mounted in 80% glycerol, and
observed under a fluorescence microscope. In Frantoio and Koroneiki, the number of ovules penetrated by a pollen tube was used to estimate the level of sexual compatibility. In Kalamata, the numbers of ovules penetrated by pollen tubes were not significantly different between treatments; therefore, the number of pollen tubes in the lower style was used. All the cultivars studied were self-incompatible. Frantoio (as a host) was incompatible with Koroneiki and Barnea but partially compatible with Mission. Koroneiki (as a host) was incompatible with Barnea but partially compatible with Frantoio and Mission. Kalamata (as a host) was compatible with Barnea, incompatible with Mission and Koroneiki in 2004, but partially compatible with them in 2005. In the second method, eight microsatellite markers were used for genotyping three Kalamata mother trees, 40 embryos per mother tree, and all the potential pollen donors. Genotyping data were analysed using FaMoz software, and the number of embryos assigned to each putative pollen donor was determined. Paternity analysis showed that Kalamata (as a host) was self-incompatible, compatible with Barnea, Benito, and Katsourela, but incompatible with Arbequina, Azapa, and Picual.

To study the gene expression in olive pistils during flowering, a genomic approach was initiated using cDNA subtractive array analysis. Total RNA was isolated from olive pistils at two developmental stages, where self-incompatibility (SI) genes are expected to be differentially expressed: 1) small green flower buds (expression of SI genes not expected) and 2) large white flower buds containing receptive pistils just prior to opening (expression of SI genes expected). From each stage, cDNA libraries were prepared and put through forward and reverse subtractive hybridisations to enrich for differentially expressed cDNAs in stage 2. Macroarrays were prepared by printing 2304 differentially expressed cDNAs onto nylon membranes and hybridised with forward- and reverse-subtracted probes. The analysis identified 90 up-regulated cDNA clones highly expressed in receptive pistils. Further subtracted and unsubtracted hybridisations confirmed up-regulation of the majority of these cDNAs. Gene expression profiles across different tissues showed that most of the genes were pistil-specific. The expression pattern of the genes showed high similarity in Kalamata, Frantoio, Barnea, and Pendolino. All the screened genes were sequenced and their similarities were searched in the NCBI database. The most redundant and interesting up-regulated clones were those similar to a receptor protein kinase-like protein. Some versions of this protein play a role in the sporophytic SI system of Brassica and the gametophytic SI system of Papaver and rye.
Declaration and Authorisation of Access to Copying

This work contains no material which has been accepted for the award of any other degree or diploma 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.

I give consent to this copy of thesis, when deposited in the University Library, being available for loan and photocopying.

Signed:                      Date:
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<table>
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<th>Definition</th>
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<tbody>
<tr>
<td>%</td>
<td>percent</td>
</tr>
<tr>
<td>°</td>
<td>degree</td>
</tr>
<tr>
<td>°C</td>
<td>degrees Celsius</td>
</tr>
<tr>
<td>µg</td>
<td>microgram</td>
</tr>
<tr>
<td>µl</td>
<td>microlitre</td>
</tr>
<tr>
<td>3'</td>
<td>3 prime end of a nucleic acid</td>
</tr>
<tr>
<td>5'</td>
<td>5 prime end of a nucleic acid</td>
</tr>
<tr>
<td>A_E</td>
<td>effective number of alleles</td>
</tr>
<tr>
<td>AFLP</td>
<td>amplified fragment length polymorphism</td>
</tr>
<tr>
<td>AGRF</td>
<td>Australian Genome Research Facility</td>
</tr>
<tr>
<td>al</td>
<td>Allele</td>
</tr>
<tr>
<td>am</td>
<td>before noon</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>A_O</td>
<td>observed number of alleles</td>
</tr>
<tr>
<td>AOA</td>
<td>Australian Olive Association</td>
</tr>
<tr>
<td>ARK</td>
<td><em>Arabidopsis</em> receptor kinase</td>
</tr>
<tr>
<td>B1</td>
<td>branch 1</td>
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<tr>
<td>B2</td>
<td>branch 2</td>
</tr>
<tr>
<td>B3</td>
<td>branch 3</td>
</tr>
<tr>
<td>B4L</td>
<td>branch 4 lateral</td>
</tr>
<tr>
<td>B4T</td>
<td>branch 4 terminal</td>
</tr>
<tr>
<td>B5L1</td>
<td>branch 5 lateral 1</td>
</tr>
<tr>
<td>B5L2</td>
<td>branch 5 lateral 2</td>
</tr>
<tr>
<td>B5T</td>
<td>branch 5 terminal</td>
</tr>
<tr>
<td>B6L1</td>
<td>branch 6 lateral 1</td>
</tr>
<tr>
<td>B6L2</td>
<td>branch 6 lateral 2</td>
</tr>
<tr>
<td>B6T</td>
<td>branch 6 terminal</td>
</tr>
<tr>
<td>BBCH</td>
<td>Biologische Bundesanstalt, Bundessortenamt, Chemische Industrie</td>
</tr>
<tr>
<td>BC</td>
<td>before Christ</td>
</tr>
<tr>
<td>BLAST</td>
<td>Basic Local Alignment Search Tool, a computer program</td>
</tr>
<tr>
<td>BLASTX</td>
<td>a computer program to search protein databases using a translated nucleotide query</td>
</tr>
<tr>
<td>bp</td>
<td>base pair</td>
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</tbody>
</table>
c column
Ca$^{2+}$ ionic solution of calcium
cDNA complementary DNA
CDP-Star disodium 2-chloro-5-(4-methoxyspiro{1,2-dioxetane-3,2'-(5'-chloro)
tricyclo[3.3.1.13,7]decan}-4-yl) phenyl phosphate
cm centimetre
CTAB cetyltrimethylammonium bromide
DEF differentially expressed fragment
df degrees of freedom
DIG digoxigenin
DNA deoxyribonucleic acid.
DNase deoxyribonuclease
dNTP a generic term referring to the four deoxyribonucleotides: dATP, dCTP, 
dGTP and dTTP
dUTP 2'-deoxyuridine 5'-triphosphate
E east
E. coli Escherichia coli
EDTA ethylenediaminetetraacetic acid
EMO2 EMO2AJ416320, a microsatellite primer
EP exclusion probability
EPD effective pollination distance
EST expressed sequence tags
et al. et alia (Latin)
E-value expect-value
FAM a fluorescent dye-labelled oligo
FAO Food and Agriculture Organisation
Fig. Figure
FS forward-subtracted
g gram
Gel Doc gel documentation system
GML generalised linear modelling
GSI gametophytic self-incompatibility
h hour
ha hectare
HE$e$ expected heterozygosity
HEX a fluorescent dye-labelled oligo
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>NS</td>
<td>not significant</td>
</tr>
<tr>
<td>®</td>
<td>trade mark</td>
</tr>
<tr>
<td>OSI</td>
<td>ovarian self-incompatibility</td>
</tr>
<tr>
<td>P</td>
<td>probability</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PD</td>
<td>power of discrimination</td>
</tr>
<tr>
<td>PF</td>
<td>all perfect flowers</td>
</tr>
<tr>
<td>pg</td>
<td>picogram</td>
</tr>
<tr>
<td>pH</td>
<td>potential of Hydrogen (-log [H⁺])</td>
</tr>
<tr>
<td>pm</td>
<td>after noon</td>
</tr>
<tr>
<td>PR</td>
<td>pathogenesis-related protein</td>
</tr>
<tr>
<td>PVP</td>
<td>polyvinylpyrrolidone</td>
</tr>
<tr>
<td>r</td>
<td>row</td>
</tr>
<tr>
<td>RAPD</td>
<td>random amplified polymorphic DNA</td>
</tr>
<tr>
<td>REML</td>
<td>restricted maximum likelihood</td>
</tr>
<tr>
<td>RFLP</td>
<td>restriction fragment length polymorphism</td>
</tr>
<tr>
<td>RFU</td>
<td>relative fluorescence unit</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>RNase</td>
<td>ribonuclease</td>
</tr>
<tr>
<td>rpm</td>
<td>revolutions per minute</td>
</tr>
<tr>
<td>RS</td>
<td>reverse-subtracted</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>reverse transcriptase polymerase chain reaction</td>
</tr>
<tr>
<td>s</td>
<td>second</td>
</tr>
<tr>
<td>S</td>
<td>south</td>
</tr>
<tr>
<td>SA</td>
<td>South Australia</td>
</tr>
<tr>
<td>SCR</td>
<td>S-locus cysteine-rich protein</td>
</tr>
<tr>
<td>SDS</td>
<td>sodium dodecyl sulphate</td>
</tr>
<tr>
<td>SE</td>
<td>standard error of the mean</td>
</tr>
<tr>
<td>SF</td>
<td>all staminate flowers</td>
</tr>
<tr>
<td>SI</td>
<td>self-incompatibility</td>
</tr>
<tr>
<td>SLF/SFB</td>
<td>S-locus F-box protein</td>
</tr>
<tr>
<td>SLG</td>
<td>S-locus glycoprotein</td>
</tr>
<tr>
<td>SNP</td>
<td>single nucleotide polymorphism</td>
</tr>
<tr>
<td>SP11</td>
<td>S-locus pollen protein 11</td>
</tr>
<tr>
<td>sPPase</td>
<td>soluble inorganic pyrophosphatase</td>
</tr>
<tr>
<td>SRK</td>
<td>S-locus receptor kinase</td>
</tr>
</tbody>
</table>
SSC  standard saline citrate
SSI  sporophytic self-incompatibility
SSR  simple sequences repeat
SSR14 ssrOeUA-DCA14 AJ279863, a microsatellite primer
SSR3 ssrOeUA-DCA3 AJ279854, a microsatellite primer
SSR4 ssrOeUA-DCA4 AJ279855, a microsatellite primer
SSR9 ssrOeUA-DCA9 AJ279859, a microsatellite primer
subsp. subspecies
t tonnes
TBLASTX a computer program to search translated nucleotide database using a translated nucleotide query
TE a buffer made of 10 mM Tris HCl, 0.1 mM EDTA, pH 8.0
TF terminal flower
TIP tonoplast intrinsic protein
TM trade mark
TP all terminal positions
TPF all terminal perfect flowers
TRIS trishydroxymethylaminomethane
TSF all terminal staminate flowers
U1 unsubtracted tester control 1
U2 unsubtracted tester control 2
UDO24 UDO99-024, a microsatellite primer
UDO6 UDO99-006, a microsatellite primer
UDO8 UDO99-008, a microsatellite primer
US upper style
USA United States of America
UV ultraviolet
v/v volume to volume
v/v/v volume to volume to volume
w/v weight to volume
WA Western Australia
X2 chi-square test
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