Self-incompatibility of Olive

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Table of Contents

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.

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List of Abbreviations

%	percent
°	degree
°C	degrees Celsius
µg	microgram
µl	microlitre
3'	3 prime end of a nucleic acid
5'	5 prime end of a nucleic acid
A_E	effective number of alleles
AFLP	amplified fragment length polymorphism
AGRF	Australian Genome Research Facility
al	Allele
am	before noon
ANOVA	analysis of variance
A_O	observed number of alleles
AOA	Australian Olive Association
ARK	*Arabidopsis* receptor kinase
B1	branch 1
B2	branch 2
B3	branch 3
B4L	branch 4 lateral
B4T	branch 4 terminal
B5L1	branch 5 lateral 1
B5L2	branch 5 lateral 2
B5T	branch 5 terminal
B6L1	branch 6 lateral 1
B6L2	branch 6 lateral 2
B6T	branch 6 terminal
BBCH	Biologische Bundesanstalt, Bundessortenamt, Chemische Industrie
BC	before Christ
BLAST	Basic Local Alignment Search Tool, a computer program
BLASTX	a computer program to search protein databases using a translated nucleotide query
bp	base pair

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
H_E	expected heterozygosity
HEX	a fluorescent dye-labelled oligo

H_O	observed heterozygosity
IP	identity probability
IPI	index of pollen-incompatibility
ISI	index of self-incompatibility
kg	kilogram
km	kilometre
LB	Luria Bertani medium
LOD	log of the odds ratio
LP	all lateral positions
LPF	all lateral perfect flowers
LS	lower style
LSD	least significant difference
LSF	all lateral staminate flowers
LSI	late-acting self-incompatibility
M	molar
m	metre
MAPK	mitogen-activated protein kinase
Max	maximum
mg	milligram
min	minute
Min	minimum
ml	millilitre
mm	millimetre
mM	millimolar
MQ	milli-Q, water that has been purified using an ion exchange cartridge
N	north
n (No)	number
NA	not available/applicable
NCBI	National Centre for Biotechnology Information
ND	no difference
NED	a fluorescent dye-labelled oligo
ng	nanogram
nm	nanometre
NOVA	National Olive Variety Assessment
NP	null allele probability
NR	non-redundant

NS	not significant
®	trade mark
OSI	ovarian self-incompatibility
P	probability
PCR	polymerase chain reaction
PD	power of discrimination
PF	all perfect flowers
pg	picogram
pH	potential of Hydrogen (-log [H^+])
pm	after noon
PR	pathogenesis-related protein
PVP	polyvinylpyrrolidone
r	row
RAPD	random amplified polymorphic DNA
REML	restricted maximum likelihood
RFLP	restriction fragment length polymorphism
RFU	relative fluorescence unit
RNA	ribonucleic acid
RNase	ribonuclease
rpm	revolutions per minute
RS	reverse-subtracted
RT-PCR	reverse transcriptase polymerase chain reaction
s	second
S	south
SA	South Australia
SCR	S-locus cysteine-rich protein
SDS	sodium dodecyl sulphate
SE	standard error of the mean
SF	all staminate flowers
SI	self-incompatibility
SLF/SFB	S-locus F-box protein
SLG	S-locus glycoprotein
SNP	single nucleotide polymorphism
SP11	S-locus pollen protein 11
sPPase	soluble inorganic pyrophosphatase
SRK	S-locus receptor kinase

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
™	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

List of Figures

List of Tables