OLIVE GENETIC DIVERSITY AND MOLECULAR INTERACTION WITH *BACTROCERA OLEAE*

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Alle mie famiglie

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1. RIASSUNTO

L’interesse globale verso la coltivazione dell’olivo è dovuto alle proprietà nutrizionali e sensoriali del suo principale prodotto: l’olio di oliva.
Dal punto di vista nutrizionale, l’olio extravergine di oliva (OEVO) è la principale fonte di lipidi della “dieta mediterranea”, illustrata dal nutrizionista Ancel Keys. Egli dimostrò che il segreto della salute e della longevità in Cilento (Campania, Italia) era da ricercarsi nella dieta, caratterizzata da un elevato contenuto in olio d'oliva, cereali, legumi, verdura, frutta e pesce e un da basso apporto di carne e grassi animali (Keys, 1970).
La dieta mediterranea è associata ad una bassa incidenza di malattie cardiovascolari e di alcuni tipi di tumori. All’OEVO è stato riconosciuto un ruolo fondamentale nella prevenzione di queste malattie (Caramia et al., 2012).
Riconoscendo il grande valore di questo modello nutrizionale, insieme al ruolo nel promuovere l'interazione sociale, la conservazione del territorio e della biodiversità e lo sviluppo delle attività tradizionali delle comunità del Mediterraneo, la dieta mediterranea è stata inclusa nella lista rappresentativa del patrimonio culturale immateriale dell'umanità dall'UNESCO nel 2010.
Dati i suoi effetti benefici sulla salute, negli ultimi 50 anni tale dieta si è diffusa in tutto il mondo. DI fatti, il consumo di olio d'oliva è aumentato da 2,6 milioni di tonnellate nel 2000 a tre milioni nel 2013 e si è registrata una crescita lenta ma costante nei paesi non tradizionalmente consumatori (Carbonari e Sarnari, 2012).
Circa il 98% delle olive da tavola e dell’olio d'oliva mondiali sono prodotti nel bacino del Mediterraneo, dove l'olivo coltivato (*Olea europaea* L.) ha trovato condizioni ambientali particolarmente favorevoli alla sua successiva evoluzione genetica e selezione varietale, allo sviluppo delle pratiche agronomiche e a quello dei sistemi di estrazione (Breton et al., 2006).
Puntando alla qualità dei prodotti agricoli e alimentari, l’Unione Europea (UE) ha rafforzato l'attuale regime per le denominazioni di origine protette e le indicazioni geografiche (DOP e IGP) con il Regolamento UE n 1151/2012. Questa politica ha contribuito all’impulso del settore olivicolo-oleario dell'Unione europea, che rappresenta circa il 66% delle esportazioni mondiali di olio di oliva. L'Italia è il secondo esportatore mondiale dopo la Spagna (European Commission, 2012).
Poiché il settore dell'olio d'oliva in Italia incontra le esigenze dei consumatori in termini di tipicità, qualità della salute, ambiente e tradizione, esso potrebbe essere strategico per lo sviluppo di attività economiche. Insieme al resto dell'Italia meridionale, la Campania ha una lunga tradizione olivicola ed è una delle regioni italiane più vocate alla produzione di olio di oliva di alta qualità, con una produzione complessiva che rappresenta il 6,59% della produzione nazionale (Carbonari e Sarnari, 2012).
Nonostante il ricco patrimonio oliovicolo-oleario della regione Campania, nessuno studio completo di genotipizzazione era stato finora prodotto.
Il primo obiettivo generale della presente tesi è stato quello di genotipizzare venti cultivar tipiche campane attraverso i loro profili SSR. I corrispondenti OEVO "monovarietali" sono stati poi caratterizzati per la loro composizione in acidi grassi e biofenoli, e per il loro profilo sensoriale. Le possibili correlazioni tra i profili SSR e le principali caratteristiche di qualità degli oli sono state studiate. L’analisi SSR è stata condotta mediante 10 microsatelliti (DCA 3, DCA 5, DCA 9, DCA 15, DCA 16, DCA 17, DCA 18, EMO 90, GAPU 71B, GAPU 103) descritti in letteratura (Sefc et al., 2000; Carriero et al., 2002; Alba et al. 2009). Tutti i loci si sono dimostrati polimorfici

ed un totale di 99 alleli è stato identificato. La frequenza allelica ha confermato l'alto livello di polimorfismo. Tale analisi ha permesso l'identificazione di un profilo genetico specifico e distintivo di quasi tutte le varietà, tranne che per 'Minucciola' e 'Nostrale'. Nuove analisi potranno chiarire questo punto.
Poiché una possibile omonimia tra le varietà 'Ortice' e 'Ravece' era già stata ipotizzata (Pugliano et al., 2000; Rao et al., 2009; Corrado et al., 2011) e potrebbe avere implicazioni commerciali (solo l'OEVO di 'Ravece' è incluso nella registro delle denominazioni di origine protette), la questione ha meritato ulteriori indagini. Il profilo SSR di 13 accessioni di 'Ortice' e 13 di 'Ravece' è stato determinato con lo stesso set di 10 SSR. L'identità genetica è stata confermata per tutti i campioni di 'Ravece'. Tra quelli di 'Ortice', sei hanno mostrato lo stesso profilo, diverso da quello di 'Ravece', mentre gli altri hanno mostrato lo stesso profilo SSR dei campioni di 'Ravece'. Ciò ha confermato un problema diffuso nella classificazione delle piante di 'Ravece' e il possibile contributo dei marcatori molecolari SSR nel chiarire possibili errori di identificazione.
La composizione in acidi grassi degli oli ottenuti dalle 20 cultivar è caratterizzata da un'elevata variabilità. I dati hanno evidenziato che il profilo degli acidi grassi degli OEVO è fortemente influenzato dal genotipo mentre le pratiche culturali, le condizioni pedoclimatiche e la fase di maturazione, sono fattori secondari (Aparicio e Luna, 2002; Di Vaio et al., 2012).
Le distanze Euclidee hanno permesso la suddivisione delle venti varietà in tre gruppi, caratterizzati rispettivamente da un basso contenuto in C 18:1n-9 (<65%) e un alto contenuto in C 18:2 e C 18:3, da un alto contenuto in C 18:1n-9 (>70%) e un contenuto in C 18:1n-9 compreso fra il 65% e il 70%.
Poiché il rapporto C 18:1n-9/C 18:2 è indicativo della stabilità ossidativa degli OEVO, (Aparicio et al., 1999), è possibile predire una minore stabilità degli OEVO appartenenti al primo gruppo (C 18:1n-9/C 18:2 < 5).
Questo studio ha evidenziato la possibilità di caratterizzare le 20 cultivar campane sulla base della loro composizione in acidi grassi (Diraman et al., 2010). È interessante notare come 'Minucciola' e 'Nostrale' si distinguano per la loro composizione in acidi grassi. La composizione in acidi grassi di 'Ortice' e 'Ravece' ha, invece, confermato la forte somiglianza fra le due cultivar.
I valori medi delle concentrazioni in secoiridoidi e lignani si differenziano fortemente nelle 20 varietà. Le alte deviazioni standard relative ai dati raccolti hanno evidenziato l'alta variabilità della composizione fenolica degli OEVO. Questa dipende da variabili connesse all'olivo (varietà, stadio di maturazione, il suolo e la zona climatica, tecniche agronomiche e la salute delle piante) e da variabili tecnologiche, quali le condizioni di conservazione delle olive, la tecnologia di estrazione e lo stoccaggio dell'olio (Aparicio e Luna, 2002; El Riachy et al., 2011).
Nonostante la forte influenza delle variabili ambientali, lo studio della composizione in biofenoli può dare un supporto alla caratterizzazione del germoplasma olivicolo in Campania. Per quanto riguarda il contenuto fenolico totale (somma dei composti identificati mediante HPLC-DAD) degli OEVO estratti dalle 20 varietà, sono stati distinti due gruppi principali in base al basso (<100 ppm) e alto (> 100 ppm) contenuto fenolico medio.
A causa delle connessioni esistenti fra le loro vie biosintetiche (Ryan et al., 2002), la distribuzione delle cultivar secondo la composizione fenolica ha evidenziato una forte correlazione positiva.
La maggior parte delle varietà caratterizzate da un elevato (> 40 ppm) contenuto in P-AP è diffusa nell'area di Salerno. La quantità di lignani consente, fra l'altro, la

discriminazione di ‘Minucciola’ e ‘Nostrale’, mentre ‘Ortice’ e ‘Ravece’, simili sia in termini di profilo SSR che in composizione degli acidi grassi, mostrano anche una simile composizione in biofenoli. L'analisi dei biofenoli può dare un contributo alla differenziazione delle 20 varietà di olive e alla caratterizzazione degli OEVO estratti in condizioni standardizzate da diverse varietà di olive (Japón-Luján et al., 2006; de Medina et al., 2015).
I 20 OEVO hanno mostrato un profilo sensoriale specifico. Quelli con maggiore intensità di amaro e piccante sono caratterizzati anche da un'elevata intensità delle note di fruttato di oliva e verdi, quali quelle di pomodoro (varietà ‘Ravece’ e ‘Ortice’) e di carciofo (‘Biancolilla’, ‘Sessana’ e ‘Nostrale’). Tali aromi sono originati attraverso la via delle lipossigenasi a partire dagli acidi grassi polienoici specifici di ciascuna varietà in un dato ambiente (Di Vaio et al., 2012; Issaoui et al., 2010).
Una tipica nota di rosmarino è stata rilevata negli OEVO di ‘Minucciola’ provenienti dall’area DOP Penisola Sorrentina. Quanto detto ha confermato che l'analisi del profilo sensoriale può fornire informazioni utili a discriminare gli OEVO ottenuti da differenti varietà di olive (Tura et al., 2008).
Infine, la correlazione profilo genetico e composizione chimica e profilo sensoriale degli OEVO è stata studiata mediante il test di Mantel. Una correlazione bassa ma significativa ($r = 0,209$; $p = 0,018$) è stata rilevata solo tra il profilo SSR delle varietà di olivo e i profili sensoriali dei relativi OEVO. Tali risultati sono coerenti con i dati riportati da diversi autori in piante medicinali e aromatiche (Mochida et al., 2009; Tonk et al., 2010).
Essendo i microsatelliti marcatori neutrali, essi sono in gran parte indipendenti da tratti genetici specifici. Questo potrebbe spiegare la mancanza di correlazione fra profili SRR e profili metabolici (Laurentin et al., 2008).

Un fattore determinante per la qualità dell'olio d'oliva è rappresentato dall'interazione fra l’olivo e il suo nemico chiave: il dittero *Bactrocera oleae*. La storia della mosca dell'olivo si è evoluta insieme a quella dell’olivo, determinando una stabile e precoce associazione pianta-insetto (Nardi et al., 2010). La produzione mondiale di olive da olio e da tavola è costantemente minacciata da *Bactrocera oleae*.
Alimentandosi, le larve distruggono la polpa dell’oliva fino a determinarne la cascola. Gli effetti diretti e indiretti della loro masticazione producono perdite quantitative, abbassamento della resa in olio, aumento dell’acidità e del numero di perossidi dell'olio, alterazioni nella sua composizione fenolica e lipidica e provoca la comparsa di difetti. Questo determina il declassamento dell’OEVO ad altre categorie commerciali meno pregiate (Gómez-Caravaca et al., 2008; Angerosa et al., 1992).
A seconda delle condizioni climatiche annuali, *Bactrocera oleae* può determinare gravi danni e perdite economiche nel settore oleario e delle olive da tavola. Nel corso degli ultimi anni, probabilmente per effetto dei cambiamenti climatici che interessano il nostro pianeta (Benelli, 2014), si è assistito ad un consistente aumento del numero di generazioni annuali della mosca dell’olivo e al conseguente aumento delle perdite di produzione. Nella stagione 2014-2015, l'Italia ha registrato il 35% di perdite (con punte del 45% in alcune regioni) nella produzione di olio d'oliva rispetto alla stagione precedente (fonte: ISTAT e ISMEA).
I metodi convenzionali di controllo di *Bactrocera oleae* in campo impiegati nel corso degli ultimi decenni, che consistono nell'uso di insetticidi, stanno determinando l’acquisizione di forme di resistenza da parte della mosca dell'olivo a tali formulazioni (Hawkes et al., 2005; Vontas et al., 2002) e rappresentano una fonte di inquinamento ambientale, di contaminazione delle olive e dell’olio e di distruzione degli insetti utili.

Oggi la difesa dalla mosca dell'olivo è in gran parte basata su metodi alternativi integrati o biologici (Daane e Johnson, 2010).
Con il rinnovato interesse del mercato globale verso l'OEVO e le sue proprietà nutritive, si è determinato anche un maggiore impegno nell'analisi del genoma dell'olivo. La genomica funzionale dell'olivo si è basata principalmente sullo studio delle EST (Bracci et al., 2010). La caratterizzazione metabolica e trascrittomica ha consentito l'identificazione dei geni differenzialmente espressi durante lo sviluppo del frutto (Alagna et al., 2009). L'analisi di diversi tessuti di olivo e stadi di sviluppo ha portato all'assemblaggio *de novo* e all'annotazione funzionale del trascrittoma di olivo (Muñoz-Mérida et al., 2013). Il sequenziamento del genoma completo di olivo, invece, è ancora incompleto. Solo pochi studi sono stati effettuati sulla risposta molecolare di *Olea europaea* all'attacco del suo parassita chiave, *Bactrocera oleae* (Corrado et al., 2012; Alagna et al., 2015).
Nonostante le importanti scoperte che ne sono derivate, molti aspetti concernenti tale interazione sono ancora da chiarire. Tra questi, i meccanismi alla base della suscettibilità all'infestazione della mosca dell'olivo (Loscalzo et al., 1994; Scarpati et al., 1996). Gli studi trascrittomici possono dare un nuovo impulso alla comprensione dei meccanismi molecolari della resistenza genetica dell'olivo e delle difese attivate dalla pianta a seguito dell'attacco della mosca al fine di sviluppare nuove strategie e metodi di controllo.
Nella presente tesi è stata condotta uno studio trascrittomico (con tecnologia CombiMatrix CustomArray ™ 90K) in drupe sane e danneggiate dalla mosca dell'olivo per ottenere una comprensione più completa dei cambiamenti molecolari che si verificano nelle olive dopo l'attacco. Per estendere tale ricerca ai meccanismi che possono innescare la resistenza alla mosca dell'olivo, sono state scelte due varietà precedentemente descritte per il loro diverso livello di suscettibilità alla mosca: 'Ruveia' (resistente) e 'Ortice' (suscettibile) (Pugliano et al., 2000).
Il software Sma3s ha permesso l'annotazione funzionale di 14209 trascritti differenzialmente espressi almeno una volta (FC ≠ 1, p-value <0,05 test t di Student), corrispondenti al 22,98% dei trascritti rappresentati sul microarray. L'analisi multivariata dei quattro punti sperimentali ha evidenziato che i profili trascrittomici delle drupe danneggiate differiscono nettamente rispetto a quelli dei loro omologhi non danneggiati. Le alterazioni trascrittomiche più rilevanti sono indotte dall'attacco di *Bactrocera oleae* in drupe di 'Ruveia'. Un totale di 2497 sequenze differenzialmente espresse caratterizzate da un fold-change> 2 e <0,5 e un p-value p <0,05 (test t di Student) è stato riscontrato in olive danneggiate di 'Ortice' e 'Ruveia' rispetto alle controparti non danneggiate.
Ciò indica che l'oliva è in grado di riconoscere e attivare risposte trascrizionali in risposta agli attacchi di *Bactrocera oleae*, come ampiamente riportato in altre specie (Appel e Cocroft, 2014; Mescher e De Moraes, 2014). Di questi trascritti, Sma3s ne ha consentito l'annotazione funzionale di 1427. L'annotazione dei trascritti non annotati da Sma3s è stata eseguita mediante Blast2GO, che ha permesso l'annotazione di ulteriori 167 sequenze.
I geni differenzialmente espressi sono stati ragguppati in dieci categorie funzionali concernenti l'organizzazione cellulare (113 geni), la trascrizione (189 geni), la modifica della parete cellulare (44 geni), lo stress e la risposta di difesa (151 geni), la trasduzione del segnale (120 geni), la fotosintesi (24 geni), il metabolismo primario (178 geni), il metabolismo proteico (213 geni), il metabolismo secondario (44 geni) e il trasporto (162 geni). Il numero relativamente elevato di trascritti con funzione

sconosciuta è da attibuirsi alla mancanza della sequenza completa del genoma dell'olivo.
l dataset annotati dei 2.497 trascritti espressi in modo differenziale (FC> 2 e <0,5 e p-value <0,05 Test t) in almeno una delle due varietà 'Ortice' e 'Ruveia' sono stati elaborati dall'analisi K-Means / K-mediane Clustering. Il grafico di espressione dei dieci gruppi risultanti ha mostrato profili di espressione distinti in base alla varietà e al danno in quasi tutti i cluster.
Gli otto gruppi più significativi sono stati sottoposti ad arricchimento funzionale mediante BinGO. Anche se in tutti cluster la maggior parte dei processi biologici GO è rappresentata, alcuni gruppi mostrano una maggiore complessità, rivelando ampie modifiche trascrittomiche in tutti i principali processi biologici a seguito dell'attacco della mosca.
La letteratura riporta estesamente l'esistenza di variazioni intraspecifiche a livello genetico e fenotipico in procarioti ed eucarioti (Koornneef et al., 2004; Keurentjes et al., 2006; Dicke e Baldwin, 2010).
Non sorprende, pertanto, che le due varietà di olivo analizzate, 'Ortice' e 'Ruveia', hanno mostrato grandi differenze nei meccanismi attivati dopo l'attacco della mosca. La discrepanza evidente nel numero dei geni differenzialmente espressi nelle olive attaccate delle due varietà, 52 in 'Ortice' e 2477 in 'Ruveia', indica che quest'ultima è in grado di attivare una risposta molto più ampia rispetto alla prima a seguito dell'attacco della mosca.
Le olive danneggiate di 'Ortice' mostrano poche sequenze sovraespresse. Tra queste non si sono riscontrati geni di difesa e risposta a stress né geni legati alla trasduzione del segnale. Tra le sequenze sottoespresse, cinque sono coinvolte nell'attivazione di difesa e risposta a stress. Questo risultato può essere indicativo del sovvertimento delle difese dell'oliva da parte della mosca, come riportato in altre interazioni pianta-insetto (Diezel et al., 2009; Musser et al., 2005; Smith et al., 2009; Walling et al., 2008; Zhu-Salzman et al., 2005).
Al contrario, 'Ruveia' mostra una vasta gamma di cambiamenti trascrittomici a seguito dell'attacco di *Bactrocera oleae*. Cinquanta geni difesa e risposta a stress sono risultati sovraespressi. Tra questi, vari geni noti in altre interazioni pianta-patogeno (Du et al., 2009) e nella biosintesi delle oxylipine (Vellosillo et al., 2007).
Novantacinque geni della stessa categoria sono sottoespressi, venti dei quali coinvolti nella regolazione negativa della risposta di difesa, indicando così che l'attacco della mosca provoca l'attivazione di risposte di difesa che, in condizioni fisiologiche normali, vengono represse. Questo risultato è coerente con il fatto che le piante abbiano evoluto difese inducibili da attivare solo in caso di attacco insetti o patogeni per risparmiare energia e nutrienti (Baldwin et al., 1998; Zavala et al., 2004).
Tra gli elementi regolatori che inducono risposte di difesa, sono stati riscontrati 21 geni di risposta al flusso di ioni calcio differenzialmente espressi in 'Ruveia' (Dodd et al., 2010). L'induzione di noti sensori dei livelli intracellulari di calcio (calmoduline, CDPKs, etc.) evidenzia come le cellule delle olive di 'Ruveia' traducano le alterazioni intracellulari di Ca^{+2} in modifiche dei meccanismi di difesa a valle (Lecourieux et al., 2006; Steinhorst e Kudla, 2014).
Gli ioni calcio sono anche associati alle specie reattive dell'ossigeno (ROS) (Wu e Baldwin, 2010). La trasduzione del segnale a seguito di danni biotici e abiotici inizia con il rapido rilascio di ROS nel cosiddetto burst ossidativo. La quantità di geni espressi in modo differenziale (40 geni) coinvolti sia nel metabolismo delle specie

reattive dell'ossigeno che nella risposta allo stress ossidativo indica l'alterazione dello stato redox delle cellule 'Ruveia' dopo l'attacco mosca dell'olivo.
I risultati sopra indicati evidenziano l’incapacità della varietà suscettibile, 'Ortice', ad attivare una risposta adeguata a *Bactrocera oleae* (Pugliano et al., 2000).
In conclusione, il presente lavoro di tesi rappresenta un avanzamento nella comprensione dei fattori che caratterizzano la qualità dell'olio d'oliva, sia dal punto di vista varietale che ambientale. Inoltre, il lavoro svolto ha approfondito i meccanismi di interazione tra olivo e mosca dell’olivo, aprendo nuovi scenari di approfondimento relativi alla selezione di materiale vegetale da adattare alle diverse condizioni ambientali e all’implementazione di sistemi di gestione e di controllo dei parassiti che possano contribuire a limitare le perdite annuali di produzione di olio e olive da tavola causate da *Bactrocera oleae*.

2. SUMMARY

The worldwide interest in olive (*Olea europaea* L.) growing is due to the extraordinary nutritional and sensory quality of its main product: olive oil. From a nutritional point of view, extra virgin olive oil (EVOO) is the main lipid source of the so-called "Mediterranean diet", stated by the nutritionist Ancel Keys (Keys, 1970). Around 98% of the global table olives and olive oil is produced in the Mediterranean basin.Italy is the world's second largest exporter after Spain (European Commission, 2012).
Campania region has a long tradition in olive growing, with an overall production that represents 6.59% of national production (Carbonari and Sarnari, 2012). In spite of the rich olive and olive oil heritage of Campania region, until now a comprehensive genotyping study was still lacking. The first overall objective of the present thesis was to genotype twenty cultivars growing in Campania through 10 SSR markers. SSR fingerprinting allowed identification of a distinctive profile for all the studied varieties except 'Minucciola' and 'Nostrale', which did not display allelic differences. A widespread problem in classifying the 'Ravece' and 'Ortice' plants was pointed out. SSR analysis may contribute to eliminate the misidentification problem. The corresponding "monovarietal" EVOOs from each variety were then characterized for their fatty acid, biophenol composition and sensory profile. This study highlighted the possibility to characterize the 20 olive varieties from Campania on the basis of their FA composition (Diraman et al., 2010). Moreover, the analysis of biophenols sensory profile can give useful information to discriminate the EVOOs obtained from different olive varieties (de Medina et al., 2015; Tura et al., 2008). A low but significant correlation was found between olive SSR profiles and olive oil sensory profiles.
A determining factor on olive oil quality is represented by the plant interaction with its key enemy: the diptera *Bactrocera oleae*. The evolutionary history of the olive fly came along with that of *Olea europaea*, indicating an early and stable plant-insect association (Nardi et al., 2010). Despite global production of virgin olive oil and table olives is threatened by *Bactrocera oleae*, only few studies focused on *Olea europaea* molecular response to olive fly attack (Corrado et al., 2012; Alagna et al., 2015).
In this thesis a transcriptomic analysis was performed to achieve a more complete understanding of the molecular changes occurring once *Bactrocera oleae* attacks *Olea europaea* drupes. In order to explore the mechanisms that may trigger resistance to olive fly, two varieties were chosen with different levels of susceptibility to olive fly, namely 'Ruveia' and 'Ortice' (Pugliano et al., 2000). 'Ortice' and 'Ruveia' showed large differences in the mechanisms activated following the attack of the fly. The noticeable discrepancy in the number of the differentially expressed genes (FC > 2 and < 0.5; p-value < 0.05 Student's t-test) in the attacked olives of the two varieties, 52 in 'Ortice' and 2477 in 'Ruveia', indicates that the latter is able to activate a much broader response than the first as a result of the attack of the fly. The differentially expressed genes were recorded and discussed in ten functional categories concerning cellular organization, transcription, cell wall modification, stress and defense response, signal transduction, photosynthesis, primary metabolism, protein metabolism, secondary metabolism and transport.
On one hand, this thesis represents an advancement in the comprehension of the features characterizing olive oil quality in terms of variety and environment. On the other, an improvement in the knowledge of the interaction between olive and olive fly that largely influence olive production.

3. BACKGROUND

Olive (*Olea europaea* L.) cultivation began in the Middle East and Eastern Mediterranean during the fourth millennium BC. The olive was spread throughout the Mediterranean basin as a result of anthropogenic factors because of the interest in its multiple uses. Nowadays, the Mediterranean countries still remain the largest olive oil producers and consumers (around 98% of the world's olives and olive oil is produced in the Mediterranean basin). In recent years there has been increasing interest worldwide in the domestic production and consumption of olive oil. The reason of the worldwide interest in the cultivation of olive primarily lies in the nutritional properties and sensory characteristics of olives and olive oil. The importance of extra virgin olive oil (EVOO) for human nutrition has been stated by Ancel Keys in the '60s as it represents the main lipid source of the so-called "Mediterranean diet".

Olive oil quality depends on a number of factors and their interactions such as the plant variety, agronomic management, environment, extraction system, storage, etc. Although considerable progress has been made in understating the factors that influence olive oil quality, several aspects remain to be clarified. One largely unexplored area relates to the influence of genetic and environmental factors (and their interaction) on EVOO organoleptic and nutritional properties. The studies of olive genetic characterization and related olive oil analyses provide advances in that knowledge. The first aim of this thesis was to contribute to the understanding of these aspects through a study of genetic and EVOO diversity. As Campania region (Italy) has a long tradition in olive growing and a rich olive genetic heritage, twenty cultivars growing in Campania were genotyped through the SSR profiles and monovarietal EVOOs from each variety were characterized for their fatty acid and biophenol composition, and sensory profile. In addition, SSR analysis was employed to contribute to the clarification of misidentification problems of some varieties.

Another determining factor on olive oil quality is represented by the plant interaction with its key pest, *Bactrocera oleae* (Diptera: Tephritidae). This insect threatens the global production of virgin olive oil and table olives. To date, very few studies focused on the *Olea europaea* molecular response to olive fly attack and many aspects concerning olive response to olive fly need further research efforts. Among these, the mechanisms that are responsible for the different level of susceptibility to olive fly infestation in different olive varieties may provide an interesting outlook. Transcriptomic studies may give new insights into molecular mechanisms of genetic resistance to olive fruit fly and defense responses activated by the plant as the result of the attack of its key enemy. This knowledge could be important to select plant material to be adapted to different environmental conditions and implement the current strategies and tools aimed at the control of olive fly population and related olive production loss. For these reasons, the second research area of this thesis was to achieve a deeper understanding of the molecular changes occurring once *Bactrocera oleae* attacks *Olea europaea* drupes. To shed light into the different mechanisms employed by genetically different plants, a transcriptomic approach based on microarrays was employed.

4. CHARACTERIZATION OF TWENTY OLIVE (OLEA EUROPAEA L.) VARIETIES SPREAD IN CAMPANIA (ITALY) AND OF THEIR VIRGIN OLIVE OILS

4.1 Abstract

Campania is one of the main Italian regions for the production of typical extra virgin olive oils (EVOOs). Since 1998 the study of the regional olive germplasm and of the resulting EVOOs has provided considerable information about olive varieties and EVOO typicality. Here we report on the characterization of the 20 traditional olive varieties most widely grown in Campania, previously morphologically characterized, based on simple sequence repeat (SSR) profiling and characterization of 382 oil samples (by fatty acid, and phenols and sensory profile analysis). Similarities and differences among 20 varieties were found in fatty acid composition, phenolic compounds and the sensory profile. Molecular biochemistry, chemical composition and sensory analysis allowed EVOO identity parameters to be established. A correlation between the cultivar SSR profiles and the organoleptic properties of EVOOs was verified. To our knowledge, this study represents the first attempt to achieve complete characterization of the Campanian olive germplasm and their respective EVOOs.

4.2 Introduction

Following the worldwide economic trend, there has been a slowdown in the growth of global consumption of oils and fats in recent years. Conversely, olive oil is attracting ever-increasing interest both in terms of consumption and production (IOOC, 2014). Olive oil global consumption rose from 2.6 million tons in 2000 to three million in 2013 and a slow but constant rise in non-traditionally consumer countries has been recorded (Carbonari and Sarnari, 2012). Around 98% of the world's olives and olive oil is produced in the Mediterranean basin. Indeed, though the origin of the cultivated olive tree (*Olea europaea* L.) has not yet been ascertained, it is widely accepted that olive cultivation began in the Middle East and Eastern Mediterranean during the fourth millennium BC (Zohary and Spiegel–Roy, 1975). Then, mainly as a result of anthropogenic factors such as migration and trade, the olive was spread throughout the Mediterranean basin, where this species found the appropriate environmental conditions which led to its subsequent genetic evolution, varietal selection, development of the related agronomic practices and oil extraction systems (Breton et al., 2006).

Although the Mediterranean countries still remain the largest olive oil producers and consumers, in recent years there has been increasing interest worldwide in the domestic production and consumption of olive oil (IOOC, 2014). The worldwide interest in the cultivation of *Olea europaea* L. is primarily due to the nutritional and sensory quality of olives and olive oil. From a nutritional point of view, extra virgin olive oil (EVOO) is the main lipid source of the so-called "Mediterranean diet", noted in the Cilento area of Campania by the nutritionist Ancel Keys as long ago as the 1960s. He ascribed the secret of health and longevity of Italian rural people to their diet rich in olive oil, cereals, legumes, vegetables, fruit and fish, and poor in meat and animal fats (Keys, 1970). Given its beneficial effects on health, this diet has been spreading around the world in the last 50 years together with olive oil consumption

and in 2010 was inscribed on the UNESCO Representative List of the Intangible Cultural Heritage of Humanity.

For its part, the European Union (EU) has increasingly focused on the quality of agricultural products and foodstuffs. One of the key elements of this commitment, detailed in EU Regulation No 1151/2012, is the reinforcement of the existing scheme for protected designations of origin and geographical indications (PDOs and PGIs). This policy has contributed to the improvement of the EU's olive oil sector, which accounts for about 66% of olive oil world exports. Italy is the world's second largest exporter after Spain (European Commission, 2012). In recent decades, the Italian olive oil sector has been merging the new structural changes resulting from mechanization with its unique pedoclimatic conditions and traditional olive growing systems. The know-how of traditional olive growing, together with the large number of local cultivars (Bartolini et al.,1998), represents the strengths of the Italian olive oil sector. Consumers increasingly seek agricultural goods differentiated on the basis of their typicality, health quality and environmental value, such that domestic and foreign markets are constantly shifting their interest toward PDOs, PGIs and organic EVOOs. As the traditional Italian olive oil sector meets all these requirements, it could be strategic for developing economic activities in rural regions. Like the whole of southern Italy, Campania has a long tradition in olive growing and is one of the most suitable Italian regions for the production of high quality olive oil, with an overall production that represents 6.59% of national production (Carbonari and Sarnari, 2012). Although Campania's olive oil sector is based around small farms which does not promote competitiveness, it ensures the conservation of local varieties and the environment, as well as the production of EVOOs with typical organoleptic and nutritional properties. The efforts of farmers and operators have been rewarded with five PDOs approved by the European Union.

Olive diversity is usually assessed by considering the main morphological and agronomic traits (Pugliano et al.,2000), the DNA polymorphism (Corrado et al., 2009; Rao et al., 2009) or both (Trujillo et al., 2014). Some authors (Aparicio and Luna, 2002) additionally evaluated chemical parameters, while others assessed olive genetic variability by enzyme markers (Ouazzani et al., 1993). To characterize olive oils from different varieties, the chemometric analysis of fatty acids (Di Bella et al., 2007), content in triterpenes (Allouche et al., 2009), volatile compounds (Berlioz et al., 2006), and spectrometric determinations compounds (Forina et al., 2007) have all been employed.

In the present study, we genotyped twenty cultivars growing in Campania through the SSR profiles and produced "monovarietal" EVOOs from each variety. The EVOOs (382 in all) were then characterized for their fatty acid and biophenol composition, and sensory profile. Possible correlations between the SSR profiles and the main quality traits of the oils were investigated.

4.3 Materials and methods

4.3.1 Plant material

Twenty olive varieties were selected as the most significant both in terms of geographical spread and olive oil market interest. The selected varieties are predominantly distributed in different areas of Campania (Table 4.1).For the DNA

extraction, young, undamaged leaves were collected from two olive trees per variety. To assess a possible homonymy for 'Ortice' and 'Ravece' varieties, leaf samples for these two varieties were collected from 13 different trees. Leaves were stored under N_2 until DNA extraction.

Table 4.1. List of the 20 olive varieties used in this study with indication of farm, location and province (AV, Avellino; BN, Benevento; CE, Caserta; NA, Napoli; SA, Salerno) where leaves were collected to perform SSR fingerprinting[a] and where olives were harvested for the production of N EVOOs[b]

Variety	Leaves [a]	Olives [b]	N
'Asprinia'	Improsta, Battipaglia, SA	Cassone, Santa Maria a Vico, CE	18
'Biancolilla'	Improsta, Battipaglia, SA	Pipolo, Laurino, SA	9
'Caiazzana'	Improsta, Battipaglia, SA	Mastroianni, Caiazzo, CE	19
'Carpellese'	Improsta, Battipaglia, SA	Naimoli, Campagna, SA	26
'Femminella'	Guerrera, San Lupo, BN	Guerrera, San Lupo, BN	8
'Marinese'	Martone, Paternopoli, AV	Martone, Paternopoli, AV	19
'Minucciola'	Le Tore, Massa Lubrense, NA	Pane, Massa Lubrense, NA	13
'Nostrale'	Capo, Castel San Lorenzo, SA	Capo, Castel San Lorenzo, SA	11
'Ogliarola'	Improsta, Battipaglia, SA	Abruzzese, Carife, AV	21
'Olivella'	Tiso, Ariano Irpino, AV	Tiso, Ariano Irpino, AV	9
'Ortice'	Nista, Buonalbergo, BN	Nista, Buonalbergo, BN	16
'Ortolana'	Improsta, Battipaglia, SA	Di Lorenzo, San Lupo, BN	9
'Pampagliosa'	Di Cosmo, Casalduni, BN	Di Cosmo, Casalduni, BN	8
'Pisciottana'	Improsta, Battipaglia, SA	Sacchi, Pisciotta, SA	41
'Racioppella'	Improsta, Battipaglia, SA	Di Biase, Guardia Sanframondi, BN	7
'Ravece'	Tiso, Ariano Irpino, AV	Tiso, Ariano Irpino, AV	25
'Rotondella'	Improsta, Battipaglia, SA	Maglio, Campagna, SA	78
'Salella'	Improsta, Battipaglia, SA	Vassallo, San Mauro Cilento, SA	31
'Sessana'	Badevisco, Capua, CE	Iameo, Alife, CE	3
'Tonda'	Improsta, Battipaglia, SA	Ferrante, Alife, CE	11

4.3.2 DNA isolation and SSR amplification

Genomic DNA was extracted from the olive leaves with the Gen Elute kit (Sigma, Milan, Italy) following the manufacturer's instructions. Genetic analysis was performed using 10 SSR loci (Table 4.2) selected on the basis of previous descriptions (Sefc et al., 2000; Alba et al., 2009; Carriero et al., 2002) using forward primers carrying VIC, FAM, PET or NED labels at their 5'-end. DNA samples were amplified by PCR in a final volume of 25 µl containing 20 ng genomic DNA, 0.1 µM dNTPs, 0.2 µM forward primer labeled with fluorochrome, 0.2 µM unlabeled reverse primer, and 0.5 U of *Taq* DNA polymerase in 1X Buffer (Promega, Milan, Italy). All amplifications were performed by an Eppendorf (Milan, Italy) thermocycler at the following PCR conditions: 5 min denaturation at 95°C; 35 cycles composed of 30 sec denaturation at 95 °C, 30 sec annealing at Ta (Table 4.2) and 1 min and 30 sec extension at 72°C; a final incubation step of 10 minutes at 72°C.

Table 4.2. List of SSR loci.

SSR locus	Core [a]	Range [b]	Primer sequences (5'→3') [c]	F [d]	T_a [e]
DCA 3	$(GA)_{19}$	228-250	CCCAAGCGGAGGTGTATATTGTTAC TGCTTTTGTCGTGTTTGAGATGTTG	FAM	50
DCA 5	$(GA)_{15}$	195-211	AACAAATCCCATACGAACTGCC CGTGTTGCTGTGAAGAAAATCG	NED	50
DCA 9	$(GA)_{23}$	161-205	AATCAAAGTCTTCCTTCTCATTTCG GATCCTTCCAAAAGTATAACCTCTC	PET	50
DCA 15	$(CA)_3G(AC)_{14}$	242-266	GATCTTGTCTGTATATCCACAC TATACCTTTTCCATCTTGACGC	FAM	50
DCA 16	$(GT)_{13}(GA)_{29}$	120-178	TTAGGTGGGATTCTGTAGATGGTTG TTTTAGGTGAGTTCATAGAATTAGC	FAM	50
DCA 17	$(GT)_9(AT)_7AGATA(GA)_{38}$	101-183	GATCAAATTCTACCAAAAATATA TAAATTTTTGGCACGTAGTATTGG	FAM	51
DCA 18	$(CA)_4CT(CA)_3(GA)_{19}$	168-184	AAGAAAGAAAAAGGCAGAATTAAGC GTTTTCGTCTCTCTACATAAGTGAC	NED	50
EMO 90	$(CA)_{10}$	183-193	CATCCGGATTTCTTGCTTTT AGCCGAATGTAGCTTTGCATGT	PET	55
GAPU 71B	$GA(AG)_6(AAG)_8$	150-165	GATCAAAGGAAGAAGGGGATAAA ACAACAAATCCGTACGCTTG	NED	56
GAPU 103 A	$(TC)_{26}$	170-200	TGAATTTAACTTTAAACCCACACA GCATCGCTCGATTTTTATCC	FAM	54

[a] Core sequence. [b] Expected length range (bp). [c] Primer sequences (forward and reverse). [d] Label fluorochrome (5'). [e] Temperature of annealing (°C).

To discriminate the alleles and determine their length, the fluorescent fragments were separated by capillary electrophoresis using an ABI Prism 3100-Avant (Applied Biosystems, Milan, Italy) at 15 kV for 45 minutes with the POP4 polymer (Applied Biosystems, Milan, Italy). The height of the peaks and the size of the alleles were calculated through the ABI Prism Genotyper 2.5 software (v. 3.7) by comparison with the standard GeneScan 500HD [Liz] (Applied Biosystems, Milan, Italy). The length of the alleles was rounded off on the basis of the structure of the core and, when present, of the allelic series described in the literature (Baldoni et al., 2009).

4.3.3 EVOO production

In all, 382 virgin olive oil samples were obtained from 20 olive varieties, harvested in Campania (southern Italy) during four crop seasons (from 1998 to 2001). For each variety the number of samples obtained ranged from 3 ('Sessana') to 78 ('Rotondella') according to local availability. Before oil extraction, the Jaén ripening index (JRI) (Uceda and Frías, 2009), based on the evaluation of the olive skin and pulp color, was determined on a representative sample of each olive batch. Batches of nearly 20 kg of olives with a JRI ranging between 2 and 4 were processed, within 24 hours, in a micro-extraction mill (OLIOMIO® Baby 50, TEM, Toscana Enologica Mori, Florence, Italy) operating under controlled conditions (hammer crusher, malaxation for 30 minutes at 18 ± 2 °C) at the Azienda Improsta (Battipaglia, Salerno, Italy). Oils were filtered on paper and stored in filled glass bottles at 16 °C in the dark until analyses. All the olive oil samples were submitted to the analysis of the quality

indexes (free acidity, peroxide number, spectrophotometric absorbance and panel test) and were graded in extra virgin quality according to EEC Regulation No 2568/91 and subsequent amendments (data not shown).

4.3.4 Chemicals

Methanol (>99.9%), n-hexane (99.0%), acetonitrile (>99.9%) and HPLC-grade water were purchased from Romil (Cambridge, UK). Trifluoroacetic acid, potassium hydroxide and p-hydroxyphenyl ethanol (p-HPEA) were obtained from Sigma-Aldrich srl (Milan, Italy). FAME multistandards were purchased from Larodan (Malmoe, Sweden).

4.4 Analytical methods

4.4.1 Determination of fatty acid composition

Fatty acids (FA) were determined as methyl ester (FAMEs), by gas chromatography. FAMEs were prepared according to the official method (EEC Regulation No 2568/91). FAME patterns were assessed with a Shimadzu GC-17A equipped with an FID detector, acquisition software (Class-VP Chromatography data system 4.6 – Shimadzu, Milan, Italy) and FAME column (60 m length; 0.25mm i.d.) coated with stationary phase 50% Cyanopropyl-Methyl Phenyl Silicone (0.25 mm thickness; Quadrex Corporation, New Heaven, USA). The following operating conditions were employed: injector and FID temperature 250 °C; the initial column temperature was set at 170 °C for 20 min, then raised at a gradient of 10 °C/min to 220 °C, held for 5 min; helium as carrier gas with a flow through the column of 2 ml/min; helium as auxiliary gas; splitting ratio 1:60; injected volume 1 µl. Peaks were identified by comparing their retention times with those of a pure standard FAME mixture analyzed under the same conditions. Thirteen fatty acids were quantified according to the percentage area, obtained by the integration of the peaks.

4.4.2 Extraction of Phenols from VOO

Phenolic compounds were extracted from virgin olive oil as described by Vasquez-Roncero (Vasquez Roncero, 1978). Olive oil (10 g) was dissolved in 10 ml hexane, and polar compounds were extracted with methanol/water (60:40, v/v, 3 x 7 ml). The final extract was washed with 5 ml hexane and, after solvent evaporation in a vacuum evaporator (40°C), the residue was dissolved in 2 ml methanol.

4.4.3 HPLC analysis of phenolic compounds

Individual phenolic compounds were separated by a HPLC system consisting of a Shimadzu liquid chromatograph (LC-10AD, Shimadzu Italy, Milan) coupled to a Diode Array Detector (DAD SPD M10A VP) and with Class-VP Chromatography data systems 4.6 (Shimadzu Italy, Milan) software. Analytical separation was achieved on a Prodigy ODS-3 (Phenomenex, Macclesfield, UK), 5 µm column (250 mm x 4.6 mm i.d.) using a binary gradient elution (Sacchi et al., 2002). Major phenol compounds were tentatively identified by comparison with the retention times of pure standards or comparing the relative elution order and UV spectra with those previously reported, and identification was subsequently confirmed by LC-MS (Sacchi et al.,

2002; Montedoro et al., 1993). Quantification of phenolic compounds was carried out at 279 nm using tyrosol (2-(p-hydroxyphenyl) ethanol) as an external standard according to the procedure described by Tsimidou et al. (1992). Phenol composition, expressed as milligrams of tyrosol per kilogram of oil, was reported considering the main eight compounds.

4.4.4 Sensory analysis

Analysis of the sensory profile was performed by a panel of 12 assessors trained according to European regulation (EEC Reg. 2568/91). The different oil samples were sensory profiled according to the intensity of 13 sensory attributes: almond, green almond, apple, artichoke, bitter, fruity, grass, leaf, pungent, rosemary, sweet, tomato and bitter vegetable.

4.4.5 Data analyses

All data were reported as mean values ± standard deviation (SD) of the determinations in the four years. Statistical analyses of chemical and sensory data were computed using analysis of variance (ANOVA, $p<0.05$). The fatty acid, biophenol and sensory profile data of the olive oils were analyzed by the Addinsoft XLSTAT version 6.1 (Paris, France). The distances (GD*ij*) between each pair of genotypes (*i*,*j*) were obtained using the Euclidean correlation coefficient. Euclidean distances of the three above olive oil parameters were employed to perform the principal component analysis (PCA) and to sort cultivars into hierarchical groups through the average-linkage method of agglomeration. The data collected from the genetic analysis of cultivars were employed to calculate the following genetic indices for each SSR locus: number of alleles; allele frequency; number of effective alleles ($N_e=1/\Sigma p_i^2$); observed heterozygosity (H_o=number of heterozygotes/number of samples); polymorphism information content (PIC) (Botstein et al., 1980), which is equivalent to the expected heterozygosity ($H_e= 1 - \Sigma p_i^2$) (Nei, 1973); fixation index ($FI = 1 - H_o/H_e$) (Hartl et al., 1997); probability of identity [$P_{(ID)}= \Sigma p_i^4 + \Sigma\Sigma (2p_ip_j)^2$] (Paetkau et al., 1995); estimated frequency of null alleles [$EFNA= (H_e - H_o)/(1 + H_e)$] (Brookfield, 1996). Genetic distances (GD*ij*) were obtained by the Peakall and Smouse coefficient (Peakall and Smouse, 2006). The dissimilarity matrix was employed to obtain the Principal Component Analysis (PCA). Genetic data processing was performed using Identity 4.0 software (Wagner and Sefc, 1999) and GenAlEx software (Peakall and Smouse, 2006). The Mantel test (Mantel, 1967) between the genetic dissimilarity matrix and the dissimilarity matrices related to the olive oil quality traits (fatty acids, biophenols and sensory profiles) was chosen to investigate the correlation between the genetic distances and the distances determined on the basis of the olive oil parameters. Statistical significance was evaluated with 1000 permutations.

4.5 Result and Discussion

4.5.1 Molecular identification of olive cultivars

DNA profiling was performed by using 10 SSR markers. All loci were polymorphic. A total of 99 alleles were identified (Table 4.3). The allele frequency confirmed the high level of polymorphism: its value, whose average was 10.1%, varied from a maximum of 55% (1 allele of the locus DCA 5) to a minimum of 2.5% (1 allele of the locus DCA 3; 3 of DCA 5; 1 of DCA 15; 4 of DCA 16; 4 of DCA 17; 1 of DCA 18; 1 of EMO 90 and 1 of GAPU 71B). The probability of identity (PI), i.e. the probability that two randomly chosen individuals in a population have identical genotypes at one locus, ranged from 0.013 (DCA 9) to 0.141 (DCA 5 and DCA 15). The observed heterozygosity (Ho), ranged from 0.150 at locus DCA 15 to 0.917 at DCA 9. The expected heterozygosity (He), which can be approximated to polymorphism information content (PIC) varied from 0.659 at locus DCA 5 to 0.917 at DCA 9. Loci DCA 15, DCA 16 and GAPU 103 showed a positive fixation index (FI). In particular, a considerable positive value with a significant probability of heterozygote deficiency was found for DCA 15 and GAPU 103. For these loci a similar number of alleles was detected to those found for other loci with an FI value around 0. Moreover, GAPU 103 was the locus with the highest number of alleles, after DCA 9 (Table 4.3). Because of the high heterozygosis level, it may be hypothesized that the lack of heterozygosis at those two loci may be due to the presence of null alleles or the fact that the two loci are fixed in our population. The estimated frequency of null alleles (EFNA) 33 strengthens the possibility of the presence of null alleles in DCA 15 (r= 0.319) and GAPU 103 (r= 0.244) (Table 4.3).

Table 4.3. Heterozygosity, F statistics and polymorphism determined for 10 microsatellite loci in 20 olive accessions from Campania Region (Italy).

SSR locus	N_a [a]	N_e [b]	H_o [c]	H_e=PIC [d]	FI [e]	PI [f]	EFNA [g]
DCA3	11	8,511	0,900	0,883	-0,020	0,025	-0,009
DCA5	8	2,930	0,700	0,659	-0,063	0,141	-0,025
DCA9	17	12,033	1,000	0,917	-0,091	0,011	-0,014
DCA15	6	3,200	0,150	0,688	0,782	0,141	0,319
DCA16	11	6,780	0,700	0,853	0,179	0,037	0,082
DCA17	10	4,571	0,900	0,781	-0,152	0,076	-0,067
DCA18	8	5,755	0,900	0,826	-0,089	0,053	-0,040
EMO90	7	3,846	0,750	0,740	-0,014	0,107	-0,006
GAPU71B	5	4,000	0,900	0,750	-0,200	0,106	-0,086
GAPU103	16	11,368	0,500	0,912	0,452	0,012	0,244

[a] Number of alleles. [b] Number of effective alleles. [c] Observed heterozygosity. [d] Expected heterozygosity = Polymorphic index content. [e] Fixation index. [f] Probability of identity. [g] Estimated frequency of null alleles.

SSR data were used to calculate pairwise genetic distances, whose average was 18.16. Genetic distances were used for PCA (Figure 4.1). The first two components explained 43.37% of the variance. The distribution of varieties resembles their geographical distribution in some instances. The majority of the varieties from the province of Salerno (‘Carpellese’, ‘Biancolilla’, ‘Pisciottana’, ‘Rotondella’, ‘Salella’), as

well as four out of five varieties from Benevento ('Femminella', 'Ortolana', 'Pampagliosa', 'Racioppella'), clustered together. SSR fingerprinting allowed identification of a distinctive profile for all the studied varieties except 'Minucciola' and 'Nostrale', which did not display allelic differences. Further investigations are needed to address this point.

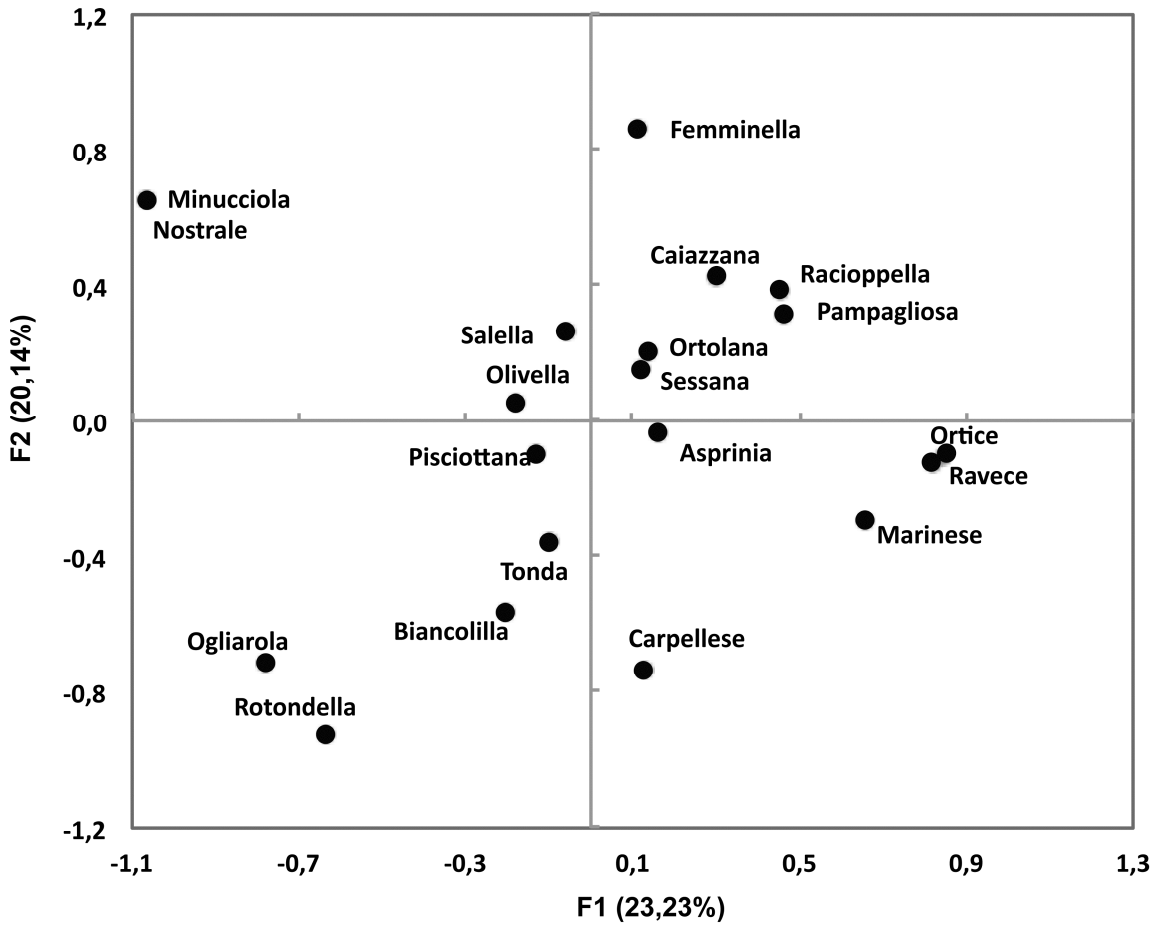

Figure 4.1. PCA of the genetic profile of 20 olive varieties from Campania (Italy) determined by SSR data.

Our study also illustrated the genetic similarity between 'Ortice' and 'Ravece'. It was previously shown10 that different trees of these two local cultivars analyzed by AFLP share a large number of AFLP loci, proving very similar, while a clear distinction was achieved by SSR analysis of the same plants (Corrado et al., 2011). This may be due to their similar phenotypic traits (Pugliano et al., 2000) which leads to misidentification of the trees occurring in the region. Only 'Ravece' virgin oil is included in the register of protected designations of origin (PDO Irpinia - Colline dell'Ufita, EC Regulation 203/2010). As the presence of a possible homonymy could have commercial implications, this issue deserved further investigation. Thus the SSR profile of 13 'Ortice' accessions (collected in seven farms in the province of Benevento) and 13 'Ravece' accessions (collected in six farms in the province of Avellino) was determined with the same 10 SSR set. The unrooted dendrogram of their genetic distances (Figure 4.2) shows that all 'Ravece' accessions form a cluster and confirms their genetic identity. Among the 'Ortice' accessions, only six are characterized by the same profile, different from that of 'Ravece', while the others present the same SSR profile as 'Ravece'. This evidence confirms that there is a

widespread problem in classifying the ‘Ravece’ plants, which are sometimes identified with ‘Ortice’. SSR analysis may contribute to eliminate the misidentification problem.

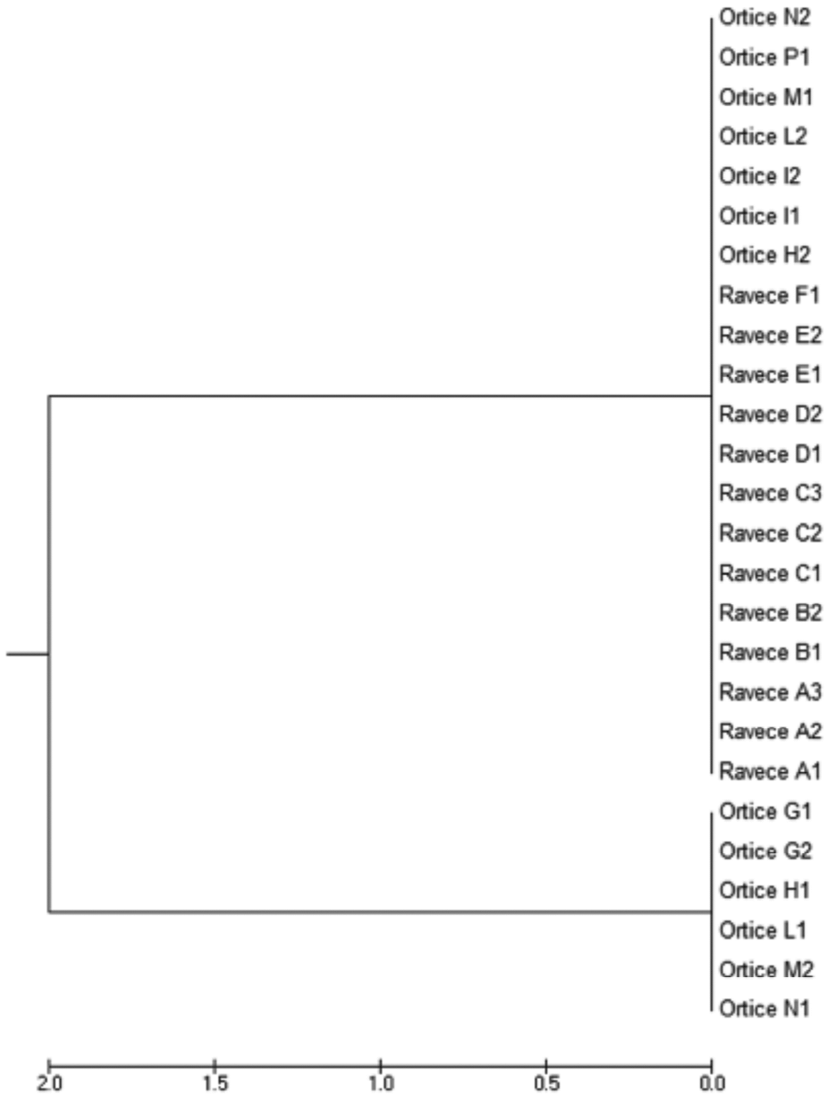

Figure 4.2. Unrooted dendrogram (UPGMA algorithm) of the genetic distances (Peakall and Smouse coefficient) of 13 ‘Ortice’ and 13 ‘Ravece’ trees determined by SSR data.

4.5.2 Fatty acid composition

The fatty acid (FA) composition of the oils obtained from the 20 olive varieties (average, standard deviation and ANOVA) is shown in Table 4.4. A high variability was observed, the amount of oleic acid (C 18:1*n*-9) ranging from 58.89 % (‘Caiazzana’) to 75.97 % (‘Biancolilla’). The low standard deviations of these data, collected during four years, highlights the fact that the FA profile of EVOOs is strongly influenced by genotype while cultural practices, pedoclimatic conditions (especially altitude and water availability) and stage of ripeness are minor factors (Aparicio and Luna, 2002; Di Vaio et al., 2012). The Euclidean distances of the FA profiles were employed to build up the phylogeny tree (Figure 4.3), which illustrates the arrangement of three main clusters. The first, characterized by a low content in C 18:1*n*-9 (<65%) and a high content in C 18:2 and C 18:3, includes ‘Salella’,

‘Caiazzana’, ‘Racioppella’, ‘Pampagliosa’ and ‘Femminella’ vaieties. The second group, characterized by a high content in C 18:1*n*-9 (>70%), comprises ‘Nostrale’, ‘Carpellese’, ‘Ogliarola’, ‘Marinese’, ‘Ortolana’, ‘Olivella’ and ‘Biancolilla’. The other varieties (‘Ravece’, ‘Ortice’, ‘Minucciola’, ‘Sessana’, ‘Asprinia’, ‘Tonda’, ‘Rotondella’ and ‘Pisciottana’) are characterized by a C 18:1*n*-9 content between 65% and 70%.

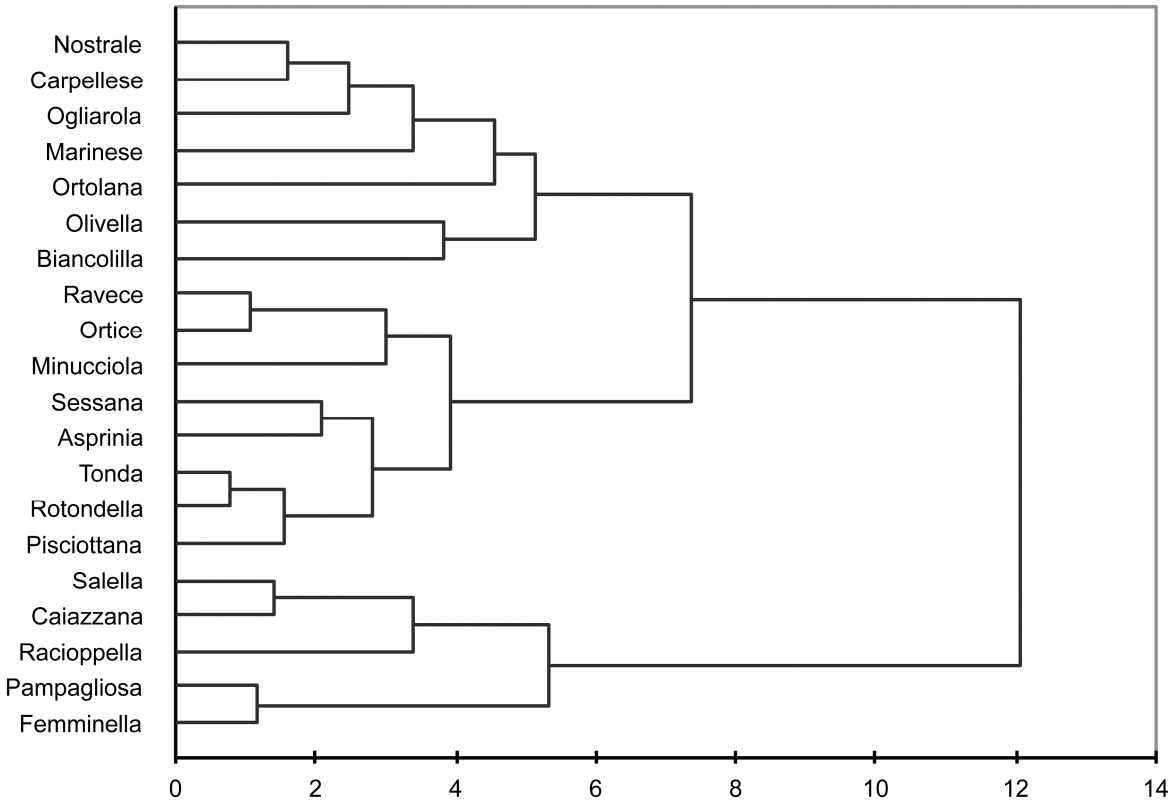

Figure 4.3. Unrooted dendrogram (Average Linkage Method of Agglomeration) of the Euclidean distances of 20 olive varieties from Campania (Italy) determined by fatty acid methyl esters composition of the respective EVOOs.

As the C 18:1*n*-9/C 18:2 ratio is a parameter related to EVOO oxidation stability (Aparicio et al., 1999) , it is possible to forecast that EVOOs obtained from the varieties in the first group are potentially less stable (C 18:1*n*-9/C 18:2 < 5). Conversely, more stable EVOOs are extracted from the varieties belonging to the third group (C 18:1*n*-9/C 18:2 > 9) with the exception of ‘Ortolana’ (C 18:1*n*-9/C 18:2 = 7.92). The biplot resulting from the PCA (Figure 4.4) shows the contribution of each fatty acid to the characterization of the 20 varieties. The first two principal components explained 74% of the observed variance: the first component discriminates the EVOOs mainly on the basis of C 18:0, C 20:0, C 22:0, C 18:1*n*-9, C 16:0, C 16:1*n*-9, C 18:1*n*-7, while the second component is mainly affected by C 17:0, C 17:1*n*-8, C 18:2 and C 18:3. The distribution of FAs in the plot also indicated a positive correlation between C 20:0 and C 18:0 and among C 16:0, C 16:1 and C 18:1*n*-7, as well as an inverse correlation between C 18:1 and both C 18:2 and C 18:3, as expected from the fatty acid biosynthetic pathway (Cook and McMaster, 2002).

Table 4.4. Means, ANOVA test ($p<0.05$) and standard deviations of 13 fatty acids relative to the 20 monovarietal EVOOs from Campania region.

Variety	C 16:0 [a]		C 16:1n9 [b]		C 17:0 [c]		C 17:1n9 [d]		C 18:0 [e]		C 18:1n9 [f]		C 18:1n7 [g]	
	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
'Asprinia'	15.03 f,g	1.44	2.29 f	0.51	0.03 a	0.01	0.08 a	0.01	1.73 a	0.24	67.06 c,d,e	2.90	3.64 i	0.24
'Biancolilla'	10.58 a,b	0.37	0.59 a,b,c	0.06	0.04 a	0.01	0.07 a	0.03	2.17 a	0.20	75.97 i	1.38	2.07 c,d,e	0.09
'Caiazzana'	14.98 f,g	1.88	1.42 d,e	0.47	0.10 b,c	0.04	0.25 c	0.05	1.69 a	0.30	58.89 a	2.60	3.08 h	0.44
'Carpellese'	12.73 d	0.74	0.85 c	0.18	0.05 a	0.03	0.10 a	0.05	1.83 a	0.18	71.93 g,h	2.67	2.62 f,g	0.29
'Femminella'	12.37 b,c,d	0.64	1.02 c,d	0.19	0.13 c,d	0.02	0.28 c,d	0.02	1.78 a	0.25	64.11 b,c	2.20	2.75 f,g,h	0.35
'Marinese'	12.42 c,d	0.80	1.70 e	0.26	0.03 a	0.01	0.08 a	0.02	2.09 a	0.34	71.48 f,g,h	2.20	3.51 i	0.37
'Minucciola'	14.22 e,f,g	1.83	1.16 d	0.36	0.04 a	0.01	0.06 a	0.01	3.10 b,c,d	0.46	65.99 b,c,d	4.10	2.28 d,e,f	0.28
'Nostrale'	12.25 b,c,d	0.52	0.76 b,c	0.12	0.06 a	0.01	0.06 a	0.01	3.05 b,c,d	0.35	72.42 g,h,i	2.09	1.83 a,b,c,d	0.21
'Ogliarola'	11.12 b,c	0.96	0.69 b,c	0.13	0.05 a	0.03	0.07 a	0.02	3.01 b,c,d	0.48	73.95 h,i	2.76	1.77 a,b,c	0.15
'Olivella'	8.78 a	1.56	0.42 a,b	0.14	0.14 c,d	0.08	0.17 b	0.08	5.38 e	2.50	75.81 i	1.38	1.37 a	0.51
'Ortice'	12.75 d,e	0.85	0.73 b,c	0.17	0.06 a,b	0.01	0.07 a	0.01	3.13 c,d	0.44	67.84 c,d,e	2.76	1.73 a,b,c	0.36
'Ortolana'	9.33 a	0.51	0.30 a	0.09	0.16 d	0.09	0.23 b,c	0.08	2.27 a	0.82	73.75 g,h,i	2.43	1.30 a	0.12
'Pampagliosa'	11.83 b,c,d	0.99	0.72 b,c	0.09	0.18 d	0.06	0.32 d	0.06	2.38 a,b,c	0.56	64.29 b,c	2.22	1.98 b,c,d	0.66
'Pisciottana'	13.86 e,f	1.23	1.38 d	0.30	0.10 c	0.04	0.23 b,c	0.07	2.06 a	0.31	68.62 d,e	3.52	2.81 g,h	0.36
'Racioppella'	14.65 f,g	1.11	1.20 d	0.27	0.17 d	0.06	0.29 c,d	0.09	2.29 a,b	0.31	61.34 a,b	2.91	2.34 d,e,f	0.36
'Ravece'	12.19 b,c,d	0.71	0.56 a,b	0.06	0.06 a	0.02	0.07 a	0.01	3.24 d	0.47	68.69 d,e,f	1.39	1.50 a,b	0.18
'Rotondella'	14.36 f,g	1.56	0.80 c	0.21	0.04 a	0.02	0.08 a	0.04	1.73 a	0.22	69.41 e,f	2.89	2.44 e,f	0.32
'Salella'	15.13 g	1.06	1.28 d	0.24	0.04 a	0.02	0.08 a	0.03	1.68 a	0.21	60.08 a	3.66	2.92 g,h	0.40
'Sessana'	15.07 f,g	0.66	0.89 c,d	0.01	0.03 a	0.01	0.06 a	0.01	1.97 a	0.21	67.17 c,d,e	0.52	2.68 f,g,h	0.16
'Tonda'	14.25 e,f,g	1.25	0.83 c	0.21	0.04 a	0.01	0.07 a	0.01	1.65 a	0.23	69.96 e,f,g	2.65	2.53 e,f,g	0.42

Table 4.4. Continue

Variety	C 18:2n6 [h]		C 20:0 [i]		C 18:3n3 [l]		C 20:1 [m]		C 22:0 [n]		C 24:0 [o]		O/L [p]	
	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
'Asprinia'	8.28 c,d	1.38	0.29 a,b	0.07	0.54 a,b	0.11	0.20 a	0.04	0.08 a,b	0.02	0.02 a	0.01	8.35 c,d	1.64
'Biancolilla'	6.12 a,b,c	0.94	0.37 b,c,d	0.02	0.55 a,b	0.06	0.27 a	0.04	0.14 c,d	0.02	0.05 a	0.01	12.69 e,f	2.09
'Caiazzana'	17.25 g	2.76	0.25 a	0.09	1.09 h	0.20	0.18 a	0.06	0.07 a	0.04	0.03 a	0.02	3.53 a	0.81
'Carpellese'	7.53 b,c,d	1.57	0.34 b,c	0.03	0.64 b,c	0.06	0.29 a	0.05	0.12 b,c,d	0.02	0.04 a	0.02	10.04 d	2.58
'Femminella'	14.92 f,g	1.93	0.29 a,b	0.03	0.94 f,g,h	0.14	0.22 a	0.03	0.09 a,b	0.02	0.03 a	0.01	4.38 a,b	0.76
'Marinese'	5.22 a	0.85	0.26 a	0.05	0.78 d,e,f	0.13	0.30 a	0.48	0.10 a,b,c,d	0.02	0.04 a	0.02	14.10 f	2.60
'Minucciola'	11.04 e	2.27	0.43 d	0.06	0.56 a,b	0.08	0.19 a	0.04	0.13 b,c,d,	0.03	0.04 a	0.02	6.32 b,c	1.87
'Nostrale'	7.59 b,c,d	1.85	0.44 d	0.04	0.59 a,b	0.05	0.22 a	0.02	0.12 b,c,d	0.02	0.04 a	0.02	10.03 d	2.23
'Ogliarola'	7.31 b,c,d	1.53	0.40 c,d	0.06	0.53 a	0.03	0.20 a	0.04	0.11 b,c,d	0.01	0.02 a	0.02	10.60 d,e	2.44
'Olivella'	5.34 a,b	0.49	0.43 d	0.14	0.61 a,b,c	0.08	0.19 a	0.01	0.13 b,c,d	0.11	0.02 a	0.02	14.32 f	1.59
'Ortice'	10.85 e	1.73	0.42 d	0.06	0.75 c,d,e	0.08	0.19 a	0.02	0.10 a,b,c,d	0.02	0.04 a	0.02	6.53 b,c	1.98
'Ortolana'	9.67 d,e	1.90	0.36 b,c,d	0.08	0.98 g,h	0.07	0.32 a	0.05	0.11 a,b,c,d	0.02	0.03 a	0.02	7.92 c,d	1.67
'Pampagliosa'	15.06 f,g	1.44	0.35 b,c,d	0.03	0.87 e,f,g	0.06	0.25 a	0.07	0.15 d	0.08	0.04 a	0.04	4.32 a,b	0.57
'Pisciottana'	8.88 d	2.34	0.30 a,b	0.04	0.60 a,b	0.08	0.21 a	0.04	0.10 a,b,c	0.03	0.04 a	0.06	8.34 c,d	2.51
'Racioppella'	14.35 f	1.60	0.36 b,c,d	0.06	1.08 h	0.17	0.21 a	0.06	0.12 b,c,d	0.06	0.04 a	0.04	4.35 a,b	0.75
'Ravece'	10.91 e	0.92	0.40 d	0.06	0.75 c,d,e	0.17	0.18 a	0.04	0.10 a,b,c,d	0.02	0.03 a	0.02	6.34 b,c	0.63
'Rotondella'	8.46 d	1.62	0.29 a,b	0.08	0.71 c,d	0.11	0.26 a	0.07	0.09 a,b	0.04	0.04 a	0.02	8.56 d	1.94
'Salella'	16.67 f,g	2.48	0.27 a	0.06	0.75 c,d,e	0.08	0.21 a	0.06	0.09 a,b	0.03	0.03 a	0.02	3.77 a	1.25
'Sessana'	9.44 d,e	0.94	0.35 b,c,d	0.02	0.78 c,d,e,f	0.06	0.26 a	0.02	0.11 a,b,c,d	0.01	0.05 a	0.00	7.17 b,c,d	0.73
'Tonda'	7.97 b,c,d	1.22	0.28 a,b	0.08	0.77 c,d,e,f	0.11	0.27 a	0.05	0.10 a,b,c,d	0.04	0.04 a	0.02	8.99 d	1.55

Values are reported as percentage of the total fatty acid content. [a] Hexadecanoic acid. [b] Cis-9-hexadecenoic acid. [c] Heptadecanoic acid. [d] Cis-9-heptacecenoic acid. [e] Octadecanoic acid. [f] Cis-9-octadecenoic acid. [g] Cis-11-octadecenoic acid. [h] Cis-9,cis-12-octadecadienoic acid. [i] Eicosanoic acid. [l] Cis-9,cis-12,cis-15-octadecatrienoic acid. [m] Cis-11-eicosenoic acid. [n] Docosanoic acid. [o] Tetracosanoic acid. [p] C 18:1n-9/C 18:2 ratio.

The varieties characterized by a high content of the unsaturated fatty acids C 17:1*n*-8, C 18:3*n*-3 and C 18:2*n*-6, form a cluster in the PCA plot and are mainly distributed in the province of Benevento (‘Femminella’, ‘Pampagliosa’, ‘Racioppella’ and ‘Ortolana’). The cluster of the varieties with a high content of C 18:1*n*-9 includes ‘Biancolilla’, ‘Nostrale’ and ‘Carpellese’, all belonging to the province of Salerno. ‘Pisciottana’, ‘Sessana’, ‘Tonda’, ‘Rotondella’, ‘Marinese’, ‘Asprinia’ and ‘Salella’ form a group of varieties characterized by a high content of C 16:0, C 16:1*n*-9, C 18:1*n*-7. The above findings highlight the possibility to characterize the 20 olive varieties from Campania on the basis of their FA composition, as shown by other authors for other varieties (Diraman et al., 2010) . Interestingly, as shown in Table 4.4 and in the FA plots (Figure 4.3 and Figure 4.4) ‘Minucciola’ and ‘Nostrale’ can be distinguished by the FA composition of their EVOOs. On the other hand, the FA composition in ‘Ortice’ and ‘Ravece’ confirmed the high similarity of the cultivars. A naturally high content of linolenic acid was found in ‘Racioppella’.

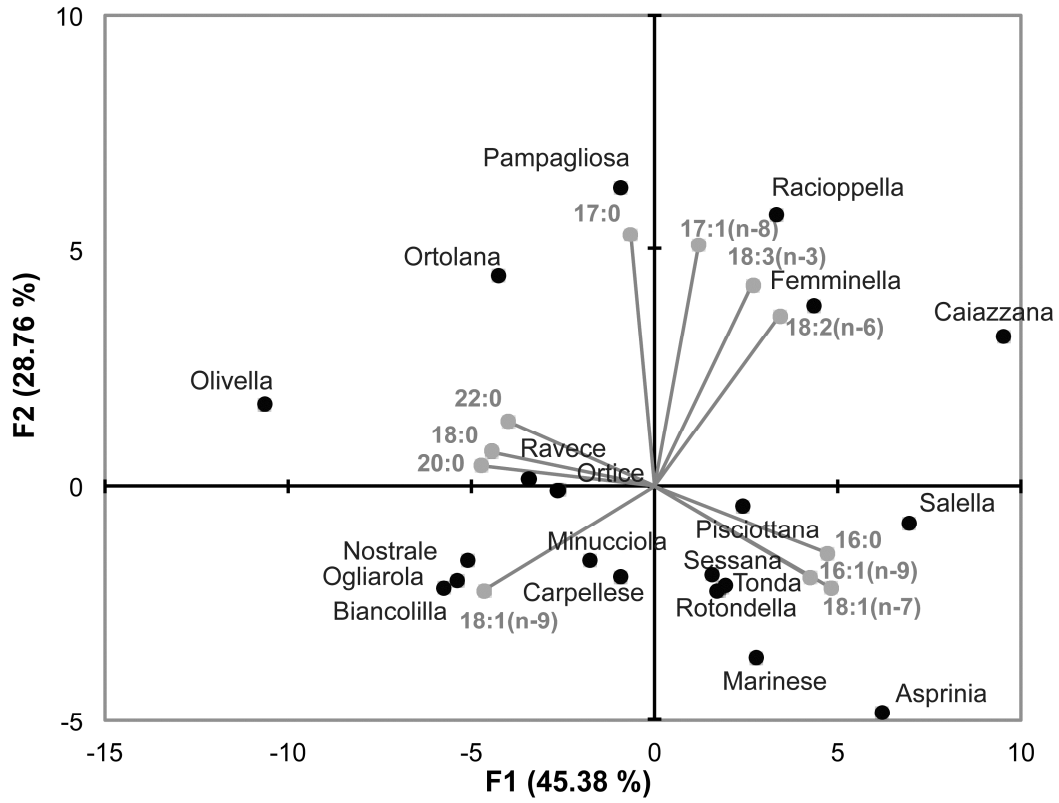

Figure 4.4. PCA (Pearson) biplot of the fatty acid composition of the EVOOs extracted from 20 olive varieties from Campania (Italy).

4.5.3 Biophenols

As shown in Table 4.5 average amounts of both secoiridoid and lignan wildly differ in the 20 varieties (for example, Ty-EDA varies from 12.00 ppm for ‘Racioppella’ to 80.16 ppm for ‘Ortolana’). The high standard deviations of data collected during four years highlight the variability of the biophenol composition of EVOOs. EVOO phenolic composition depends on olive-related variables (variety, stage of ripening, soil and climatic area, agronomic techniques and plant health) and also on technological variables such as olive storage conditions, extraction technology and oil storage (Aparicio and Luna, 2002; El Riachy et al., 2011) .

Table 4.5. Means, ANOVA test (p<0.05) and standard deviations of 8 biophenols relative to the 20 monovarietal EVOOs from Campania region.

Variety	OHTy [a]		Ty [b]		OHTy-EDA [c]		Ty-EDA [d]		P-AP [e]		OHTy-EA [f]		Total [g]	
	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
'Asprinia'	3.14 a	3.65	5.16 a	5.38	10.39 a,b	16.10	15.66 a	10.55	9.38 a	9.48	6.78 a,b	7.68	50.53 a,b	41.10
'Biancolilla'	0.93 a	1.35	13.82 a,b,c	5.47	2.28 a,b	3.78	33.84 a,b	15.68	54.98 d,e	33.94	7.80 a,b	6.19	113.66 b,c	51.27
'Caiazzana'	4.56 a	4.72	6.47 a	3.76	23.01 a,b,c	36.21	31.40 a,b	30.92	21.00 a,b	24.62	9.61 a,b	12.16	96.05 a,b,c	81.32
'Carpellese'	1.59 a	3.05	15.17 a,b,c	9.85	1.34 a	2.99	29.30 a	17.66	80.36 e	19.40	7.99 a,b	5.11	135.76 b,c,d	35.59
'Femminella'	7.68 a,b,c	7.00	6.75 a	4.62	22.41 a,b,c	23.76	24.10 a	13.04	13.09 a,b	6.79	12.18 a,b	7.70	86.21 a,b,c	42.63
'Marinese'	2.65 a	2.74	15.54 a,b,c	12.79	25.30 a,b,c	38.42	57.68 b,c,d	32.77	9.86 a	3.26	7.60 a,b,c	6.80	118.63 b,c	73.22
'Minucciola'	14.79 a,b,c,d	13.01	16.78 a,b,c	7.90	29.63 a,b,c	46.11	24.97 a	13.50	31.28 a,b,c,d	28.12	29.29 b,c	27.11	146.74 b,c,d	92.96
'Nostrale'	11.79 a,b,c,d	7.59	27.93 c,d	18.09	20.43 a,b,c	34.60	25.98 a	16.68	51.88 c,d	10.62	35.37 b,c	35.87	173.37 b,c,d	77.17
'Ogliarola'	19.28 c,d	13.37	21.47 b,c	12.92	31.96 a,b,c	24.52	38.15 a,b	17.88	46.18 c,d	20.82	44.05 c	22.14	201.10 d	61.00
'Olivella'	7.45 a,b,c	6.22	13.94 a,b,c	5.04	35.30 a,b,c	29.28	74.77 c,d	49.63	29.37 a,b,c,d	33.42	26.73 a,b,c	13.24	187.56 c,d	79.55
'Ortice'	21.97 d	16.72	39.36 d	27.59	41.47 b,c	33.17	79.70 d	46.88	24.64 a,b,c	11.38	32.98 b,c	18.81	240.12 d	90.16
'Ortolana'	6.58 a,b	8.76	18.06 a,b,c	12.30	31.51 a,b,c	32.46	80.16 d	32.45	4.67 a	3.51	9.98 a,b	5.09	150.96 b,c,d	66.72
'Pampagliosa'	13.33 a,b,c,d	16.22	7.20 a	4.56	51.79 c	31.49	38.17 a,b,c	24.91	15.01 a,b	6.62	49.95 c	34.23	175.45 b,c,d	88.24
'Pisciottana'	5.84 a	10.69	6.71 a	5.23	24.09 a,b,c	47.05	17.16 a	19.34	11.59 a	11.45	13.25 a,b	18.48	78.64 a,b	96.95
'Racioppella'	4.93 a	11.04	8.04 a	16.06	8.09 a,b	11.81	12.00 a	6.96	8.83 a	9.76	6.60 a,b	8.84	48.50 a,b	56.48
'Ravece'	15.98 b,c,d	13.25	30.36 c,d	21.46	47.74 c	43.72	69.20 c,d	31.63	32.47 b,c,d	37.26	39.16 c	37.25	234.91 d	120.57
'Rotondella'	5.98 a	6.45	8.20 a	5.47	13.84 a,b	15.24	25.04 a	15.39	43.13 c,d	13.56	13.46 a,b	8.67	109.65 b,c	44.23
'Salella'	2.08 a	3.05	4.03 a	3.28	2.10 a	4.66	13.18 a	9.78	16.77 a,b	15.93	4.03 a	6.44	42.19 a	37.76
'Sessana'	3.43 a	1.47	12.93 a,b,c	13.69	23.30 a,b,c	25.35	33.30 a,b	21.79	38.07 b,c,d	6.76	16.97 a,b,c	12.92	128.00 b,c,d	54.36
'Tonda'	4.37 a	4.24	9.56 a,b	7.71	18.93 a,b,c	23.59	32.54 a,b	22.73	34.52 b,c,d	13.09	13.03 a,b	12.33	112.96 b,c	62.55

Values are expressed as milligrams of tyrosol per kilogram of oil. [a] Hydroxytyrosol. [b] Tyrosol (Ty). [c] Dialdehydic form of elenolic acid linked to OHTy. [d] Dialdehydic form of elenolic acid linked to Ty (ligstroside aglycone or oleocanthal). [e] Pinoresinol and acetoxypinoresinol. [f] Aldehydic form of elenolic acid linked to OHTy (oleuropein aglycone). [g] Total phenols.

For this reason, all EVOO samples were extracted by the same micro-mill operating at the same technological conditions, in order to minimize the influence of the variables other than olive variety. Despite the strong influence of environmental variables, the study of biophenol composition could give an insight into the characterization of the olive germplasm in Campania. Concerning the total phenolic content (sum of the compounds identified by HPLC-DAD) of the EVOO samples extracted from the 20 varieties, which ranges from a minimum of 42.19 ppm in 'Salella' to a maximum of 240.12 ppm in 'Ortice', two main groups may be distinguished. The varieties 'Asprinia', 'Caiazzana', 'Femminella', 'Pisciottana', 'Racioppella' and 'Salella' show a low average phenolic content (<100 ppm). All the other EVOOs present a total phenolic content higher than 100 ppm. PCA analysis (Figure 4.5) shows the distribution of the 20 olive varieties according to their biophenol composition.

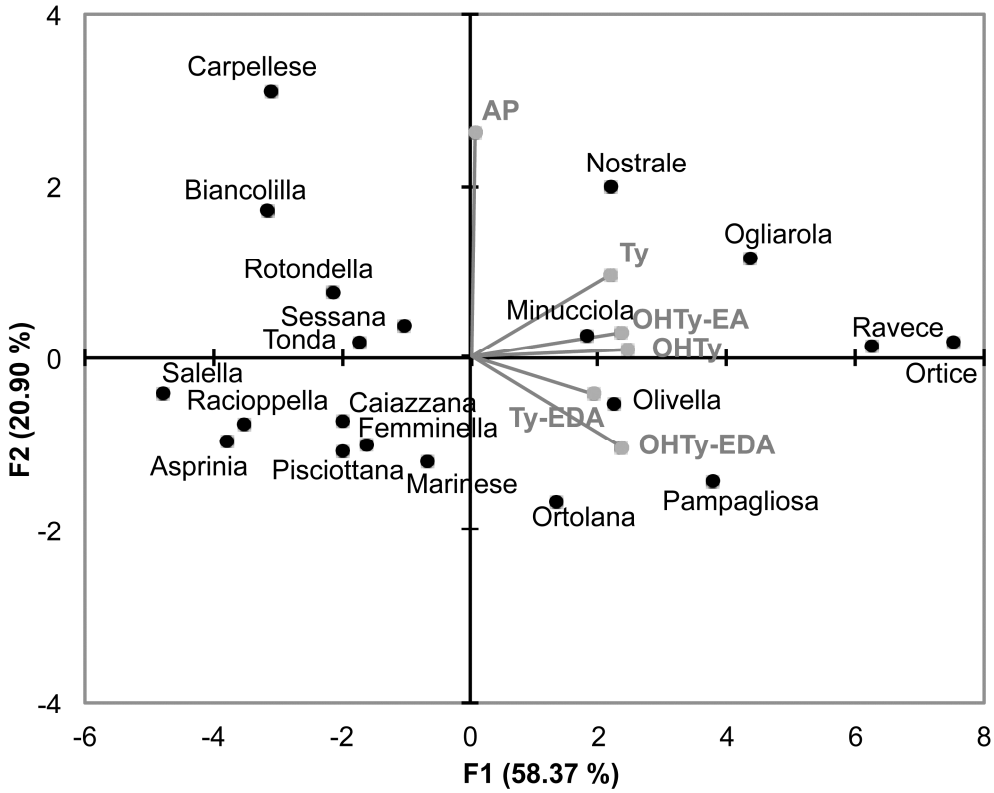

Figure 4.5. PCA (Pearson) biplot of the biophenol composition of the EVOOs extracted from 20 local varieties widely grown in Campania (Italy).

Because of the links among their biosynthetic pathways (Ryan et al., 2002) , the distribution of all biophenols in the plot indicated a positive correlation among all of them. The first two principal components explained 77.84% of the observed variance. F1 discriminates the EVOOs mainly for the OHTy-EA content, which was positively correlated to all the other secoiridoid derivatives. F2 differentiated them on the basis of lignan (P-AP) content. Thus, 'Carpellese', 'Biancolilla', 'Nostrale', 'Rotondella' and 'Ogliarola' (P-AP content >40 ppm) are located in the upper part of the graph, with 'Carpellese' showing a noticeable high content (80.36 ppm) in P-AP with respect to all the other varieties. These varieties, with the exception of 'Ogliarola', are found throughout the Salerno area. The amount of lignans also allows discrimination of 'Minucciola' and 'Nostrale', while 'Ortice' and 'Ravece', which are similar both in terms of SSR profile and FA composition, show a similar biophenol composition too.

The metabolic pathways of biophenols (shikimate pathway and phenylpropanoid metabolism) are particularly complex with multiple alternative metabolic fates that may vary from tissue to tissue, from one growing condition to another, and in response to environmental stimuli (Ryan et al., 2002). The analysis of biophenols can give a contribute in the differentiation of the 20 olive varieties and in the characterization the EVOOs extracted under standardized conditions from different olive varieties (Japón-Luján et al., 2006; de Medina et al., 2015) .

4.5.4 Sensory profile

The PCA biplot in Figure 4.6 shows the distribution of the EVOOs from the 20 varieties on the basis of the sensory profile (n attributes). The first two principal components explained 53.50% of the observed variance. F1 discriminates the EVOOs according to the attributes of bitterness, pungency, leaf, artichoke and tomato. F2 discriminates them on the basis of sweet, almond, green almond, vegetable, and rosemary flavors. Each of the 20 EVOOs showed a specific sensory profile. The EVOOs with higher intensity of bitterness and pungency were characterized by high intensity of olive fruity and by green notes, such as tomato (‘Ravece’ and ‘Ortice’ varieties) and artichoke (‘Biancolilla’, ‘Sessana’ and ‘Nostrale’). These flavors are originated by the lypoxygenase pathway from the particular polyenoic fatty acids of a given variety in a given environment (Di Vaio et al., 2012; Issaoui et al., 2010) . A typical note of ‘rosemary’ was detected in ‘Minucciola’ EVOOs from the Sorrento peninsula PDO area. The above findings confirm that sensory profile analysis can give useful information to discriminate the EVOOs obtained from different olive varieties, as reported elsewhere (Tura et al., 2008).

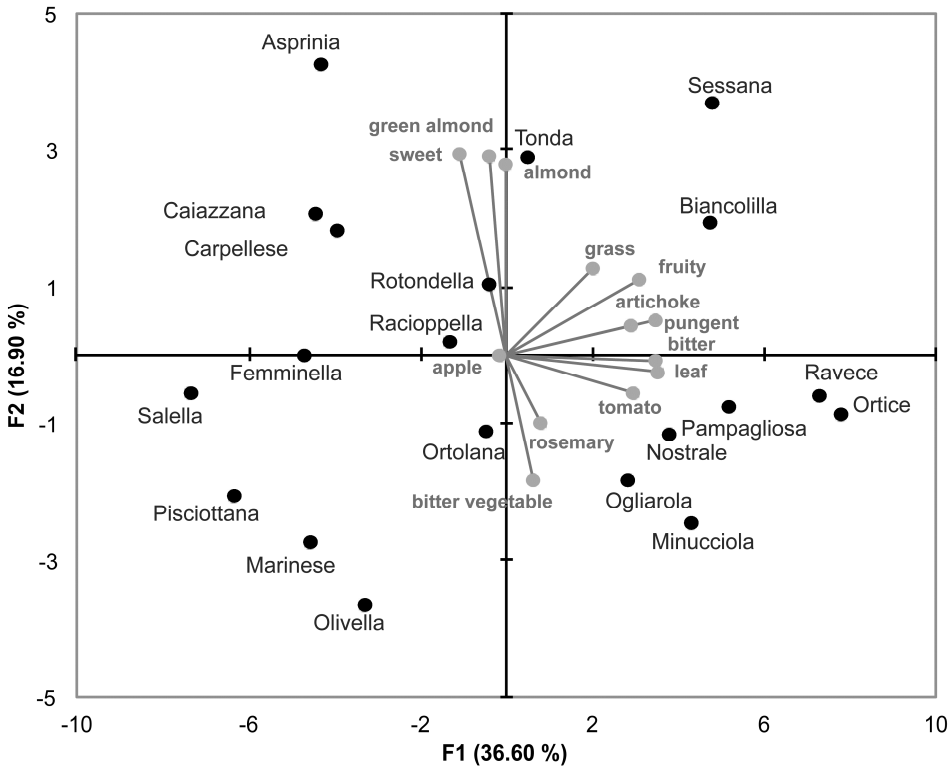

Figure 4.6 : PCA (Pearson) biplot of the sensory profiles of the EVOOs extracted from 20 local varieties widely grown in Campania (Italy).

4.5.5 Correlation between genetic fingerprinting, EVOO chemical composition and sensory profiles

The correlations between the genetic dissimilarity matrix of the 20 olive varieties of Campania and the dissimilarity matrices determined on the basis of the whole pool of fatty acids, biophenols and sensory profiles of the relative olive oils was verified by applying the Mantel test. A low but significant correlation (r= 0.209, p= 0.018) was revealed only between the SSR profile of the olive varieties and sensory profiles of the relative EVOOs. Conversely, no correlation was found with fatty acids (r= 0.029, p= 0.396) or biophenols (r= 0.054, p= 0.301). Our findings are in contrast with those of previous research (Rotondi et al., 2011; Echeverrigaray et al., 2001) which found a positive correlation between chemical parameters and genetic profiles, but are consistent with data reported by several authors for medicinal and aromatic plants (Mochida et al., 2009; Tonk et al., 2010). As microsatellites are neutral markers, they could be mostly independent of specific genetic traits. This could explain the lack of correlation of SRR patterns with metabolic profiles (Laurentin et al., 2008).

4.5.6 Abbreviations

EVOO, extra virgin olive oil; SSR, simple sequence repeats; FA, fatty acid; FAME, fatty acid methyl ester; PDO, protected designation of origin; PGI protected geographical indication; JRI, Jaén ripening index; HPLC, high-performance liquid chromatography; IOC, International Olive Council; ANOVA, analysis of variance; GD, genetic distance; PCA, principal component analysis; N_e, number of effective alleles; H_o, observed heterozygosity; PIC, polymorphism information content; H_e, expected heterozygosity; FI, fixation index; $P_{(ID)}$, probability of identity; EFNA, estimated frequency of null alleles.

4.5.7 Acknowledgments

The authors are grateful to the olive farmers who kindly provided plant materials, to Grazia Stanzione (STAPA-CePICA of Regione Campania, NA), Angelo Lo Conte and Tommaso Vitale (to whose memory this paper is dedicated) for their contribution in selecting farms and sampling. This work was supported by the Regione Campania - Assessorato Agricoltura (OMeGaProject), MIUR (PRIN 09 Project) and MIPAAF (RIOM Project - Innovazione in Olivicoltura).

5. EFFECTS OF THE STING OF BACTROCERA OLEAE ON OLIVE TRANSCRIPTOME

5.1 Introduction

5.1.1 Olive and olive fly

The olive fly, *Bactrocera oleae* (Diptera: Tephritidae) (Clarke et al. 2005) was described for the first time in the 3rd century by Theophrastus. Its evolutionary history is characterized by the differentiation of three phylogenetic groups (Africa, Mediterranean, Pakistan; Nardi et al., 2005) and by its coevolution with *Olea europaea*, indicating an early and stable plant-insect association (Nardi et al., 2010). In particular, it has been suggested that the Mediterranean group might have established firstly on wild olive of *Olea europaea* subsp. *europaea* and then, once cultivated olive spread in the Mediterranean basin, olive fly adapted to it (Nardi et al., 2010). In the Mediterranean basin, the olive tree found the right environmental conditions to diffuse along with the olive fly. During the centuries the olive oil, typically employed as a main lipid source in the Mediterranean diet, has been also diffusing as an important cosmetic and basic medical matrix. Nowadays, extra virgin olive oil (EVOO) is considered as an effective element in prevention and reduction of several ailments such as cardiovascular diseases, oxidative stress, obesity, diabetes, inflammatory processes and cancer (Caramia et al., 2012). The nutritional and sensory interest in EVOO has increased during the last decades, together with its economic importance. Even though Mediterranean Countries (especially Spain, Italy and Greece) still held over 98.0% of global production, the olive growing has been diffusing in several Countries of Africa, Americas, Asia and Oceania. As the invasion in California suggests (Zygouridis et al., 2009), olive fly is following its host.
Global production of virgin olive oil and table olives is threatened by *Bactrocera oleae* as its feeding is strictly dependent to the presence of olive fruits. While the adult flies can survive feeding on several organic sources, larvae can only feed on olives (Fletcher, 1987). To enhance the access to food of the young larvae, adult females puncture the olive to place the egg in the pulp (Tzanakakis, 2003). By chewing and feeding, larvae destroy the olive pulp and may determine the fruit drop. As a direct effect, it produces quantitative loss and fall in oil yield. Moreover, it increases olive oil acidity and number of peroxides, phenol and sterol fraction modifications and causes the occurrence of musty and earthy off-flavours, which can considerably reduce oil quality and determine the downgrading of extra virgin olive oil to other less valuable categories. Indirect effects are also determined because of microorganisms that grow in the feeding tunnels and necrotic areas (Gómez-Caravaca et al., 2008; Angerosa et al., 1992). Autumn is usually the most tricky period of the year in terms of olive fly infestation as climate conditions (mild temperature and high humidity) and olive fruit ripening stage are optimal for larval growth and adult reproduction (Tzanakakis, 2006; Wang et al., 2009).
Depending on the yearly climate conditions, *Bactrocera oleae* may determine severe damages and economic losses in the olive oil and table olive sectors. During the last years, possibly because of global warming (Ponti et al., 2014), we have witnessed a consistent increase in the number of olive fruit fly generations per year and the consequent increase of olive production loss. In the 2014-2015 season, Italy

registered 35% fall (with peaks of 45% in some Regions) in olive oil production compared to the previous season (source: ISTAT and ISMEA).

5.1.2 Control strategies

Taking into account that the negative impact of olive fly on table olive and olive oil markets is increasing, more efforts are needed to contain olive fly population and attack.
The conventional *Bactrocera oleae* management programs employed during the last decades, is based on the use of several insecticides (e.g., organophosphates and pyrethroids; Daane and Johnson, 2010). It has been reported that olive fly frequently acquires resistance to those formulations (Hawkes et al., 2005; Vontas et al., 2002).
In addition to the high costs and the difficulty to successfully adapt the conventional control strategies to different environments and varieties, chemical methods represent a source of environmental pollution, olive and olive oil contamination and destruction of useful insects. Currently, the defense from the olive fly is mostly based on integrated or biological methods (Daane and Johnson, 2010).
A first aid to reduce the presence of olive fly in the olive grove can come by the agronomic treatments, such as the removal of the residual olives at the end of the season, the targeted pruning and the early harvest.
An important method employed in olive fly control programs consists in the use of traps (Yellow Sticky, McPhail, OLIPE, Attract and Kill, etc.) to monitor the olive fly population, together with the visual detection of the presence of stings on the drupes. Mass trapping, relying on the use of traps placed at a high density, may also be employed as a support for olive fly control to improve the effect of bait sprays (Broumas et al., 2002).
Recently, less dangerous insecticides have been developed, like bait sprays containing Spinosad, that attract the flies and determine their death by feeding (Thomas and Mangan, 2005; Wang et al., 2011), or kaolin clay barrier sprays, which repel olive flies (Saour and Makee, 2004).
Several biological control methods using a wide range of natural enemies have been developing too, with the employment of several introduced parasitoids (Daane and Johnson, 2010; Hoelmer et al., 2011; Sirjani et al., 2009).
A promising method (Knipling, 1955) is the SIT (sterile insect technique), together with its advanced alternative known as RIDL (release of insects carrying a dominant lethal; Alphey et al., 2002). Whole transcriptome analyses, together with the existing molecular knowledge, are providing new insights into the olive fly biology to develop new tools for a successful SIT protocol (Sagri et al., 2014).

5.1.3 Olive genome and transcriptome

Although the first olive DNA sequence was released in NCBI database in 1994, a real involvement in olive genome analysis has beginning only few years ago with the renewed interest of global market in extra virgin olive oil and its nutritional properties (Bracci et al., 2011).
Olive functional genomics has been mainly based on EST identification, especially those related to pollen allergens and olive fruit characteristics. Several studies on genes involved in fatty acid, triacylglycerols, minor components (especially phenolic compounds) and antioxidant biosynthetic pathways have been carried out, as well as

investigations about olive response to water stress and olive chloroplast genome sequencing (Bracci et al., 2011).
Metabolic and transcriptional profiling research allowed the identification of differentially expressed genes during fruit development, particularly of those involved in lipid and phenolic metabolism (Alagna et al., 2009).
The largest research aimed at identifying olive expressed sequence tags (ESTs) was focused on several olive tissues and developmental stages and resulted in the *de novo* assembly and functional annotation of olive transcriptome (Muñoz-Mérida et al., 2013).
Unlike other tree species of interest, such as *Vitis vinifera* (Velasco et al., 2007; Jaillon et al., 2007) and *Populus trichocarpa* (Tuskan et al., 2006), olive complete genome sequencing is still incomplete. Hence, transcriptome sequencing is still the most reliable tool to investigate on olive gene, transcripts and biological processes (Muñoz-Mérida et al., 2013).

5.1.4 Molecular basis of the interaction between olive and olive fly

The importance of *Olea europaea* and its key enemy *Bactrocera oleae*, does not match research efforts to examine the interaction between olive and olive fly in depth and to shed light on the mechanisms underlying olive defense and resistance.
Despite of the wide knowledge of other plant-pest systems (Engelberth et al., 2013; Kant et al., 2004; Coppola et al., 2014; Coppola et al., 2013), only few studies focused on olive molecular response to the attack of its key pest.
A first investigation on the subject indicated, in agreement with what occurs in other plant-insect systems, a wide modification of both transcriptomic and proteomic profiles of the drupe once the olive fly attacks it. Different genes involved in stress responses and signaling pathways were identified, as well as those responsible for the production of direct defense compounds and ROS (Corrado et al., 2012).
A recent study confirmed the complexity of the response of *Olea europaea* to *Bactrocera oleae*, highlighting the activation of several defense mechanisms. Along with the production of defense proteins and phytohormones, a strong induction of ethylene burst was revealed, together with a deep modification of the volatile organic compound blend emitted by the drupe (Alagna et al., 2015).
Despite this important findings, many aspects of the olive response to olive fly need further research. For instance, the mechanisms that are responsible for the different susceptibility to olive fly infestation of the olive varieties has never been addressed in molecular terms. Tolerance has been observed and reported in some olive varieties (Daane and Johnson, 2010; Iannotta and Scalercio, 2012). Although it was hypothesized that it could rely on mechanical barriers (e.g. aliphatic waxes) and chemical factors (e.g. oleuropein, cyanidine), the responsible features remain unclear (Loscalzo et al., 1994; Scarpati et al., 1996).
Transcriptomic studies may give new insights into molecular mechanisms of genetic resistance to olive fruit fly and defense responses activated by the plant as the result of the attack of its key enemy. This knowledge could be important to implement current strategies and develop new tools aimed at the control of olive fly population and related olive production loss.

5.2 Materials and methods

5.2.1 Plant material

Two olive varieties, included in the germplasm of Campania region (Italy), were selected on the basis of their different response to the attack of *Bactrocera oleae*. In fact, while 'Ortice' is known to be susceptible to the olive fly, 'Ruveia' is listed as tolerant to it.
A pool of undamaged drupes and olives with olive fly egg-laying punctures were hand-harvested for both varieties at the Azienda Improsta (Battipaglia, Salerno). Olives with symptoms of the attack of other pathogens as well as those with adult olive fly emergence holes were excluded from the analysis.
Olives were sliced under a light microscope within 6 hours of harvesting and slices were frozen in liquid nitrogen immediately after the incision. Damaged ones were dissected in order to eliminate the larvae (first to third instar) and collect only the olive material around the feeding tunnels. The drupes with inactive stings were discarded.
Samples were kept at -80 °C until use.

5.2.2 RNA isolation

Total RNA was extracted from 200 mg of tissue (previously grinded in liquid nitrogen) by the RNeasy plant Mini Kit (Qiagen, Milano, Italy). To optimize the protocol to the chemical composition of the olives, the disruption of the tissue as indicated by the manufacturer's instructions was replaced by two steps with a 1:1 solution of extraction buffer (1 M Tris-HCl pH 8.5, 5M NaCl, 0.5 M EDTA, 10% SDS, water) and phenol–chloroform (1:1) followed by one purification step with chloroform. RNA size, quantity and quality parameters were assessed using the 2100 Bioanalyzer system (Agilent Technologies). Samples characterized by a 260/280 nm absorbance higher than 1.8 and a 260/230 nm absorbance higher than 2 were selected to perform the array experiment and purified with an additional phenol–chloroform (1:1) step.
Three technical replicates of the experimental points were prepared pooling the extracted RNA.

5.2.3 Microarray layout

CombiMatrix CustomArray ™ 90K (CombiMatrix Corporation) arrays had been previously developed at the ENEA - TRISAIA Research Center (Rotondella, Matera, Italy). Probes were designed with the CombiMatrix Probe Weaver software for specificity with a pool of cDNA libraries obtained by 454 pyrosequencing. To this end, olives of 'Ortice' and 'Ruveia' varieties with the following five features were collected: undamaged, punctured, damaged with 1st instar larvae, damaged with larvae from 2st to 3rd instar, and damaged with the adult olive fly emergence hole. Only the areas around the punctures and tunnels, excluding necrotic tissues, were dissected and harvested to obtain three biological replicates for each of the five conditions both for 'Ortice' and 'Ruveia'. Then, the corresponding replicates were pooled and a total of 10 cDNA libraries for 454 sequencing were prepared and subsequently pooled to obtain a total of four samples: 'Ortice' undamaged, 'Ortice' damaged (different attack stages), 'Ruveia' undamaged and 'Ruveia' damaged (different attack stages).

The chip layout counted 61.,825 probes (oligonucleotide length of 35-40mers with a melting temperature of 70-75 °C) out of the 87,720 sequences of the pooled library. Among the array probes, 60,706 were non redundant.
The probes were synthesized on microarrays through the CustomArray Synthesizer (CombiMatrix Corporation), including quality control sequences.

5.2.4 cRNA amplification and labeling

According to the RNA ampULSe Amplification and Labeling Kit for CombiMatrix arrays (Kreatech Biotechnology, Amsterdam, The Netherlands), the reverse transcription of 2 µg RNA was carried out, followed by the cDNA purification, the cRNA synthesis and purification. Labeling was carried out with the Cy5-ULS (Cyanine-Universal Linkage System). Dye was removed with KREApure and Cy5-cRNA yield and quality were assessed by a NanoDrop 1000 (Thermo Scientific). To provide better binding specificity and improve detection sensitivity, the resulting cRNAs were fragmented for 20 min at 95°C with Tris Acetate pH 8.1 (200 mM), KOAc (500 mM) and MOAc (150 mM).

5.2.5 Array hybridization and imaging

The fragmented Cy5-cRNA were added to the hybridization solution prepared with 6X SSPE, 0.05% Tween-20, 20mM EDTA, 25% DI formamide, 100 ng/µL salmon sperm DNA and 0.04% SDS and poured in the hybridization chambers of the pre-hybridized microarrays, following the CombiMatrix Hybridization Protocol. After an overnight incubation at 45°C in a rotating rotisserie oven, the microarrays were treated with six washing steps: firstly in 6X SSPE and 0.05% Tween-20 for 5’ at 45°C, then with 3X and 0.5X SSPE and 0.05% Tween-20 for 1’ at room temperature, with 2X PBS and 0.1% Tween-20 and finally with 2X PBS for 1’ at room temperature for two times.
The imaging of the microarrays was performed by an high-resolution fluorescent microarray scanner (GenePix® Pro microarray scanner, Axon Instruments, Inc.) and the spot intensities were detected by the CombiMatrix Microarray Imager software (CombiMatrix Corporation).
Intra-chip hybridization quality was detected by the coefficient of variance (CV), calculated on the basis of the spots corresponding to identical probes in every microarray, with a maximum threshold of 0.20. The correlation among the three replicates for each experimental point was assured by a minimum Pearson coefficient of 0.99 ($R \geq 0.99$).
As three biological replicates per each experimental point were analyzed, the denaturation and detachment of the labeled aRNAs from the probes was necessary to allow the reuse of the microarrays, as indicated in the CustomArray™ stripping protocol.

5.2.6 Microarray data analyses

Raw values were normalized by the CombiMatrix Probe Weaver software using the quantile algorithm. Quantitative gene expression analysis of the microarray was processed using GeneSpring GX 10 (Agilent Technologies).
In order to get a visualization of the effect of the attack of the olive fly on the whole differentially expressed gene sets (FC≠1, p-value < 0.05 Student’s t-test) of ‘Ortice’

and ‘Ruveia’ drupes, the multivariate analysis Principal Component Analysis (PCA) was done using the Log_{10}-transformed data by GeneSpring GX 10 (Agilent Technologies).
Differentially expressed ESTs were identified as the ones whose corresponding expression value ratios in damaged samples respect to undamaged ones showed at least a 2-fold change. The Student’s *t*-test with a 0.05 false discovery rate ($p < 0.05$) was employed to filter the differentially expressed gene lists.
In order to show the possible overlaps and identify the number of genes expressed in multiple conditions, the microarray data sets of differentially expressed genes with FC > 2 and FC < 0.5 (p-value < 0.05 *t*-test) were compared using the freeware SERIAL LIST 2.3 (http://serialbasics.free.fr/Serial_List.html). The results were manually represented in a Venn diagram.

5.2.7 Functional annotation

The whole array database was functionally annotated by Sma3s (Muñoz-Mérida et al., 2014). Functional annotation of the differentially expressed genes was firstly accomplished by the Sma3s algorithm. Based on a restrictive BLAST (Altschul et al., 1990), it gets sequence annotations in three sequential steps. The first is based on a similarity search against the existing annotated sequences with a sequence identity threshold of 90%. The second examines the similarity against orthologous sequences with at least the 75% of sequence identity. The third looks for all statistically significant alignments (Sander et al., 1991; Rost, 1999). The Sma3s analysis finally released the Gene ontology (GO) terms, Swiss-Prot keywords and pathways, InterPro domains, and IntAct interactions (UniProt Knowledgebase Release 2014_04) for each sequence.
All sequences that were not annotated by Sma3s were processed in a further annotation step by Blast2GO (Götz et al., 2008), which provides a BlastX similarity search against the NCBI database.
Additional information about protein domains, sequence function, pathways and biological processes was obtained through the NCBI RefSeq, UniProtKB and Swiss-Prot, TAIR, InterPro, AmiGO and KEGG databases.

5.2.8 Data mining

The annotated datasets of differentially expressed genes (FC > 2 and < 0.5 and p-value < 0.05 Student’s t-test) were imported into the MeV microarray analysis software (Multi Experiment Viewer, www.tm4.org; Saeed et al., 2003) to perform the KMC: K-Means/K-Medians Clustering analysis (Soukas et al. 2000). To get an hypothesis about the number of clusters to partition the genes into, 8 to 16 cluster numbers were compared. Finally, dataset grouping in ten clusters by Euclidian average linkage clustering was chosen and the corresponding expression graph was visualized in MeV.
Eight clusters were selected for further analysis to assess over-represented Gene Ontology (GO) terms by the Cytoscape plugin BiNGO (Biological Networks Gene Ontology) (Maere et al., 2005).
Additional statistics and charts were produced by XLSTAT™ software.

5.3 Results

5.3.1 Analysis of the microarray datasets

DNA microarrays were hybridized with labeled cRNA prepared from undamaged olives of the varieties ‘Ortice’ and ‘Ruveia’ and corresponding samples damaged by the olive fly. Three RNA technical replicates were reverse transcribed, amplified, labelled and hybridized on CombiMatrix CustomArray ™ 90K arrays (CombiMatrix Corporation).
A total of 14209 transcripts were identified as differentially expressed (FC≠1, p-value < 0.05 Student’s t-test), corresponding to 22.98% of all transcripts represented on the microarray.
Applying a fold-change cut-off > 2 and < 0.5 and a p-value < 0.05 (Student’s t-test), 52 differentially expressed transcripts were identified in ‘Ortice’ and 2477 in ‘Ruveia’ after the attack of *Bactrocera oleae*.
The PCA (Figure 5.1) of the four experimental conditions allowed the visualization of the changes that occur in ‘Ortice’ and ‘Ruveia’ varieties at the transcriptional level once the olive is actively damaged by the olive fruit fly larva.

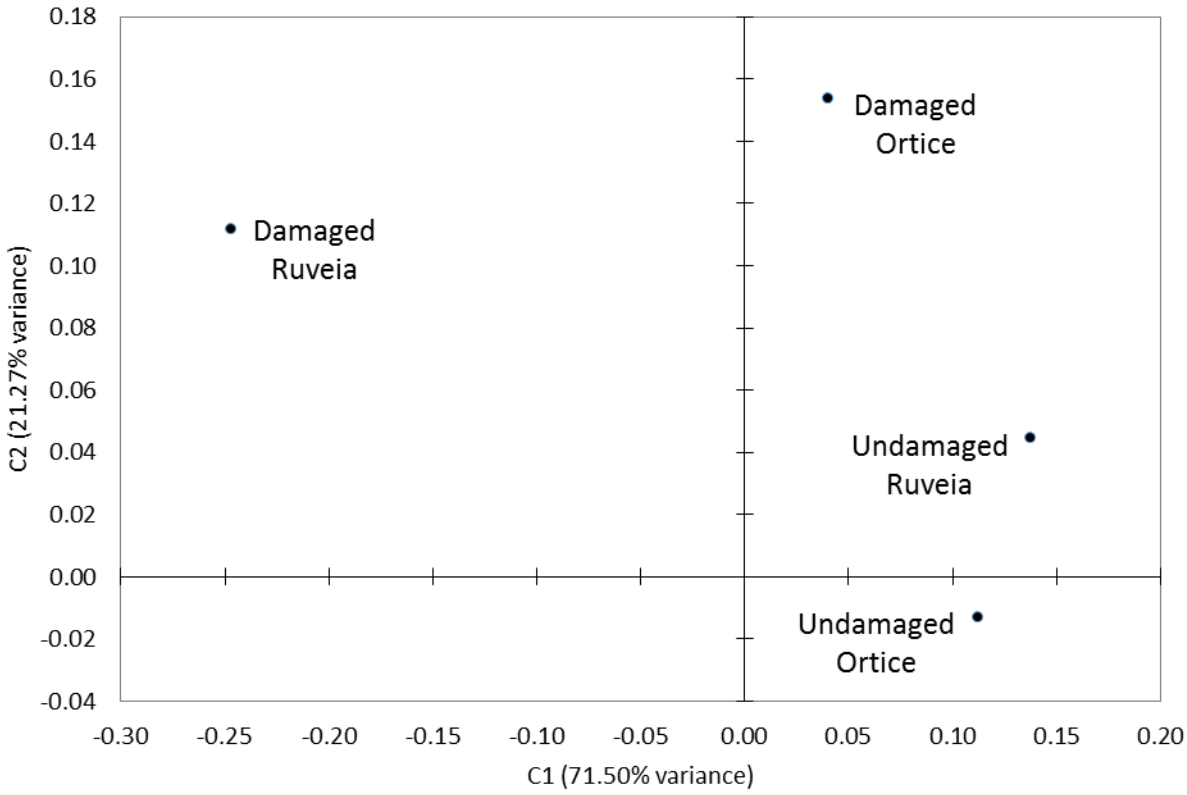

Figure 5.1. Principal Component Analysis (PCA) of differentially expressed genes (FC≠1, p-value < 0.05 Student’s t-test) obtained through the GeneSpring software. The graph represents the score plot of the eigen values determined on the basis of the averages of three array replicates for each experimental point: ‘Ruveia’ damaged, ‘Ruveia’ undamaged, ‘Ortice’ damaged and ‘Ortice’ undamaged olive samples. The variance explained by the first two principal components is 92.77% (the percentage of variance explained by each component is shown within brackets).

The PCA, whose first two principal components explained 92.77% of the variance, displayed that the transcriptomic profiles of the damaged drupes differ from their undamaged counterparts. The distribution indicates that the most severe transcriptomic changes are induced by the attack of *Bactrocera oleae* in 'Ruveia' drupes. Furthermore, the PCA analysis indicated that, as expected, the two control (undamaged olive) transcriptomic data cluster together. Finally, the analysis indicated that *B. oleae* attack increases the transcriptomic response in the two cultivars under examination, underlying that these different olive varieties have different response to the fruit fly.

To further investigate the commonality and specificity between the olive varieties and their response, differentially expressed genes were first identified in all pairwise comparisons. Their presence in more than one comparison is reported in Figure 5.2

Venn diagrams depict the overlap between differentially expressed genes for a FC > 2, in panel A, and for FC < 0.5, in panel B (p-value < 0.05 Student's t-test). In other words, the image illustrates what happens at a transcriptomic level when olive fly larvae damage the drupes in both 'Ortice' and 'Ruveia' varieties, but also what are the differences in the transcriptomic pattern of the undamaged olives as well as the ones between damaged olives of the two varieties. Panel A of the picture, showing up-regulated genes (FC>2; p<0.05 Student's t-Test), displays how only B (DR/UR) and C (DO/UO) as well as B (DR/UR) and D (DR/DO) have overexpressed genes in common. The most relevant differences arise from the first intersection (B ∩ C), where only 3 genes result, indicating that damaged 'Ruveia' drupes up-regulate more unique genes (354) than the 'Ortice' counterpart (43) when attacked by the olive fly. The second comparison point out that, among the 1010 overexpressed genes characterizing DR, 654 are also overexpressed respect to DO, while 353 only in 'Ruveia' olives after the damage. The 51 genes that discriminate UR from UO, don't have interactions with the other conditions, thus demonstrating that they don't contribute to the defense response to the insect.

Panel B of Figure 5.2 depicts down-regulated genes (FC<0.5; p<0.05 Student's t-Test) and reveals more complex intersections than for up-regulated genes. The absolute number of underexpressed genes in 'Ruveia' (1543) after the damage of *Bactrocera oleae* is much higher than in 'Ortice' (187). Most of the genes (141) that are down-regulated in 'Ortice' have the same trend also in 'Ruveia' after the insect damage, as B ∩ C, (B ∩ C) ∩ D, (A ∩ B) ∩ C, and (A ∩ B) ∩ (C ∩ D) intersections reveal. Among these genes, 2 are also down-regulated in UR respect to UO and 2 are underexpressed in all comparisons, revealing their role in the response to the olive fly. Conversely, 'Ruveia' drupes down-regulate much more unique genes (878) than the 'Ortice' counterpart (41) when attacked. B ∩ D intersection shows that, among the total number of down-regulated genes in DR, 524 are also underexpressed respect to DO. The A area that doesn't intersect any of the others include only 3 genes, revealing that these don't play a role in plant response to the insect. Overall, the analysis indicated that the olive response in the susceptible and tolerant olive varieties under investigation is different in quantitative (i.e. the number of genes) and qualitative (i.e. the genes) terms.

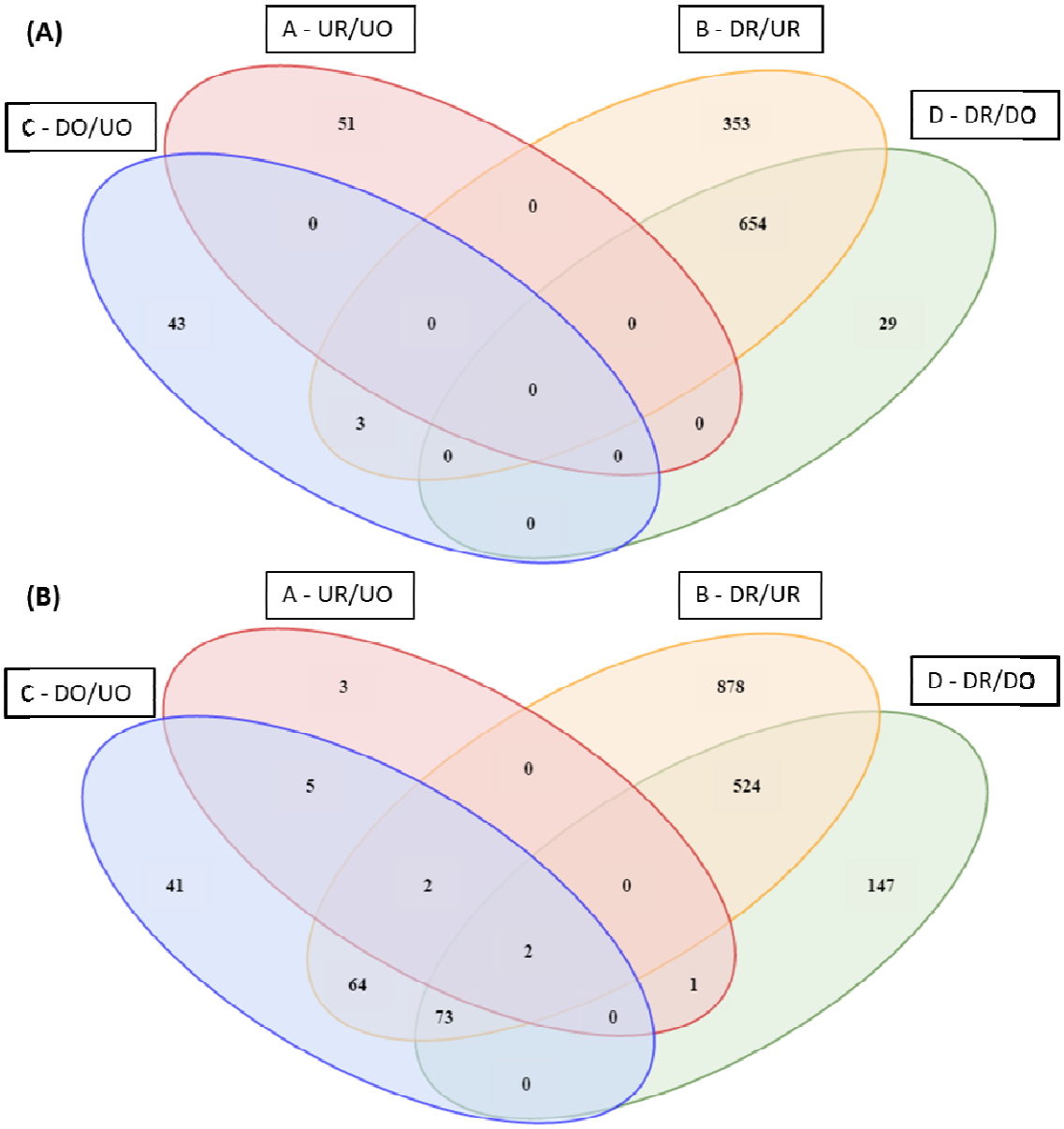

Figure 5.2. Venn diagram of the differentially expressed transcripts in 'Ruveia' and 'Ortice' olive samples after the attack of *Bactrocera oleae*: upregulated (A) and down-regulated (B). The areas correspond to the transcriptomic changes occurring in damaged 'Ortice' olives (DO) vs undamaged ones (UO), in undamaged 'Ruveia' olives (UR) vs undamaged 'Ortice' ones (UO), in damaged 'Ruveia' olives (DR) vs undamaged 'Ruveia' olives (UR) and in damaged 'Ruveia' olives (DR) vs damaged 'Ortice' olives (DO). (I) Number of specific and shared up-regulated genes (FC>2; p<0.05 Student's t-Test). (II) Number of specific and shared down-regulated genes (FC<0.5; p<0.05 Student's t-Test).

5.3.2 Transcript clustering

To group differentially expressed genes across the four experimental conditions according to their expression pattern, the dataset of the 2497 differentially expressed genes (FC > 2 and < 0.5 and p-value < 0.05 Student's t-test) were processed by the K-Means/K-Medians Clustering analysis (Figure 5.3). In each box, grey lines represent genes, whose expression levels varies in the four experimental conditions, namely undamaged 'Ortice', damaged 'Ortice', undamaged 'Ruveia' and damaged 'Ruveia'. A centroid line is overlaid on the individual expression graphs (pink), to

show the mean expression level of genes belonging to each cluster. The plot indicates distinct expression patterns: cluster 1 consists of transcripts which are expressed at low levels in undamaged olives of both ‘Ortice’ and ‘Ruveia’ varieties, at quite higher levels in damaged ‘Ortice’ and very high levels in damaged ‘Ruveia’; clusters 6 and 7 group transcripts are characterized by a low expression in all experimental points but in damaged ‘Ruveia’, where they are highly expressed; cluster 2 contains transcripts that are constitutively transcripted in all conditions with the exception of damaged ‘Ruveia’ where they are highly underexpressed; cluster 3 includes transcripts that are overexpressed only in damaged ‘Ortice’; cluster 4, 8 and 9 include those transcripts that are expressed at high to moderate levels in undamaged olives of both ‘Ortice’ and ‘Ruveia’ and are underexpressed in damaged olives of both varieties with much strengthens in damaged ‘Ruveia’; cluster 5 and 10 with no clear trend.

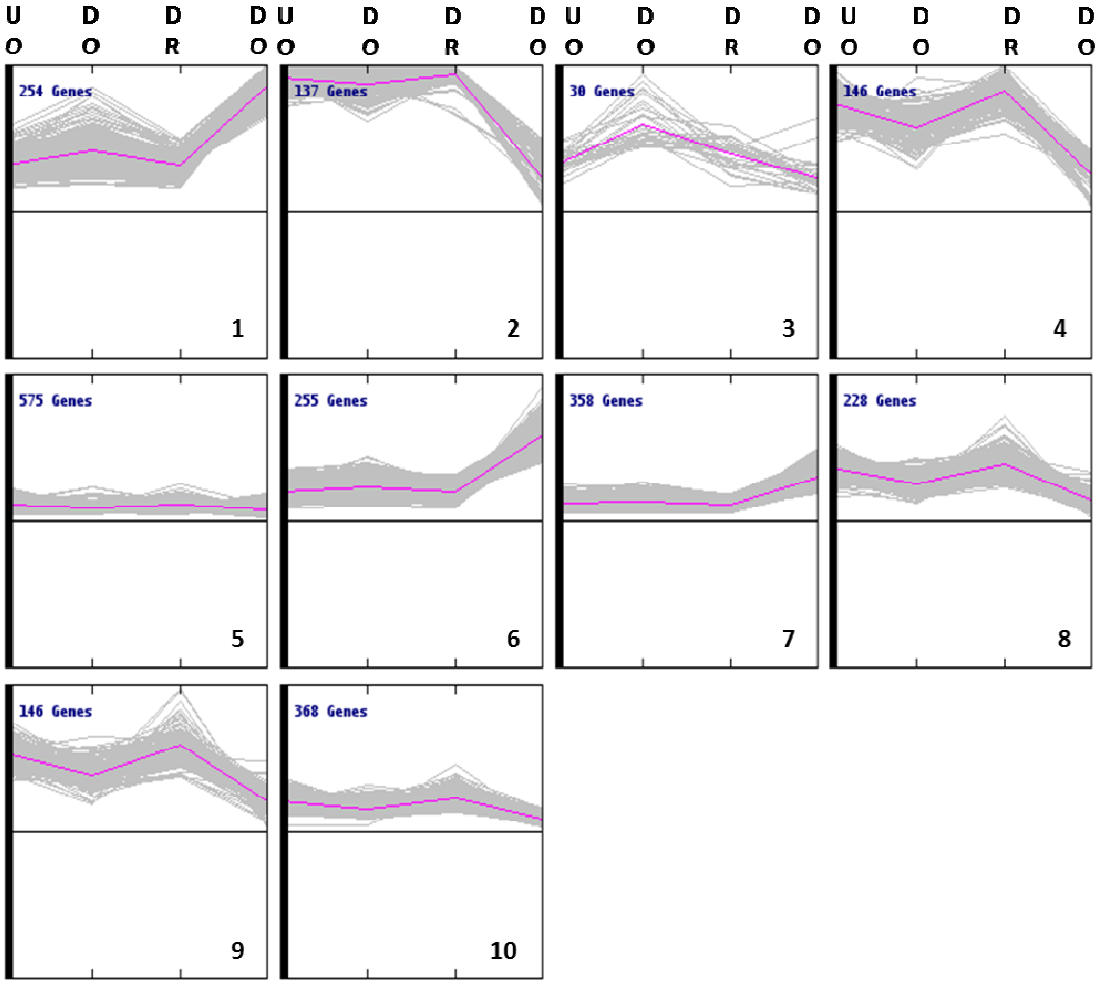

Figure 5.3. Expression graphs of 2497 genes that were differentially expressed (FC > 2 and FC < 0.5; $p < 0.05$ Student's t-test) in ‘Ortice’ and ‘Ruveia’ olives as a consequence of the attack of the olive fly. The ten patterns, inferred by K-means clustering, show the average expression pattern of all genes in each cluster (pink line) and the expression pattern of each gene belonging to the cluster (grey lines). In the left upper part of the boxes the total number of genes in each cluster is indicated. In the right lower part, the progressive number of clusters is reported.

Hence, only eight clusters (corresponding to the numbers 1, 2, 3, 4, 6, 7, 8, 9 reported in the right lower part of the boxes) were chosen to assess over-represented Gene Ontology (GO) terms. The results of the functional categorization performed by BINGO are depicted in Figure 4.

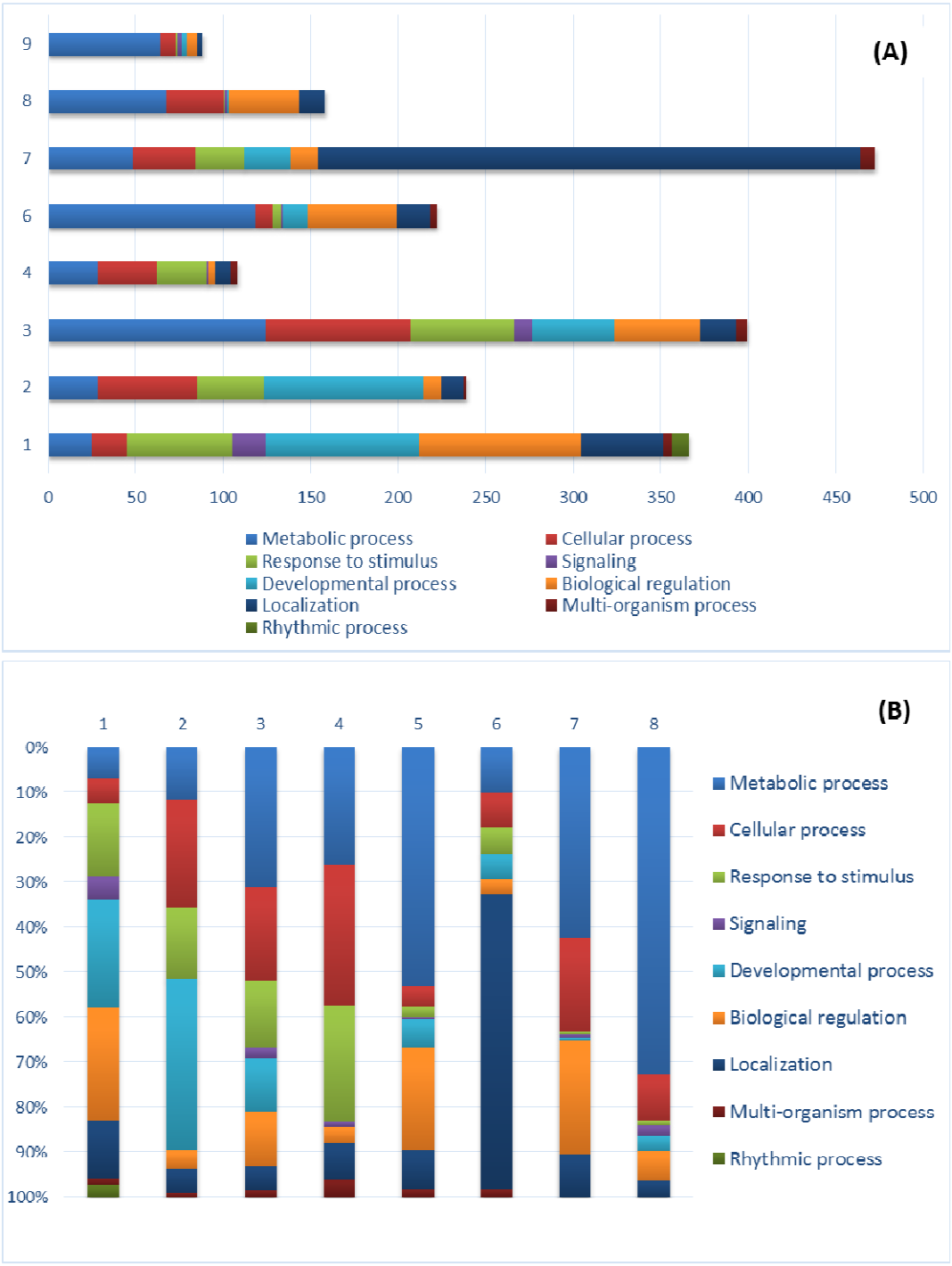

Figure 5.4. Over-represented functional categories for significantly differentially expressed genes (FC > 2 and FC<0.5; $p < 0.05$ Student's t-test) in 'Ortice' and 'Ruveia' olives after the attack of *Bactrocera oleae*. The bars represent the 8 clusters (numbered 1, 2, 3, 4, 6, 7, 8 and 9) chosen from those inferred by K-means clustering. Colored boxes indicate the GO biological processes summarized by the indicated terms. (A) Absolute number of biologically enriched genes for each GO biological process. (B) Relative number of biologically enriched genes for each GO biological process.

Although in the other clusters the majority of GO biological processes are represented, some clusters (1 and 3) are more complex. Only cluster 1 (genes overexpressed in 'Ortice' or 'Ruveia' after fruit fly attack) includes all 9 GO categories considered. Cluster 2 (characterized by genes that are highly underexpressed in 'Ruveia' after attack) is mainly characterized by an high percentage of genes involved in developmental processes. In cluster 6 (gene overexpressed in 'Ortice' after attack), most of the genes belongs to the localization category.
The representation of the absolute number of biologically enriched genes (Figure 5.4 A), gives an idea of the size of the clusters, together with the representativeness of each GO biological process. The histogram of the relative number of biologically enriched genes (Figure 5.4 B), shows the contribution of GO biological process in each clusters.

5.3.3 Identification of functional categories

The investigation of the olive transcriptome following the attack of *Bactrocera oleae* highlighted the activation of a wide and complex response, as the transcriptional reconfiguration involved a broad range of biological processes in a different way.
To underline the biological objective to which the differentially expressed genes contribute, we used the Sma3s tool to provide Gene Ontology (GO) terms association. Sma3s functionally annotated 35866 sequences out of the 39740 with blast hits represented in the whole microarray database. Among the differentially expressed transcripts represented on the microarray with FC > 2 and < 0.5 and p-value < 0.05 (Student's t-test), 1427 genes were annotated by Sma3s. The remaining sequences were processed by a further annotation step with the Blast2GO tool, which allowed the annotation of 167 ESTs more. The results (Appendix: Tables 5.1 to 5.4), whose processing included a manual search step of information about each differentially expressed gene, were finalized with the manual classification of the genes according to the functions they are involved in. For sake of simplicity, genes that participate in more than one biological processes are presented only once considering their prevalent role in plant metabolism. Ten functional categories were identified: cellular organization, transcription, cell wall modification, stress and defense responses, signal transduction, photosynthesis, primary metabolism, protein metabolism, secondary metabolism and transport.
A total of 113 genes coding for proteins putatively involved in development, maintenance and cellular organization were differentially expressed in at least one of the two varieties 'Ortice' and 'Ruveia' after the attack of the olive fly. The cellular organization category includes genes involved in the organization of the cytoskeleton (e.g. *Profilin*), circadian rhythm, control system of the cell cycle (e.g. *Cyclin-B1-1*), organelle division and morphology (e.g. *Dynamin*), cell mobility (e.g. *Actin*), etc.
The transcription functional category, which is the second largest functional category with 189 genes, comprised genes related to regulation of transcription (WRKYs, ERFs, bHLHs, bZIPs, MYBs, etc.), histone modification (*Histone regulator protein*, *Histone deacetylase 19*, etc.), splicing factors (*Pre-mRNA branch site p14-like protein*, *Chloroplastic group IIA intron splicing facilitator CRS1*, etc.), etc.
Genes affected by *B. oleae* infestation were also related to cell wall modification. This category included 44 genes involved in cellulose biosynthesis (e.g. *CESA3*), pectin modification (*Glycoside hydrolase family 28 family protein*, *Pectinesterase 1*, *AXY3* , etc.), extension of plant cell wall (e.g. *Alpha-expansin-1*), etc.

One hundred fifty differentially expressed genes were annotated as involved in stress and defense responses, namely those genes whose function is related to oxidative stress (e.g. *G-type lectin S-receptor-like serine/threonine-protein kinase B120*), ROS detoxification (*Glutathione S-transferase*, *PRXIIC*, etc.), biotic and abiotic stress responses (e.g. *Heat shock protein 82*) and defense responses (e.g. *Ethylene response factor 3*).
Following *B. oleae* attack, 120 genes related to signal transduction were differentially expressed. Among these, several genes that play a role in the signaling pathways activated by ethylene (e.g. *Serine/threonine-protein kinase CTR1*), jasmonic acid (e.g. *Peptidyl-prolyl cis-trans isomerase CYP38*), abscisic acid (e.g. CBL-interacting protein kinases), phosphatidylinositol (e.g. *Phosphoinositide phospholipase C 2*), calcium (e.g. *Glutamate receptor 2.2*), etc.
Differentially expressed genes included also those associated with photosynthesis (24 genes), such as *PSI type II chlorophyll a/b-binding protein* and *Cytochrome c oxidase assembly protein SURF1*.
Primary metabolism category was the third largest functional category, after that of protein metabolism and transcription. The 178 differentially expressed genes belonging to this group are involved in the metabolism of carbohydrates (*Beta-galactosidase 1*, *Sucrose phosphate synthase 1F*, etc.) and lipids (*Sphinganine C(4)-monooxygenase 1*, *3-ketoacyl-CoA synthase 19*, etc.), as well as nucleoside metabolic processes (e.g. *5'-methylthioadenosine nucleosidase*), biosynthesis of amino acids (e.g. *Glutamine synthetase cytosolic isozyme 2*), etc.
Protein metabolism is the largest functional category, with 213 represented genes involved in translation (ribosomal proteins, *Elongation factor 2*, *Aminoacyl-tRNA synthetase*, etc.), protein modification (A*spartyl aminopeptidase*, *Subtilisin-like protease*, etc.), ubiquitination (*Plant U-box protein 34*, *Ubiquitin-conjugating enzyme E2 12*, *SKP1-like protein 16*, etc.) and autophagy (transducin family proteins, *Autophagy protein 5*, etc.).
In secondary metabolism group were included 44 genes related to the production of metabolites that, not essential for plant growth, may act as signal or defense molecules, or be related to other functions (Chalcone synthase, Violaxanthin de-epoxidase, Cytochrome P450 77A4, etc.).
The transport category included 162 genes coding for proteins involved in metal ion transport (e.g. *Copper chaperone ATX1*), vescicle-mediated transport (e.g. Coatomer subunit gamma), protein transport (*Short-chain dehydrogenase TIC 32*, *SEC13-like protein*), carbohydrate transport (*Probable plastidic glucose transporter 1*), etc.
The relatively high number of ESTs with unassigned or unknown function (365 transcripts), which were omitted from the present thesis, may be explained by the lack of the olive complete genome sequence.

5.3.4 Functional classification of differentially expressed genes in 'Ortice' and 'Ruveia'

Functional classification of differentially expressed genes in 'Ortice'

'Ortice' damaged olives showed thirteen upregulated transcripts, seven of which were annotated and showed fold changes (FC) around 2 (Appendix: **Errore. L'origine riferimento non è stata trovata.**). Among these, two transcription related genes: *Hydroxyproline-rich glycoprotein*, which acts as a GR-protein (RNA polymerase II-associated factor 1), and *Pentatricopeptide repeat-containing protein*,

whose gene family in plants is known to code for proteins involved in a broad range of functions related to transcription (Barkan and Small, 2014).
The genes *Protein argonaute 1* (translation initiation factor 2c), *JCGZ_10098* (whose sequence was cited in *Jatropha curcas* L. seedlings response to salt stress; Zhang et al., 2014) and *SUN1*, involved in maintaining the elongated nuclear shape of epidermal cells, were upregulated following olive fly attack too.
The attack of *Bactrocera oleae* determined the down-regulation of thirty-eight 'Ortice' transcripts, all repressed around two times in damaged drupes (Appendix: **Errore. L'origine riferimento non è stata trovata.**). Among the thirty genes with known function, five are involved in the activation of stress and defense responses: *Blight resistance protein RPI* (G0MWCVW04IKBOH), that triggers a defense system which restricts the pathogen growth; *Glutathione transferase* (G0MWCVW03GLQFI), involved in systemic acquired resistance (SAR) and oxidation-reduction processes; *Universal stress protein A-like protein* (contig12675), whose role is to enhance the rate of cell survival during prolonged exposure to stress conditions, and *Desiccation protectant protein Lea14 homolog* (contig10312). Two genes are involved in signal transduction: *Regulator of G-protein signaling 1* (G0MWCVW03F14TN) and *Serine-threonine protein kinase* (G0MWCVW04H37A1). Five differentially expressed genes were annotated as involved in primary metabolism, like *Glycerate dehydrogenase* (G0MWCVW04JAZS7), and two in photosynthesis: *Monogalactosyldiacylglycerol synthase 2* (contig06309), involved in the synthesis of the major structural component of photosynthetic membranes, and *Ferrochelatase- chloroplastic-like* (G0MWCVW01BXEOG) which play a role in dealing with oxidative stress and wound-induced supply of heme to defensive hemoproteins. Two genes were related to secondary metabolism: *S-adenosylmethionine-dependent methyltransferases* (G0MWCVW03G7TPC), which is involved in ethylene biosynthesis, and *Cytochrome p450 family member* (G0MWCVW02DQHWH), which induces synthesis of volatile compounds that affect chemical ecology and insect interactions. Down-regulated list included also three genes associated with transcription, two with cellular organization, one with transport (*Sorting nexin 2A* - G0MWCVW04JWUKU - involved in vesicular trafficking from endosomes to the vacuole) and five with protein metabolism.

Functional classification of differentially expressed genes in 'Ruveia'

Conversely, 'Ruveia' shows a wide range of transcriptomic changes as a consequence of the attack of *Bactrocera oleae* (Appendix: **Errore. L'origine riferimento non è stata trovata.** and **Errore. L'origine riferimento non è stata trovata.**).
Remarkably, fifty stress and defense response genes are up-regulated. Ninety-five genes of the same category are down-regulated, twenty of these involved in the negative regulation of defense response. Signaling that follows biotic or abiotic damages starts with the rapid release of ROS in the so-called respiratory (or oxidative) burst. *Translation initiation factor IF-2* (G0MWCVW03G1GN3), overexpressed 3.5 times after the attack of *B. oleae*, is involved in hydrogen peroxide biosynthetic process. The amount of differentially expressed genes (40 genes) involved both in metabolism of reactive oxygen species and in response to oxidative stress indicates the alteration of redox state of olive cells after olive fly attack. Among those genes, *NADPH:quinone oxidoreductase* (G0MWCVW02D8P26) is down-regulated in 'Ruveia'. *NADH dehydrogenase b2* (G0MWCVW01ANRL0), involved in

oxidation-reduction processes, is induced 5.7 times following the attack of olive fly. Many genes are involved in the regulation of the respiratory burst involved in defense response, for instance the transcriptional repressor *Cold induced zinc finger protein 2* (contig05089), the transcription factor *WRKY11* (contig03271), the *ERF5 Ethylene-responsive transcription factor 5* (G0MWCVW01BLALZ), the *probable serine threonine-protein kinase at1g18390* (G0MWCVW02EATOM), all over-expressed in 'Ruveia', and the down-regulated *G-type lectin S-receptor-like serine/threonine-protein kinase B120* (G0MWCVW03F56U6) and *MPK3* (G0MWCVW03GUTP2), involved in oxidative stress-mediated signaling cascade and innate immune MAP kinase signaling cascade.

As a consequence of the respiratory burst, cellular concentration of hydrogen peroxide increases to induce the defense responses. At the same time it represents a potential risk because of its oxidative power. Plant cell may activate enzymes to reduce the excess internal H_2O_2 level. Thirteen cell redox homeostasis genes are differentially expressed in 'Ruveia'. Among these, *Glutaredoxin family protein* (G0MWCVW03GOEZU) is induced more than 4.5 times after the attack of olive fly. Other over-expressed glutaredoxins are *Monothiol glutaredoxin-S14* (contig07474), *Monothiol glutaredoxin-S16* (G0MWCVW04I40Q4) and *Thioredoxin H4* (contig13866). Among the others, *Glutathione S-transferase* (contig05145) and *Glutathione reductase* (contig00953) are underexpressed in 'Ruveia'. Only five out to twenty-three genes with peroxidase activity are up-regulated, including *PRXIIC* (G0MWCVW01AR7YB) induced in 'Ruveia' with a FC of 4.2.

Due to the oxidation of membrane lipids, ROS may produce aldehydic toxic products to the plant cell, which in turn regulates the expression of detoxifying genes like aldehyde dehydrogenase and aldo-keto reductase. To this end, 'Ruveia' up-regulates *ALDH* (contig07747).

One up-regulated gene (with FC of 2.4), *Probable WRKY transcription factor 15* (G0MWCVW02D8S2N), plays a role in both respiratory burst involved in defense response and ethylene biosynthetic process interacting with an elicitor-responsive cis-acting element. Conversely, Acc synthase (G0MWCVW03HHIVR), involved in ethylene biosynthesis via S-adenosyl-L-methionine, is repressed 3 times in damaged 'Ruveia' olives. Nine up-regulated and eleven down-regulated genes, belonging to different functional categories, are involved in response to ethylene and ethylene-activated signaling pathway. As an example, *Histone deacetylase 19* (contig14107), contribute to the ethylene dependent pathogen resistance, and *Serine/threonine-protein kinase CTR1* (G0MWCVW02DW2GF), which acts as a negative regulator in the ethylene response pathway, are overexpressed, while *bZIP transcription factor 60* (contig09713) and *Constitutive triple response 3* (G0MWCVW02DSO30) are down-regulated in 'Ruveia' olives after olive fly attack.

Genes affected by *B. oleae* infestation were also related to the biosynthetic processes of other stress response signal molecules: salicylic acid (4 up-regulated and 10 down-regulated genes) and jasmonic acid (6 overexpressed and 1 underexpressed genes). For instance, *Probable methylenetetrahydrofolate reductase* (contig00213) is up-regulated 4 times after olive fly damage. A wide range of up and down regulated genes involved in response to salicylic acid or in salicylic acid mediated pathways (26 down and 8 up-regulated genes), as well as in response to jasmonic acid or in jasmonic acid mediated pathways (22 down and 12 up-regulated genes) were detected.

Among the salicylic and jasmonic acid mediated genes, *Peptidyl-prolyl cis-trans isomerase CYP38* (G0MWCVW03F6342), up-regulated with a FC of 3.6, is involved

in several processes related to response to chitin, response to cold, systemic acquired resistance, salicylic acid mediated signaling pathway, jasmonic acid mediated signaling pathway, etc.
Among the salicylic acid response genes, *26S proteasome non-ATPase regulatory subunit 2 homolog A* (G0MWCVW01CBLZH), required for plant growth, stress responses and innate immunity, is overexpressed. Conversely, *wall-associated receptor kinase 2-like* (G0MWCVW01BLTD5), which have significance in the control of cell expansion, morphogenesis and development, and G0MWCVW01B4IV6 are underexpressed. *Ankyrin repeat protein* is known to be involved in innate immune response, jasmonic acid mediated signaling pathway, negative regulation of defense response, negative regulation of programmed cell death, systemic acquired resistance, etc.
Among the response to jasmonic acid genes, *IAA-amino acid hydrolase ILR1-like 6* (G0MWCVW02DIEZV), involved in response to wounding, and *Chalcone synthase* (contig02051) involved in anthocyanin and flavonoid biosynthetic processes, are induced. *Ribulose bisphosphate carboxylase/oxygenase activase* (G0MWCVW02EBQF8) is repressed 20 times in damaged olives. Through the activation of RuBisCO, it is involved in several functions related to negative regulation defense response, detection of biotic stimulus, jasmonic acid mediated signaling pathway, systemic acquired resistance, etc.
Several disease resistance proteins (van Ooijen et al., 2007) were also differentially expressed after 'Ruveia' olive damage. *NBS-LRR type disease resistance protein* (G0MWCVW04IRIVQ), whose gene family is well known in plant-pathogen interactions (Belkhadir et al., 2004), was induced after the attack of *B. oleae*. *Disease resistance protein* (G0MWCVW01AK8QJ), belonging to TIR-NBS class involved in signal transduction, apoptosis, innate immune response, was down-regulated as well as *Disease resistance protein RGH4* (G0MWCVW01BQ2OX) and *Disease resistance protein BS2* (G0MWCVW02EDVOG), containing an NB-ARC domain and acting in the formation of the apoptosome.
Other relevant overexpressed genes related to defense and response to stress are *Serine carboxypeptidase-like 50* (G0MWCVW03FXPRU) and *Serine carboxypeptidase 3* (contig09176), both involved in proteolysis, and *Serine carboxypeptidase-like 51* (G0MWCVW04JXGC8), also required for response to endoplasmic reticulum stress and systemic acquired resistance. Conversely, *Serine carboxypeptidase-like 45* (G0MWCVW01CDW0O) is underexpressed.
Two heat shock proteins were induced too, the molecular chaperone *Protein BOBBER 1* (contig04057) required for the establishment of auxin gradients and *Luminal-binding protein 5* (contig01799), whose role is to facilitate the assembly of multimeric protein complexes inside the ER. The molecular chaperone *Heat shock protein 82* (G0MWCVW02DY1A6), involved in cell cycle control and signal transduction in response to stress, was instead repressed.

A total of 118 genes related to signal transduction were differentially regulated in 'Ruveia' olives after the attack of *B. oleae*, of which 48 were induced.
Plant mitogen-activated protein kinase (MAPK) cascades play key roles in signaling plant defense against pathogen attack and activate transcription factors associated with defense genes (Meng and Zhang, 2013).
After the attack of the olive fly, 'Ruveia' olives showed positive and negative regulation of several genes coding for these proteins. *MAP3K epsilon protein kinase* (G0MWCVW01A0W3Y) was induced by olive fly damage, while *MAPKKK*

serine/threonine-protein kinase EDR1 (G0MWCVW01BEOJA), *MAPKKK ANP1* (contig02350) and *MAPKKKe* (G0MWCVW03GFE73) were repressed. Interestingly, the second (*EDR1*) is involved in the regulation of a MAP kinase cascade that negatively regulates salicylic acid dependent defense responses, abscisic acid (ABA) signaling, and ethylene-induced senescence and its repression could induce the activation of these functions. The same goes for *Protein SGT1 homolog B* (contig02820), which play a role in the negative regulation of defense response, in plant innate immunity and plant disease resistance signaling by activating a MAPK cascade.

Among the regulatory elements inducing defense responses, 21 calcium ion-related genes are differentially regulated in 'Ruveia'. As an example, *Cyclic nucleotide-gated ion channel 1* (G0MWCVW04H7GKZ), a cation channel responsible for cAMP-induced calcium entry in cells and involved in the calcium signal transduction, is over-expressed in 'Ruveia'. *Calcium-dependent protein kinase 13* (G0MWCVW04IZTSX), which is repressed 25 times in damaged olives, plays a role in signal transduction pathways that involve calcium as a second messenger, while *Calcium-transporting ATPase 10* (G0MWCVW03FN16H), activated by calmodulin, translocate calcium from the cytosol into the endoplasmic reticulum. Among the proteins that are directly modulated by calcium ions, the voltage-gated cation channel *TPK1* (G0MWCVW03G478Y) and *ANN4* (contig05212), an annexin family protein involved in Golgi mediated secretion.

Because of the role of Calmodulin as an intermediate messenger protein that transduces calcium signals, Calmodulin-binding protein genes are also differentially expressed. The transcription factor Calmoduline binding WRKY11 (contig03271), which acts in intracellular signal transduction and respiratory burst involved in defense response, is induced 3 times in damaged 'Ruveia' olives. On the contrary, *Calmodulin-binding transcription activator 4* (contig12508) and *Calmodulin-binding transcription activator 3* (G0MWCVW01A584F) are repressed.

Five CBL-interacting serine/threonine-protein kinases were induced in damaged 'Ruveia' olives, namely *CIPK12* (contig01285), *CIPK1* (contig02784), *CIPK10* (G0MWCVW01AQSQY), *CIPK1* (contig05243) and *CIPK21* (G0MWCVW02EAM50). This kinases are activated in a calcium-dependent manner and are involved in signal transduction.

Three protein phosphatase 2c were overexpressed: *Probable protein phosphatase 2C 59* (contig12817), which modulates defense responses; *Protein phosphatase 2C 32* (G0MWCVW04JL9W0), involved in the regulation of meristem structural organization; *Probable protein phosphatase 2C 55* (G0MWCVW04I94BM). Protein phosphatase 2C 70 (KAPP) (contig07360), involved in protein dephosphorylation, and Protein phosphatase 2C 56 (G0MWCVW02DCBZJ), a key repressor of the abscisic acid (ABA) signaling pathway that regulates numerous ABA responses, were repressed.

Remarkably, *Ethylene receptor 1* (G0MWCVW01B931E) and *Ethylene receptor 2* (G0MWCVW04JLM0P), which act as negative regulators of ethylene signaling, are both repressed in damaged 'Ruveia' olives more than 2 times respect to the undamaged ones, indicating the unlocking of ethylene signaling after the attack of the olive fly.

Transcription factors associated with auxin-activated signaling pathway are also repressed: *Auxin-responsive family protein* (contig07763), *Auxin response factor* (G0MWCVW01B706O), *Auxin-responsive protein* (G0MWCVW01ASYLD) and *Auxin-responsive protein IAA8* (contig07467).

A total of 184 differentially expressed genes were annotated as for their involvement in functions related to transcription in ‘Ruveia’ olives after the attack of *B. oleae*. The induced genes were 73.

WRKY family, one of the largest of plant transcriptional regulators, includes important actors of signaling pathways that modulate plant processes (Rinerson et al., 2015; Rushton et al., 2010). Among the transcription factors included in this functional category, eight WRKY a listed.

Four WRKY proteins were induced: *WRKY dna-binding protein* (G0MWCVW04H4H4U); *Putative uncharacterized protein WRKY_12* (G0MWCVW04IWW1M), related to anthocyanin-containing compound biosynthetic process; *WRKY11* (contig03271), involved in respiratory burst involved in defense response; *Probable WRKY transcription factor 15* (G0MWCVW02D8S2N), associated to response to chitin, ethylene biosynthetic process and respiratory burst involved in defense response.

WRKY transcription factor 23 (G0MWCVW04JZHSX), WRKY transcription factor IId-4, WRKY53-superfamily (G0MWCVW04IO9ZZ) and Probable WRKY transcription factor 71 (contig05996) are repressed.

Several AP2 transcription factors, belonging to the ERF (ethylene response factor) family (Nakano et al., 2006) are also induced: *WIN1-like protein* (G0MWCVW04I1K6H), involved in wax and cutin biosynthesis; *ERF5* (G0MWCVW01BLALZ) which plays a role in intracellular signal transduction, respiratory burst involved in defense response, response to chitin and response to cold; *Dehydration-responsive element-binding protein 2C* (contig05908), required for abscisic acid (ABA) signal transduction, ABA-mediated glucose response, and hexokinase-dependent sugar responses in defense response, resistance to drought and salt (Sakuma et al., 2002); *Ethylene response factor 3* (contig13605) involved in defense response, ethylene-activated signaling pathway and response to abscisic acid. Conversely, *Constitutive triple response 3* (G0MWCVW02DSO30) is repressed.

Other transcription factor families are represented among those up or down-regulated after the attack of the olive fly, such as bHLH and MYB plant transcription factors (Feller et al., 2011). Among the basic helix-loop-helix (bHLH) DNA-binding superfamily proteins, *AtbHLH103* (G0MWCVW02EJ217), which is a positive regulation factor of flavonoid biosynthetic process, protein targeting to membrane and regulation of plant-type hypersensitive response, was induced, along with *Anthocyanin regulatory Lc protein* (G0MWCVW02DXP39), involved in leaf morphogenesis and regulation of flower development. *bHLH140* (G0MWCVW01AVHT8), related to cell cycle and xylan biosynthetic functions, was down-regulated. Among the MYB proteins, transcription factor *MYB44-like* (G0MWCVW04JTISI) was overexpressed while transcription repressor *MYB4* (G0MWCVW03G6AFB), involved in regulation of protection against UV which responde to UV-B, jasmonic acid, salicylic acid and wounding, was repressed 2.5 times in damaged olives.

NAC family proteins act as transcription factor for a wide range of responses to biotic and abiotic stress (Hegedus et al., 2003). Following *B. oleae* attack, *Nac domain-containing protein 100-like* (G0MWCVW04JNCQV) transcription factor, involved in senescence and transition between active cell division and cell expansion, was induced. *Nac domain-containing protein isoform 1* (contig05850) and *Early-responsive to dehydration protein* (contig07007), involved in response to desiccation, were instead repressed.

Ten pentatricopeptide repeat-containing proteins, involved in a broad range of functions related to transcription (Barkan and Small, 2014), were differentially expressed too.

B. oleae infestation determined modulation of the expression of 44 genes involved in the metabolism and remodeling of the cell wall, 18 of which were overexpressed.
Following *B. oleae* attack, *CESA3* (G0MWCVW03FWP1T) is induced with a FC of 3.4. This gene codes for a cellulose synthase isomer, which plays an important role in the synthesis of cellulose. Four polygalacturonase (G0MWCVW03HEZXX, G0MWCVW01BQVZ3, G0MWCVW04JFEB7, contig07350), which play a role in carbohydrate metabolic process and cell wall organization through the hydrolysis of galactosiduronic linkages in pectate and other galacturonans, were induced. Another protein of the same family, *Polygalacturonase ADPG2* (G0MWCVW01BR1T6), with a role in cell wall modification involved in floral organ abscission and fruit dehiscence, was underexpressed.
The repression of *Pectinesterase 1* (G0MWCVW02DR0YF), which plays a role in cell wall metabolism during fruit ripening, was revealed, as well as those of two xyloglucan metabolism genes: *Alpha-xylosidase 1* (contig14286), which is involved in starch metabolic process, xylan catabolic process, xyloglucan metabolic process and plant-type cell wall organization, and *Xyloglucan galactosyltransferase KATAMARI1* (G0MWCVW04JTW7H), which codes for a dual-function protein responsible for actin organization and synthesis of cell wall materials and is involved in salicylic acid mediated signaling pathway.
Two expansin family genes are also induced (G0MWCVW02EXH06 and contig10205) and the corresponding proteins cause loosening and extension of plant cell walls by disrupting non-covalent bonding between cellulose microfibrils and matrix glucans. These findings are consistent with previous studies indicating that the thickness of cell walls could be the initial barrier against insect attack (Barros-Rios et al., 2011, Vorwerk et al., 2004). It is possible to hypothesize that the strengthening of the cell wall is induced as a direct consequence of the insect attack. To this end, the alteration of chemical composition of the wall (pectins, cellulose, etc.) occurs in order to reinforce the external barrier against pathogens and insects (Kuśnierczyk et al., 2008; Hückelhoven, 2007).

After the attack of the olive fly, the ‘Ruveia’ drupe responds by modulating the expression of genes that code proteins involved in photosynthesis, with a total of 22 differentially expressed genes.
Although the literature reports the trend of plants attacked by insects to decrease the expression of genes related to photosynthesis (Bilgin et al., 2010), half of the related genes were induced and half repressed in damaged ‘Ruveia’ olives.
Among the overexpressed genes there were *Protein PHYLLO* (G0MWCVW03GZUSA), required for phylloquinone (vitamin K1) biosynthesis; *Photosystem II 22 kDa protein* (contig04024), coding for a protein associated with photosystem II (PSII) involved in non-photochemical quenching to protect the plant against photo-oxidative damage; *Protein Low PSII Accumulation 1* (G0MWCVW04IKGLQ), coding for a chaperone required for photosystem II (PSII) assembly; *AT1G67700 protein* (contig07687) involved in chlorophyll biosynthetic process, photosynthesis and photosystem II assembly; *Metalloprotease m41 ftsh* (G0MWCVW02DIUOO), involved in PSII associated light-harvesting complex II catabolic process and photosystem II repair; *60S ribosomal protein L10*

(G0MWCVW03HJU0Z), which plays a role in photosystem II assembly and thylakoid membrane organization.
Conversely, other genes of the same functional category were underexpressed: *Photosystem I reaction center subunit V* (G0MWCVW04I2ME0), *Photosystem I assembly protein Ycf3* (G0MWCVW02EZOWE); *Protease Do-like 2* (G0MWCVW02DPB4E), a serine protease involved in photosystem II repair; *Fructose-1,6-bisphosphatase* (contig03119), related to chlorophyll biosynthetic process, photosynthetic electron transport in photosystem I, photosystem II assembly, and thylakoid membrane organization; PsbP-like protein 1 (PPL1), which plays a role in photosystem II assembly; PSII 43 kDa protein (contig00007), coding for one of the components of the core complex of photosystem II.
It was also observed the down-regulation of *Phytochrome B* (G0MWCVW01B50GX), a regulatory photoreceptor responsible for many functions, among which phosphorelay signal transduction system, photomorphogenesis and photosynthesis, and *ACD1-like protein* (G0MWCVW03GTT6Z), involved in the protochlorophyllide-dependent import of the precursor *NADPH:protochlorophyllide oxidoreductase A*.

One hundred seventy-three differentially expressed genes were annotated as involved in primary metabolism in 'Ruveia' olives following the attack of the olive fly. A total of 61 genes were overexpressed. This indicates that almost twice as many transcripts are down-regulated. The data indicated that at least some components of primary metabolic pathways are directly or indirectly involved in the defense responses in olive.
Regarding the metabolism of carbohydrates, genes coding for several enzyme families are represented. Taking into account epimerases, *UDP-D-glucuronate 4-epimerase* (G0MWCVW04H5VIF), involved in the synthesis of pectic cell wall components, was induced by olive fly attack. Conversely, *Aldose 1-epimerase family protein* (contig01584), which plays a role in carbohydrate metabolic process, and *UDP-glucuronate 4-epimerase 2* (contig13982), whose function is related to the synthesis of pectic cell wall components, were repressed. *Isoamylase 2* (G0MWCVW03HE3VI), related to amylopectin, glucosinolate and starch biosynthetic processes was induced. Among the beta-galactosidases, *Beta-galactosidase 1, glycosyl hydrolase family 35* (G0MWCVW01BBBJT), involved in carbohydrate metabolic process and hydrogen peroxide catabolic process, was induced, while *Beta-galactosidase 5* (G0MWCVW03G134V), whose role is related to acetyl-CoA metabolic process, brassinosteroid and sterol biosynthetic processes, was repressed. A raffinose synthase family gene (G0MWCVW04JOEWG) was overexpressed. This genes are also known as seed imbibition (Sip1) proteins and play critical roles in the acquisition of tolerance to desiccation and seed longevity. Two alpha-galactosidases (G0MWCVW01B5FFD and G0MWCVW04IWYNP) with raffinose alpha-galactosidase activity, acting in carbohydrate metabolic process and cell wall organization, were underexpressed.
Five beta-glucosidases were repressed: *Glucan endo-1,3-beta-glucosidase-like protein 2* (G0MWCVW01BIJUF) and *Beta-glucosidase G1* (G0MWCVW04JSR4T), involved in carbohydrate metabolic process; *Beta-glucosidase-like SFR2* (G0MWCVW04IMYFE), which plays a role in carbohydrate metabolic process, freezing tolerance and chloroplast protection; *Glucan endo-1,3-beta-glucosidase 5* (G0MWCVW04IYRVX), acting in carbohydrate metabolic process and defense response; *Glycosyl hydrolase family 1 beta glucosidase protein* (G0MWCVW04IX936), which plays a role in abscisic acid-activated signaling

pathway, carbohydrate metabolic process, defense response to insect and response to salt stress. *Alpha-xylosidase 1* (contig14286) involved in xylan catabolic process, xyloglucan metabolic process and cell wall biogenesis and organization, and *Alpha glucosidase-like protein* (G0MWCVW04IXBFB), related to carbohydrate metabolic process, were repressed too.
For what concerns lipid metabolism, several lipases and hydrolases were deregulated. Epoxide hydrolase (contig03186), Alpha/beta hydrolase fold protein (G0MWCVW02DTIVD) and Hydrolase, alpha/beta fold family protein (contig03772) were overexpressed after the attack of *Bactrocera oleae* in 'Ruveia' drupes. GDSL esterase/lipase At1g28580 (G0MWCVW02EOVXE), Lipase class 3 family protein (G0MWCVW04JHSUL), Lipase (contig10025) and Lipase class 3 family protein (G0MWCVW01BNTS0) were underexpressed.
Among the induced genes are included: *3-ketoacyl-CoA synthase 19* (contig00669), whose corresponding protein mediates the synthesis of VLCFAs from 22 to 26 carbons in length; *Reduced Oleate Desaturation 1* (contig04233) involved in triacylglycerols synthesis pathway; *Lecithin-cholesterol acyltransferase-like 3* (contig01824) related to lipid catabolic process; *Glycosyl transferase family 17 protein* (contig08228), whose product plays a role in galactolipid biosynthetic process; *Sphinganine C(4)-monooxygenase 1* (contig05404), involved in sphingolipid trihydroxy long-chain base biosynthesis; *Biotin carboxyl carrier protein of acetyl-CoA carboxylase* (contig05566), involved in several functions, such as fatty acid, brassinosteroid and polysaccharide biosynthetic processes, regulation of hormone levels, regulation of meristem growth, and cell wall biogenesis and organization; *C-14 sterol reductase* (G0MWCVW03GQWUV) involved in sterol and brassinosteroid biosynthetic processes and pentacyclic triterpenoid biosynthetic process.
Repressed genes comprise: *Enoyl-[acyl-carrier-protein] reductase* (contig02248), required for fatty acid and brassinosteroid biosynthetic process in normal plant growth; *Predicted protein* (G0MWCVW01BWQYJ), which plays a role in phosphatidylinositol biosynthetic process; *Phospholipase C* (G0MWCVW03GYVZ0) involved in lipid metabolic process; *Omega-6 fatty acid desaturase* (G0MWCVW04H3AH0), whose related protein acts in biosynthesis of 18:3 fatty acids, important constituents of plant membranes. A deeply repressed gene (5.2 times respect to the undamaged olive) was *Chromodomain-helicase-DNA-binding protein* (G0MWCVW03FXTRX), whose corresponding protein mediates glycerolipid biosynthetic process.

Protein metabolism is the largest functional category of differentially expressed genes in 'Ruveia' olives following the attack of *B. oleae*, with 75 up-regulated and 132 down-regulated genes. Most of the corresponding gene products play a role in translation. Eight ribosomal proteins were induced, such as the structural constituent of ribosome *60S ribosomal protein L10* (G0MWCVW03HJU0Z), and 17 were repressed, such as the *40S ribosomal protein SA* (G0MWCVW03GJHF4), required for the assembly and stability of the 40S ribosomal subunit and involved in response to osmotic and salt stress and response to temperature stimulus. Several aminoacyl-tRNA synthetase were also differentially expressed.
Twelve genes coding for proteasome proteins were differentially expressed. As an example, among the 6 overexpressed genes was included *26S proteasome non-ATPase regulatory subunit 2 homolog A* (G0MWCVW01CBLZH), acting in the proteasome-mediated ubiquitin-dependent protein catabolic process in response to misfolded protein and to salicylic acid. On the contrary, *20S proteasome subunit*

beta-3 (contig06857) was underexpressed. Twenty-eight genes coding for ubiquitin proteins were also differentially expressed. Five autophagy proteins were differentially expressed. For instance, among the three induced genes there was *Autophagy-related protein 18a* (G0MWCVW02DAOYC), whose coded protein is required for autophagy by autophagosome formation during nutrient deprivation, senescence and under abiotic stress, including oxidative, high salt and osmotic stress conditions. This protein cooperates with jasmonate and WRKY33 mediated signaling pathways in the regulation of plant defense responses. Quite a lot of genes belonging to the protein metabolism functional category coded for protein modification enzymes.
Among the others, the repressed *Probable aspartyl aminopeptidase* (contig01943) is likely to play an important role in intracellular protein and peptide metabolism. *Urease accessory protein UREG* (G0MWCVW01B2HS2), required for the maturation and activation of urease, was underexpressed too. Ten endopeptidase were deregulated. Among the metalloendopeptidase, *Probable mitochondrial intermediate peptidase* (G0MWCVW01CD3UM), involved in methylglyoxal catabolic process to D-lactate and proteolysis, was up-regulated. *Major surface glycoprotein-like* (G0MWCVW01BIJXG), *Protease ecfE* (G0MWCVW03HDZXI) and *Peptidase M50 family protein* (G0MWCVW02D68N7) were down-regulated.

The attack of the olive fly determines the alteration of genes involved in the secondary metabolism of the plant, with a total of 20 up and 23 down-regulated genes. Although secondary metabolism leads to the production of metabolites that are not essential for the normal growth and reproduction of the plant, it has a great ecological importance because it intervenes in the activation of several functions, such as defense mechanisms against herbivores and pathogens. The biosynthesis of secondary metabolites occurs when *Bactrocera oleae* attacks ‘Ruveia’ drupes, as the induction of several related genes showed.
Four Cytochrome P450 related genes are differentially expressed. The coded enzymes have a relevant defense role, as they are required for both the detoxification of toxic molecules and the biosynthesis of defense molecules (Mizutani and Ohta, 2010). *Cytochrome P450 77A4* (contig10995), which catalyzes the epoxidation of unsaturated fatty acids and is involved in cutin biosynthetic process, and *NADPH-cytochrome P450 reductase* (G0MWCVW02EOCAP), required for electron transfer from NADP to cytochrome P450, which mediates ER to Golgi vesicle-mediated transport and phenylpropanoid metabolic process in response to abscisic acid, were overexpressed. Conversely, *NADPH--cytochrome P450 reductase 1* (contig00497), whose role is to transfer electron from NADP to cytochrome P450 in microsomes and is involved in phenylpropanoid metabolic process in response to abscisic acid and oxidative stress, was underexpressed along with *Cytochrome P450 97B2* (G0MWCVW02EMLM7), involved in the oxidative degradation of various compounds.
G10H gene (contig00982), coding for an hydroxylase involved in monoterpenoid biosynthetic process and specifically in the biosynthesis of hydroxygeraniol, a precursor of the terpenoid indole alkaloids such as vinblastine and vincristine, was up-regulated after the attack of the olive fly. On the contrary, *Geraniol 8-hydroxylase* (G0MWCVW04H7D2K), required for the biosynthesis of hydroxygeraniol in monoterpenoid biosynthetic process, was down-regulated.
Twelve genes coded for protein related to polyamine biosynthesis and catabolism. Among those two arginine decarboxylase. On one hand, *ADC1*

(G0MWCVW02EDFRK), which encodes an arginine decarboxylase (ADC) that catalyzes the first step of polyamine (PA) biosynthesis and play a role in spermidine and putrescine biosynthetic processes in response to cold, oxidative stress and salt stress, was induced. On the other, *Spermine synthase* (G0MWCVW02EW8DW) involved in defense response to bacterium, polyamine and spermine biosynthetic processes, was repressed.
At1g55290 (contig08219), which encodes a protein required in scopoletin (coumarin) biosynthesis, was overexpressed, as well as Tropinone reductase (contig12711), which plays a role in alkaloid biosynthetic process. *Tropinone reductase 2* (G0MWCVW01AOCDA), involved in tropane alkaloid biosynthetic process, was instead underexpressed.
2-succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexene-1-carboxylate synthase (G0MWCVW03GZUSA), required for phylloquinone (vitamin K1) biosynthesis, was induced along with Oxoglutarate dehydrogenase (contig13252), required for tricarboxylic acid cycle.
After the attack of *B. oleae*, 'Ruveia' induces the production of oxylipins, as shown by the overexpression of *Violaxanthin de-epoxidase* (G0MWCVW03G9TNB) and *Beta-ketoacyl-ACP synthase II* (contig01714). Oxylipins represent an important response to insects (Vellosillo et al., 2007). Convesely, *Linoleate 9S-lipoxygenase A* (G0MWCVW03GOPJS), involved in oxylipin biosynthetic process, was repressed. Due to its multiple functions in fatty acid and lipid biosynthesis and metabolism, this enzyme plays a role in a number of diverse aspects of plant physiology, including growth and development, pest resistance, senescence and responses to wounding.

A total of 42 over and 68 underexpressed genes related to cellular organization were found in 'Ruveia' drupes damaged by the olive fly compared to undamaged ones.
Several of those genes are known to be involved in cytoskeleton organization. Profilin gene(contig09415) is up-regulated. The corresponding protein affects the structure of the cytoskeleton by influencing actin polymerization or depolymerization.
The gene coding for *Cyclase-associated protein 1* (G0MWCVW04IA4R1), an actin monomer binding protein that is involved in actin cytoskeleton organization and unidimensional cell growth, was underexpressed.
AtMYA1 Myosin heavy chain (G0MWCVW03GW2MT), involved in the cell cycle-regulated transport of various organelles and proteins for their segregation, was induced, as well as *Myosin-9 isoform x3* (G0MWCVW03GZU5O), required for the cell cycle-regulated transport of various organelles and proteins for their segregation. Other two genes (G0MWCVW03GBKZ5, G0MWCVW04IMZ6Z) coding for myosin heavy chains involved in Golgi localization, actin filament-based movement, root hair elongation, mitochondrion and peroxisome localization, were repressed, along with Myosin heavy chain IB (contig06614), which has motor activity activated by F-actin. Especially for the plant-pathogen interaction, many studies indicated that actin plays an important role in formation of physiological barrier in the site of infection and that actin dynamics has also a role in plant defense signaling.
1,3-beta-glucan synthase (G0MWCVW04IZVZG) was overexpressed. This enzyme is required for callose deposition in cell wall, microsporogenesis and regulation of cell shape but is supposed not to be involved in callose formation after wounding or pathogen attack.
Six induced and nine repressed genes encoding cyclin or cyclin-dependent proteins were found after the attack of *B. oleae*. These proteins are important in the control system of the cell cycle.

The gene for *Kinesin-like protein* (G0MWCVW01B07G3), involved in cell proliferation, microtubule-based movement and cytokinesis by cell plate formation was induced. Conversely the genes coding for *Kinesin-related protein* (G0MWCVW01BQA8W) and *Kinesin-related protein 11* (G0MWCVW01B80CK), both involved in microtubule-based movement, were repressed along with *125 kDa kinesin-related protein* (G0MWCVW02DOLZL), responsible for microtubule translocation by organizing the phragmoplast-specific arrays of microtubules.

A total of 73 and 88 genes coding for proteins putatively involved in transport were up-regulated and down-regulated respectively in 'Ruveia' damaged olives. A wide range of transport activities is represented, consistent with the wide physiological functions that are expected to be altered. For instance, two aquaporin genes were induced, coding for: *Aquaporin protein AQU20* (G0MWCVW01AKYVN), a membrane channel with transporter activity that selectively transport water, small neutral molecules, and ions out of and between cells, and *SYP61* (G0MWCVW04JVEU2), a syntaxin that coordinates the trafficking of plasma membrane aquaporin. The gene coding for the *Vacuolar protein sorting 55 family protein* (G0MWCVW02EKJNP), involved in phosphatidylinositol biosynthetic process and transport, was also up-regulated. Three genes that code for components of the *COPII coat* (contig00259, G0MWCVW02DGAB1, contig05454), which is required for promoting the transport of secretory, plasma membrane, and vacuolar proteins from the endoplasmic reticulum to the Golgi complex, were down-regulated. Another (G0MWCVW03F6PKR) was up-regulated. *Phosphoenolpyruvate/phosphate translocator 1* (contig12736), coding for an antiporter protein involved in anthocyanin-containing compound biosynthetic process and transport of phosphoenolpyruvate and purine nucleobases, was induced, as well as *Triose phosphate/phosphate translocator* (contig01124), which encodes a chloroplast translocator that transports triose phosphates derived from the Calvin cycle in the stroma to the cytosol for use in sucrose synthesis and other biosynthetic processes. On the contrary, *xylulose 5-phosphate phosphate* (G0MWCVW04IW4ER), required for phosphoglycerate transport and triose phosphate transmembrane transport, was repressed.
Many genes for carbohydrate transporters were up-regulated, such as those coding for: *Chloroplastic outer envelope pore protein of 21 kDa A* (G0MWCVW03GTHJD), a voltage-dependent regulator of anion channel that facilitates the translocation between chloroplast and cytoplasm of phosphorylated carbohydrates; *Polyol transporter 6* (contig11096), a plasma membrane sugar-proton symporter involved in amino acid and cation transport; *Dicarboxylate/tricarboxylate carrier* (contig03012), a mitochondrial carrier that catalyzes dicarboxylates and tricarboxylates transport.

5.4 Discussion

Plant growth and survival depend on the ability to sense and respond to specific stimuli in complex environments. External signals produced by biotic factors are thought to be detected by plant receptors and to induce complex responses that involve the whole plant (Appel and Cocroft, 2014; Mescher and De Moraes, 2014). Different cellular processes activate a cascade of genes, leading to the alteration of plant transcriptomic profile in order to stimulate adequate stress responses (Fujita et al., 2006). When the attack of pathogens or insects occurs, the transcriptomic reorganization takes place both in the areas affected by the damage and in undamaged tissues, thanks to the production and circulation of signal molecules

(Taylor et al., 2004; Zebelo and Maffei, 2015). Signals are also transmitted to neighbor undamaged plants through volatile organic compounds (VOCs; Kessler and Baldwin, 2001) and common mycelial networks (CMNs) interconnecting plants (Johnson and Gilbert, 2014).
During the last years, different approaches were used to study the gene expression changes in plant-insect interactions (Barah and Bones, 2015; Giri et al., 2006; Kessler and Baldwin, 2002; Wu and Baldwin, 2010). Although *Olea europaea* is a major woody crop in the Mediterranean basin and is diffused worldwide, to date very few studies have investigated its response to biotic or abiotic stress. Some research was carried out on the molecular changes derived from olive-fungus interaction (Benitez et al., 2005) and olive response to salt stress (Bazakos et al., 2012). Only two studies examined the interaction between olive and its key pest, *Bactrocera oleae*, with different approaches (Corrado et al., 2012; Alagna et al., 2015).
Olive fly is the most harmful species that compromises olive worldwide. Its direct and indirect damages on olives, and consequently on table olive and olive oil production, are very well known (Daane and Johnson, 2010). Conversely, the molecular mechanisms associated with its attack are still largely unexplored. Understanding the basis of the defense mechanisms employed by the plant could help the identification of genes that play a key role in olive defense. New insights into molecular mechanisms of olive genetic resistance to olive fruit fly and related defense responses could implement the pest control management systems and contribute to limit the yearly olive production losses caused by *Bactrocera oleae*. Moreover, a research area that may provide important application, is the study of the effect of the fruit fly on olive oil composition and quality.
Besides, transcriptomic studies allow the simultaneous analysis of a wide set of sequences, the in-depth study of the extensive reprogramming of the plant transcriptional profile under stress conditions and the improvement of the knowledge related to plant defense mechanism against insects (Heidel-Fischer, 2014; Zheng and Dicke, 2008).

5.4.1 Olive transcriptional reprogramming following the attack of Bactrocera oleae

It is currently widely accepted that transcriptomic approaches are effective tools to investigate on molecular mechanisms involved in plant defense (Kuhn and Schaller, 2004). The first attempt to accomplish a comprehensive investigation of the molecular basis and related signaling pathways involved in olive interaction with olive fly was done by PCR-based Suppression Subtractive Hybridisation (SSH) and gel-based proteomic techniques (Corrado et al., 2012).
The SSH method has been effectively employed to investigate on plant response to biotic and abiotic stress (Estrada-Hernandez et al., 2009; Jin et al., 2010; Ouyang et al., 2007). Although the large amount and quality of information revealed by the SSH study of the interaction between olive and olive fly, some glitches emerged, related to the large number of not annotated transcripts, also because of the relatively short length of the sequences (an unavoidable feature of the SSH) along with the lack of the olive genome sequence and comprehensive ESTs databases.
Thus, in this study we performed a transcriptomic analysis to achieve a more complete understanding of the molecular changes occurring once *Bactrocera oleae* attacks *Olea europaea* drupes. Moreover, to extend our research at the mechanisms that may trigger resistance to olive fly, we chose two varieties that were previously

described as having very different level of susceptibility to olive fly, namely ‘Ruveia’ and ‘Ortice’ (Pugliano et al., 2000).
The transcriptional changes induced in olive following the attack of olive fly were here evaluated through a microarray analysis. This allowed the identification of several transcripts whose expression changed significantly in response to *Bactrocera oleae* damage: a total of 2497 sequences were differentially expressed in damaged olive of ‘Ortice’ and ‘Ruveia’ varieties respect to the undamaged counterparts. This finding highlighted an extensive transcriptional reprogramming occurring in olive as a consequence of the attack of olive fly. A high number of differentially expressed genes is reported in several studies in other insect-plant interactions, where a wide transcriptional regulation occur after the damage (De Vos et al., 2005; Gutsche et al., 2009).
The differentially expressed genes were recorded in ten functional categories. The most represented one was that of protein metabolism, followed by those of transcription, primary metabolism, transport, stress and defense response, signal transduction, cellular organization, secondary metabolism, cell wall modification and photosynthesis. The relatively high number of ESTs with unassigned or unknown function (365 out of a total of 1603 annotated ESTs) can be justified in the lack of the olive complete genome sequence. This finding suggests that olive is able to recognize and activate transcriptional responses against the attacks of *Bactrocera oleae*, as reported in other species (Appel and Cocroft, 2014; Mescher and De Moraes, 2014). According to the GO ontology of the differentially expressed genes, several biological functions were affected, implying that the effect of the fruit fly on drupes should affect a wide range of features, chemical compounds and physiological processes. Considering that well-developed fruits are sink organs, the most differentially expressed categories suggest that olive defense responses may be characterized by a reconfiguration of resource allocation, along with the involvement of genes directly or indirectly affecting pest growth or survival. Especially in plant-pathogen interactions, proteins are often reallocated away from pathogen-infected tissues, as well as carbohydrates. This is interpreted as a defense response in which plant withdraws critical resources to limit pathogen or pest growth, while minimizing the loss of limiting nutrients. It is not surprising that sink creation is one of the features of the attack of insects that establish a long-lasting interaction with plants. Many studies have been conducted for galling insect species but this phenomenon has also been described for herbivore and phloem–feeding pests. The competitive nature of the plant-insect interaction is a likely explanation on why genes belonging to the different GO categories were at the same time overexpressed and down-regulated After the damage of *B. oleae*, olive primary and protein metabolism functional categories include approximately double of under-expressed genes than the overexpressed ones. This suggests that olive fly attack may induce the plant to isolate the damaged sites (attacked olives), possibly inducing premature senescence and fruit drop.

5.4.2 Resistance and susceptibility to olive fly: transcriptional changes in ‘Ruveia’ and ‘Ortice’

Literature extensively indicates that intraspecific variations occur at genetic and phenotypic level in both prokaryotes and eukaryotes. In *Arabidopsis*, the model organism in plant biology, a wide diversity was reported in both phenotypic traits (Koornneef et al., 2004) and metabolic profiles (Keurentjes et al., 2006). In *Nicotiana*

attenuata, deep differences were found in regulatory signals (Wu et al., 2008) and defense compounds (Dicke and Baldwin, 2010) produced after herbivory damages.
Therefore, it is not surprising that the two olive varieties analyzed, 'Ortice' and 'Ruveia', have shown differences in the mechanisms activated following the attack of the fly. Moreover, the data indicated that there is a very limited overlap between the response of the two varieties. The noticeable discrepancy in the number of the differentially expressed genes (fold-change > 2 and < 0.5 and p-value < 0.05, Student's t-test), in the attacked olives of the two varieties, 52 in 'Ortice' and 2477 in 'Ruveia', indicates that the latter is able to activate a broader response. This is also supported by the fact that, taking into account the functional categories in which the differentially expressed genes were classified, some of these are not or poorly represented in 'Ortice'. In contrast, 'Ruveia' shows a wide range of differentially expressed transcripts related to all functional categories. The specificity of a particular genotype in activating gene expression is an important element of plant resistance management. The data indicated that higher induction of several compounds in the insect resistant variety, not limited to genes involved in secondary metabolites and other defensive compounds, should explain the molecular basis of why insect damage might result in reduced damage in 'Ruveia'. Further studies should then elucidate at chemical and biochemical level the link between a more complex gene expression and the impact on plant resistance level. Nonetheless, the data offer interesting perspectives on olive resistance to the fruit fly. Firstly, considering the wide genetic variability on the olive cultivated germplasm, there is the concrete possibility to test for a correlation between resistance level and genetic variation in gene expression to identify candidate genes or genome regions that contribute to olive resistance, in absence of a reference genome. Moreover, the data open the possibility that it may be possible to screen and select at molecular level the tolerance in olive germplasm. The wide qualitative and quantitative difference in gene expression between the two olive varieties, if confirmed in a more complex panel of varieties, also pave the way for the development of gene-expression-based predictive markers. One of the problem of fruit fly-drupe interaction is related to the difficulties of having controlled testing conditions and the use of predictive markers will represent a cost-efficient way to screen large germplasm collections.
Specifically, the defense of 'Ortice' drupes, activated as a consequence of the olive fly attack, counts on a few elements. The induction of *Hydroxyproline-rich glycoprotein*, a component of the cell wall involved in the strengthening of cell wall in response to oxidative stress (Deepak et al., 2010), suggests the attempt of the olive to reinforce its external barrier against the fly. Five transcripts that were down-regulated after the attack of the olive fly are involved in the activation of stress and defense responses. Among those, *Blight resistance protein RPI* (G0MWCVW04IKBOH) codes for a disease resistance protein involved in the recognition and growth restriction of the pathogen (Song et al., 2003); *Cytochrome p450 family member* (G0MWCVW02DQHWH) induces the synthesis of volatile compounds responsible for defense responses against insects (Matthes et al., 2011); *Glutathione transferase* (G0MWCVW03GLQFI) plays a role in SAR and oxidation-reduction process (Nutricati et al., 2006). This finding could be explained by the subversion of olive defenses carried out by the olive fly, as reported in other plant-insect interactions due to hormone cross-talk (Diezel et al., 2009; Musser et al., 2005; Smith et al., 2009; Walling et al., 2008; Zhu-Salzman et al., 2005). On the other hand, 'Ruveia' olives dramatically reshape their transcriptome following the

attack of *Bactrocera oleae*, indicating the activation of a wide range of inducible defenses against its key pest.
The data available so far in the literature and those obtained in this project do not allow the definition of the molecular basis of olive perception of olive fly attack, the putative insect elicitors or plant herbivory-induced molecules (Heil, 2009). Anyway, deregulated genes coding for receptors involved in the recognition of pathogens were found, such as *Flagellin-sensing 2-like protein* (G0MWCVW01ARDS3), a LRR receptor-like serine/threonine-protein kinase which plays a role in plant-pathogen interaction through the perception of flagellin (flg22), an elicitor of the defense response to pathogen-associated molecular patterns (PAMPs).
Whatever the olive fly elicitors and relative olive receptors are, a great amount of early signaling events are triggered by their interaction in 'Ruveia' olives. In fact, several differentially expressed transcripts involved in signal transduction were found. Ca^{2+} signals are an early response to the damage of cell membranes and recognition of microbial-associated molecular patterns (MAMPs). Calcium ion stores are mobilized and determine alterations in nuclear and cytosolic calcium, which act in the maintenance of cellular homeostasis during plant-insect interaction and lead to the activation of different classes of protein kinases involved in the triggering of defense responses (Maffei et al., 2007; Ma et al., 2007). Conversely, Ca^{2+} signals also suppress SA-mediated acquisition of systemic acquired resistance (Du et al., 2009). This suggests that multiple and complex calcium functions play a role in defense responses Furthermore, the fact that several Ca^{2+} defense responses are both down and up-regulated suggests the presence of Ca^{2+}-responsive but antagonistic signaling mechanisms (Dodd et al.; 2010). Several transcripts were found to be involved in calcium entry in 'Ruveia' cells following the attack of *B. oleae* and consequently in calcium signal transduction. Among these, *Glutamate receptor 2.2* (G0MWCVW04H69Q9), involved in light-signal transduction and calcium homeostasis via the regulation of calcium influx into cells, and *Cyclic nucleotide-gated ion channel 1* (G0MWCVW04H7GKZ), which acts as cyclic nucleotide-gated ion channel responsible for cAMP-induced calcium import. To reduce the efflux of calcium ions from cytosol, *Calcium-transporting ATPase 10* (G0MWCVW03FN16H), which catalyzes the hydrolysis of ATP coupled with the translocation of calcium from the cytosol into the endoplasmic reticulum, was repressed. Moreover, several genes coding for proteins that are directly modulated by calcium ions were up-regulated, such as the voltage-gated cation channel *TPK1* (G0MWCVW03G478Y), the annexin family protein *ANN4* (contig05212) and the transcription factor Calmoduline binding WRKY11 (contig03271). On the contrary, *Calmodulin-binding transcription activator 4* (contig12508) and *Calmodulin-binding transcription activator 3* (G0MWCVW01A584F) are repressed. In confirmation of the key role of calcium in signal transduction, five CBL-interacting serine/threonine-protein kinases (*CIPK)*, activated in a calcium-dependent manner, were induced in damaged 'Ruveia' olives. Conversely, three calcium-dependent protein kinase (contig01004, G0MWCVW04IZTSX, G0MWCVW03GUPQ9), belonging to the CDPK family proteins induced by calcium and involved in abscisic acid-activated signaling pathway (Romeis and Herde, 2014), were repressed.
Plant MAPK signaling plays a central role in early stress response, especially biotic stress, and regulates transcription factors associated with defense genes (Meng and Zhang, 2013). A number of studies indicate that, although other proteins may be their substrates, the major targets of plant MAPKs are transcription factors (Wu and Baldwin, 2010) and have established the key role of MAPKs in regulating plant

transcriptomes (Kim and Zhang, 2004; Wu et al., 2007). In effect, 'Ruveia' olives showed positive and negative regulation of several genes coding for these proteins following the attack of the olive fly. Among those, the induced *MAP3K epsilon protein kinase* (G0MWCVW01A0W3Y) and the repressed *MAPKKK serine/threonine-protein kinase EDR1* (G0MWCVW01BEOJA) and *Protein SGT1 homolog B* (contig02820). *EDR1* and *SGT1* are involved in the negative regulation of and their repression could induce the activation of these functions. Interestingly, a complete MAPK and WRKY pathway was identified in *Arabidopsis*, which is activated by the flagellin receptor FLS2 (Asai et al., 2002), overexpressed in 'Ruveia' after the attack of the olive fly.

ROS (singlet oxygen, superoxide and hydrogen peroxide) production is an essential part of plant stress responses, specifically of responses to pathogens (Lamb and Dixon, 1997) and herbivores (Bi and Felton, 1995). Signaling that follows biotic or abiotic damages starts with the rapid release of ROS in the so-called respiratory (or oxidative) burst. This kind of reaction was attributed exclusively to plant-insect interaction in *Medicago truncatula*, were wounding did not produce noticeable amounts of ROS (Leitner et al., 2005). Each type of ROS determines different responses (Gadjev et al., 2006). After the attack of *B. oleae*, the *Translation initiation factor IF-2* (G0MWCVW03G1GN3), involved in hydrogen peroxide biosynthetic process was overexpressed, indicating the role of H_2O_2 in 'Ruveia' signaling. As 40 differentially expressed genes were involved both in metabolism of reactive oxygen species and in response to oxidative stress after olive fly attack, it is possible to state that the alteration of redox state of olive cells plays an important role even in olive interaction with olive fly. Among these, many genes are involved in the regulation of the respiratory burst involved in defense response, such as the overexpressed transcription factor *WRKY11* (contig03271) and *ERF5 Ethylene-responsive transcription factor 5* (G0MWCVW01BLALZ), and the underexpressed *G-type lectin S-receptor-like serine/threonine-protein kinase B120* (G0MWCVW03F56U6) and *MPK3* (G0MWCVW03GUTP2). The latter was counted among the genes that are down-regulated when oxidative stress tolerance is positively regulated in *Arabidopsis* (Lee and Ellis, 2007).

Although their important role in the respiratory burst involved in defense response, ROS may cause oxidative damages. Thus, plant cell may activate enzymes to reduce the excess internal ROS level. Thirteen cell redox homeostasis genes were differentially expressed in 'Ruveia'. Among these, there were several over-expressed glutaredoxins. Glutaredoxin family proteins play a direct role in plant cell antioxidant system and are involved in the salicylic acid signaling pathway (Rouhier et al., 2008). *Glutaredoxin family protein* (G0MWCVW03GOEZU) was highly induced, indicating 'Ruveia' attempt to control ROS damages. Interestingly, *Glutathione S-transferase* (contig05145) was underexpressed. This enzyme is known for its function in counteracting the effect of higher ROS production in stressed plants (Bianchi et al., 2002) and was associated with defense responses in other plant-insect interactions (Park et al., 2006). On the contrary, *Superoxide dismutase* (G0MWCVW02DKXNQ), involved in the catabolism of superoxide, was induced.

Five out of twenty-three genes with peroxidase activity were up-regulated in 'Ruveia', suggesting that peroxidation is an important component of the response to olive fly. Peroxidases are glycoproteins whose main function is to oxidize a wide range of molecules, employing either H_2O_2 or O_2 (Yoshida et al., 2003). Several studies confirmed their role in oxidative signal transduction that regulates cell redox homeostasis and the downstream control of cellular calcium levels and expression of defense genes (Kawano, 2003). Peroxidases were described in induced and

constitutive defenses against leaf feeding insects in poplar, where their activity was induced between 24 and 72 h from the attack (Barbehenn et al. 2010).
The cascade of responses triggered during plant recognition of herbivore elicitors should also include changes in plasma membrane potential and activation of networks of phytohormones (Maffei et al., 2007). Jasmonic acid, salicylic acid, and ethylene play a key role in herbivore-induced defense responses acting in a complex regulatory network of plant signaling pathways (Pieterse et al., 2012). Their concentrations undergo dynamic changes after the damage and reveal the crosstalk among these three hormones (Stam et al., 2014). Cytokinins, abscisic acid, gibberellins and auxins also play a role in defense signaling (Erb et al., 2012).
The literature highlights jasmonic acid (JA) key role in response to biotic stress (Wasternack and Hause, 2013; Howe and Jander, 2008). Microarray transcriptomic analyses indicated that jasmonic acid is the main regulator of the responses to wounding and herbivory (Reymond et al., 2000; Reymond et al., 2004). 'Ruveia' responses to olive fly included 6 overexpressed and 1 underexpressed genes involved in jasmonic acid biosynthetic process. Interestingly, the down-regulated gene is the *Jasmonate ZIM domain-containing protein 2* (contig11024), which code for a repressor of jasmonate responses. In response to auxin, ethylene, jasmonic acid and wounding, JAZ is repressed and JA-responsive genes are activated, including genes encoding JAZ proteins, genes involved in the abscisic acid signaling pathway, salicylic acid mediated signaling pathway and jasmonic acid biosynthetic process (Mewis et al., 2006). The induction of *COLD INDUCED ZINC FINGER PROTEIN 2* (contig05089), a transcriptional repressor involved in abiotic stress responses and jasmonate early signaling response, which controls the expression of TIFY10A/JAZ1, suggests that JAZ may play a key role in jasmonate responses induced by the attack of the olive fly in 'Ruveia'. Many differentially expressed genes (22 down and 12 up-regulated genes) code for response to jasmonic acid or involved in jasmonic acid mediated pathways proteins. This result indicates the important role that JA plays in the response of 'Ruveia' against *B. oleae*.
JA signaling pathway has two antagonistic branches (Paschold et al., 2007; Paschold et al., 2008). Whether in 'Ruveia' occurs the activation of the MYC2 branch, that positively regulates the expression of wound-inducible JA-responsive marker genes (such as LOX), or the ethylene response factor (ERF) branch, in which JA and ET induce the expression of JA/ET-responsive transcription factors, including ERFs, is not clear. The repression of *Serine/threonine-protein kinase EDR1* (G0MWCVW01BEOJA) involved in the regulation of a MAP kinase cascade that negatively regulates salicylic acid defense responses, abscisic acid signaling and ethylene-induced senescence and represses MYC2, may suggest that the MYC2 branch, which is known for its defense against herbivorous insects, could be activated. Conversely, the overexpression of four ethylene response factors, may propose the activation of the ERF branch, known to be especially involved in the induced defense against necrotrophic pathogens (Stam et al., 2014).
Alagna and collaborators (Alagna et al., 2015) reported a strong increase in olive fruits ethylene production following the attack of *B. oleae*. That result was confirmed by the increase in the mRNA level of genes involved in ethylene (ET) biosynthesis. The biosynthesis of ET follows rapidly after herbivore attack and is specific for insect-induced damages (von Dahl et al., 2007).
The outcomes of the transcriptomic study developed in the present thesis indicated the up-regulation of the *WRKY transcription factor 15* (G0MWCVW02D8S2N), which plays a role in both respiratory burst involved in defense response and ethylene

biosynthetic process. Conversely, 1-aminocyclopropane-1-carboxylate synthase (G0MWCVW03HHIVR), involved in ethylene biosynthesis via S-adenosyl-L-methionine, was repressed in damaged 'Ruveia' olives. This finding is in contrast with what was reported by Alagna et al. (2015). Nine up-regulated and eleven down-regulated genes, belonging to different functional categories, are involved in response to ethylene and ethylene-activated signaling pathway. Three ERFs (ethylene responsive transcription factors), namely ERF3 (contig13605), ERF5 (G0MWCVW01BLALZ) and ERF/AP2 (G0MWCVW04I1K6H), were induced. ERFs play a relevant role in ethylene signaling pathway, as they respond to ethylene to induce the production of secondary metabolites involved in defense response (Erb et al. 2012). Interestingly, Ethylene receptor 1 (G0MWCVW01B931E) and Ethylene receptor 2 (G0MWCVW04JLM0P), which act as negative regulators of ethylene signaling, were both repressed in damaged 'Ruveia' olives, indicating the unlocking of ethylene signaling after the attack of the olive fly.

The salicylic acid (SA) pathway is known for its pivotal role in the plant responses against phloem-feeding insects and biotrophic pathogens (Glazebrook, 2005; Walling, 2000), yet alteration of SA levels was reported in some plant interactions with chewing insects (De Vos et al., 2005; Leitner et al., 2005). Moreover, it was suggested that the ET burst may play a role in SA pathway regulation (Diezel et al., 2009). The damage of *B. oleae* larva in olive drupe determined the differential regulation of several transcripts related to salicylic acid biosynthetic processes (4 up-regulated and 10 down-regulated genes) and response to salicylic acid or salicylic acid mediated pathways (26 down and 8 up-regulated genes). Although the majority of the SA-induced genes was down-regulated, some transcripts are clearly involved in stress responses. This is the case of the induced *26S proteasome non-ATPase regulatory subunit 2 homolog A* (G0MWCVW01CBLZH).

The above described complex regulatory component activated following the *B. oleae* attack modulates the biosynthesis of defense and response to stress metabolites (Wu and Baldwin, 2010).

The disease resistance proteins (van Ooijen et al., 2007) that were differentially expressed belong to well-known protein families: NBS-LRR, TIR-NBS, NB-ARC, carboxypeptidase and heat shock proteins. Among defense and response to stress metabolites, *NBS-LRR type disease resistance protein* (G0MWCVW04IRIVQ), involved in plant-pathogen interactions (Belkhadir et al., 2004), was induced along with *Serine carboxypeptidase-like 51* (G0MWCVW04JXGC8), required for response to endoplasmic reticulum stress and systemic acquired resistance.

The resistance to herbivores is strongly influenced by plant secondary metabolism (Mithöfer and Boland, 2012). Although secondary metabolism leads to the production of metabolites that are not essential for the normal growth and reproduction of the plant, it has a great ecological importance in defense mechanisms against herbivores. The up-regulation of several transcripts involved in the biosynthesis of secondary metabolites was evidenced in 'Ruveia' drupes infested by olive fly. Four Cytochrome P450 related genes were differentially expressed. These genes code for enzymes with a relevant defense role as they are required for both for the detoxification of toxic molecules and the biosynthesis of a defense molecules (Mizutani and Ohta, 2010). For instance, *Cytochrome P450 77A4* (contig10995), which catalyzes the epoxidation of unsaturated fatty acids and is involved in cutin biosynthetic process (Sauveplane et al., 2009), and *NADPH-cytochrome P450 reductase* (G0MWCVW02EOCAP), which mediates ER to Golgi vesicle-mediated transport and phenylpropanoid metabolic process in response to abscisic acid, were

overexpressed. In citrus, phenylpropanoids, together with ethylene, were highlighted for their important roles in the induction of resistance (Ballester et al., 2011). Interestingly, *G10H* (contig00982), involved in monoterpenoid biosynthetic process and specifically in the biosynthesis of hydroxygeraniol, a precursor of the terpenoid indole alkaloids, was up-regulated after the attack of the olive fly. On the contrary, Geraniol 8-hydroxylase (G0MWCVW04H7D2K), required for the biosynthesis of hydroxygeraniol in monoterpenoid biosynthetic process, was down-regulated. Several studies demonstrated that herbivore oviposition and the secretions released from oviducts stimulate the induction of genes involved in the biosynthesis of terpenoids and the release of volatile compounds (Köpke et al., 2008 ; Schröder et al., 2007). Twelve genes coding for protein related to biosynthesis and catabolism of polyamines, known for their role in the protection against a wide range of stress, including oxidative damage (Alcázar et al., 2006). The repression of genes involved in their biosynthesis, such as *Spermine synthase* (G0MWCVW02EW8DW), may modulate the presence of ROS and their downstream regulation pathways. *At1g55290* (contig08219), which encodes a protein required in scopoletin (coumarin) biosynthesis (Kai et al., 2008), was overexpressed, as well as *Tropinone reductase* (contig12711), which plays a role in alkaloid biosynthetic process. *Tropinone reductase 2* (G0MWCVW01AOCDA), involved in tropane alkaloid biosynthetic process, was instead underexpressed. After the attack of B. oleae, 'Ruveia' induces the production of oxylipins, which represent an important response to insects (Vellosillo et al., 2007). Two transcripts involved in their biosynthetic process were overexpressed: Violaxanthin de-epoxidase (G0MWCVW03G9TNB) and Beta-ketoacyl-ACP synthase II (contig01714). Convesely, Linoleate 9S-lipoxygenase A (G0MWCVW03GOPJS), involved in multiple functions in plant physiology, including growth and development, pest resistance, senescence and responses to wounding, was underexpressed.

Several microarray studies demonstrated that the transcriptomic rearrangements that occur in the plant-herbivore interaction involve all aspects of metabolism, including a remarkable component of primary metabolism (Schwachtje and Baldwin, 2008). Plant defense responses are associated with increased demands for energy and carbon skeletons that are provided by primary metabolic pathways (Bolton, 2009; Bergen et al., 2007). Several hypothesis were produced to explain how the assimilation and partitioning of assimilates are altered by herbivory and how primary metabolites function as signals or defenses. Several aspects of photosynthesis, assimilate partitioning, and source–sink regulation were involved (Schwachtje and Baldwin, 2008). That was confirmed in the transcriptomic study of the interaction between olive and olive fly larva, where a considerable portion of the transcriptomic changes were involved in primary and protein metabolism as well as in metabolite transport. Carbohydrate metabolism, along with that of amino acids and lipids, represents the unique source of nutrients for the larvae. It is possible to hypothesize that olive sink/source relations are altered by the feeding of the larva, which represents a new sink site, thus determining a deep transcriptomic rearrangement for what concerns primary metabolism. It was also proposed that such a deep metabolic alteration may be involved in a downstream signal pathway (Schwachtje and Baldwin, 2008). The metabolism of carbohydrates was strongly affected, with several enzyme families represented. Some of this were clearly regulating specific functions related to plant-insect interaction. For instance, the induced *UDP-D-glucuronate 4-epimerase* (G0MWCVW04H5VIF) is involved in the synthesis of pectic cell wall components. Similarly, *Beta-galactosidase 1, glycosyl hydrolase family 35* (G0MWCVW01BBBJT),

involved in carbohydrate metabolic process and hydrogen peroxide catabolic process, was induced, indicating that primary metabolism may support defense mechanisms. Several alpha-, beta-glucosidases and xylosidase were repressed. For what concerns lipid metabolism, several lipases and hydrolases were repressed. Other lipid metabolism transcripts were induced, such as *Biotin carboxyl carrier protein of acetyl-CoA carboxylase* (contig05566), involved in brassinosteroid, polysaccharide biosynthetic processes and cell wall biogenesis and organization or *C-14 sterol reductase* (G0MWCVW03GQWUV) involved in sterol and brassinosteroid biosynthetic processes and pentacyclic triterpenoid biosynthetic process. For what concerns protein metabolism, it is important to emphasize the differentially expression of several transcripts involved in the proteasome-mediated and ubiquitin-dependent protein catabolic process, as well as in autophagy. This functions indicate the effort of the plant in facing the metabolic alterations following olive fly attack and maybe the attempt to degrade exogenous proteins.
The thickness of cell walls could be the initial barrier against insect attack (Barros-Rios et al., 2011, Vorwerk et al., 2004). The strengthening of the cell wall is induced as a consequence of the attack of pathogens and insect herbivores. To this end, the alteration of chemical composition of the wall (pectins, cellulose, etc.) occurs in order to reinforce the external barrier against pathogens and insects (Kusnierczyk et al., 2008; Hückelhoven, 2007). Following *B. oleae* attack, CESA3 (G0MWCVW03FWP1T), coding for a cellulose synthase isomer which play an important role in the synthesis of cellulose, was induced, along with four polygalacturonase involved in cell wall organization. Two expansins (G0MWCVW02EXH06 and contig10205), which cause loosening and extension of plant cell walls by disrupting non-covalent bonding between cellulose microfibrils and matrix glucans, were up-regulated too. Other enzymes involved in cell wall organization or biosynthesis, as *Polygalacturonase ADPG2* (G0MWCVW01BR1T6), *Pectinesterase 1* (G0MWCVW02DR0YF) and Alpha-xylosidase 1 (contig14286) were repressed. The cell wall-degrading mechanisms found in plant-herbivore interactions could be ascribed to the induction of plant defense responses (Vidal et al., 1998).

5.4.3 Future perspectives

The research on transcriptomic responses of ‘Ortice’ and ‘Ruveia’ olive varieties to the attack of *Bactrocera oleae* here described opens up a number of perspectives.
To validate the expression data obtained through the transcriptomic study of the molecular responses to the olive fly, the validation by Real-time PCR analysis of selected genes should be carried out. This activity, besides being an independent verification of the microarray analysis, should also allow to test some of the genes in different olive varieties in order to develop transcriptional markers. These should be selected among those differentially expressed of particular interest on the basis of their function, preferably those related to stress and defense responses or those involved in the biosynthesis of relevant secondary metabolites that could have implications on olive oil quality.
The results obtained so far could also be processed in the direction of the comparison between the experimental points related to ‘Ortice’ and ‘Ruveia’ undamaged olives. This comparison may, in fact, clarify the mechanisms of constitutive defense with which the two varieties face the attack of the olive fly. Such a study would broaden the knowledge on the molecular mechanisms underlying the interaction between the olive and the olive fly.

An interesting future perspective would be to expand the analysis presented here with the study of the transcriptomic modifications determined by the puncture, oviposition and different larval stages.
In this way it could be possible to differentiate the early olive mechanisms of response to puncture and oviposition from those determined by the feeding of the larvae.
Another significant aspect that could be investigated is the overall impact of olive fly on olive oil quality. If olive response seems to be largely dependent on the genotype under investigation, it is likely that some of the differences in extra virgin-olive oil may be also dependent on the specific reaction of the drupe to the fruit fly. In fact, even though the negative effects of *B. oleae* damage on olive and olive oil productions are very well known, no information is available to date on the possible production of metabolites and volatile compounds that could positively influence the quality of olive oil as a consequence of the presence of the fly in the olive grove. To this end, transcriptomic and expression profiling, along with metabolomics approaches like the analysis of volatile compounds emitted in the interaction between olive and olive fly, may open broad possibilities for in-depth analyses of the effects of the presence of the fly in the olive grove as well as its direct attack.
A long term perspective is related to the current lack of the complete genome sequence of *Olea europaea*. Currently, due to the insufficient knowledge of the olive genome, the opportunity to explore the molecular mechanisms of the interaction between olive and olive fly is quite limited. Once the complete olive genome sequence will be available, it will allow to perform a Gene Ontology Enrichment Analysis (GOEA) on the whole genome. This will open new possibilities to deepen the current knowledge of this important plant-insect interaction, as well as of many other molecular mechanisms of olive biology.

5.4.4 Conclusions

The main aim of this study was to investigate the response of two olive varieties to the attack of the olive fly. To this end, two varieties characterized by very different levels of susceptibility were selected: ‘Ortice’, known for its sensitivity to the attack, and ‘Ruveia, known for its tolerance.
The analysis indicated that the olive response in the susceptible and tolerant olive varieties under investigation is quantitatively and qualitatively different. The study of the differentially expressed genes following the attack of *B. oleae* sheds light on the specific mechanisms that the two varieties deploy to face the attack of the olive fruit fly.
This study confirms that the variability observed in different olive varieties not only involves phenotypic traits and quality of olive and olive oil productions, but also the responses to olive key pest. The great variability in defense resistance mechanisms revealed in olive varieties gives the opportunity to design *ad hoc* studies on the selection of plant material to be adapted to different environmental conditions.
This study represents an advancement in the existing knowledge about the interaction between olive and olive fly. The obtained outcomes could have a relevant value in further studies aimed at the comprehension of this interaction and provide with useful insights into olive defense responses in order to develop new tools for olive fly control.

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7. APPENDIX

Table 7.1. List of differentially expressed genes with fold-change > 2 and p-value < 0.05 (Student's t-test) in 'Ortice' olives after the attack of *Bactrocera oleae*.

Seq. Name	Seq. Codes	Seq. Description	FC Od/Ou
Cellular organization			
G0MWCVW02EBJ9B	AMTR_s00101p00088850	AT5g04990/MUG13_15	2,712
Transcription			
G0MWCVW01A1C2H	ARALYDRAFT_340237	Hydroxyproline-rich glycoprotein	2,749
G0MWCVW01B7PC8	At5g46100	Pentatricopeptide repeat-containing protein At5g46100	2,537
Stress and defense response			
G0MWCVW02ESD70		SUN 1. Hypothetical protein JCGZ_10098	2,386
Protein metabolism			
G0MWCVW02DHPDY	ago-01	Protein argonaute 1	2,067

Table 7.2. List of differentially expressed genes with fold-change < 0.5 and p-value < 0.05 (Student's t-test) in 'Ortice' olives after the attack of *Bactrocera oleae*.

Seq. Name	Seq. Codes	Seq. Description	FC Od/Ou
Cellular organization			
contig11001	APY1	ADPase	0,468
contig00318	At1g79280	AtTPR	0,413
Transcription			
G0MWCVW04I7LD4	At1g55310	At-SCL33	0,460
G0MWCVW04JH5DH	AMTR_s00058p00198960	Uncharacterized protein	0,425
G0MWCVW03GWEF4		protein ros1	0,387
Stress and defense response			
G0MWCVW04IKBOH	177O13.40	Blight resistance protein RPI	0,416
G0MWCVW02DQHWH	At3g28740	1.14.-.-	0,379
G0MWCVW03GLQFI	At2g30860	2.5.1.18	0,493
G0MWCVW04H37A1	CICLE_v10014317mg	Serine-threonine protein kinase, plant-type, putative	0,460
contig10312	LEA14-A	Desiccation protectant protein Lea14 homolog	0,493
Signal transduction			
G0MWCVW03F14TN	At3g26090	AtRGS1	0,457
contig12675	PRUPE_ppa012490mg	Universal stress protein A-like protein	0,487
Photosynthesis			
contig06309	MGD2	Monogalactosyldiacylglycerol synthase 2, chloroplastic	0,499
G0MWCVW01BXEOG		ferrochelatase- chloroplastic-like	0,456
Primary metabolism			
G0MWCVW01BW7ZS	AMTR_s00046p00133890	AT5g35440/MOK9_2	0,488
G0MWCVW04JAZS7	AT2G45630	Glycerate dehydrogenase, putative	0,424
G0MWCVW04I7HF2	AMTR_s00002p00257980	Polyadenylate binding protein	0,486
G0MWCVW02EENTC	AMTR_s00024p00234750	MTD1 family protein	0,483
G0MWCVW02EUHWL	PHAVU_007G180900g	Indole-3-acetamide hydrolase	0,422
Protein metabolism			
contig02047	At4g05000	Vacuolar protein sorting-associated protein 28 homolog 1	0,393
contig05516	UBC12	6.3.2.19	0,439
G0MWCVW02DVGCU	HAL	Histidyl-tRNA synthetase	0,491
G0MWCVW02DE30D	F441_00054	Pseudouridine synthase and archaeosine transglycosylase domain-containing protein	0,486
G0MWCVW02DJ33C		pentatricopeptide repeat-containing protein mitochondrial	0,409
Secondary metabolism			
G0MWCVW03G7TPC	PGSC0003DMG400030659	Uncharacterized protein	0,497
Transport			
G0MWCVW04JWUKU	At5g07120	Sorting nexin 2A	0,347

Table 7.3. List of differentially expressed genes with fold-change > 2 and p-value < 0.05 (Student's t-test) in 'Ruveia' olives after the attack of *Bactrocera oleae*.

Seq. Name	Seq. Codes	Seq. Description	FC Rd/Ru
Cellular organization			
contig01252	At5g62810	AtPEX14	2,195
contig08796	At2g46180	AtGC3	2,189
G0MWCVW03FVO0Z	At5g59560	Protein SENSITIVITY TO RED LIGHT REDUCED 1	4,848
contig09578	ADO3	Adagio protein 3	2,334
G0MWCVW03G3H24	CYCB1-1	CycB1	2,285
G0MWCVW01BJMMV	AT4G38360	Uncharacterized protein	2,093
G0MWCVW01B7WZU	ADL2	Dynamin-like protein 2	4,368
G0MWCVW03FYZBR	CYCB1-1	CycB1	2,209
contig10389	At2g17090	Probable inactive receptor-like kinase SSP	3,069
contig06084	At2g24940	AtMP1	3,894
G0MWCVW03HKC64	AMTR_s00133p00038030	Uncharacterized protein	2,738
G0MWCVW01BWIQQ	MOR1	Protein GEM1	2,885
G0MWCVW01BSF4K	PGSC0003DMG400002138	Putative double-strand telomere binding protein 2	4,821
G0MWCVW01BTI2K	ARPC3-1	ARP2/3 complex, subuit 3	2,046
contig04057	At4g27890	Protein BOBBER 1	3,083
contig07186	At1g14740	Protein OBERON 3	2,007
contig07291	ADF1	ADF-1	2,706
contig04664	At5g06830	CDK5RAP3-like protein	3,831
G0MWCVW01BSD8U	At5g65770	NMCP1-like	2,134
contig00350	CAFP	Cell division cycle protein 48 homolog	2,042
G0MWCVW02DQYKS	ACT-1	Actin	2,052
G0MWCVW02EE8KM	PGSC0003DMG400027010	At5g62575	3,046
G0MWCVW03GW2MT	At1g04160	AtMYA1	2,043
contig00505	PVIP	OBERON-like protein	2,024
G0MWCVW01CD1JD	ARALYDRAFT_495454	Nucleotidyltransferase family protein	3,458
contig09415	PRO1	Profilin	2,002
contig08335	A_IG002N01.18	Protein CURVATURE THYLAKOID 1A, chloroplastic	2,183
G0MWCVW04ICMMI	VIT_15s0046g02270	Putative uncharacterized protein	3,177
G0MWCVW01CAXJU	APEM3	AtPMP38	2,988
G0MWCVW01B1B4F	At2g31970	AtRAD50	2,099
contig04024	PSBS	CP22	2,639
G0MWCVW04IZVZG	At1g05570	1,3-beta-glucan synthase	2,390
G0MWCVW04JSPH2	At1g16710	2.3.1.48	3,962
G0MWCVW02EW1PV	PHAVU_011G113500g	At1g03620	2,487
G0MWCVW04JXKAZ	At1g71440	AtTFCE	4,007
G0MWCVW04JB1DN	At3g07650	Putative zinc finger protein CONSTANS-LIKE 11	4,714
G0MWCVW02EYL1Y	DET1	Light-mediated development protein DET1	2,875
contig14107	HDA19	Histone deacetylase 19	2,298
contig11060	PGSC0003DMG400029287	Os07g0656600 protein	2,793
G0MWCVW01AX443	CICLE_v10016602mg	Uncharacterized protein	2,606
contig07732	PGSC0003DMG400024347	Cytokinesis negative regulator RCP1	2,553
contig01427	At3g57060	Condensin complex subunit 1	3,216
Transcription			
contig08862	AMTR_s00119p00102250	At2g21530	4,424
G0MWCVW01BV46T	At2g31370	AtbZIP59	3,380
G0MWCVW01APJYG	At3g44530	Histone regulator protein	2,517
contig07862	At1g04950	AtTAF6	2,785
contig09865	At5g12190	Pre-mRNA branch site p14-like protein	2,365
contig03824	PCF1	Protein CCA1 HIKING EXPEDITION	2,261
G0MWCVW02DDKQG	At1g20920	DEAD-box RNA helicase RCF1	2,309
contig03583	AMTR_s00066p00163770	HIV Tat-specific factor 1-like protein	3,614
contig06235	At1g07090	Protein ELONGATED EMPTY GLUME	2,268
G0MWCVW04JTISI	PGSC0003DMG400024983	Tuber-specific protein	3,085
G0MWCVW02DNEYD	PHAVU_004G133700g	At5g08430	2,011
contig04535	At4g14300	Heterogeneous nuclear ribonucleoprotein 1	2,368
G0MWCVW04IWW1M	M569_15845	Putative uncharacterized protein WRKY_12	2,300
contig13292	MTR_4g100970	CWC15-like protein	2,099
G0MWCVW04INCNP	PRP3	U4/U6 small nuclear ribonucleoprotein Prp3	2,082
G0MWCVW02EJ217	At1g61660	AtbHLH103	4,921
G0MWCVW03GU012	AMTR_s00004p00093740	CCR4-Not complex component, N-terminal	3,158
G0MWCVW02DIZ0D	AMTR_s00033p00116200	Poly(RC)-binding protein, putative	3,574
contig04385	At1g56280	AtDi19-1	2,038
G0MWCVW04H5D1R	PCL1	OsPCL1	3,154
contig06811	At1g27050	Homeobox transcription factor	4,939
G0MWCVW04IDV4M	At4g30220	Probable small nuclear ribonucleoprotein F	3,237
G0MWCVW04I23YE	M569_15845	Putative uncharacterized protein Sb01g027770	4,102
contig13184	AG1	Floral homeotic protein AGAMOUS	4,191
G0MWCVW02C3XH9	CFM2	Chloroplastic group IIA intron splicing facilitator	2,088

		CRS1, chloroplastic	
G0MWCVW02C217V	AMTR_s00040p00227330	PHD finger family protein	2,084
G0MWCVW02DM6G7	MTR_1g025500	Uncharacterized protein	3,190
contig06175	AMTR_s00024p00238750	Uncharacterized protein	2,081
G0MWCVW04JJ4VT	AMTR_s00066p00089100	Os02g0167500 protein	2,505
G0MWCVW01BLCM0	At2g37340	At-RS2Z32	2,577
G0MWCVW03HER5U	rpoC1	DNA-directed RNA polymerase	3,212
contig07114	AT4G16830	Nuclear RNA binding protein, putative	4,594
G0MWCVW02DYVDC	Solyc03g112350.2	-	2,070
G0MWCVW03GC3VD	rpa1	DNA-directed RNA polymerase	3,086
contig00549	At1g03790	Zinc finger CCCH domain-containing protein 2	2,275
contig01776	NAC1	NAC transcription factor NAM-B1	2,094
G0MWCVW02DUL28	AMTR_s00143p00072740	AT5g67320/K8K14_4	3,928
contig00705	AMTR_s00003p00244690	COP1-interacting protein 7	2,712
G0MWCVW04IWT45	At4g11130	AtRDRP2	2,292
contig08540	AMTR_s00103p00136100	Heterogeneous nuclear ribonucleoprotein, putative, expressed	5,238
contig10849	At1g56280	AtDi19-1	2,047
G0MWCVW03GVRQ6	AMTR_s00143p00072740	AT5g67320/K8K14_4	2,778
G0MWCVW04I1K6H	AMTR_s00058p00066390	WIN1-like protein	2,147
G0MWCVW03GB2IT	At1g52520	Protein FAR1-RELATED SEQUENCE 6	3,746
G0MWCVW04I96CY	AMTR_s00143p00072740	AT5g67320/K8K14_4	2,605
G0MWCVW04JVUNL	NAC1	Protein BEARSKIN1	2,183
G0MWCVW02DXP39	At1g63650	Anthocyanin regulatory Lc protein	3,377
G0MWCVW02C6YS8	At2g41720	Pentatricopeptide repeat-containing protein At2g41720	3,082
G0MWCVW02DPNGI	At4g39620	Pentatricopeptide repeat-containing protein At4g39620, chloroplastic	2,174
contig08244	AMTR_s00002p00272190	Os07g0175100 protein	3,340
G0MWCVW02D79WE	At3g18110	Pentatricopeptide repeat-containing protein At3g18110, chloroplastic	2,434
contig11547	POPTR_0001s04510g	-	4,641
contig03271	WRKY11	WRKY transcription factor	2,916
contig05908	ABI4	Dehydration-responsive element-binding protein 2C	2,941
G0MWCVW01BLALZ	ERF5	EREBP-4	3,333
G0MWCVW03GK2OM	23.t00008	Protein vip1	2,263
G0MWCVW02D8S2N	At2g23320	Probable WRKY transcription factor 15	2,419
G0MWCVW02DWOEN	RCOM_0792940	Putative uncharacterized protein	2,034
G0MWCVW03GHKM7	At2g29760	Pentatricopeptide repeat-containing protein At2g29760, chloroplastic	3,191
contig10143	CID9	Splicing regulatory glutamine/lysine-rich protein 1	2,549
G0MWCVW03GKMWD	MTR_2g042550	Pentatricopeptide repeat-containing protein	2,659
G0MWCVW04H50NZ	At3g46870	Pentatricopeptide repeat-containing protein At3g46870	2,476
G0MWCVW04JHV2S	AMTR_s00023p00182890	Nfrkb, putative	2,089
G0MWCVW01CGUA1		DOF transcription factor 6	2,250
G0MWCVW02EXZEX		glutamine-rich protein 23	2,154
G0MWCVW04JWZ7W		zinc finger ccch domain-containing protein 12-like	2,886
G0MWCVW02EKS5O		paired amphipathic helix protein sin3-like 4 isoform x1	3,256
G0MWCVW04H4H4U		wrky dna-binding protein	2,534
G0MWCVW04I6DNK		nuclear transcription factor y subunit a-1	2,026
G0MWCVW01B0FGZ		myb-related protein 3r-1	2,714
G0MWCVW04JNCQV		nac domain-containing protein 100-like	2,185
G0MWCVW01AQAOW		transcription repressor kan1 isoform x2	2,217
G0MWCVW03GL7W8		ubp1 interacting protein 1a	2,317
Cell wall modification			
G0MWCVW01A83OM	At3g55990	Protein ESKIMO 1	2,813
G0MWCVW02EXH06	EXP1	Alpha-expansin-1	2,318
G0MWCVW01B07G3	AMTR_s00033p00236820	Kinesin-like protein	2,275
G0MWCVW02DBDGK	AMTR_s00132p00031640	Uncharacterized protein	2,443
G0MWCVW02EIA6M	PGSC0003DMG400031385	Pectin acetylesterase	2,045
G0MWCVW03HEZXX	MTR_4g120730	Glycoside hydrolase family 28 family protein	2,042
G0MWCVW01BQVZ3	GSVIVT00026920001	Probable polygalacturonase	2,191
G0MWCVW02EHPC0	BRADI1G35900	Putative uncharacterized protein Sb06g032340	2,770
G0MWCVW04H5VIF	At1g02000	5.1.3.6	3,484
G0MWCVW01CEJ9S	CICLE_v10019694mg	Fringe-related family protein	4,801
G0MWCVW04JFEB7	GSVIVT00026920001	Probable polygalacturonase	4,043
contig06480	At1g69420	Probable palmitoyltransferase At1g69420	4,406
contig10205	EXP1	Alpha-expansin-1	2,255
G0MWCVW01CGQD2	At1g60790	Protein trichome birefringence	3,620
G0MWCVW01BSBA7	AMTR_s00056p00069610	Exostosin family protein	2,825
G0MWCVW02EZ5HU	CICLE_v10015386mg	Feruloyl esterase A	2,004
G0MWCVW03FWP1T	CESA3	2.4.1.12	3,428

contig13005	AMTR_s00032p00169660	Glycosyltransferase CAZy family GT32	2,118
Stress and defense response			
contig03672	Si017605m.g	Quinone oxidoreductase PIG3	2,005
G0MWCVW04JTRDG	At5g47120	BI-1	3,547
G0MWCVW02DIEZV	ILL6	3.5.1.-	2,735
contig03988	GLB1	Non-symbiotic hemoglobin 2	2,208
G0MWCVW02DPHE6	ARALYDRAFT_489601	DNAJ heat shock N-terminal domain-containing protein	2,006
contig08219	At1g55290	1.14.11.-	5,418
G0MWCVW01A4LV2	ARALYDRAFT_327188	Os08g0474600 protein	3,086
contig09531	NDPK1	2.7.4.6	2,287
G0MWCVW01B3N80	ldh	1.1.1.27	2,671
contig00213	Os03g0815200	Probable methylenetetrahydrofolate reductase	4,182
contig04144	At1g25520	GDT1-like protein 5	2,238
G0MWCVW04JXGC8	At2g27920	Serine carboxypeptidase-like 51	2,457
contig11523	At2g39260	AtUpf2	2,131
G0MWCVW03GOEZU	AMTR_s00063p00014550	Glutaredoxin family protein	4,478
G0MWCVW03HI9O3	MTR_6g087000	Putative cyclic nucleotide-dependent protein kinase isoform B variant 2	3,459
G0MWCVW03HJBHB	ZEAMMB73_458792	Oxidoreductase family protein	2,186
G0MWCVW01APIJI	POPTR_0009s13840g	At2g20940	2,095
G0MWCVW04JWBNH	At1g70520	2.7.11.-	3,104
G0MWCVW04JP5K5	AKR1	GmAKR1	2,217
contig09046	46C02.5	SAP1 protein	2,900
G0MWCVW02DAOYC	ATG18A	AtATG18a	2,850
G0MWCVW04IX936	bGlu	Glycosyl hydrolase family 1 beta glucosidase protein	2,963
G0MWCVW03FY4LV	PGSC0003DMG400028740	Os02g0147800 protein	2,583
G0MWCVW01AR7YB	PRXIIC	1.11.1.15	4,217
contig13866	At1g19730	Trx-H	4,500
contig07474	At3g54900	AtGRXcp	3,478
contig07747	ALDH	1.2.1.3	2,927
G0MWCVW03HBFPH	ARALYDRAFT_491904	Thioredoxin family protein	2,034
contig01645	GAPC	1.2.1.12	3,525
G0MWCVW02DKXNQ	SODB	1.15.1.1	2,036
G0MWCVW01CCCFC	ARALYDRAFT_348499	Putative uncharacterized protein	3,340
G0MWCVW03GJLCY	ARALYDRAFT_488004	Alpha/beta fold hydrolase family protein	2,068
G0MWCVW03HHLP5	ATMIN7	ARF guanine-nucleotide exchange factor BIG1	2,172
G0MWCVW01ARDS3	FLS2	Flagellin-sensing 2-like protein	2,683
G0MWCVW01ASR74	At1g08450	Calreticulin	2,380
G0MWCVW04IRIVQ	POPTR_0001s01660g	NBS-LRR type disease resistance protein	2,971
contig12817	At4g31750	3.1.3.16	2,147
contig09176	CBP3	Serine carboxypeptidase 3	2,004
G0MWCVW02EATOM		probable serine threonine-protein kinase at1g18390 isoform x1	2,377
G0MWCVW02EVKS6		lysm domain-containing gpi-anchored protein 2-like	2,722
G0MWCVW03GNMO5		regulatory protein npr3-like	2,822
contig13605		ethylene response factor 3	2,017
contig11855		wound-responsive family isoform 1	3,154
G0MWCVW01ANRL0		nad h dehydrogenase b2	5,694
G0MWCVW02D29CY		nbs domain resistance protein	2,281
G0MWCVW01AQ58A		cyst nematode resistance protein	2,191
G0MWCVW01BRY7J	POPTR_0011s07810g	Apoptosis inhibitor, putative	2,522
contig03623	AMTR_s00002p00260390	Glycine-rich family protein	2,066
G0MWCVW01APYY6	M569_09562	Late blight resistance protein, putative	3,277
G0MWCVW02DZA41	PI206	Dirigent protein PI206	2,716
Signal transduction			
G0MWCVW03GEGBG	PRUPE_ppa005552mg	-	3,069
contig09953	AFRR	Monodehydroascorbate reductase	2,826
contig01285	CIPK12	CBL-interacting serine/threonine-protein kinase 12	2,364
G0MWCVW03FWFBQ	AT3G28510	Mitochondrial chaperone BCS1	2,112
contig10222	At1g12340	Probable protein cornichon homolog 2	2,166
contig02784	CIPK1	2.7.11.1	2,889
G0MWCVW01AQSQY	CIPK10	2.7.11.1	3,769
contig10835	XCT	Protein XAP5 CIRCADIAN TIMEKEEPER	2,093
contig06118	NORK	2.7.11.1	2,612
contig06341	RAB11A	Ras-related protein Rab11A	2,286
G0MWCVW02D017Y	At4g14480	Protein BLUE LIGHT SIGNALING 1	2,036
G0MWCVW02DJH53	AMTR_s00016p00218760	GYF domain-containing protein	3,823
G0MWCVW04IXLUQ	At3g08510	3.1.4.11	2,824
G0MWCVW04H5MC8	AUR1	AtAur1	2,725
G0MWCVW03HDTLC	AMTR_s00268p00016240	FAT domain-containing protein	2,690
contig05243	CIPK1	2.7.11.1	2,137
G0MWCVW02EAM50	At1g48260	CBL-interacting protein kinase 21	3,041
G0MWCVW04I60F1	At1g66150	Probable receptor protein kinase TMK1	2,314

G0MWCVW02DMST4	AMTR_s00024p00252010	Serine/threonine-protein kinase PBS1, putative	2,386
contig07934	AMTR_s00003p00020850	Mitochondrial Rho GTPase	2,564
G0MWCVW03HIA2C	AMTR_s00095p00175220	SRP68	2,118
G0MWCVW04JLMPP	PARP1	ADPRT-1	2,565
contig09804	CARUB_v10022521mg	Serine/threonine protein kinase, putative	2,365
G0MWCVW04H69Q9	At2g24720	Glutamate receptor 2.2	2,260
G0MWCVW02DW2GF	At5g03730	Serine/threonine-protein kinase CTR1	2,215
G0MWCVW01CDGLV	WNK2	2.7.11.1	4,279
G0MWCVW03G8VVW	CICLE_v100184241mg	Pleckstrin homology (PH) domain-containing protein	2,817
G0MWCVW03GT5R4	ARALYDRAFT_489466	Uncharacterized protein	2,952
contig12437	At3g05140	2.7.11.1	2,803
G0MWCVW01BUG5K	AHK2	2.7.13.3	2,036
G0MWCVW02D7RTW	APK1A	2.7.11.1	2,443
G0MWCVW02DSIBX	ARALYDRAFT_471824	Serine/threonine protein kinase, putative	2,398
G0MWCVW04JAU3F	AHK2	2.7.13.3	2,112
G0MWCVW02DBNYT	ARALYDRAFT_484442	Serine/threonine protein kinase, putative	2,004
G0MWCVW02D3ZTS	At3g27580	2.7.11.1	3,382
G0MWCVW03F6342	At3g01480	5.2.1.8	3,580
G0MWCVW04I0V4R	M569_08172	-	2,393
G0MWCVW02EW4Z6	At1g49340	AtPI4Kalpha1	2,025
G0MWCVW01A0W3Y	24K23.24	MAPKKKe	2,123
contig11674	At3g02410	3.1.1.n2	2,285
G0MWCVW02EDJY6	At2g41210	2.7.1.68	2,521
G0MWCVW03FYZNQ	GA2OX1	1.14.11.13	2,272
contig02720	AMTR_s00177p00067830	RING zinc finger protein-like	3,632
G0MWCVW04H2TVG	At2g18730	2.7.1.107	3,093
G0MWCVW01B0M4T		probable lrr receptor-like serine threonine-protein kinase at3g47570 isoform x2	2,235
G0MWCVW03F88VA		probable serine threonine-protein kinase nak isoform x1	2,028
contig05089	At1g27730	COLD INDUCED ZINC FINGER PROTEIN 2	2,428
G0MWCVW01CBACJ	PRUPE_ppa001780mg	WD and tetratricopeptide repeats protein 1	2,994
Photosynthesis			
G0MWCVW03G04CP	AMTR_s00131p00114510	At1g19150	2,115
contig01470	RCA	RA	2,590
G0MWCVW04IKGLQ	At1g02910	Protein LOW PSII ACCUMULATION 1, chloroplastic	2,873
G0MWCVW04ISX6E	At3g17910	Cytochrome c oxidase assembly protein SURF1	2,047
G0MWCVW02D6E2U	ARALYDRAFT_491679	Putative oxidoreductase/electron carrier	2,266
contig07687	BRADI3G47890	AT1G67700 protein	3,927
G0MWCVW03HI5AM	PPOX2	1.3.3.4	3,795
G0MWCVW02DIUOO	ARALYDRAFT_671183	Metalloprotease m41 ftsh, putative	3,545
G0MWCVW03GZUSA	At1g68890/At1g68900	2-succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexene-1-carboxylate synthase	3,017
G0MWCVW03GW350	Si030097m.g	Os09g0436900 protein	4,910
G0MWCVW01A7DH4	PGSC0003DMG400021145	At5g45170	3,043
Primary metabolism			
G0MWCVW03GLZR5	PGSC0003DMG400020916	-	2,143
G0MWCVW02EGWEZ	CICLE_v10001735mg	ERI1 exoribonuclease	2,081
G0MWCVW02EQVEK	At1g30660	2.7.7.-	2,415
contig12825	At2g13560	1.1.1.39	2,077
G0MWCVW01BM71Y	At3g06310	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8-A	3,640
G0MWCVW01CD0TO	At3g21070	2.7.1.23	2,086
G0MWCVW02DXNLO	At4g34840	5'-methylthioadenosine nucleosidase	2,359
contig02161	GS1-2	Glutamine synthetase cytosolic isozyme 2	2,004
G0MWCVW03HEL3A	At5g52100	HTPA reductase 3	2,279
G0MWCVW01CB281	DHPS1	4.3.3.7	3,189
contig02231	LOC_Os03g19930	Adenylosuccinate lyase	2,770
contig03186	PGSC0003DMG402023438	Epoxide hydrolase	3,224
contig00669	KCS19	3-ketoacyl-CoA synthase 19	2,083
contig04233	At3g15820	2.7.8.-	2,564
contig13030	SPS1	2.4.1.14	6,663
contig01824	At3g03310	Lecithin-cholesterol acyltransferase-like 3	2,090
G0MWCVW04I77W1	AAE1	6.2.1.-	2,863
G0MWCVW04JOEWG	AMTR_s00166p00054410	2.4.1.67	2,884
G0MWCVW03G46WC	AT1G62305	Core-2/I-branching beta-1,6-N-acetylglucosaminyltransferase family protein	2,192
contig01912	At1g06410	2.4.1.15	2,065
G0MWCVW01AUDB7	isi1	Impaired sucrose induction 1-like protein	2,955
contig08228	BRADI5G13460	Glycosyl transferase family 17 protein	2,326
contig05404	SBH1	Sphinganine C(4)-monooxygenase 1	2,399
contig13252	AMTR_s00168p00055310	1.2.4.2	3,369
G0MWCVW03HE3VI	At1g03310	AtISA2	2,099
contig05936	AMTR_s00066p00106720	Poly(ADP-ribose) glycohydrolase	4,563

contig05566	ACCB-1	AtBCCP1	2,921
G0MWCVW03GQWUV	At3g52940	C-14 sterol reductase	2,741
G0MWCVW03F8WGO	AT5G65685	Starch synthase V	2,329
contig06785	At2g32260	2.7.7.15	2,014
G0MWCVW01BBBJT	At3g13750	3.2.1.23	2,042
contig07350	GSVIVT00026920001	Probable polygalacturonase	2,274
G0MWCVW03HCJYY	PHO1	Alpha-1,4 glucan phosphorylase L isozyme, chloroplastic/amyloplastic	2,061
contig07239	At5g47030	ATP synthase subunit delta', mitochondrial	2,127
G0MWCVW02DP6QZ	APFI	4.2.1.-	2,782
contig00046	RCOM_1509320	Copper amine oxidase, putative	3,141
G0MWCVW01BUNVU	AAT	2.6.1.1	3,271
G0MWCVW01BHZR6	AMTR_s00154p00074010	Asparagine synthetase domain-containing protein 1	3,320
G0MWCVW03FVZ3X	Solyc07g066030.2	-	2,768
G0MWCVW02EXQLF	PGSC0003DMG400022270	Sucrase-like protein	2,043
G0MWCVW02C10Z7	At4g25434	3.6.1.-	2,979
G0MWCVW03FXD1G	M569_04012	DEAD box ATP-dependent RNA helicase, putative	3,601
G0MWCVW03GO8P7	LOC_Os03g42110	AGPR	2,318
G0MWCVW03G4QTG	AMTR_s00045p00197400	Alanyl-tRNA synthetase	2,704
contig04715	AT3G47560	Esterase/lipase/thioesterase	3,193
contig07546	POPTR_0009s04590g	Helicase, putative	2,410
contig12130	ARALYDRAFT_496448	AT5g62650/MRG21_7	2,003
G0MWCVW01BUUU5	ALNC14_094960	Nicotinate phosphoribosyltransferase	2,693
contig08430	NSGT2	UDP-glucose:flavonoid glucoside 1,6-glucosyltransferase	2,425
G0MWCVW02C8PFW	MTR_5g030130	Zinc-binding family protein	3,354
G0MWCVW02DTIVD	AMTR_s00099p00159350	Alpha/beta hydrolase fold protein	3,005
G0MWCVW03GZ4PD	ARALYDRAFT_323061	Uncharacterized protein	2,142
G0MWCVW04JNMNI	POPTR_0015s08180g	ADP-glucose pyrophosphorylase family protein	5,200
G0MWCVW02EEC7M	PGSC0003DMG401002553	Putative uncharacterized protein At3g16260/MYA6_19	2,565
G0MWCVW04IONUN	AMTR_s00010p00264160	HNH endonuclease domain-containing protein, putative, expressed	2,369
G0MWCVW04I94F6	AMTR_s00066p00202260	Putative uncharacterized protein F4F15.160	2,362
contig03772	Si001586m.g	Hydrolase, alpha/beta fold family protein	2,597
G0MWCVW02DHRGN		dolichol-phosphate mannosyltransferase subunit 1	2,516
G0MWCVW02EKRT5		ribonuclease h protein at1g65750-like	2,264
G0MWCVW01A54IM		ribonuclease h protein at1g65750-like	2,065
G0MWCVW04IOUFJ		nudix hydrolase mitochondrial-like	2,057
Protein metabolism			
contig08438	At3g12340	Rotamase	2,267
G0MWCVW03GZ7N9	ATG18G	AtATG18g	2,041
contig06992	ATG18B	AtATG18b	3,916
G0MWCVW02DY0EB	At1g22760	PABP-2	2,086
contig02035	CTU1	Cytoplasmic tRNA 2-thiolation protein 1	3,996
G0MWCVW03G1GN3	AMTR_s00059p00132740	Translation initiation factor IF-2	3,492
G0MWCVW02E0GI8	At1g17220	Translation initiation factor IF-2, chloroplastic	2,664
contig07443	rpl2	50S ribosomal protein L2, chloroplastic	2,907
G0MWCVW02D105G	PGSC0003DMG400017174	Uncharacterized protein	2,166
contig12565	At1g13950	eIF-4D	2,003
G0MWCVW01A5BE5	EFTS	EF-TsMt	3,151
G0MWCVW01B3PCP	AMTR_s00010p00234800	Mitochondrial transcription termination factor family protein	3,822
contig04930	RPL5	60S ribosomal protein L5	2,069
contig09837	RPL27	60S ribosomal protein L27	3,309
contig03359	At1g48970	Translation initiation factor eIF-2B delta subunit	2,364
contig08695	At4g18100	60S ribosomal protein L32	2,147
G0MWCVW02DSV0Q	RPS3A	40S ribosomal protein S3a	2,864
contig02824	At3g58140	Phenylalanine--tRNA ligase, chloroplastic/mitochondrial	2,411
contig03821	VIP3	At4g29830	2,264
contig01799	BIP5	Luminal-binding protein 5	2,244
G0MWCVW02D9SVR	PGSC0003DMG400014817	-	4,439
G0MWCVW03HJU0Z	RPL10	60S ribosomal protein L10	2,186
contig13074	BRADI4G14150	Peptidyl-prolyl cis-trans isomerase cyclophilin-type family protein	2,322
contig10129	CLPB-M	ATP-dependent Clp protease ATP-binding subunit ClpB homolog 3	2,513
contig06863	At3g52300	ATP synthase subunit d, mitochondrial	2,184
contig07084	AHUS5	AtSCE1	2,584
contig03460	SPPL2	3.4.23.-	2,179
G0MWCVW04IAGS7	AMTR_s00268p00016240	FAT domain-containing protein	2,338
G0MWCVW04I22Z8	At1g32530	AtMIP1	2,243
G0MWCVW02DVYL1	CICLE_v10030698mg	Armadillo repeat-containing protein-like	4,049
contig05678	PRUPE_ppa003477mg	-	2,144

G0MWCVW02ESQEP	ARI10	6.3.2.-	2,062
contig02958	At2g17190	Ubiquitin domain-containing protein DSK2a	2,626
contig00649	ARALYDRAFT_484511	F-box family protein	2,747
G0MWCVW02DZLNJ	At1g26830	AtCUL3a	3,286
G0MWCVW01BMIBT	ASK16	AtSK16	2,149
G0MWCVW01AY0PB	mgp1	Male gametophyte defective 1	2,082
contig10639	AMTR_s00025p00160940	Fiber protein Fb15	2,822
contig03818	At4g19006	26S proteasome non-ATPase regulatory subunit 13 homolog A	2,451
contig00506	At3g11910	3.4.19.12	2,409
G0MWCVW02DCN0Q	UBC12	6.3.2.19	2,282
G0MWCVW03FR6NK	At3g61590	F-box/kelch-repeat protein At3g61590	3,840
G0MWCVW02ELRZW	At4g17270	Degreening-related gene dee76 protein	3,214
G0MWCVW03FXPRU	At1g15000	Serine carboxypeptidase-like 50	2,866
contig03987	At3g11910	3.4.19.12	2,615
G0MWCVW01BUKB8	ASK16	AtSK16	2,226
G0MWCVW02DZ5LW	AMTR_s00080p00063640	Ubiquitin protein ligase E3a, putative	2,082
G0MWCVW01CBLZH	At2g20580	26S proteasome non-ATPase regulatory subunit 2 homolog A	2,033
contig06693	At1g02560	3.4.21.92	3,227
contig08799	CICLE_v10032685mg	Ubiquitin family protein	2,480
contig09116	PGSC0003DMG400027415	Ubiquitin-associated domain-containing family protein	3,266
contig08638	PDIL5-4	AtPDIL5-3	2,289
G0MWCVW02DLKL9	PGSC0003DMG400029704	30S ribosomal protein S5, putative	2,129
G0MWCVW01CD3UM	At5g51540	Probable mitochondrial intermediate peptidase, mitochondrial	2,247
G0MWCVW04H30SM	At5g26240	AtCLC-d	6,123
G0MWCVW01AWAEJ	PHAVU_009G195600g	Putative uncharacterized protein AT4g28080	2,549
G0MWCVW04IPDJU	RPS3A	40S ribosomal protein S3a	2,376
G0MWCVW02DAGCS	At1g77810	2.4.1.-	2,616
G0MWCVW03GSPMD	At2g41790	Peroxisomal M16 protease	2,085
contig11425	AMTR_s00001p00272380	At1g49850	4,319
contig03641	At3g49720	Uncharacterized protein At3g49720	2,717
contig12178	BSL2	3.1.3.16	2,274
contig09299	AMTR_s00002p00253520	Aspartyl protease family protein	2,215
contig03947	At2g26850	F-box protein At2g26850	3,162
G0MWCVW04IYCY0	AMTR_s00007p00259420	Similar to late embryogenesis abundant proteins	2,001
G0MWCVW03F87HG	AMTR_s00169p00022730	Methyltransferase, FkbM family protein, expressed	2,313
G0MWCVW02EGMWT	BRA013454	Uncharacterized protein	3,881
G0MWCVW04JL9W0	pol	Pol protein integrase region	3,027
G0MWCVW02EADK4	AMTR_s00023p00093390	Uncharacterized protein	4,128
G0MWCVW04I94BM	At4g16580	AtPP2C55	2,395
G0MWCVW02EZBGX	POPTR_0013s06250g	Glycosyltransferase family 14 protein	4,924
G0MWCVW01BHZA7		protein skip34	2,180
G0MWCVW02C8MSR		ring-h2 finger protein atl56-like	2,840
G0MWCVW02DBBV0		ring-h2 finger protein atl56-like	2,309
G0MWCVW02DFUZO		otu domain-containing protein at3g57810-like isoform x2	2,102
Secondary metabolism			
contig14444	SAMDC	4.1.1.50	2,548
G0MWCVW03F9K6P	PDT1	4.2.1.91	4,964
contig02051	CHS	Chalcone synthase	2,201
G0MWCVW02EDFRK	ADC1	4.1.1.19	4,501
contig00982	G10H	1.14.-.-	3,312
G0MWCVW03G9TNB	VDR	Violaxanthin de-epoxidase	2,530
contig14240	SAMDC	4.1.1.50	4,163
G0MWCVW01ASUZI	AMTR_s00012p00210330	CER3 protein	4,484
G0MWCVW02DU22A	At1g01610	2.3.1.15	2,207
G0MWCVW02EKH1A	RCOM_1173780	Putative uncharacterized protein	4,297
contig01714	At1g74960	2.3.1.41	2,336
contig10995	At5g04660	1.14.-.-	2,508
G0MWCVW03FV5GN	SRT1	NAD-dependent protein deacetylase SRT1	4,781
G0MWCVW01AMDX4	CRTISO	5.2.1.13	2,426
contig13802	CHLP	Geranylgeranyl diphosphate reductase, chloroplastic	2,295
G0MWCVW04IFGQG	ARALYDRAFT_478046	GTP cyclohydrolase I	2,131
contig04908	FL	Flavonol synthase/flavanone 3-hydroxylase	2,819
G0MWCVW02EOCAP	PGSC0003DMG400009340	NADPH--cytochrome P450 reductase	2,349
contig03771	EUTSA_v10000343mg	AT4g30620/F17I23_40	5,133
contig12711	F13M22	1.1.1.-	2,787
Transport			
contig13494	At2g35190	AtNPSN11	3,300
contig01124	TPT	Triose phosphate/phosphate translocator, chloroplastic	2,583
contig14143	EUTSA_v10019295mg	F9L1.32 protein	2,251

G0MWCVW01AKYVN	CICLE_v10021916mg	Aquaporin protein AQU20	4,247
G0MWCVW04IJBGE	AP22.86	AtPLT5	2,712
G0MWCVW04JS16B	B1120F06.131	S-adenosylmethionine mitochondrial carrier protein	2,571
G0MWCVW02ECD34	RAB11A	Ras-related protein Rab11A	2,164
G0MWCVW01B5KO7	PGSC0003DMG400029682	AP-3 complex subunit beta-2	2,977
contig12742	At1g66240	Copper chaperone ATX1	2,354
G0MWCVW04JDWLP	SPX4	Protein SPX DOMAIN GENE 4	2,138
G0MWCVW01B09LB	BRADI1G54510	Coatomer subunit gamma	2,730
G0MWCVW03F3CX1	CICLE_v10004887mg	Ras-GTPase-activating protein-binding protein, putative	2,509
G0MWCVW04JVEU2	At1g28490	AtSYP61	2,309
G0MWCVW02D5L31	TIC32	Short-chain dehydrogenase TIC 32, chloroplastic	2,182
contig08669	At1g05030	AtpGlcT	2,602
contig03012	At5g19760	Dicarboxylate/tricarboxylate carrier	2,073
G0MWCVW02DRWAB	AT3G47550	Membrane associated ring finger 1,8, putative	2,220
contig00554	PGSC0003DMG400027696	Auxin efflux carrier family protein	2,114
G0MWCVW01CIKHQ	SEC13	Protein transport SEC13-like protein	4,339
G0MWCVW03GMEY6	ARALYDRAFT_493322	CASP-like protein ARALYDRAFT_493322	2,082
G0MWCVW04JFR8W	At4g10770	AtOPT7	5,516
G0MWCVW03GTHJD	At1g20816	Chloroplastic outer envelope pore protein of 21 kDa A	3,575
G0MWCVW04JK7Y5	At3g60600	AtPVA11	4,091
G0MWCVW04H7GKZ	At1g15990	AtCNGC1	3,950
G0MWCVW02EKJNP	AMTR_s00040p00119530	AT3g11530/F24K9_21	2,070
G0MWCVW01BVXIG	MRP10	3.6.3.44	2,138
G0MWCVW04JKJJU	At1g13980	ARF guanine-nucleotide exchange factor GNL1	2,049
contig09955	ZEAMMB73_304784	Fcf2 pre-rRNA processing protein	2,774
G0MWCVW02EDS8M	AMTR_s00039p00231550	Gb|AAF04433.1	2,389
G0MWCVW03F6PKR	Si016087m.g	RGPR-related protein	2,032
G0MWCVW01B1JN2	AMTR_s00036p00206680	Putative developmental protein	4,211
G0MWCVW03G8LF5	At3g21865	AtPEX22	2,040
G0MWCVW01A4DTN	ARALYDRAFT_495677	Putative uncharacterized protein At5g55950	2,284
contig00246	NPF6.1	Protein NRT1/ PTR FAMILY 6.1	3,493
G0MWCVW01A03EW	At3g11397	AtPRA1.A1	3,125
G0MWCVW03GG076	CARUB_v10002124mg	Uncharacterized protein	2,296
contig00628	sec61	Sec61 alpha subunit	2,517
contig03727	At2g15290	AtCIA5	2,170
contig08143	At5g14880	AtHAK8	2,900
contig09779	1A7.6	Importin beta-3, putative	2,010
G0MWCVW04JLUZH	ADNT1	Adenine nucleotide transporter 1	2,411
contig12736	PPT2	Phosphoenolpyruvate/phosphate translocator 1, chloroplastic	2,008
contig03869	ADNT1	Adenine nucleotide transporter 1	2,034
G0MWCVW02DPZS2	AMTR_s00007p00267150	Nuclear pore complex protein Nup107	2,005
G0MWCVW03GDQH1	BRA040967	Uncharacterized protein	2,519
G0MWCVW04INCFN	At5g11490	AP complex subunit beta-A	2,180
G0MWCVW02DULQG	At1g62020	Alpha-COP 1	2,285
G0MWCVW01B7933	At1g78900	V-ATPase 69 kDa subunit	2,256
G0MWCVW02EV795	PGSC0003DMG400000734	-	2,518
G0MWCVW04IOQSH	PGSC0003DMG400007865	Putative polyol/monosaccharide transporter	2,173
contig00110	At5g49730	1.16.1.7	2,608
contig12777	AMTR_s00044p00119220	Chloroplast inner membrane localized protein	3,573
G0MWCVW03GSRE1	AMTR_s00107p00047780	NIC-domain-containing protein	2,034
G0MWCVW03GNHUC	At1g01960	ARF guanine-nucleotide exchange factor BIG1	5,030
G0MWCVW04H6ZYU	AMTR_s00138p00044110	F5A9.22 family protein	2,153
G0MWCVW01AZ85Y	ABCG11	ABC transporter ABCG.11	2,950
G0MWCVW01CAT8S	AHA1	3.6.3.6	2,530
G0MWCVW03GLVCH	POPTR_0005s27320g	Patched family protein	2,039
G0MWCVW04I7S7F	AHA1	3.6.3.6	2,341
G0MWCVW02DA315	AMTR_s00095p00175220	SRP68	2,792
G0MWCVW02DUUD3	AGD4	ADP-ribosylation factor GTPase-activating protein AGD2	2,078
G0MWCVW02C5N6Q	ABCB27	ABC transporter ABCB.27	3,194
contig11096	AP22.86	AtPLT5	3,181
G0MWCVW03HCS46	At1g63440	3.6.3.54	3,552
G0MWCVW04I40Q4	At2g38270	AtGrxS16	2,747
G0MWCVW01ALGRL	At4g24730	ADPRibase-Mn	3,265
contig14196	M569_05335	F-ATPase delta' subunit	3,337
G0MWCVW02DO2FL		k(+) efflux antiporter 3	2,071
G0MWCVW04IRPCQ		trafficking protein particle complex subunit 11 isoform x2	2,016
G0MWCVW03GZU5O		myosin-9 isoform x3	2,446
G0MWCVW03GCN17		calcium-transporting atpase plasma membrane-type-like	2,313
contig14714		plasma membrane atp adp transporter tlc1	3,779
G0MWCVW04JD2CC		glutamate receptor -like	2,337

Table 7.4. List of differentially expressed genes with fold-change < 0.5 and p-value < 0.05 (Student's t-test) in 'Ruveia' olives after the attack of *Bactrocera oleae.*

Seq. Name	Seq. Codes	Seq. Description	FC Rd/Ru
Cellular organization			
G0MWCVW04JWQF3	ALH3-1.1	Histone H3	0,421
G0MWCVW01B7QPL	At1g14690	65-kDa microtubule-associated protein 6	0,423
G0MWCVW03GBKZ5	At1g04160	AtMYA1	0,430
G0MWCVW04IMZ6Z	At1g04160	AtMYA1	0,263
G0MWCVW04IBLU4	At2g28370	CASP-like protein 1	0,240
G0MWCVW02EAUL2	ATJ20	AtDjC20	0,451
G0MWCVW02D7RZJ	At5g59560	Protein SENSITIVITY TO RED LIGHT REDUCED 1	0,420
contig05154	CYCD3-2	CycD3	0,452
contig08932	TUBB4	Tubulin beta-4 chain	0,330
G0MWCVW02C5ZJA	CYCH1-1	CycH1	0,158
G0MWCVW04IDBP4	At3g06400	ISW2-like	0,399
G0MWCVW04ICTO0	At1g71440	AtTFCE	0,331
G0MWCVW03GW6FB	BRADI1G48350	Putative far-red impaired response protein	0,475
G0MWCVW03FP5OY	At3g23400	AtPGL30.4	0,451
G0MWCVW02EYMMP	TUBB	Tubulin beta chain	0,095
G0MWCVW02ERD9R	At3g10380	AtSec8	0,382
G0MWCVW01BCT4W	PGSC0003DMG400028814	WD repeat-containing protein 26	0,337
contig06334	PGSC0003DMG400004723	SDA1 family protein	0,423
contig04898	ADL3	3.6.5.5	0,483
contig05168	At2g34680	Os07g0148800 protein	0,494
contig05437	ACT-1	Actin	0,496
G0MWCVW03GX24U	At4g24900	TITAN-like protein	0,350
contig04949	POPTR_0013s06350g	Zinc finger protein VAR3, chloroplastic	0,333
G0MWCVW02EX0ZF	At1g73970	Uncharacterized protein	0,285
contig09826	At5g53280	Plastid division protein PDV1	0,062
contig09203	At1g22690	GAST1 protein homolog 14	0,202
G0MWCVW04IEUPW	CALS10	Callose synthase 10	0,485
G0MWCVW04IFL6E	ARP4	Actin-related protein 4	0,379
G0MWCVW02DQDXF	AT1G65810	P-loop containing nucleoside triphosphate hydrolases superfamily protein	0,406
G0MWCVW03GGR7M	NAP1	NAP of plants	0,269
G0MWCVW04ILT1H	CjBAp12	EG45-like domain containing protein	0,485
G0MWCVW02DPB4E	At2g47940	Protease Do-like 2, chloroplastic	0,442
G0MWCVW04H23J6	At2g41740	Villin-2	0,321
G0MWCVW01AZLUH	DEK1	Calpain-type cysteine protease DEK1	0,336
G0MWCVW03GH1AA	At1g22060	Uncharacterized protein	0,275
G0MWCVW02ELGDW	TUBB	Tubulin beta chain	0,269
G0MWCVW01BIJUF	At1g69295	Glucan endo-1,3-beta-glucosidase-like protein 2	0,094
G0MWCVW01BQA8W	PHAVU_011G040700g	Kinesin-related protein	0,149
contig10733	ARALYDRAFT_495910	FIP1 [V]-like protein	0,427
G0MWCVW02ER45E	ACL5	Thermospermine synthase ACAULIS5	0,410
G0MWCVW02DAVGA	VITISV_032223	-	0,500
contig06285	SEC10	Exocyst complex component 5	0,491
G0MWCVW03HIYD7	ARP7	Actin-related protein 7	0,457
contig12286	AT4G37890	Protein binding protein, putative	0,087
G0MWCVW02EHR2Z	At4g13590	GDT1-like protein 2, chloroplastic	0,498
G0MWCVW02EJ9CC	At1g58470	AtRBP1	0,459
G0MWCVW02C60C0	M569_07809	Uncharacterized protein	0,204
G0MWCVW02DPG35	At2g41740	Villin-2	0,460
G0MWCVW01BOS36	AMTR_s00024p00172850	Gamma-tubulin complex component 3-like protein	0,479
G0MWCVW02DGSXQ	AGD10	ADP-ribosylation factor GTPase-activating protein AGD10	0,422
contig06614	MTR_3g090670	Myosin heavy chain IB	0,442
G0MWCVW03G1UVT	BRADI2G23277	Putative auxin-independent growth promoter	0,056
G0MWCVW04JPU8F	At3g58100	(1->3)-beta-glucan endohydrolase 12	0,405
G0MWCVW03GQHZ1	At1g64090	AtRTNLB1	0,371
G0MWCVW02DAXUL	BRADI4G35720	UBX domain-containing protein	0,345
G0MWCVW04JNCD5	AMTR_s00099p00142390	Enzyme of the cupin superfamily	0,395
G0MWCVW02C96RU	At1g04990	Zinc finger CCCH domain-containing protein ZFN-like 1	0,489
G0MWCVW01B80CK	AT3G12020	Kinesin-related protein 11	0,455
contig13519	PGSC0003DMG400005910	Cellular nucleic acid binding protein, putative	0,436
G0MWCVW03G1LCW	AMTR_s00077p00157770	At3g56430	0,467
G0MWCVW03HC1RE	AMTR_s00013p00248880	Zinc finger CCCH domain-containing protein ZFN-like	0,334
contig03730	ZEAMMB73_406719	Cov1	0,479
G0MWCVW02DR7X0	At5g16730	WEB family protein At5g16730, chloroplastic	0,398
G0MWCVW02EA2V5	At1g63260	Tetraspanin-10	0,297

G0MWCVW02DT1XY	At2g19950	Golgin-84	0,452
G0MWCVW02DOLZL	TKRP125	125 kDa kinesin-related protein	0,469
G0MWCVW04IA4R1		cyclase-associated protein 1	0,384
G0MWCVW02DD065		microtubule-associated protein rp eb family member 1	0,486
Transcription			
G0MWCVW03GJ190	M569_07124	Uncharacterized protein	0,313
G0MWCVW03F23NB	At5g16180	Chloroplastic RNA splicing factor 1	0,493
G0MWCVW03FWYD8	Si030327m.g	Putative aspartate-arginine-rich mRNA binding protein mRNA	0,285
G0MWCVW04JJVS9	POPTR_0007s15090g	TAZ zinc finger family protein	0,500
G0MWCVW01BJCJB	At1g27660	AtbHLH103	0,342
G0MWCVW03F1PFR	ARALYDRAFT_913702	At4g28200	0,475
contig02569	AMTR_s00154p00086540	At3g26850	0,461
contig09740	HAP3A	Nuclear transcription factor Y subunit B-2	0,470
G0MWCVW04JZHSX	Solyc01g095100.2	-	0,432
G0MWCVW02ETJOO	At2g27100	Serrate RNA effector molecule	0,467
G0MWCVW01BARA8	CICLE_v10011338mg	Transcription factor, putative	0,495
G0MWCVW03GBK1I	AMTR_s00010p00243220	Pre-mRNA-splicing factor SYF1	0,456
G0MWCVW02EN9ZK	AMTR_s00143p00072740	AT5g67320/K8K14_4	0,345
contig13082	POPTR_0012s09850g	AT5g63440/MLE2_7	0,209
G0MWCVW03FM6WG	POPTR_0005s10780g	Scaffold attachment factor B1	0,343
G0MWCVW02DNMNH	ARALYDRAFT_496142	DNA-directed RNA polymerase	0,466
G0MWCVW02E0TZ1	GF14A	14-3-3-like protein	0,270
G0MWCVW01BPHF2	At5g42920	THO complex subunit 5B	0,399
contig11666	AMTR_s00032p00242160	Plus-3 domain-containing family protein	0,443
G0MWCVW01AZBGH	HD2b	Type 2 histone deacetylase a	0,302
contig00993	AMTR_s00001p00251940	Trihelix transcription factor GT-2	0,474
G0MWCVW01BCRGL	ARALYDRAFT_911877	At4g03250	0,415
contig10132	AT5G51170	U6 snRNA phosphodiesterase	0,040
G0MWCVW04IRRTU	NtEIG-D48	WRKY transcription factor IId-4	0,497
G0MWCVW02DS6M8	At1g30500	AtNF-YA-1	0,478
contig14660	NAM-B1	NAC transcription factor NAM-B1	0,331
G0MWCVW01AU3PC	MTR_5g029470	MYB transcription factor MYB34	0,410
G0MWCVW03GVISE	ASHH1	ASH1 homolog 1	0,262
G0MWCVW02DC7Y5	PGSC0003DMG400002481	Myb family transcription factor family protein	0,316
G0MWCVW03GNYBF	AMTR_s00024p00227830	Os07g0476200 protein	0,131
G0MWCVW03GIWJ9	At1g14650	Probable splicing factor 3A subunit 1	0,325
contig04744	DIVARICATA	Transcription factor DIVARICATA	0,208
G0MWCVW03HH9AZ	At1g53230	Plastid transcription factor 1	0,254
contig11936	34G24.3	AT5G51300 protein	0,182
contig11258	BRADI1G52060	Uncharacterized protein	0,271
contig10678	At3g01770	AtBET10	0,339
contig07378	At1g17590	AtNF-YA-1	0,452
contig12508	At1g67310	AtFIN21	0,318
contig13373	At4g03120	U1 small nuclear ribonucleoprotein C	0,455
contig01478	At2g48110	AtREF4	0,403
G0MWCVW03G6AFB	MYB4	ATR1	0,410
contig13418	At2g04630	DNA-directed RNA polymerases II and V subunit 6B	0,318
contig04796	At1g03840	ID1-like zinc finger protein 3	0,471
G0MWCVW02DIA2K	At2g15400	DNA-directed RNA polymerase II 36 kDa polypeptide A	0,472
G0MWCVW02C8ZN2	CICLE_v10020379mg	Aquarius	0,479
contig12905	AMTR_s00066p00163770	HIV Tat-specific factor 1-like protein	0,455
G0MWCVW02EE0LX	AMTR_s00010p00182210	Intron-binding protein aquarius	0,419
contig07763	AMTR_s00027p00198570	Auxin-responsive family protein	0,358
G0MWCVW01B706O	CICLE_v10007292mg	Auxin response factor	0,421
contig00111	At1g23860	At-RSZ21	0,489
G0MWCVW03GAJZJ	ENBP1	Lysine-specific demethylase 3B	0,066
G0MWCVW02EAJEW	PGSC0003DMG400009473	-	0,416
G0MWCVW03GM4W3	At1g55750	AtTFB1-1	0,297
G0MWCVW04IGSAX	At2g19910	2.7.7.48	0,314
G0MWCVW03GTDLE	ATX3	Histone-lysine N-methyltransferase ATX3	0,390
G0MWCVW02EVJWT	myb	MYB transcription factor	0,434
contig14165	NRPB6A	DNA-directed RNA polymerases II, IV and V subunit 6A	0,475
contig06772	MTR_7g089140	Transcription elongation factor-related family protein	0,329
G0MWCVW04JSR6T	Solyc08g029090.2	-	0,148
G0MWCVW01CAIG6	AMTR_s00072p00015740	COL domain class transcription factor	0,452
G0MWCVW01A9ERZ	At4g18930	3.1.4.-	0,334
G0MWCVW04IO9ZZ	CICLE_v10000654mg	WRKY53-superfamily of TFs having WRKY and zinc finger domains	0,439
G0MWCVW02E1PGN	At2g15400	DNA-directed RNA polymerase II 36 kDa	0,484

		polypeptide A	
G0MWCVW01A98S9	TULP1	Tubby-like F-box protein 1	0,424
G0MWCVW01B15CL	POPTR_0007s15090g	TAZ zinc finger family protein	0,461
G0MWCVW01ASYLD	PRUPE_ppa002077mg	Auxin-responsive protein	0,323
contig07467	At2g22670	Auxin-responsive protein IAA8	0,462
contig05996	At1g29860	AT.I.24-4	0,411
contig07007	PGSC0003DMG400004926	Early-responsive to dehydration protein, putative, expressed	0,176
G0MWCVW02EAJPJ	AMTR_s00001p00273050	Retinoblastoma-binding protein-like	0,460
contig03456	ANL2	GLABRA 2-like homeobox protein 1	0,220
G0MWCVW01A3RJQ	At5g13010	Pre-mRNA-splicing factor ATP-dependent RNA helicase PRP16	0,359
G0MWCVW03GPUYR	ANL2	GLABRA 2-like homeobox protein 1	0,491
contig09466	At1g14685	AtBPC1	0,041
contig11271	At2g21060	Glycine-rich protein 2	0,259
G0MWCVW02EA5ZV	CICLE_v10023767mg	Pentatricopeptide repeat-containing protein, putative	0,417
G0MWCVW01AV7OI	RCOM_0992720	Pentatricopeptide repeat-containing protein, putative	0,498
G0MWCVW01BCMPV	PGSC0003DMG400023145	Pentatricopeptide repeat-containing protein, putative	0,453
G0MWCVW01A3KKL	AMTR_s00004p00115360	DEA(D/H)-box RNA helicase family protein	0,421
contig06940	POPTR_0031s00240g	Uncharacterized protein	0,371
G0MWCVW01CEM7Q	At3g22430	XS domain containing protein, expressed	0,082
contig10014	PGSC0003DMG400023341	AG-motif binding protein-1	0,142
G0MWCVW03F921G	ALNC14_033250	DNA-directed RNA polymerase	0,426
G0MWCVW01APVY9	POPTR_0008s07350g	ATP-dependent helicase, putative	0,260
G0MWCVW02EFJTI	CICLE_v10029330mg	At2g27290	0,486
contig07602	AMTR_s00010p00175790	VQ motif-containing family protein	0,388
contig01369	SAHH	Adenosylhomocysteinase	0,409
G0MWCVW03GPUTW	CICLE_v10011236mg	Pentatricopeptide repeat-containing family protein	0,328
G0MWCVW04I3L2U	MTR_7g080630	Poly(RC)-binding protein	0,387
contig04520	PGSC0003DMG400033662	Calcineurin-like metallo-phosphoesterase family protein	0,342
contig03252	At1g04050	2.1.1.43	0,427
G0MWCVW01CBN2N	MTR_3g110650	Poly(RC)-binding protein, putative	0,457
G0MWCVW03GPQAS	At3g02490	Pentatricopeptide repeat-containing protein At3g02490, mitochondrial	0,335
contig05850		nac domain-containing protein isoform 1	0,491
G0MWCVW01ASHRI		t-box transcription factor isoform 1	0,362
G0MWCVW01BGBSY		telomere repeat-binding protein 6	0,084
G0MWCVW02C48OI		protein phr1-like 1-like isoform x1	0,492
G0MWCVW04IY90T		nuclear transcription factor y subunit a-1	0,277
contig12996		transcription factor hbp-1b -like	0,367
G0MWCVW03F2WW5		sequence-specific dna binding transcription factors	0,441
G0MWCVW03F8PE8		mitochondrial transcription termination factor family isoform 1	0,301
G0MWCVW04JFMA0		b3 domain-containing protein os03g0622200-like isoform x2	0,470
G0MWCVW01AVHT8		transcription factor bhlh140	0,337
G0MWCVW02DSAGF		t-box transcription factor isoform 1	0,452
G0MWCVW04JJIOP		cleavage stimulation factor subunit 2	0,334
G0MWCVW01BITJ3		b3 domain-containing transcription repressor val1-like	0,051
G0MWCVW01B3F9J		wd repeat-containing protein 82	0,385
contig10396		nuclear receptor binding set domain containing protein isoform 1	0,361
G0MWCVW03F1KZ4		probable lysine-specific demethylase jmj14-like	0,154
G0MWCVW04JRTVG		pentatricopeptide repeat-containing protein mitochondrial-like	0,365
G0MWCVW03GCNY7	At2g36740	SWR1 complex subunit 2	0,353
Cell wall modification			
G0MWCVW03GN12O	At3g10540	3-phosphoinositide-dependent protein kinase 1	0,171
G0MWCVW03G79JA	MAP70.1	70 kDa microtubule-associated protein 1	0,459
contig14286	AXY3	Maltase	0,389
G0MWCVW01BU8AL	CESA3	2.4.1.12	0,404
G0MWCVW04JTW7H	At2g20370	Protein MURUS 3	0,387
G0MWCVW01B5FFD	AGAL1	Alpha-galactosidase	0,435
contig04270	EXP1	Alpha-expansin-1	0,070
G0MWCVW04IWYNP	AGAL1	Alpha-galactosidase	0,442
G0MWCVW04JH974	ARALYDRAFT_321547	CASP-like protein ARALYDRAFT_321547	0,430
contig13524	EXP1	Alpha-expansin-1	0,493
G0MWCVW01BOR21	ARAF	Beta-D-xylosidase	0,073
G0MWCVW02EZ1W7	AT1G29890	O-acetyltransferase Cas1p-like protein	0,060
G0MWCVW03GHNH0	At5g23450	AtLCBK1	0,432

G0MWCVW02C6IDT	At5g03795	2.4.-.-	0,422
G0MWCVW01BV1HF	At1g18580	2.4.1.-	0,195
contig07916	At1g49710	AtFUT11	0,304
G0MWCVW02ECW6F	At2g46480	2.4.1.-	0,454
contig10920	At1g62990	Homeobox protein knotted-1-like 3	0,437
G0MWCVW01BR1T6	ADPG2	3.2.1.15	0,403
G0MWCVW02DR0YF	PME1.9	3.1.1.11	0,406
contig00455	BRADI1G27290	7-deoxyloganetic acid UDP-glucosyltransferase-like protein	0,349
G0MWCVW02ERTBR	AT4g38040	AT4G38040 protein	0,488
G0MWCVW02D9WJW	AMTR_s00033p00229880	Exostosin family protein	0,487
contig00044	AMTR_s00002p00253220	Exostosin-2	0,211
G0MWCVW02C17YS	M569_14508	-	0,306
G0MWCVW01BBYNH		probable pectinesterase pectinesterase inhibitor 13	0,433
Stress and defense response			
G0MWCVW02DY1A6	HSP82	Heat shock protein 82	0,441
contig07843	At3g54900	AtGRXcp	0,458
contig00497	AR1	1.6.2.4	0,468
G0MWCVW02DRW6S	AMTR_s00092p00163760	D-amino acid oxidase	0,431
contig02799	GLO1	1.1.3.15	0,427
contig05145	F3F9.11	2.5.1.18	0,464
contig04361	At1g75280	1.3.1.-	0,278
contig06319	At3g54900	AtGRXcp	0,454
contig04291	At3g54900	AtGRXcp	0,489
G0MWCVW04JA5PN	AMTR_s00049p00109360	Putative polyketide hydroxylase	0,446
G0MWCVW02E09NA	AT1G31600	AT1G31600 protein	0,416
G0MWCVW03GVIJJ	CICLE_v10028812mg	Putative 2-nitropropane dioxygenase	0,230
contig07666	PRUPE_ppa008397mg	Prostaglandin E synthase	0,376
contig00953	GR	GR	0,438
contig03701	At5g16970	1.3.1.74	0,229
G0MWCVW04IZJS6	M569_12985	Thioredoxin	0,488
contig05877	AMTR_s00016p00259900	2-oxoglutarate-iron(II)-dependent oxygenase	0,376
contig09478	GPX1	PHGPx	0,371
contig02396	At1g63010	SPX domain-containing membrane protein At1g63010	0,489
contig00259	CEF	Protein transport protein Sec24-like CEF	0,476
G0MWCVW02C7UZO	F28P5.2	AtNPF5.1	0,476
contig04123	AGAA.4	GTP-binding protein SAR1	0,073
G0MWCVW02DSO30	PHAVU_009G035800g	Constitutive triple response 3	0,325
G0MWCVW02D8P26	At3g27890	1.6.5.2	0,299
contig05212	ANN4	AnnAt4	0,309
G0MWCVW01BI622	At1g77100	1.11.1.7	0,237
contig04621	ANN1	AnnAt1	0,470
G0MWCVW02D7DUC	AMTR_s00002p00267790	NAD(P)H dehydrogenase 18	0,486
G0MWCVW01ARCBR	At2g40690	1.1.1.8	0,115
G0MWCVW04I7ISU	At4g21670	AtCPL1	0,446
G0MWCVW02EM7GX	At2g38840	Guanylate-binding protein 4	0,370
contig00936	ALAAT1	2.6.1.2	0,483
contig11138	ANN1	AnnAt1	0,259
contig04240	SRK	2.7.11.1	0,292
contig02760	P4H2	Oxidoreductase	0,463
G0MWCVW02ERK13	Solyc05g008260.2	Peptidyl serine alpha-galactosyltransferase	0,078
G0MWCVW04IMYFE	SFR2	Beta-glucosidase-like SFR2, chloroplastic	0,353
contig11024	At1g74950	Jasmonate ZIM domain-containing protein 2	0,426
contig08542	At2g30140	UDP-glycosyltransferase 87A1	0,425
contig05051	HSC80	Heat shock cognate protein 80	0,356
G0MWCVW01A1C1V	At2g40690	1.1.1.8	0,471
G0MWCVW03GZRZ7	AWI31	3-oxo-Delta(4,5)-steroid 5-beta-reductase	0,368
G0MWCVW04IVDUO	ATG18A	AtATG18a	0,447
contig04492	At2g20340	4.1.1.25	0,486
G0MWCVW02C66G6	At3g01480	5.2.1.8	0,308
G0MWCVW02DXWHF	MET	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	0,471
G0MWCVW03FYTYK	DAD1	DAD-1	0,242
G0MWCVW02DCBZJ	ABI1	3.1.3.16	0,469
G0MWCVW02DHC24	ATPK1	Ribosomal-protein S6 kinase homolog 1	0,370
G0MWCVW02DSWR1	RAPTOR1	Protein RAPTOR 1	0,303
G0MWCVW01AK8QJ	6J23.20	Putative uncharacterized protein AT4g23440	0,440
G0MWCVW03F56U6	At4g21390	G-type lectin S-receptor-like serine/threonine-protein kinase B120	0,101
contig02121	AT3G12050	Activator of 90 kDa heat shock protein ATPase	0,369
G0MWCVW01A584F	At2g22300	AtER66	0,050
G0MWCVW01BDF3S	HSP70-6	Heat shock 70 kDa protein 6, chloroplastic	0,445
G0MWCVW04IYRVX	At4g31140	3.2.1.39	0,484
G0MWCVW02E1RDB	AMTR_s00005p00261220	AT5g40470/K21I16_20	0,496

G0MWCVW02C86LA	CICLE_v10016944mg	Multiple stress-responsive zinc-finger protein	0,474
G0MWCVW01B4IV6	AMTR_s00069p00197120	Ankyrin repeat and zinc finger domain-containing protein	0,489
G0MWCVW01AXCXE	BRADI3G58920	Elicitor-inducible protein EIG-J7	0,480
G0MWCVW02EF6KA	PGSC0003DMG400007818	Putative adiponectin receptor 1	0,323
G0MWCVW01B931E	ETR1	2.7.13.3	0,411
contig00451	At1g08450	Calreticulin	0,399
G0MWCVW04I3BPD	AGC2-1	Protein OXIDATIVE SIGNAL-INDUCIBLE 1	0,392
G0MWCVW04JLUP1	At2g39260	AtUpf2	0,418
G0MWCVW02EDVOG	BS2	Disease resistance protein BS2	0,445
contig01374	AMC4	3.4.22.-	0,488
contig04053	DGK2	Diacylglycerol kinase 2	0,419
contig09997	At4g17040	ATP-dependent Clp protease proteolytic subunit-related protein 4, chloroplastic	0,405
G0MWCVW03GZCS3	At2g33580	LysM domain receptor-like kinase 5	0,232
G0MWCVW01BQ2OX	NBS226-4	Disease resistance protein RGH4	0,354
G0MWCVW02DUHIV	At5g48380	Probably inactive leucine-rich repeat receptor-like protein kinase At5g48380	0,477
G0MWCVW01CGKCX	MLP	Putative major latex-like protein	0,480
G0MWCVW02EW8DW	CICLE_v10006705mg	Spermine synthase	0,223
G0MWCVW04IWLPF	At1g08450	Calreticulin	0,396
G0MWCVW03GOPJS	LOX1.1	1.13.11.58	0,464
G0MWCVW01BEOJA	EDR1	EDR1	0,302
contig04042	ATHCOR1	3.1.1.14	0,305
contig02820	At4g11260	AtSGT1a	0,208
contig08983	CARUB_v10010521mg	Putative drought-induced protein SDi-6-like	0,385
contig10675	HSP17.9-D	17.9 kDa class II heat shock protein	0,078
G0MWCVW04I2IUY	PAD4	Phytoalexin deficient 4	0,374
contig06521	At1g25520	GDT1-like protein 5	0,365
contig04940	AMTR_s00068p00201560	Os06g0530300 protein	0,385
G0MWCVW04H3Q5Q	BSP	Putative bark storage protein	0,230
contig10931	At3g29760	Genomic DNA, chromosome 3, P1 clone:MOD1	0,439
G0MWCVW04I2PZA	M569_04847	Haloacid dehalogenase superfamily protein	0,456
G0MWCVW04I7DW2	PGSC0003DMG400028740	-	0,395
G0MWCVW04I7ULV		protein mks1-like	0,456
G0MWCVW03GK88T		diacylglycerol kinase 2	0,437
G0MWCVW02C8JCL		nbs-lrr resistance partial	0,475
G0MWCVW02DHIAR		annexin isoform a	0,340
G0MWCVW03GX7EC		udp-glucose glycoprotein:	0,350
G0MWCVW04H2NMW		salicylic acid-binding protein 2-like	0,344
contig05205	CICLE_v10021291mg	Desiccation-related protein PCC13-62	0,398
Signal transduction			
G0MWCVW01CE0BF	A_TM018A10.18	2.7.11.-	0,422
G0MWCVW01BS8C4	AMTR_s00021p00241290	A_IG002N01.30 protein	0,467
G0MWCVW02EBQF8	RCA	RA	0,050
contig07538	SPPL1	Signal peptide peptidase-like 1	0,349
contig12834	At2g21480	2.7.11.-	0,475
G0MWCVW04IMBJ5	CARUB_v10016673mg	FERONIA receptor-like kinase	0,392
G0MWCVW01A86LV	At1g17350	Probable complex I intermediate-associated protein 30	0,245
contig09713	At1g42990	AtbZIP60	0,368
G0MWCVW03GUS22	M569_14433	Putative mitogen-activated protein kinase 1	0,477
contig09543	CICLE_v10014466mg	ATP binding protein, putative	0,319
G0MWCVW04JVEHI	A_IG005I10.19	2.7.11.-	0,258
G0MWCVW02DNJOY	At1g60490	2.7.1.137	0,050
contig13221	At1g06840	Probable LRR receptor-like serine/threonine-protein kinase At1g06840	0,465
contig01004	CPK2	2.7.11.1	0,366
contig06515	At5g21040	F-box/WD-40 repeat-containing protein At5g21040	0,187
G0MWCVW02EL9P8	GAI	Gibberellic acid-insensitive mutant protein	0,408
contig00986	EF-TU	Elongation factor 2	0,375
G0MWCVW02D1SUH	MPK1	2.7.11.24	0,378
G0MWCVW01CFIYX	AMTR_s00009p00267490	Putative LRR receptor-like serine/threonine-protein kinase	0,376
G0MWCVW03HGACR	MTR_5g068770	Lectin-domain containing receptor kinase A4.2	0,344
contig06837	At2g31400	Pentatricopeptide repeat-containing protein At2g31400, chloroplastic	0,366
contig02350	ANP1	2.7.11.25	0,492
G0MWCVW02DF0F2	At1g06840	Probable LRR receptor-like serine/threonine-protein kinase At1g06840	0,374
G0MWCVW04JPCJS	At1g48480	Probable inactive receptor kinase At1g48480	0,321
contig09491	ATMRK1	Serine/threonine-protein kinase HT1	0,339
G0MWCVW03GQJKE	At1g63430	2.7.11.1	0,266
contig11085	At1g56130	Probable LRR receptor-like serine/threonine-protein kinase At1g56130	0,454

G0MWCVW01AO1SK	At1g63430	2.7.11.1	0,454
G0MWCVW01B4OR0	At1g34210	2.7.10.1	0,499
G0MWCVW04IE6LU	CICLE_v10014549mg	Receptor serine/threonine kinase, putative	0,427
contig08357	At1g02090	Protein FUSCA 5	0,493
G0MWCVW03GFE73	BRADI5G24870	MAPKKKe	0,366
contig08627	PP1	3.1.3.16	0,420
G0MWCVW03GUTP2	MPK3	2.7.11.24	0,488
contig05976	ARCA	Guanine nucleotide-binding protein subunit beta-like protein	0,482
contig01494	NORK	2.7.11.1	0,416
G0MWCVW02ELAVX	At1g17230	Leucine-rich repeat receptor-like serine/threonine-protein kinase At1g17230	0,357
G0MWCVW03GSXJ6	At2g01690	Protein VAC14 homolog	0,281
G0MWCVW04IZTSX	ATEM1.10	Calcium-dependent protein kinase 13	0,040
G0MWCVW04IPPSE	ARALYDRAFT_470697	F4H5.16 protein	0,406
G0MWCVW01BEL1G	Af12-aa	Af12-amino acid	0,341
G0MWCVW02DEB60	POPTR_0008s22990g	Putative B-type response regulator 12	0,472
G0MWCVW01BCRO9	BRA007921	-	0,231
G0MWCVW01BPGC8	At1g17230	2.7.11.1	0,300
G0MWCVW04JLM0P	ETR2	Ethylene receptor	0,436
contig01919	PP1	3.1.3.16	0,153
G0MWCVW03HIROW	PLD1	3.1.4.4	0,435
G0MWCVW03GUPQ9	CPK2	2.7.11.1	0,325
G0MWCVW02EPKV3	AMTR_s00058p00164840	Os02g0796700 protein	0,297
contig06456	ABIP1	ABI1-binding protein 6	0,371
G0MWCVW01BKT5T	M569_02160	-	0,409
G0MWCVW03HGO25	AHK2	Arabidopsis histidine kinase 2	0,371
G0MWCVW01BW5K2	At1g09020	AKIN subunit betagamma	0,332
G0MWCVW03GIM70	170F8.3	36I5.3	0,478
contig07360	At5g19280	AtPP2C70	0,413
contig06842	NORK	2.7.11.1	0,406
G0MWCVW04I88M7	T5I8.2	2.7.11.1	0,277
G0MWCVW04JDRLA	PRUPE_ppa010514mg	-	0,247
G0MWCVW01A9Z16	IP3K	2.7.1.151	0,470
contig08156	AMTR_s00032p00023260	Cell-cell signaling protein csgA-like	0,340
G0MWCVW03F8C6H	POPTR_0013s01200g	Calmodulin-binding protein 60-C	0,302
G0MWCVW03GCYM2	PHAVU_002G172800g	Calmodulin-binding family protein	0,343
G0MWCVW04IAIDH	AMTR_s00057p00187090	Serine/threonine protein kinase, putative	0,467
G0MWCVW04IBIAZ	CICLE_v100278361mg	SIT4 phosphatase-associated family protein	0,322
G0MWCVW01BLTD5		wall-associated receptor kinase 2-like	0,403
G0MWCVW03G9HQV		protein kinase pvpk-1-like	0,445
G0MWCVW04ILTLZ		tbc domain-containing partial	0,349
G0MWCVW02DG52Z		serine threonine-protein kinase atm	0,460
G0MWCVW02D61ZV		protein kinase chloroplastic-like	0,472
G0MWCVW04I37QW	At1g64210	Inactive leucine-rich repeat receptor-like serine/threonine-protein kinase At5g24100	0,478
Photosynthesis			
contig07655	ccsA	Cytochrome c biogenesis protein CcsA	0,406
contig03119	FBP	Fructose-1,6-bisphosphatase, chloroplastic	0,457
G0MWCVW01B50GX	PHYB	Phytochrome B	0,364
contig07119	ND5	NADH-ubiquinone oxidoreductase chain 5	0,381
contig04575	CAB8	Chlorophyll a-b binding protein 8, chloroplastic	0,333
G0MWCVW04I2ME0	PSAG	PSI-G	0,383
G0MWCVW02DIHSK	At3g55330	OEC23-like protein 4	0,428
G0MWCVW03G9UZ4	ccsA	Cytochrome c heme attachment protein	0,433
G0MWCVW01BUTF3	At1g02910	Protein LOW PSII ACCUMULATION 1, chloroplastic	0,478
G0MWCVW02EZOWE	ycf3	Photosystem I assembly protein Ycf3	0,358
contig00007	psbC	PSII 43 kDa protein	0,488
Primary metabolism			
G0MWCVW02EZSUF	POPTR_0013s06730g	5'->3' exoribonuclease, putative	0,327
contig06996	AMTR_s00092p00137780	At3g57000	0,438
G0MWCVW02D6Z8O	AMTR_s00058p00171240	Florfenicol resistance protein-like	0,494
G0MWCVW04IUZUO	PGSC0003DMG400025975	Os10g0555200 protein	0,169
G0MWCVW02EU07Z	At4g16390	Chloroplastic RNA-binding protein P67	0,452
contig10467	At5g14620/At5g14630	DNA (cytosine-5)-methyltransferase DRM2	0,048
G0MWCVW02DY5TG	PGSC0003DMG400006257	-	0,456
G0MWCVW01A4Z2H	At1g15710	1.3.1.78	0,459
G0MWCVW02DBSTF	PGSC0003DMG400014173	Aspartate semialdehyde dehydrogenase, putative	0,194
G0MWCVW04IMQRT	GYRB	5.99.1.3	0,455
G0MWCVW02EX576	At3g09650	Pentatricopeptide repeat-containing protein At3g09650, chloroplastic	0,262
contig07486	At3g26780/MDJ14_6	Putative uncharacterized protein At3g26780/MDJ14_6	0,260

G0MWCVW02EQ9AS	AMTR_s00004p00267320	Neuroblastoma-amplified protein	0,492
contig12838	ARALYDRAFT_321659	Putative uncharacterized protein At2g39740	0,436
contig08545	At3g13290	Enhancer of mRNA-decapping protein 4	0,357
G0MWCVW01CAQ0W	At5g22750	3.6.4.-	0,338
G0MWCVW03F8IBG	AT1G65070	DNA mismatch repair protein MutS2	0,326
G0MWCVW03FPUA2	At3g56120	2.1.1.228	0,439
G0MWCVW03HE5IK	AMTR_s00004p00267320	Neuroblastoma-amplified protein	0,053
G0MWCVW01CHEOC	PGSC0003DMG400024951	1.14.13.89	0,455
G0MWCVW03G7NQR	FTRC	Ferredoxin-thioredoxin reductase catalytic chain, chloroplastic	0,488
G0MWCVW03GNK1U	At1g51720	NADP-specific glutatamate dehydrogenase, putative	0,480
G0MWCVW03G41GO	At3g08610	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 1	0,217
G0MWCVW03F79IN	TPP5	Trehalose 6-phosphate phosphatase	0,280
G0MWCVW01CE2AF	FTSH4	3.4.24.-	0,399
contig12799	SIR	1.8.7.1	0,365
G0MWCVW01CF143	SIR	1.8.7.1	0,408
contig07537	At1g56190	2.7.2.3	0,409
G0MWCVW03G18BR	ADK-A	ATP:AMP phosphotransferase	0,124
contig13000	At1g16350	1.1.1.205	0,383
G0MWCVW02D4VWX	CICLE_v10004614mg	Flap endonuclease GEN-like protein	0,347
G0MWCVW03HEM5C	FPGS2	FPGS	0,288
contig11631	At5g36700	3.1.3.18	0,118
G0MWCVW03GU87C	At4g12420	Monocopper oxidase-like protein SKU5	0,350
G0MWCVW01BRZFA	PGSC0003DMG400000466	Lecithin:cholesterol acyltransferase family protein	0,185
contig13982	At1g02000	5.1.3.6	0,428
G0MWCVW03G93N9	AMTR_s00010p00199710	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase	0,390
G0MWCVW03FXTRX	PGSC0003DMG400007878	Chromodomain-helicase-DNA-binding protein	0,190
G0MWCVW04IXBFB	AMTR_s00048p00176710	Alpha glucosidase-like protein	0,350
contig02248	At2g05990	1.3.1.9	0,187
contig02018	MDH	1.1.1.37	0,282
contig04025	PHI-1	Putative phi-1	0,475
contig07889	At2g22480	2.7.1.11	0,429
G0MWCVW03G1763	At4g26270	2.7.1.11	0,389
G0MWCVW02EOVXE	At1g28580	3.1.1.-	0,493
contig10025	AMTR_s00057p00060720	Lipase	0,469
G0MWCVW01BWQYJ	BRA037418	Predicted protein	0,314
contig01939	At2g05990	1.3.1.9	0,254
G0MWCVW03GYVZ0	PHAVU_008G031000g	Phospholipase C, putative	0,450
contig04646	MOD1	1.1.1.40	0,317
G0MWCVW04JSR4T	BRADI5G13270	Beta-glucosidase G1	0,411
contig11017	LPAAT2	2.3.1.51	0,441
G0MWCVW01BNTS0	AMTR_s00020p00187990	Lipase class 3 family protein	0,306
contig10851	At1g10150	AtPP2-A10	0,419
contig11707	POPTR_0019s04970g	-	0,413
contig14684	At3g15820	2.7.8.-	0,405
G0MWCVW04H3AH0	At3g12120	1.14.19.-	0,464
G0MWCVW02DYBRU	AUD1	UGD	0,282
contig11793	MOD1	1.1.1.40	0,279
contig02451	At5g51970	L-iditol 2-dehydrogenase	0,452
G0MWCVW04JHSUL	At2g42450	Lipase class 3 family protein	0,299
G0MWCVW02DZ137	ARA1	L-arabinokinase	0,104
G0MWCVW01B3UUV	At1g06410	AtTPS7	0,413
G0MWCVW02DHGLR	At4g27700	AtStr14	0,249
contig13335	AMTR_s00001p00155640	Prolyl 4-hydroxylase alpha-2 subunit	0,299
G0MWCVW02EEU55	PGSC0003DMG400001921	RNase H domain-containing protein	0,344
contig04151	PGSC0003DMG400001921	RNase H domain-containing protein	0,394
contig12520	POPTR_0014s01620g	At5g63905	0,428
contig00790	At2g37500	Arginine biosynthesis bifunctional protein ArgJ, chloroplastic	0,269
G0MWCVW01BYHEE	KS1	Ent-kaur-16-ene synthase, chloroplastic	0,401
G0MWCVW04IBXZI	At1g78960	5.4.99.39	0,402
G0MWCVW03G134V	At1g45130	3.2.1.23	0,295
contig00777	At1g22020	2.1.2.1	0,492
G0MWCVW01BQMC8	AMTR_s00058p00101170	Methionyl-tRNA synthetase	0,212
contig11955	At3g22425	4.2.1.19	0,338
G0MWCVW01B3REY	M569_14363	Cytidine deaminase	0,444
G0MWCVW02D49JV	AMTR_s00066p00202260	DNA polymerase I	0,500
G0MWCVW01BKNN8	BRA010863	Uncharacterized protein	0,458
contig04537	AMTR_s00004p00174320	Core-2/I-branching beta-1,6-N-acetylglucosaminyltransferase family protein	0,157
G0MWCVW04I3OV3	At1g72810	4.2.3.1	0,389
G0MWCVW01BA6WP	DNMT2	DNA methyltransferase-2	0,222
G0MWCVW04H2QVI	AMTR_s00068p00169260	-	0,339

G0MWCVW02EZBS6	CICLE_v10024989mg	Rela/spot homolog 3 family protein	0,280
contig00229	CICLE_v10025634mg	La domain-containing family protein	0,411
G0MWCVW04I02KH	AT1G72880	Acid phosphatase	0,413
contig03755	SR	Serine racemase	0,261
contig08035	At4g39280	Phenylalanyl-tRNA synthetase alpha subunit	0,475
G0MWCVW02C2HJE	CICLE_v10018683mg	Poly (ADP-ribose) polymerase family protein	0,339
G0MWCVW01CIQPG	BRA006893	Uncharacterized protein	0,413
G0MWCVW02DDHLW	MTR_4g123970	tRNA pseudouridine synthase	0,489
G0MWCVW03FK547	AMTR_s00010p00243220	OJ1458_B07.103 gene product (ISS)	0,490
G0MWCVW04H9G08	CICLE_v10019671mg	U3 small nucleolar RNA-associated protein-like protein	0,394
G0MWCVW03GN4MP	ALNC14_094960	Nicotinate phosphoribosyltransferase	0,035
contig02803	At1g19190	3.1.1.1	0,452
G0MWCVW02EVMK9	At2g04530	Ribonuclease Z, chloroplastic	0,342
contig00572	EBM	Endo-beta-mannosidase	0,495
contig01584	CICLE_v10025909mg	Aldose 1-epimerase family protein	0,237
G0MWCVW01BDNK5	PGSC0003DMG401002553	Putative uncharacterized protein At3g16260/MYA6_19	0,479
G0MWCVW04J0F8W	RECA	DNA repair protein recA homolog 1, chloroplastic	0,434
contig12253	CICLE_v10008145mg	Sucrase-related family protein	0,449
G0MWCVW03HE02N	PHAVU_011G206500g	Putative amidohydrolase ytcJ	0,246
G0MWCVW04I2JDY		dna polymerase theta	0,414
G0MWCVW04IQBOJ		cardiolipin synthetase 1 isoform partial	0,035
G0MWCVW02EOL7O		box c d snorna protein 1-like	0,432
G0MWCVW02E0DFC		ribonuclease h protein at1g65750	0,455
G0MWCVW02DTP8B		ribonuclease h protein at1g65750	0,499
G0MWCVW02DMOVK		ribonuclease h protein at1g65750- partial	0,385
G0MWCVW03FZ1DR		phytoene desaturase 3	0,382
G0MWCVW04IAI6D		pseudouridine synthase isoform 3	0,460
G0MWCVW04IQ4D6		beta- -n-acetylglucosaminyltransferase lunatic isoform 1	0,474
G0MWCVW02DO130		pap-specific phosphatase hal2-like	0,382
G0MWCVW01CCMPQ		dna mismatch repair protein type isoform 2	0,175
Protein metabolism			
contig07796	At2g47570	60S ribosomal protein L18	0,068
G0MWCVW04I4MD9	TyrS	Tyrosyl-tRNA synthetase	0,045
G0MWCVW02D1XQH	RPL9	CL9	0,382
G0MWCVW04I6Z3Y	RPL13A	60S ribosomal protein L13a	0,154
G0MWCVW04JCA2M	AMTR_s00169p00043580	Putative uncharacterized protein At5g66860	0,292
G0MWCVW02EM989	At5g43710	Probable alpha-mannosidase I MNS4	0,477
G0MWCVW02DX6PZ	GATB	6.3.5.-	0,362
contig01943	RCOM_1506700	Probable aspartyl aminopeptidase	0,364
G0MWCVW04JKWJX	AMTR_s00056p00136230	Initiator tRNA phosphoribosyl transferase family protein	0,299
contig10227	At2g40510	40S ribosomal protein S26	0,481
G0MWCVW03GNRZC	RPS5	40S ribosomal protein S5	0,468
G0MWCVW04JZRD0	AMTR_s00049p00157500	AT5g63200/MDC12_17	0,472
contig12954	At2g46280	Eukaryotic translation initiation factor 3 subunit 2	0,486
G0MWCVW01A8RHB	At3g25220	Rotamase	0,458
contig02226	At2g40010	60S acidic ribosomal protein P0	0,104
G0MWCVW04JI7AT	EF2	Elongation factor 2	0,388
G0MWCVW03HCZIC	CICLE_v10005159mg	40S ribosomal protein S1	0,224
G0MWCVW04JXNGM	AMTR_s00016p00259000	Nucleotide exchange factor SIL1	0,400
contig10677	EUTSA_v10010482mg	Hsp70 nucleotide exchange factor fes1	0,487
G0MWCVW03F8W9P	rpl20	50S ribosomal protein L20, chloroplastic	0,425
contig07257	At1g54290	Protein translation factor SUI1 homolog	0,381
G0MWCVW02DSDR4	AMTR_s00143p00072740	F-box-like/WD repeat-containing protein TBL1XR1	0,477
G0MWCVW04I8U7P	ARALYDRAFT_470307	Uncharacterized protein	0,463
contig05825	At1g08360	60S ribosomal protein L10a	0,370
G0MWCVW02DLMVU	RPS25	40S ribosomal protein S25	0,215
G0MWCVW01B40Z4	At3g06530	Uncharacterized protein At3g06530	0,337
G0MWCVW04I731P	At5g08180	H/ACA ribonucleoprotein complex subunit 2-like protein	0,402
G0MWCVW02C8VGG	RPS25	40S ribosomal protein S25	0,353
contig01900	EUTSA_v10010482mg	Hsp70 nucleotide exchange factor fes1	0,471
G0MWCVW01ARS9B	At5g39780	Gb|AAF22924.1	0,353
contig02177	At3g25220	Rotamase	0,424
contig09122	RPL34	60S ribosomal protein L34	0,481
G0MWCVW01BN9N2	At3g60360	Probable U3 small nucleolar RNA-associated protein 11	0,443
contig05259	Solyc09g074400.2	Probable cytosolic iron-sulfur protein assembly protein CIAO1 homolog	0,332
G0MWCVW03GSQFO	At1g53290	2.4.1.-	0,383
contig02919	POPTR_0001s15440g	Palmitoyl protein thioesterase family protein	0,455
contig11006	At3g04920	40S ribosomal protein S24-1	0,406

contig05889	ZEAMMB73_510741	Ribosomal protein S8e family protein	0,109
contig01275	AUL1	Auxilin-like protein 1	0,362
contig11496	FKBP15-1	Peptidyl-prolyl cis-trans isomerase FKBP15-1	0,410
contig02061	ARALYDRAFT_325654	At5g11240	0,457
G0MWCVW01AL5EP	HDC	HDC	0,330
G0MWCVW02DRYQ0	AP22.85	CDK5RAP1-like protein	0,208
G0MWCVW01BIJXG	AMTR_s00059p00146300	Major surface glycoprotein-like	0,468
contig05237	PCM	L-isoaspartyl protein carboxyl methyltransferase	0,461
G0MWCVW02EZZV4	At5g22130	GPI mannosyltransferase 1	0,426
G0MWCVW01CIWVD	CICLE_v10000364mg	Subtilisin-like protease	0,477
G0MWCVW02ELLLC	ATJ15	AtARG1	0,341
G0MWCVW01BZV72	At5g23320	2.1.1.100	0,063
G0MWCVW04IETMM	AMTR_s00030p00241220	Nucleolar GTP-binding protein 1	0,318
G0MWCVW02EMN2F	ARALYDRAFT_483465	Uncharacterized protein	0,455
G0MWCVW02DSNOM	CICLE_v10024676mg	Midasin	0,134
contig10035	At3g01020	AtISU1	0,486
G0MWCVW04JRCK1	At2g19410	Plant U-box protein 34	0,407
contig08296	UBC12	6.3.2.19	0,475
G0MWCVW01BEP8W	M569_03611	Putative uncharacterized protein Sb09g023650	0,427
contig05907	ARI1	6.3.2.-	0,425
contig09030	AMTR_s00133p00094810	Transducin family protein	0,489
G0MWCVW02EHJ3M	At5g64920	COP1-interacting protein 8	0,318
G0MWCVW02EJPSF	M569_08869	Plant ubiquilin, putative	0,087
G0MWCVW03FXDXB	At4g38600/At4g38610	E3 ubiquitin-protein ligase UPL3	0,330
contig05805	At2g41980	6.3.2.-	0,396
contig07995	At1g23410	Ubiquitin-related 4	0,361
G0MWCVW02DTV3J	PGSC0003DMG400030407	Protein spotted leaf 11	0,354
contig06857	PBC1	20S proteasome subunit beta-3	0,397
G0MWCVW01CDW0O	At1g28110	3.4.16.-	0,419
contig03719	CICLE_v10026172mg	Transducin family protein	0,263
G0MWCVW02DLX9J	At4g17270	Degreening-related gene dee76 protein	0,414
G0MWCVW04I7PGA	At1g28320	AtDEG15	0,496
G0MWCVW02DNRVW	CICLE_v10004383mg	Transducin family protein	0,242
G0MWCVW04IG0UT	At2g45910	Plant U-box protein 32	0,471
contig14699	BRADI3G06080	Ubiquitin fusion degradation UFD1 family protein	0,397
G0MWCVW02D3G2J	At2g17190	Ubiquitin domain-containing protein DSK2a	0,434
G0MWCVW02C8D5Z	At3g11910	3.4.19.12	0,392
G0MWCVW02DGMYR	CICLE_v10032987mg	RING-H2 type zinc finger	0,463
G0MWCVW02DSCWW	MKRN	E3 ubiquitin-protein ligase makorin	0,216
contig14044	At1g14400	6.3.2.19	0,109
contig12223	PR46a	Ubiquitin-fold modifier 1	0,129
contig14822	At5g46210	AtCUL4	0,468
contig03809	At4g06599	Ubiquitin-like domain-containing CTD phosphatase	0,278
G0MWCVW04IJG88	At1g12760	6.3.2.-	0,300
G0MWCVW04ITVE1	At4g15475	AtFBL4	0,124
G0MWCVW03GB6TB	At2g39760	AtBPM1	0,356
G0MWCVW04I3BMM	At1g04810	26S proteasome non-ATPase regulatory subunit 1 homolog A	0,477
G0MWCVW01BB7CG	At4g07400	F-box protein SKIP2	0,390
contig10220	At1g17280	6.3.2.19	0,286
contig11068	At3g03380	Protease Do-like 7	0,296
G0MWCVW04J1BF4	CICLE_v10004383mg	Transducin family protein	0,488
contig00974	At1g53025	6.3.2.19	0,449
G0MWCVW04I7PHW	At2g22310	3.4.19.12	0,427
G0MWCVW02EBL8M	At3g11910	3.4.19.12	0,425
G0MWCVW02DMY18	PDIL6-1	AtPDIL6-1	0,387
G0MWCVW01BVF5M	MTR_5g088750	Ubiquitin-associated domain-containing family protein	0,448
G0MWCVW03GJHF4	179B	40S ribosomal protein SA	0,459
contig01271	CARUB_v10004926mg	Latex abundant protein 1	0,328
contig08774	rps12	ATP-dependent Clp protease proteolytic subunit	0,395
G0MWCVW03F81AT	At3g60240	Eukaryotic translation initiation factor 4G	0,370
G0MWCVW02DA5YC	POPTR_0018s11390g	SKP1 INTERACTING PARTNER 1 family protein	0,398
G0MWCVW01ARO3C	BRA005727	SLT1 protein	0,423
G0MWCVW01ARVFZ	At2g26590	26S proteasome non-ATPase regulatory subunit 13	0,464
G0MWCVW04H2L1V	LOC_Os03g15930	OTU-like cysteine protease family protein, expressed	0,323
G0MWCVW04JI9UT	AMTR_s00169p00022730	Methyltransferase, FkbM family protein, expressed	0,286
contig07703	At3g01650	E3 ubiquitin-protein ligase RGLG1	0,249
G0MWCVW02EFZWB	VITISV_008681	Putative uncharacterized protein	0,425
contig12994	ARALYDRAFT_472131	Inter-alpha-trypsin inhibitor heavy chain, putative	0,352
G0MWCVW01AUD9W	Solyc04g009380.1	S-acyltransferase	0,307
G0MWCVW04IHDWM	F775_23272	Putative uncharacterized protein	0,481
G0MWCVW03HDZXI	CICLE_v10015229mg	Protease ecfE, putative	0,449
G0MWCVW04IWNCY	AMTR_s00018p00189720	Autophagy-related protein	0,424

G0MWCVW04JYNPD	PGSC0003DMG400017857	C2 domain-containing family protein	0,267
contig01202	At1g13860	2.1.1.-	0,311
G0MWCVW03F1EJT	VITISV_043911	-	0,384
G0MWCVW04IFTBJ	ARA12	Cucumisin-like serine protease	0,243
contig13180	At3g49720	Uncharacterized protein At3g49720	0,398
G0MWCVW02D68N7	BRA013454	Peptidase M50 family protein	0,461
contig06655	At5g03905	Iron-sulfur assembly protein IscA-like 2, mitochondrial	0,417
contig04310	EUTSA_v10025970mg	CRUMPLED LEAF	0,056
G0MWCVW02DYO49	AT1G16570	Chitobiosyldiphosphodolichol beta-mannosyltransferase	0,445
G0MWCVW01B2HS2	CARUB_v10023247mg	Urease accessory protein	0,188
contig06629	AMTR_s00009p00268340	LON peptidase N-terminal domain and RING finger protein 1	0,481
G0MWCVW03GMXC2		pumilio homolog 5-like isoform x1	0,321
G0MWCVW02EEO6M		pre-rrna-processing protein tsr1 homolog	0,428
G0MWCVW02EUXBO		protein trichome birefringence-like 42	0,300
contig13093		peptidyl-prolyl cis-trans isomerase cyp95 isoform x3	0,326
G0MWCVW01A4B71		trinucleotide repeat-containing gene 6b	0,069
G0MWCVW02D8PRH		autophagy protein 5	0,456
contig13001		peptidase s24 s26a s26b s26c family protein isoform 1	0,355
G0MWCVW01BGIAD		glycosyltransferase family protein	0,357
G0MWCVW02C7EN0		hypothetical protein M569_07122	0,096
G0MWCVW04JP62C		e3 ubiquitin-protein ligase synoviolin-like	0,387
G0MWCVW01A9S56		glyoxysomal processing protease, glyoxysomal	0,460
G0MWCVW01AM69G		s-adenosyl-l-methionine-dependent methyltransferases superfamily protein isoform 3	0,491
Secondary metabolism			
G0MWCVW02EMLM7	CYP97B2	Cytochrome P450 97B2, chloroplastic	0,313
contig12907	M569_08686	Acyltransferase, putative	0,473
G0MWCVW02EPKRU	At4g23660	2.5.1.93	0,477
contig02055	At2g19070	BAHD-like hydroxycinnamoyl transferase	0,352
contig10296	CHLM	Magnesium protoporphyrin IX methyltransferase, chloroplastic	0,439
G0MWCVW02EIYVX	COR1.1	Aldo-keto reductase 2	0,406
contig02098	AKR1	1.1.1.-	0,435
G0MWCVW01AOCDA	TR2	TR-II	0,307
contig03738	PGSC0003DMG400014899	SEC14 cytosolic factor family protein	0,163
contig06841	UGT80B1	Sterol 3-beta-glucosyltransferase UGT80B1	0,498
G0MWCVW04JOAG5	CHLP	Geranylgeranyl diphosphate reductase, chloroplastic	0,088
G0MWCVW03G0I24	HEMA1	GluTR	0,257
contig01226	At4g34640	Squalene synthase	0,376
G0MWCVW04H7D2K	G10H	F3'5'H	0,237
G0MWCVW02ED8OY	At2g34630	All-trans-nonaprenyl-diphosphate synthase 1 (geranyl-diphosphate specific)	0,486
G0MWCVW03HHIVR	CICLE_v10006462mg	Acc synthase, putative	0,323
G0MWCVW04JMNQU	AT4G02860	Phenazine biosynthesis protein, putative	0,090
G0MWCVW02DNT3R	At1g43620	2.4.1.173	0,374
G0MWCVW02ECKDG	CAD1	1.1.1.195	0,474
G0MWCVW02EZR3U	MTR_2g094530	-	0,430
G0MWCVW04H9KIA	CICLE_v10019871mg	Flavanone 3-dioxygenase-like protein	0,371
G0MWCVW03GXJK4	AS	Hydroquinone glucosyltransferase	0,283
contig10078	PHAVU_009G191000g	2-aminoethanethiol dioxygenase	0,457
Transport			
contig02717	DIT1	Dicarboxylate transporter 1, chloroplastic	0,473
contig11076	At5g47560	AtSDAT	0,486
contig09132	At5g66030	AtGRIP	0,101
G0MWCVW03GGLC2	H257_04344	Transmembrane 9 superfamily member 3	0,176
G0MWCVW01B0GNE	At1g60070	AP-1 complex subunit gamma-2	0,487
G0MWCVW02DPB0Q	AMTR_s00046p00091860	Os02g0614100 protein	0,376
contig09463	RAB11B	Ras-related protein Rab11B	0,311
contig05629	SWEET16	Bidirectional sugar transporter SWEET16	0,483
G0MWCVW01B1F2H	At1g08820	AtPVA11	0,403
G0MWCVW04IDE74	At4g38580	AtFP6	0,088
G0MWCVW03FQJAE	EUTSA_v10021604mg	Trafficking protein	0,079
contig00674	At1g14910	Putative clathrin assembly protein At1g14910	0,397
contig10703	At5g63060	Sec14p-like phosphatidylinositol transfer family protein	0,393
G0MWCVW03GGS34	At2g04620	AtMTP12	0,386
contig05980	At2g23980	AtCNGC1	0,478
contig12912	At1g21870	At-UDP-GalT1	0,447

contig09160	ALA10	3.6.3.1	0,174
G0MWCVW04JB8N5	At1g08230	AtGAT1	0,469
G0MWCVW01ALABH	ACA3	3.6.3.8	0,275
G0MWCVW04JYVCV	At2g20780	Probable polyol transporter 4	0,464
contig05612	OEP37	Chloroplastic outer envelope pore protein of 37 kDa	0,361
G0MWCVW03FT698	At5g66380	AtFOLT1	0,457
G0MWCVW03FN16H	ACA10	3.6.3.8	0,269
contig04458	At1g48230	Probable sugar phosphate/phosphate translocator At1g48230	0,493
contig02412	MGT8	AtMGT8	0,419
G0MWCVW03G478Y	CICLE_v10015235mg	TPK1	0,460
G0MWCVW02EC05M	AMT1-2	AtAMT1	0,095
G0MWCVW04H91XA	ZEAMMB73_664224	CRT (Chloroquine-resistance transporter)-like transporter 1	0,488
contig06730	At1g07140	Ran-binding protein 1 homolog a	0,494
G0MWCVW02DZ115	NRAMP1	Metal transporter Nramp1	0,325
G0MWCVW04INZIQ	At1g12110	AtNPF6.2	0,305
contig03619	ASNAP	Alpha-SNAP	0,493
G0MWCVW03F0DRC	ALA10	3.6.3.1	0,354
contig06313	ARF1	ADP-ribosylation factor 1	0,472
G0MWCVW02C52LI	ABCE1	ABC transporter ABCE.1	0,497
G0MWCVW03GKC4J	ZEAMMB73_500395	Exportin-7, putative	0,295
G0MWCVW03GR33G	ycf2-A	Protein Ycf2	0,462
G0MWCVW01B07T6	ABCA10	ABC transporter A family member 10	0,400
G0MWCVW03F2GRK	At1g12600	AtUTr2	0,387
contig04883	CICLE_v10000945mg	At4g24330	0,440
G0MWCVW04IV517	At4g34450	Coatomer subunit gamma	0,423
contig11108	TIM21	Probable mitochondrial import inner membrane translocase subunit TIM21	0,477
G0MWCVW02DMZ8V	ACA1	3.6.3.8	0,270
contig09329	At1g08820	AtPVA11	0,456
contig08237	ARALYDRAFT_496554	Gb|AAF26070.1	0,485
G0MWCVW02EHD63	At1g55690	AtSFH13	0,497
contig08579	CARUB_v10000352mg	Glycosylphosphatidylinositol anchor attachment 1 protein	0,458
G0MWCVW02DLT6H	MRP10	3.6.3.44	0,370
contig04417	At1g11250	AtSYP121	0,424
G0MWCVW02D7LX1	AT5G22640	MORN (Membrane Occupation and Recognition Nexus) repeat-containing protein	0,433
contig01684	ZIP3	ZRT/IRT-like protein 3	0,299
G0MWCVW04JBGW8	BT1	Adenine nucleotide transporter BT1, chloroplastic/amyloplastic/mitochondrial	0,292
G0MWCVW01AYFV6	POPTR_0015s10180g	AT5g50310/MXI22_1	0,485
G0MWCVW02C41Z4	At3g06720	Importin subunit alpha	0,463
G0MWCVW02D29QT	ABCB26	ABC transporter ABCB.26	0,189
G0MWCVW01AWRUY	ABCG22	ABC transporter ABCG.22	0,435
G0MWCVW04JUMGC	AMTR_s00002p00265180	Amino acid/polyamine transporter II family protein	0,443
contig01686	MTP11	Metal tolerance protein 11	0,329
G0MWCVW02DGAB1	At4g32640	Protein transport protein Sec24-like At4g32640	0,245
contig10262	At1g25240	Putative clathrin assembly protein At1g25240	0,421
contig11283	At1g21870	At-UDP-GalT1	0,284
G0MWCVW04IS3AW	At3g51490	Monosaccharide-sensing protein 2	0,273
G0MWCVW04JSME8	At2g19600	AtKEA4	0,499
G0MWCVW03GLPMB	At2g19600	AtKEA4	0,399
G0MWCVW02DAVH6	UTR4	UDP-galactose/UDP-glucose transporter 4	0,334
G0MWCVW02DL9R9	At1g21870	At-UDP-GalT1	0,434
G0MWCVW03FZQ7H	At2g38460	Solute carrier family 40 member 1	0,445
G0MWCVW01BD60M	At1g62020	Coatomer subunit alpha-1	0,444
G0MWCVW03GTT6Z	ACD1-like	ACD1-like protein	0,498
G0MWCVW02DFU3S	PMA4	Plasma membrane ATPase 4	0,188
G0MWCVW01BDR1K	YSL1	Metal-nicotianamine transporter YSL2	0,397
contig05454	At3g07100	Protein transport protein Sec24-like At3g07100	0,471
contig06016	ASN1	6.3.5.4	0,485
G0MWCVW01BANUT	PMA1	3.6.3.6	0,402
G0MWCVW03FSE17	AMTR_s00019p00246780	ABC1 family protein	0,493
G0MWCVW02C6PHJ	At1g63440	3.6.3.54	0,285
contig00903	VHA-B2	V-type proton ATPase subunit B2	0,278
G0MWCVW02EYZTF	At5g12080	AtMSL10	0,428
contig11501	dd44Y	Oligomycin sensitivity conferring protein	0,438
G0MWCVW02EPQLN		abc transporter b family member 2-like	0,407
contig09962		mitochondrial substrate carrier family protein b-like	0,349
G0MWCVW04IW4ER		xylulose 5-phosphate phosphate chloroplastic	0,453
G0MWCVW03GZZWL		clathrin assembly protein at4g40080	0,425
G0MWCVW03GUWGJ		mate efflux family protein 5-like	0,477
G0MWCVW02DJW51		protein grip isoform x1	0,282

G0MWCVW02C2K52	hypothetical protein MIMGU_mgv1a019095mg, partial	0,337
G0MWCVW04IPK0O	mitochondrial substrate carrier family protein	0,421
G0MWCVW04JTNNQ	k+ efflux antiporter family protein	0,228

STUDY AND RESEARCH ABROAD

Study visit at the *Unit of Agronomy of the UCO (Universidad de Córdoba)* with Professors Diego Barranco Navero, Luís Rallo and Isabel Trujillo and the “Centro Alameda del Obispo” of IFAPA (Instituto de Investigación y Formación Agraria y Pesquera) with Dr. Angelina Belaj (22-25/05/2013).

Stay at *ENEA - Centro Ricerche Trisaia* to perform profiling analyses (17-28/06/2013).

Stay at the UPO-Genetics Bioinformatics group of Dr. Antonio J. Pérez-Pulido at the *Centro Andaluz de Biología del Desarrollo (CABD)*, Universidad Pablo de Olavide (Seville, Spain) (26/04/2014 - 01/08/2014).

PUBLICATIONS

Submitted paper. Characterization of twenty olive (*Olea europaea* L.) varieties grown in Campania (Italy) and their extra virgin olive oils. Filomena Grasso, Antonello Paduano, Giandomenico Corrado, Maria Luisa Ambrosino, Rosa Rao* and Raffaele Sacchi*. *Journal of Agricultural and Food Chemistry.*

Abstract. GRASSO F, RAO R, SACCHI R (2012). Olive tree genetic diversity and olive oil flavours. In: XI Corso Estivo di Approfondimento sulla Genetica Vegetale "Plants, genes and environment - Gene action, adaptation mechanisms and breeding". Gargnano (BS), 10-12 June 2012.

Poster and short oral presentation. GRASSO F, CORRADO G, PADUANO A, RAO R, SACCHI R (2012). Olive tree genetic diversity and olive oil flavours. In: D. Bosco, P.J. Mazzoglio & L. Tavella, European PhD Network in "Insect Science", 3rd Annual Meeting - Abstract Book. Ivrea, Turin, Italy, 6th – 9th November 2012.

Poster. GRASSO F., PADUANO A., CORRADO G., SACCHI R., RAO R. (2013). Exploring genetic diversity in 20 olive (*Olea europaea* L.) varieties spread in Campania (Italy) and its relationship with the olive oil profile. In: Italian Society of Agricultural Genetics. Proceedings of the 57th Italian Society of Agricultural Genetics Annual Congress. ISBN: 9788890457036, Foggia, 16-19 settembre 2013

Oral communication. GRASSO F, COPPOLA M, PADUANO A, GARONNA A P, CARBONE F, CORRADO G, PERROTTA G, SACCHI R, RAO R (2014). Effetto della mosca olearia (Bactrocera oleae) sul profilo trascrittomico e metabolomico dell'olivo (*Olea europaea*) . In: (a cura di): Mannu R, XXIV Congresso Nazionale Italiano di Entomologia. Riassunti delle comunicazioni orali. . SASSARI: Edizioni ISE-CNR Istituto per lo Studio degli Ecosistemi, Consiglio Nazionale delle Ricerche Traversa la Crucca 3, 07100 SASSARI (Italia), ISBN: 978-88-97934-04-2, Orosei (Nuoro), 13-16 Giugno.

Poster. GRASSO F, PADUANO A, GENOVESE A, CASCONE P, GUERRIERI E, RAO R, SACCHI R (2014). Composti volatili dell'oliva e loro variazioni in risposta all'attacco della mosca dell'olivo. In: Gomes T., Camposeo S., Clodoveo M.L.. Riassunti dei lavori del III Convegno Nazionale dell'Olivo e dell'Olio. ACTA ITALUS HORTUS, vol. 14, p. 51, Firenze: Società di Ortoflorofrutticoltura Italiana (SOI), ISBN: 9788894027617, Bari, 26-28 Novembre 2014.

XI Corso Estivo di Approfondimento sulla Genetica Vegetale

"Plants, genes and environment - Gene action, adaptation mechanisms and breeding"

Gargnano, Bs, June 10-12, 2012

OLIVE TREE GENETIC DIVERSITY AND OLIVE OIL FLAVOURS

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Genetic diversity, oil flavours, Oleae europeae, Bactrocera Oleae.

A very important aspect of olive oil quality is represented by the plant's interaction with his key enemy, the diptera *Bactrocera Oleae*, which, depending on the year, determines severe quantitative and qualitative production losses. It was pointed out that, following the attack of the herbivore, host plants activate a cascade of genes thus modifying the mixture of volatile compounds (VOC) emitted with the aim of activating both the direct and indirect defense mechanisms. As a part of this mechanism, lipoxygenases (LOX) are involved in the production of volatile compounds from linolenic acid some of these VOCs have an important role in defining the flavors of extra virgin olive oil, so that is possible to hypothesize that the drupe production of some aromas which give valuable characteristics of typicity to extra virgin olive oils are modulated, in part or wholly, by the attack or the presence of the olive fly in the cultivated area. During my PhD project I will study, by Gaschromatography coupled to Mass Spectrometry, VOCs emitted following the presence of *Bactrocera Oleae*. Moreover I will perform transcriptomic studies with the aim to clarify the molecular mechanisms underlying the VOC production. I will finally try to evaluate the correlation between the presence of the olive fly and the typical flavor of some typic monovarietal extra virgin olive oils produced in Campania.

Session 4: Insect control and biotechnology

Olive tree genetic diversity and olive oil flavours

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Campania is one of the Italian regions most suited to the production of olive oil. Following previous studies and in order to provide relevant information for the conservation and enhancement of its olive germplasm and corresponding typical olive oils, we have been extending the descriptive framework of genetic variability at the 20 main varieties cultivated in Campania by means of Simple Sequence Repeats (SSR). Furthermore, exploring the correlation between SSR profiles and three olive oil quality traits (fatty acids, biophenols, sensory profile), a significant correlation between SSR profile and sensory profile has been shown.

An important aspect of olive oil quality is the plant's interaction with its key enemy, the dipteran *Bactrocera oleae*. Following its attack, host plants activate a cascade of genes, thus modifying the mixture of volatile compounds (VOCs) emitted and activating the defence mechanisms. In this framework, lipoxygenases (LOX) are involved in the production of VOCs from linolenic acid. Some of these play an important role in the typicity of extra virgin olive oils, so that is possible to hypothesize that the drupe production of some aromas is somehow modulated by the attack or the presence of the olive fly. VOCs emitted following the presence of *B. oleae* have been studied by means of GC/MS. Then I will perform transcriptomic studies to clarify the molecular mechanisms underlying the VOC production and finally investigate the correlation between the presence of the olive fly and the typical flavour of some monovarietal extra virgin olive oils of Campania.

Proceedings of the 57th Italian Society of Agricultural Genetics Annual Congress
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Poster Communication Abstract – 5.31

EXPLORING GENETIC DIVERSITY IN 20 OLIVE (*OLEA EUROPAEA* L.) VARIETIES SPREAD IN CAMPANIA (ITALY) AND ITS RELATIONSHIP WITH THE OLIVE OIL PROFILE

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SSRs, olive oil, fatty acids, biophenols, sensory profile

Campania is one of the most suited Italian regions to the production of olive oil. In order to provide relevant information for the conservation and enhancement of its olive germplasm and corresponding typical olive oils, the descriptive framework of genetic diversity of the 20 main varieties cultivated in Campania was studied by means of ten SSR (Simple Sequence Repeats) loci. All loci showed a high polymorphism level: 107 different alleles were identified at all loci, resulting in a 10,7 average number of alleles per locus. The allele frequency confirmed the high level of polymorphism. The number of effective alleles was high (64,89) and equivalent to 64,6% of the total number of alleles. The average genetic distance (18,505 ± 3,817) indicated that the 10 SSRs were able to show a good level of diversity. Two of the 10 loci showed a positive fixation index (F), which could be explained by the presence of null alleles or the fixation of the two loci in the population of olive genotypes in Campania. Genetic distances were explored by PCA (Principal Component Analysis), which showed, with some exceptions, the tendency of varieties to group on the base of the geographical area. Genetic distances were also employed to build up a phylogeny tree. The tree showed that all accessions could be identified through the aforementioned 10 SSRs, but 'Minucciola' and 'Nostrale'. Moreover, the tree did not indicate the presence of evident clusters, confirming the richness of the olive biodiversity in Campania Region and underling the important role of local farmers in the conservation of olive germplasm.

The relationships among the 20 olive varieties in study were also explored on the base of three quality traits of the relative olive oils: fatty acids, biophenols and sensory profile. Oil parameters were firstly explored by PCA, which confirmed a general tendency to group on the base of the geographical area.

Mantel test allowed to verify the correlation between the genetic dissimilarity matrix and each of the three dissimilarity matrices related to the three olive oil quality traits (fatty acids, biophenols, sensory profile). A significant correlation was highlighted between SSR profile and sensory profile. The results are discussed in terms of typicality of olive oil.

Effetto della mosca olearia (*Bactrocera oleae*) sul profilo trascrittomico e metabolomico dell'olivo (*Olea europaea*)

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Introduzione

Un aspetto fondamentale dell'ecologia dell'olivo è rappresentato dall'interazione con il suo nemico chiave, *Bactrocera oleae*. La mosca dell'olivo, infatti, determina ingenti perdite quantitative e qualitative di drupe che, a seconda delle condizioni ambientali, si possono tradurre in pesanti perdite del settore olivicolo-oleario. In accordo con quanto avviene in altre specie vegetali (Engelberth et al., 2013; Kant et al., 2004), l'attacco della mosca olearia attiva una cascata di geni, determinando l'alterazione del profilo trascrittomico delle drupe (Corrado et al., 2012). Allo stesso modo, il profilo dei composti volatili (VOC) emessi dalle piante viene rimodulato a seguito dell'attacco di insetti dannosi per attivare i meccanismi di difesa indiretta (Sasso et al., 2007). Poiché alcuni di questi VOC sono anche annoverati fra quelli che caratterizzano la qualità e la tipicità degli oli extravergini di oliva, è possibile ipotizzare che la mosca olearia possa avere un effetto positivo sul profilo sensoriale degli oli (Sacchi et al., 2010). Dati gli studi sul priming (Frost et al., 2008), inoltre, è possibile ipotizzare che la sola presenza della mosca nell'oliveto possa modulare la produzione di tali composti. Lo studio dell'interazione fra mosca olearia e olivo è stato qui approfondito mediante l'esame dei profili trascrittomici e metabolomici di varietà campane di olivo.

Materiali e metodi

L'analisi trascrittomica è stata condotta con tecnologia CombiMatrix 90K su RNA di drupe sane e punte delle varietà 'Ortice' (suscettibile alla mosca) e 'Ruveia' (tollerante alla mosca). L'annotazione funzionale è stata condotta con il software Sma3s. L'esame dei VOC è stato condotto su drupe delle varietà 'Minucciola' (tollerante alla mosca) e 'Ortice' (suscettibile alla mosca) raccolte in due stadi di maturazione nei loro ambienti di origine in campi trattati e non trattati contro la mosca dell'olivo. I composti volatili sono stati estratti dai campioni mediante SPME (Solid Phase Micro Extraction) per 24 ore e analizzati con sistema di gas cromatografia accoppiata alla spettrometria di massa (GC/MS).

Risultati e discussione

Lo studio dei VOC emessi dalle drupe ha rivelato profili diversi per le due varietà. I risultati ottenuti sono stati elaborati mediante l'analisi comparativa delle aree percentuali dei picchi dei cromatogrammi ottenuti dalle olive prelevate nei campi trattati, caratterizzati da un minore attacco di mosca, e da quelli non trattati, caratterizzati da un maggiore indice di attacco. Si è così evidenziato che il profilo dei VOC di entrambe le varietà, pur mantenendo una composizione simile, varia sia in termini qualitativi (soprattutto per quel che riguarda i composti terpenici) sia in termini semi-quantitativi nelle tesi trattate rispetto a quelle non trattate. Dato che il piano sperimentale è stato disegnato in modo da minimizzare l'effetto di altri fattori, è possibile pensare che questa evidenza sia stata prodotta dalla differente pressione della popolazione di mosca negli oliveti trattati e non trattati. Infine, se è vero che il controllo della popolazione di mosca dell'olivo salvaguarda la produzione delle olive, sembrerebbe anche determinare una variazione dei VOC emessi dalle olive e con essa il profilo sensoriale dei corrispondenti oli extra vergini di oliva.

L'impatto di *Bactrocera oleae* sul trascrittoma della varietà 'Ortice' è risultato più basso rispetto a quello su 'Ruveia': sono state identificate 52 EST differenzialmente espresse nella prima e 2452 nella seconda (FC>2; p<0,05 Student's T-test). La PCA (Principal Component Analysis) delle EST differenzialmente espresse con FC > 1 e p < 0.05 (Student's t- test) mostra che i trascrittomi delle olive sane delle due varietà risultano più simili fra loro rispetto a quelli delle drupe danneggiate dalla mosca. Il trascrittoma delle olive danneggiate di 'Ruveia' si distingue dagli altri, anche rispetto a quello delle olive danneggiate di 'Ortice'. Tale evidenza è in accordo con la supposta tolleranza della varietà 'Ruveia' alla mosca dell'olivo.

Conclusioni

I risultati di questo studio hanno prodotto un avanzamento delle conoscenze dei meccanismi molecolari dell'interazione fra olivo e mosca olearia e fornito evidenze di interesse biotecnologico applicativo nel settore olivicolo-oleario.

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Composti volatili dell'oliva e loro variazioni in risposta all'attacco della mosca dell'olivo

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L'ecologia dell'olivo è fortemente influenzata dall'interazione con il suo nemico-chiave, la mosca dell'olivo (*Bactrocera oleae*). In relazione alle condizioni ambientali, infatti, la mosca dell'olivo può determinare ingenti perdite quantitative e qualitative nel settore olivicolo-oleario. In accordo con quanto avviene in altre specie vegetali, è possibile ipotizzare la rimodulazione del profilo dei composti volatili (VOCs) emessi dalla drupa, riconducibile all'attivazione di meccanismi di difesa indiretta (1). Dati gli studi sul *priming* (2), la sola presenza della mosca in oliveto potrebbe modulare la produzione di VOCs da parte dell'oliva. Inoltre, poiché alcuni di questi composti sono fra quelli che caratterizzano anche la qualità sensoriale e la tipicità degli oli extravergini di oliva (OEVO), è possibile ipotizzare che l'interazione fra olivo e mosca possa influire sulla qualità sensoriale dell'OEVO.
In questo studio, l'interazione fra mosca olearia e olivo è stata approfondita mediante l'analisi dei profili dei VOCs emessi da drupe sane, in presenza o assenza di mosca olearia. Sono state campionate drupe delle varietà campane 'Minucciola' (tollerante alla mosca) e 'Ortice' (suscettibile alla mosca) raccolte nei loro ambienti di origine in campi trattati e non trattati contro la mosca dell'olivo. La frazione volatile delle drupe intere è stata isolata con fibra SPME (Solid Phase Micro Extraction) e Tenax. L'analisi dei VOCs è stata condotta mediante Gascromatografia accoppiata alla Spettrometria di Massa (GC/MS).
I profili dei VOCs (soprattutto terpeni) hanno mostrato significative variazioni fra le olive prelevate nei campi trattati e non trattati, caratterizzati da un maggiore attacco di mosca. I profili GC/MS hanno confermato, inoltre, come il profilo dei VOCs è fortemente influenzato dalla varietà di olivo. Tali riscontri, attualmente in corso di approfondimento, rafforzano l'ipotesi che le interazioni pianta-insetto e gli stress biotici possano influire sull'espressione della tipicità aromatica delle olive e degli OEVOs prodotti da diverse varietà e in diversi ambienti di coltivazione.

Analisi metabolomica NMR di effetti ambientali sulla composizione dell'olio di Bosana

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I fattori determinanti la qualità degli oli vergini di oliva, nei suoi vari aspetti merceologico, sensoriale e nutrizionale, sono da tempo oggetto di attenzione da parte della ricerca internazionale. Chiari appaiono gli effetti del fattore genetico legato alla cultivar, di quello tecnologico di trasformazione e agronomico, meno chiare -e non sempre univoche- appaiono le influenze dell'ambiente di coltivazione e, nello specifico, della componente pedoclimatica. Un areale non troppo esteso, come il centro nord Sardegna, omogeneo sotto l'aspetto varietale, appare un ideale sito di investigazione.
La ricerca biennale si basa su una rete sperimentale rappresentata da oliveti tradizionali monovarietali di *Bosana* e suoi sinonimi (*Palma, Sassarese* e *Tondo Sassarese*), condotti in coltura asciutta e con piante di età superiore ai cinquant'anni, ricadenti su cinque tipologie geo-pedologiche: calcari, basalti, trachiti, graniti e alluvioni. I siti di studio sono stati caratterizzati con appropriati indici agrometeorologici. Su ogni oliveto si è monitorata la maturazione dei frutti per mezzo del IM di Jaen prelevandone, al raggiungimento del valore 2 (semi invaiatura), 100kg circa; tutti i campioni sono stati trasformati in un mini frantoio aziendale a due fasi (TEM Oliomio) secondo un protocollo standard.
In entrambi gli anni gli oli sono ricaduti nella categoria merceologica degli extra vergini.
Gli spettri ottenuti da analisi NMR (Spettrometro Bruker Avance II 600) sono stati digitalizzati (bucket width 0.04 ppm) e sottoposti ad analisi statistica col software SIMCA-P 13.0 (Unimetrics); i dati sono stati scalati in maniera univariata, e sono state effettuate analisi O-PLS-DA.
La classificazione tramite analisi discriminante evidenzia che le differenti tipologie di suolo influenzano la composizione degli oli soprattutto nella componente polifenolica.
L'esperienza conferma la validità del metodo NMR quale importante strumento per la caratterizzazione dell'influenza ambientale nella composizione dell'olio vergine di oliva.