Transcriptomics and Biochemical Profiling: Current Dynamics in Elucidating the Potential Attributes of Olive

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Abstract
Various transcriptome studies have remained useful in unraveling the complexity of molecular pathways regulating the oil biochemical contents and fruit characteristics of agronomic value in olive. Genes networks associated with plant architecture and abiotic stress tolerance have been constructed due to robust genomic data generated by the tools of genomics. This, familiarity will accelerate the breeding programmes in making the selection of high yielding olive genotypes promptly and efficiently. Moreover, comparative transcriptome studies for endogeneous enzymes at different expression sites explicate the contribution of various pathways in phenol and lipid oxidation in olive. Recently, non-targeted metabolomics and metabolic profiling techniques have not only made the understanding of metabolic changes easy but also elucidate biomarkers in fruits related to agronomic parameters and abiotic stresses. However, the alteration in the architectural build up of phenotypes authenticate the conservation of their potential genetic links that will invoke interest for future olive breeding.

Introduction
Olea europea is the most important tree of Mediterranean basin which consists 95% of globally cultivated olive trees (Loumou and Giourga, 2003). Oil extraction is major purpose of olive production which is the sixth abundant vegetable oil produced worldwide (Conde et al., 2008). Olive tree is a diploid specie (2n=46), whose seeds are produced by cross-pollination (Besnard et al., 2000; Doveri et al., 2006), with ability to remain viable for long interval. It is glycophytic specie exhibiting a high tolerance towards salinity and drought as compared to other salinity susceptible fruit trees. The term olive oil is simply used for the oil exclusively extracted from fruit, whereas the oil exclusively extracted from fruit by using physical or mechanical means is termed as virgin olive oil (VOO). In order to obtain virgin olive oil no treatment with the exception of washing, decantation, centrifugation and filtration should be employed, however only such conditions are provided that favour alterations (IOOC, 2014). Olive oil has been used for consumption, medical, cosmetic, textile, illumination and other purposes for centuries (Mataix and Barbancho, 2006). Nowadays, it is indeed one of the greatest valuable and standard sources of fat in Mediterranean diet accompanying with several health benefits (Riachy et al., 2011a). Olive oil consists of two types of fractions i.e major fraction and minor fraction. Major fraction is recognized as saponifiable or glyceride fraction, constituting 98 to 99% of oil's weight. It is mainly composed of triacylglycerol (TAG) with addition of some free fatty acids, monoglycerols and diglycerols. The typical fatty-acid profile of virgin olive oil embraces oleic acid (68-81. 5%), which is the predominant and classifies it among MUFA (monounsaturated fatty acid) oils, as well as others such as linoleic, palmitic, stearic acid etc. (Ramirez-Tortosa et al., 2006). The ratio between n-6 and n-3 fatty acids is not of highest, i.e. 16 (published ranges for vegetable oils: 0-738) and hence its main putative health benefits are typically linked with its minor fraction (Dubois et al., 2007). However, minor fraction
comprises more than 230 different components, constituting 1-2% of oil's weight. Several groups can be included in these compounds such as non-glyceride esters (e.g., waxes), aliphatic and triterpenic alcohols, sterols (e.g., campesterol), hydrocarbons (e.g., squalene), polar pigments (e.g., chlorophylls), tocopherols, phenolic compounds (e.g., hydroxytyrosol) and volatiles (e.g., benzaldehyde) (Ramírez-Tortosa et al., 2006). Conversely, only few of them were acknowledged as bioactive along with their benefits reviewed by Covas et al. (2006).

For the extraction of oil from olive (Olea europaea L.) fruit, only those mechanical or physical procedures are preferred which do not alter its gloceric structure in order to preserve its natural high-worth healthy compounds including vitamins. The virgin olive oil is well recognized due to its countless health benefits among which fewer like tendency to decrease low-density lipoprotein cholesterol, antioxidant and antimicrobial qualities and cancer prevention are remarkable (Owen et al., 2000; Elloumi et al., 2012). Different genetic tools could be applied in order to understand the complexity of molecular pathways determining the oil and fruit characteristics of agronomic value. Moreover, introduction of marker assisted selection and genomic tools in olive breeding programmes will be helpful in production of olive cultivars with desirable phenotypes in short time. Furthermore, the generation of sequence information of olive or closely related species will accelerate genomics research (Alagna et al., 2009; Donaire et al., 2011). Olive has genome size of about 1,800 Mb, with high intra-specific genetic variation (Loureiro et al., 2007). Advances in olive genome mapping are continuously increasing the genomic data (Dela-Rosa et al., 2003; Wu et al., 2004), whereas, whole plastome sequence of an Italian cultivar "Frantoio" has been done already (Marriott et al., 2010). Moreover, a project entitled OLEA (http://www.oleagenome.org/) has undertaken the sequencing of olive genome in Italy. This review authenticates the utility of transcriptomic, proteomics and metabolic profiling in understanding the various important biochemical and physiological dynamics of olive plant. Moreover, the pathways involved in lipid formation and oxidation has been proposed for olive by interrogating the roles of enzymes and respective genes in Arabidopsis.

Oil biosynthesis pathways in Olive and Transcriptomics

Polyunsaturated fatty acids (PFA) are the typical example of plant lipids playing vital role in metabolic processes in the form of triacylglycerol (TAG), as a precursor of signaling molecules engaged in plant stress and development response, and as a structural part of membrane lipids (Ohlrogge and Browse, 1998; Weber, 2002). Linoleic acid along with oleic acid is a main fatty acid in vegetable oils which determine their technology related aspects such as nutrition and oxidative stability (Marqez-Ruiz et al., 1990; Cunnane, 2003). Most of the plants deposit huge quantity of TAGs in their seeds, whereas some deposit in mesocarp tissues (Lung and Weselake, 2006). Amongst, olive has prime economic significance as its oil can be consumed directly. Therefore, it is highly important to trace the core features involved in oil biosynthesis and storage in olive. This, familiarity will accelerate the breeding programmes in making the selection of high yielding olive genotypes promptly and efficiently. Till now, the molecular grounds of genes underpinning oil production in olive are much away from completion. Hitherto, an extensive knowledge is available about the involvement of various genes in modification and biosynthesis of fatty acids (Doveri and Baldoni, 2007; Banillas and Hatzopoulos, 2009), however least information is on cellular processes controlling the conversion of fatty acids into stored TAGs both in olive and other plants (Shockey et al., 2006). Figure 1 is a proposed model ortholog (based on genes with respective enzymes from Arabidopsis) which describes how enzymes regulate the synthesis of TAG inside endoplasmic reticulum via glycerol-3-phosphate (GP) pathway in olive. After expulsion from plastid, fatty acids are modified into acyl-CoA at outer plastid envelope by long chain acyl-CoA synthetase (LAS). Moreover, at outer envelope lysophosphatidylcholine acyltransferase (LPCAT) do the eserification of newly synthesized fatty acids to phosphatidylcholine (PC) via acyl editing cycle (Tjellstrom et al. 2012). Therefore, it is speculated that PC act as transporter of fatty acids from plastid to endoplasmic reticulum (Andersson et al., 2007). Moreover, it is supposed that the transfer of fatty acids from plastid to ER also takes place by lipid-related ABC transporters. However, about such transporters transcriptome evidences are limited (Kim et al., 2013). In ER, firstly GP-acyltrans-
Figure 1. Proposed model ortholog for the sequential pathways involved in oil synthesis in olive, where respective enzymes of each transformation are indicated along controlling genes. Abbreviations: For Enzymes in light green oval circles: LAS, Long chain Acyl-CoA Synthtase; LPCAT, Lysophosphatidylcholine acyltransferase; FAD, Fatty acid desaturase; LPAAT, Lysophosphatidic acid acyltransferase; GPAT, Glycerol-3-phosphate acyltransferase; PP, Phosphatidic acid phosphatase; DGAT, Diglyceride acyltransferase. For Substrates in pathways: PC, Phosphatidylcholine; PFA, Polyunsaturated fatty acid; PA, Phosphatidic acid; LPC, Lysophosphatidylcholine; LPA, Lysophosphatidic acid; DAG, Diacylglycerol; TAG, Triacylglycerol.
ferase (GPAT) converts GP to lysophosphatidic acid (LPA) by acylation. Secondly, LPA-acyltransferase (LPAT) further acylates LPA to phosphatidic acid (PA) which on dephosphorylation generates diacylglycerol (DAG). Thirdly, the enzyme diacylglycerol acyltransferase (DGAT) converts DAG into TAG through acylation (Figure 1).

Studies conducted at transcriptome level reveal that there is difference in transcriptome monitoring of TAG synthesis in non seed tissues of olive and palm oil is different than that of seed tissues. TAG synthesis in seed tissues is regulated in direct or indirect way by the genes LEC1, LEC2, ABI3 and FUS3 via WRI1 which is a downstream transcription factor. Likewise, in floral and non seed tissues the biosynthesis of fatty acids is also controlled by WR11, WR13 and WR14 (To et al., 2012). Furthermore, transcription factors controlling enzymes participating in later phases of TAG deposition are highly mysterious. Therefore, it will be highly interesting to study which genes are intensively expressed in lipid synthesis and how their expression pattern in mesocarp tissues vary as compare to their expression pattern in seed tissues.

Olive Phenols profile, Transcriptome and Metabolic Association

Olive matrices often exhibit exceptionally complex phenolic composition with a great diversity in structures. A wide range of secondary metabolites accumulate in olive fruit mesocarp. The main category of secondary metabolites belongs to Oleaceae and other few dicotyledonous families, represented by a group of monoterpenoids with a cleaved methylcyclopentane skeleton called secoiridoids. Phenol conjugated compounds that may have glycoside moiety are examples of secoiridoids present in excess in olive. Moreover, oleuropein, oleurosides, demethyloleuropein, ligstroside, nuzhenide and their aglycon forms are the most eminent secoiridoids present in VOO and olive fruit. Aglycon exists in dialdehydic form of decarboxymethyl elenolic acid bonded to either p-HPEA, an isomer of ligstroside aglycon and oleuropein aglycon, or 3,4-DHPEA (Obied et al., 2008). Oleocanthal, an important secoiridoid compound was never been detected in olive fruit, however it has been diagnosed in virgin olive oil owe to post-harvest enzymatic activity (Beauchamp et al., 2005). Although, other phenolics such as phenolic alcohols, lignans, phenolic acids, flavonoids and tyrosol also exist in all components of olive fruit (Oliveras et al., 2008) but in highest amount in pulp (Owen et al., 2003). High concentrations of verbascoside and other phenolic compounds like vanillic acid, phloretic acid, p-coumaric acid, caffeic acid, homovanillic alcohol (Boskou et al., 2006) and low concentration of comselegoside are also found in fruits of numerous olive cultivars at maturity (Servili et al., 2004; Jerman et al., 2010). The phenolic profile assessment of leave, stone, seed, mesocarp and exocarp of olive demonstrates that phenolics are tissues specific, as salidroside and nuzhenide are exclusive components of olive seed (Ryan et al., 2002), whereas flavonoids like quercetin, rutin and luteolin-7-glucoside are exclusive components of fruit peel (Servili et al., 1999). Ticopherols and tri-terpenic acids such as oleanolic and maslinic acids are some other compounds present in olive drupe (Dabbou et al., 2009; Goulas et al., 2012). Squalene is a compound of high significance for human health present only in vegetable oils and olive and serve as an intermediary of sterol pathway (Waterman et al., 2007). Moreover, it is a precursor of uvaol, tri-terpenic-diols-erythrodiols and α and β-amyrins. When olive fruit attains its ultimate size and veraison starts, then progressive deposition of sterols such as 24-methylenecycloartanol and cycloartanol occurs (Striti et al., 2007). Owe to health and organoleptic features, secoiridoids are the most vital micro constituents of VOOs. These are insoluble in oil, therefore can be recovered easily after mechanical extraction (Servili et al., 2002). Olive secoiridoids not only inhibit lipoprotein peroxidation but also play crucial role in prevention of atherosclerosis (Waterman et al., 2007). Omar et al. (2010) have demonstrated that these compounds have anticancer activities, while Puel et al. (2006) have confirmed their role in nutritional prevention of osteoporosis. Many health benefits have been shown on human by oleuropein, hydroxytyrosol (Omar et al., 2010) and oleocanthal (Beauchamp et al., 2005). The secoiridoids not only determine the quality of olive oil, but also affect oil taste by developing bitterness and pungency. However, they also serve as primary antioxidants and provide oxidative stability to the oil (Servili et al., 2004).

Phenolics also invoke defensive response in plant against defoliating insect under environmental determinants (Mumm et al.,
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2006). Moreover, they provide protection to the cells by hindering the fungal entry into the cambial zone (Umehara et al., 2008) and also affect shoot branching (Beckman et al., 2000; Franceschi et al., 2005). Some facts are augmenting the idea that might be certain types of phenolics are responsible for resistance to specific pathogens (Ockels et al., 2007; Wallis et al., 2008). Phytoalexins released by Oleuropein (Kubo et al., 1985) is polyvalent alkylator that plays role as a perfect protein cross-linker, presenting the strongest activity explained for a plant metabolite, which reduces the nutritive value of dietary proteins so affects the herbivores adversely (Konno et al., 1999). Till note, secoiridoid metabolism has not been elucidated in comprehensive way, however a pathway has been projected for few Oleaceae species (Damtoft et al., 1995a; Damtoft et al., 1995b). Malik et al. (2008) concluded that Secoiridoid deposition is a controlled reaction whose manifestation and makeup fluctuate notably among cultivars, tissues, developmental phases and in response to various environmental determinants. Phenolic compounds are peculiar attributes of VOO and never found in other vegetable oils. In this review we have classified the phenolics ingredients of olive drupe into six classes as indicated in Table 1. These classes are further sub categorized on the basis of available information from literature. This classification reveals that phenolics exist in all components associated with olive fruit. The studies examining olive phenols tissue distribution are scarce, encompassing only few reports that separately monitored all the constitutual parts of fruit i.e. peel, pulp and stone (Servili et al., 1999a; Servili et al., 1999b). Other studies have focused only on a single tissue, mainly pulp and/or pulp and peel together, while stone has been rarely investigated (Silva et al., 2010; Servili et al., 2007; Silva et al., 2006; Ryan et al., 2003; Ryan et al., 2002b). However, the peel and pulp together contain more than 90% of total fruit phenols, while the rest are confined in the seed (Servili et al., 2012).

Previously, due to the unavailability of genome sequence information for olive, no notable genes controlling the formation and breakdown of secondary metabolites in olive fruits had been characterized, except few genes meant for triterpene biosynthesis (Shibuya et al., 1999; Saimaru et al., 2007). However, first olive fruit transcriptome data manifesting an eminent source for identifying the genes engaged in olive fruit metabolism has been released now (Alagna et al., 2009; Galla et al., 2009). Due to the unavailability of methodical protocols for transformation, in vitro regeneration and mutagenesis, functional genetic techniques are not pragmatic in perennial woody species. Therefore, it has become more appreciable tool to understand the natural dynamics of traits under consideration. Currently, in plant science, the metabolic data sets and gene expression are unanimously being implemented to elucidate metabolic pathways (Saito et al., 2008). Hence, in future there is a need to understand the evolution of phenols in olive fruit as well as to mine and categorize the genes determining them.

Transcriptome Based Expression profiling for Fruit Abcission Zones

High intra-specific genetic diversity in olive (Loureiro et al., 2007) helps in elucidating biological mechanisms by applying the tools of biotechnology, such as lipid and phenol metabolic pathways (Alagna et al., 2009; Bianco et al., 2013) as well as terpenoids and sterols (Stiti et al., 2007). These mechanisms directly or indirectly affect the quality of olive oil and its nutritional profile as well. Alongside, with the help of 454-pyrosequencing and Sanger technologies 2 million expressed sequence tags (ESTs) have been generated (Muñoz-Mérida et al., 2013) which supplement the datasets catalog of transcripts in olive (Mariotti et al., 2010; Bianco et al., 2013). This will facilitate molecular breeding, functional analysis and gene mining in future. Major physiological processes like fruit ripening, abscission and senescence during growth and development of higher plants create commercial scale problems for both plant and the harvest. In order to develop varieties with high tolerance against environmental stresses and extensive fruit shelf lives, the pre-requisite is to manipulate the genes controlling these phenomenons. As various genes are engaged in ripening, abscission and senescence, therefore it is not pragmatic to manipulate these traits by using single gene, consequently major research is centered on specific transcription factors that regulate entire network (Nath et al., 2007). Many factors including genotypes govern the development of olive fruit which occur in the form of double sigmoidal growth curve and lasts for 4-5 months (Conde et al., 2008; Gomez-Jimenez
<table>
<thead>
<tr>
<th>Phenols Class</th>
<th>Categories</th>
<th>Occurrence</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple Phenols</td>
<td>Hydroxytyrosol acetate / 3,4-DHPEA-AC</td>
<td>Olive fruit, Waste water, oil</td>
<td>Morello et al., 2004; Artajo et al., 2006b</td>
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<tr>
<td>Tyrosol</td>
<td>Olive fruit, stone, paste, pomace, waste water, oil</td>
<td>Ryan et al., 2003; Suarez et al., 2008; Lozano-Sanchez et al., 2011</td>
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<tr>
<td>Tyrosol glucoside</td>
<td>Olive fruit, stone, paste, pomace</td>
<td>Peralbo-Molina et al., 2012; Kanakis et al., 2013</td>
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<tr>
<td>Hydroxytyrosol</td>
<td>Olive fruit, stone, paste, pomace, waste water, oil</td>
<td>Savarese et al., 2007; Lozano-Sanchez et al., 2011; Dierkes et al., 2012</td>
<td></td>
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<tr>
<td>Hydroxytyrosol-1-β-glucoside</td>
<td>Olive fruit, stone, paste, pomace, waste water, oil</td>
<td>Savarese et al., 2007; Kanakis et al., 2013</td>
<td></td>
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<tr>
<td>Hydroxytyrosol glucoside</td>
<td>Olive fruit, oil</td>
<td>Kanakis et al., 2013</td>
<td></td>
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<tr>
<td>Benzoic acids</td>
<td>Vanillin,</td>
<td>Olive fruit, stone, paste, pomace, waste water, oil</td>
<td>Lozano-Sanchez et al., 2011; Peralbo-Molina et al., 2012</td>
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<tr>
<td>Gentic acid</td>
<td>Oil</td>
<td>De Marco et al., 2007; Peralbo-Molina et al., 2012</td>
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<tr>
<td>Gentic acid</td>
<td>Oil</td>
<td>Carrasco-Pancorbo et al., 2005</td>
<td></td>
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<tr>
<td>Flavonoids</td>
<td>Apigenin</td>
<td>Olive fruit, stone, paste, pomace, waste water, oil</td>
<td>Fu et al., 2009a; Lozano-Sanchez et al., 2011</td>
</tr>
<tr>
<td>Luteolin</td>
<td>Olive fruit, stone, paste, pomace, Waste water, oil</td>
<td>Fu et al., 2009a; Lozano-Sanchez et al., 2011</td>
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<tr>
<td>Quercitrin</td>
<td>Olive fruit, stone, paste, pomace, waste water, oil</td>
<td>Au’datt et al., 2010; Rigane et al., 2011</td>
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<tr>
<td>Apigenin-7-O-glucoside</td>
<td>Olive fruit, stone, paste, pomace</td>
<td>Suarez et al., 2008; Peralbo-Molina et al., 2012</td>
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<tr>
<td>Luteolin-3-O-glucoside</td>
<td>Olive fruit, stone, paste, pomace</td>
<td>Yorulmaz et al., 2011</td>
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<td>Rutin</td>
<td>Olive fruit, stone, paste, pomace, waste water, oil</td>
<td>Savarese et al., 2007; Gomez-Rico et al., 2009</td>
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<td>Luteolin-7-O-glucoside</td>
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<td>Savarese et al., 2007; Suarez et al., 2010; Peralbo-Molina et al., 2012</td>
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<td>Cinnamic acids</td>
<td>Verbascoside / Acteoside</td>
<td>Olive fruit, stone, paste, pomace, waste water</td>
<td>Gomez-Rico et al., 2009; Rigane et al., 2011</td>
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<td>p-Coumaric acid</td>
<td>Olive fruit, stone, paste, pomace, waste water, oil</td>
<td>Suarez et al., 2010; Peralbo-Molina et al., 2012</td>
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<td>β-OH verbascoside</td>
<td>Olive fruit, stone, paste, pomace, waste water, oil</td>
<td>Suarez et al., 2010; Peralbo-Molina et al., 2012</td>
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<td>Lignans</td>
<td>Acetoxypinoresinol</td>
<td>Olive fruit, stone, paste, pomace, waste water, oil</td>
<td>Lopez et al., 2008; Fu et al., 2009a; Suarez et al., 2010</td>
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<td>Pinoresinol</td>
<td>Olive fruit, stone, paste, pomace, waste water, oil</td>
<td>Lopez et al., 2008; Fu et al., 2009a; Suarez et al., 2010</td>
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<td>Secoiridoids</td>
<td>Oleoside</td>
<td>Olive fruit, stone, paste, pomace, oil</td>
<td>Bouaziz et al., 2010; Fu et al., 2009a; Kanakis et al., 2013</td>
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<tr>
<td>Secologanioside</td>
<td>Paste, oil</td>
<td>Fu et al., 2009a; Kanakis et al., 2013</td>
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<td>Demethyl-oleuropin</td>
<td>Olive fruit, stone, paste, oil</td>
<td>Servilli et al., 2007; Savarese et al., 2007</td>
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<td>Dihydro-oleuropin</td>
<td>Olive fruit, stone, paste, pomace</td>
<td>Peralbo-Molina et al., 2012; Kanakis et al., 2013</td>
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<td>Oleuropein dighlucoside</td>
<td>Olive fruit, stone, paste, pomace</td>
<td>Cardoso et al., 2005; Bouaziz et al., 2010</td>
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<td>Oleuropein aglycone</td>
<td>Oil</td>
<td>Dierkes et al., 2012</td>
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<td>Oleuropein</td>
<td>Olive fruit, stone, paste, pomace, waste water, oil</td>
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<td>Oleurosides</td>
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<td>Ligstoside</td>
<td>Oil</td>
<td>De-La-Torre-Carbot et al., 2005</td>
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<td>Conseulosides</td>
<td>Olive fruit, paste, pomace</td>
<td>Obied et al., 2007a; Kanakis et al., 2013</td>
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<td>Nürzhenide 11- methyl oleoside</td>
<td>Stone, paste</td>
<td>Silva et al., 2010; Kanakis et al., 2013</td>
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et al., 2010). The quality of olive fruit and oil depend upon the last mix of primary and secondary metabolites which deposit during ripening and drupe possessions at the time of harvest. Many changes occur during the development and ripening of olive-fruit as elaborated by recent metabolic and transcriptomic studies (Galla et al., 2009; Bianco et al., 2013). Advances in monitoring the transcriptome of olive in terms of gene ontology and functional annotations have made it possible to demonstrate differential expression of genes between olive tissues (Alagna et al., 2009; Galla et al., 2009). Hitherto, there is no complete transcriptome knowledge of olive fruit at full ripening stage. Intensive natural abscission take place in many fruit trees after ripening. The activation of abscission zones residing between pedicel and fruit stimulate the abscission of mature fruits in olive. Likewise, the patterns of abscission of mature fruit vary among cultivars (Gomez-Jimenez et al., 2010; Parra-Lobato et al., 2011). In olive cultivar "Picual" abscission takes place at 217 days after anthesis (Gomez-Jimenez et al., 2010; Parra-Lobato et al., 2011). Gil-Amado and Gomez-Jimenez (2013) have carried out a comparative abscission zone transcriptome study for Picual fruit at abscission and pre-abscission stages by using the RNA-Seq technique which has generated 148 Mb sequences and 4,728 differentially expressed genes were identified. Seventy transcription factor genes expressed during mature fruit abscission in olive are associated with three types of proteins e. g MYB proteins, bZIP proteins and homeobox protein domain (Gomez-Jimnez, 2013; Parra et al., 2013). Transcriptome comparisons between abscission zone and fruit facilitate us to inscribe the cluster of genes expectedly related to abscission, hence the dimension of study for this process can be more result oriented. Although, transcription factors and other signaling molecules directly or indirectly determine the crosstalk between two tissues, but there is limiting molecular genetic data on the relation between ripe fruit and abscission zone. Parra et al. (2013) have generated transcriptional catalog for fruit mesocrop of olive cultivar "Picual" by using 454-pyrosequencing technology. This technology has facilitated to establish the divergence and resemblances in transcriptional networks by comparing the transcriptomes produced from abscission zone and pericarp tissues. Moreover, it also categorized the biological activities and transcriptional modulators abundant in differentially modulated gene clusters. As a whole, 4,391 differentially expressed genes have been categorized for their biological roles using KEGG and GO pathway analysis in abscission zone and ripe fruit.

Transcriptomics and Candidate Gene Mining for olive plant architect

In agriculture plant architect is of prime importance that determines the suitability of plant for light, yield, cultivation and harvesting (Hanan et al., 2003; Badenes and Byrne, 2011). Although internal metabolic processes and environmental factors unanimously control plant architect, yet genotype is considered as supreme dynamic (Barthélémy and Caraglio, 2007; Busov et al., 2008). Plant architecture is a composite of various features like shape, size, branching prototype, and orientation of flowers and leaves (Reinhardt and Kuhlemeier, 2002; McSteen and Leyser, 2005). However, its intricacy is explained by the capability to form new axis of growth due to differentiation of axillary meristems during post embryonic development (Baldi et al., 2013; Costes et al., 2015). Therefore, vegetative branching pattern is defined by the regulation of shoot growth (Schmitz and Theres, 2005). Endogenous and environmental signaling determines the extent and interval of branching (Kwon et al., 2005). Plant hormones like cytokinins, gibberellins, auxins or strigolactones are the examples of endogenous signals (Vogel et al., 2010; Wang and Li, 2008). Moreover, meristematic activity during reproductive phase forms different structures that lead to flowering which is an important paramount of reproductive success and plant architect (Schmitz and Theres, 2005; Costanzo et al., 2014). Molecular mechanisms have been investigated in annual crops that reveal plant height is an important parameter which affects yield directly (Wang and Li, 2006). Since olive is primarily important vegetable oils source in the world, therefore it is economically significant to understand the philosophy of its plant architecture (Conde et al., 2008). But most of the current cultivars of olives are traditional (Belaj et al., 2012) that are not well acclimatized to the modern patterns of olive cultivation (Haouane et al., 2011). The main focus of modern trends is to promote intensive plantations of 2000 tree per hectare from the traditional 100 tree per hectare in hedge row growing systems (Baptista and Biswas, 2010).
However, canopy size and shape is adjusted according to high densities by pruning that aims to attain the maximum leaf to wood ratio (Rosati et al., 2013) under least shading (Haoouane et al., 2011). These methods are used for the plantations of Spanish varieties like Arbequina and Arbosana in intensive hedgerow orchards owing to their good agronomic potential (Rosati et al., 2013). As these varieties possess low to medium vigor, therefore there will be considerable yield loss at early stage due to high competition for light and nutrients if sufficient pruning practices are not implemented (Connor et al., 2009; Petersen and Kroist, 2013). Till note, two olive varieties with architecture adapted to high planting densities have been produced via breeding programmes, Chiquitita in Spain (Rallo et al., 2008) and Askal in Israel (Lavee et al., 2003). Moreover, less awareness is available on molecular dynamics that regulate plant architectures in olives (Segura et al., 2009; Hollender and Dardick, 2015). These cultivars have maximum light exposure and need less pruning. Apart from some specific motives in breeding for particular tree architecture in olive, little has been done on genetics, heritability patterns and QTL mapping (Hammami et al., 2012; Moriya et al., 2012). Nevertheless, no gene related to growth pattern has been mined in olive, which reflects how much space is available for the tools of genomics (Sadok et al., 2013). Genomics and its accessory techniques are providing new dimension to the researchers for exploring the genetic and molecular processes determining the growth habit in historically related trees (Hollender and Dardick, 2015). Undoubtedly, QTL analysis is really practical in breeding as they are cultivar specific, still the mining and categorization of genes related to particular aspect of tree architecture is crucial to explore tree genomes for crop improvement. It will boost both conventional and biotechnological approaches in accelerating plant improvement. Microarray analysis is the best choice for the identification of candidate genes particularly undermining the complex traits related to plant architect in non sequenced plant species (Utsumi et al., 2012) for example in grapevine (Diaz-Riquelme et al., 2012), Populus trichocarpa (Di Bacco et al., 2011) and olive (García-López et al., 2014). Moreover, these are also been used for exploring the genes triggering the columnar growth habit in apple (Petersen and Kroist, 2013). OLEAGEN Consortium has developed an olive microarray that is useful in mining the candidate genes determining the plant architecture of olive (Muñoz-Médica et al., 2013; García-López et al., 2014). It reflects extensive scope of transcriptomic approaches in constructing gene networks that monitor the architect of plant in olive. The breeding programme of olive is currently carried out at University of Cordoba and IFAPA where comparative RNA analysis of varieties with different architectural phenotypes and common genetic background was done to investigate the genes endorsing the variations. Chiquitita is one of the selected varieties within this breeding programme was bred to incorporate the features like, shrubby growth and compact weeping canopy that make it highly desirable for high density cultivation and mechanical harvesting with straddle (Rallo et al., 2008). Likewise, the trees with non standard growth patterns can serve as an excellent choice for understanding the complicated developmental mechanisms regulating the architectural features of plants (Hollender and Dardick, 2015). Approximately, 2252 differentially expressed genes shaping plant architect were identified in olive by González-Plaza et al., 2016, by transcriptome analysis of generated microarray data. Further confirmation of microarrays findings was carried out by qRT-PCR to link phenotypic expression in selected varieties and their seedlings. Orthologs of selected candidate genes sought a functional proof by co-relating phenotypic architect of Arabidopsis mutants with explored genetic networks (González-Plaza et al., 2016). However, the alteration in the architectural build up of phenotypes authenticates the conservation of their potential genetic links that will invoke interest for future olive breeding.

Endogenous Enzymes and Biochemical Attributes of Oil and Fruit

Due to its nutritional value and healthful attributes virgin olive oil (VOO) has remained a part of human relict history and culture (Clodoveo et al., 2014a). Mechanical techniques are used for the extraction oil from olive fruits. Many endogeneous enzymes like polyphenol oxidases, pectinases, peroxidases, hydroperoxide lyases, lipases, β-glucosidases and lipoxygenases work in composite medium which constitutes olive (Clodoveo et al., 2014b). Theses enzymes get discharge when fruit tissues undergo pathogenic attacks during storage, extraction or mechanical damage during harvesting. The organoleptic properties of VOO
are fundamentally associated with its volatile and phenolic contents that provide oil fruity essence and stability against autoxidation respectively (Angerosa, 2002; Fregapane and Salvador, 2013). Phenolic compounds in olive fruit are determined by intrinsic factors like enzymatic activities and glycosides amount (Romero-Segura et al., 2012) and technological factors involved during oil extraction (Fregapane and Salvador, 2013). The pioneer endogeneous enzymes in olive fruit are oxidoreductase and β-glucosidase that oxidize phenolic compounds and hydrolyze phenolic glycosides respectively. Furthermore, the oxidoreductase activity is highly dependent on the olive genotype, maturation stage, locality and altitude (Clodoveo et al., 2014a). The major phenolic glycosides isolated from various olive genotypes at different maturation stages are oleuropein, demethyl-oleuropin, elenolic acid glucoside, apigenin-7-glucoside, luteolin-7-glucoside, verbascoside, quercetin-3-rutinoside and rutin (Gomez-Rico et al., 2008; Clodoveo et al., 2014a). Beta-glucosidase hydrolyzes phenolic glycosides into secoiridoid during extraction process which is an important constituent of VOO (Romero-Segura et al., 2009).

Moreover, during kneading and milling phases of oil extraction the oxidoreductases particularly, polyphenol oxidase (PPO) and peroxidase (POX) stimulate the oxidation of phenolic compounds (Servili et al., 2008). However, peroxidases use oxidizing agents like organic peroxides or hydrogen peroxide (H₂O₂) for the oxidation of phenolic compounds to generate free radicals and reactive intermediates with ability of polymerization (Gajhede, 2001). Availability of H₂O₂ determines POX activity as autoxidation of phenolic compounds in damaged tissue of olive generates H₂O₂ which is used by POX for further oxidation of phenolics (Takahama and Oniki, 2000). The polyphenol peroxidase containing copper as a cofactor is widely present in nature which not only triggers many metabolic activities (Waliszewski et al., 2009) but also induce browning in vegetables and plants damaged by poor handling, bruising and compression (Zawitowski et al., 1991). PPO catalyse di phenolase and monophenolase activities which include the oxidation of o-diphenols to o-quinones and o-hydroxylation of monophenols to di-phenols respectively. The large amount of phenolic compounds in VOO at higher malaxation temperature indicates the greater stability and functionality of peroxidase at 37°C as compare to lower stability of polyphenol peroxidase at 40°C (Taticchi et al. 2013). The virgin olive oil extracted from Spanish variety Arbequina is strongly preferred by consumers due to its organoleptic characteristics (Yousfi et al., 2012). These characteristics are dependent upon the fruit maturation stage. The decline in photosynthetic pigments and phenolic compounds in overripe fruit is associated with decline in organoleptic attributes and oxidative stability of VOO (Morello et al., 2004). Therefore, it is suggested that for short storage period Arbequina fruit should be collected at early maturation stage (Romero et al., 2002). But, it raises constraint where we have to store the fruit due to insufficient milling capacity which hinders the processing of available fruit simultaneously. Different studies have been conducted to explore the relationship between fruit storage and oil quality. However, it has been found if Picual fruit is stored at 5°C the peroxide value, free acidity, sensory quality and ultraviolet absorbance of obtained oil will remain within prescribed limits even at 45 day interval of fruit storage (Garcia et al., 1996a). The refrigeration of olive cultivars Blanqueta and Villalonga at 5°C on an industrial scale hindered the spoilage of their chemical, physical and sensorial attributes related to oil quality even during 30 days interval of storage. Chemical oxidation happening during extensive storage of either the oil or the fruit is lethal as it causes sensory abnormalities (Kirtitsakis et al., 1998). Olive oil extracted from Coratina has kept its preliminary chemical qualities during 30 days interval of olives storage at 5°C under humid aeration with 3% oxygen and 5% carbon dioxide Clodoveo et al. (2007), though, at room temperature the oil quality deteriorates after after 15 days of storage. On the other hand Yousfi et al. (2012) found that VOO extracted from mechanically harvested fruit of Arbequina retains chemical composition and best quality at 3°C. Hence, mechanical harvesting hinders the deterioration process and increases the shelf life of olive fruits. The nutritional and organoleptic aspects of VOO are based upon phenolics, therefore, to gain a product of economic worth it is important to understand the origin of enzymatic activities undermining phenols metabolism during fruit storage period (Lazzez et al., 2011). Thus, it is important to understand basic biochemistry and molecular dynamics of olive fruit and oil for making its better commercialization in international markets.
note, conducted studies are insufficient to investigate the activities of endogenous enzymes during fruit maturation, harvesting, storage and their effect on the quality of VOO.

Lipid oxidation is one of the major causes of olive oil quality deterioration. During extraction the damage in cell wall activates endogenous enzymes. The molecular oxygen generated by peroxidases accelerates the oxidation of polyunsaturated fatty acids and stored TAGs by LOX enzyme. It results in the generation of hydroperoxides and other volatile compounds, which produce unpleasant odor in oil (Figure 2). For systematic explanation of lipid oxidation pathway in olive the concerned enzymes have been comprehensively (Table 2) interrogated through literature. The enzymes like polygalactouranses or pectinases are compartmentalized in the sites such as endomembrane system, extracellular region, cell wall and apoplast (Li et al., 2013; Park et al., 2015; Mabuchi et al., 2016). Moreover, these enzymes are actively involved in carbohydrate metabolism (Kim et al., 2006), loosening of cell wall connection associated with fruit firmness (Park et al., 2015) and cell wall organization (Keunen et al., 2013) to some extent. The genetic determinants of these enzymes manifest themselves in different parts and cells of plants during different stages as indicated in table 2. Moreover, LOXs are located in chloroplast (Thieme et al., 2015) and cytoplasm (Mira et al., 2016) where they govern the processes like membrane disassembly (Marcos et al., 2015; Nalam et al., 2015), lipid oxidation and jasmonites biosynthesis. The genetic determinants of LOXs are expressed at different sites during different stages (Table 2). Furthermore, other enzymes like lipases, peroxidases, β-glucosidases and polyphenol oxidase are also indicated with their respective genes, roles, activities, expression sites and stages in Table 2. Comparative transcriptome studies for these enzymes at different expression sites can elucidate the different enzymatic pathways involved in lipid and phenol oxidation. However, in this review we proposed a schematic pathway of lipid oxidation in olive by exploring the functionality and genomics of olive endogenous enzymes in Arabidopsis (Figure 2). The oxidation of membrane lipid and TAG take place by endogeneous enzymes inside cells of olive fruit mesocarp. LOX compartmentlize in vacuole and chloroplast possibly do the oxidation of polyunsaturated fatty acids from membrane and TAG from ER into hydroxypolyunsaturated fatty acids when ROS are generated by peroxidases. These HPFAs are converted into products like EHPFs, jasmonic acids and other volatiles aldehydes and ketones, whereas pectinase enzyme compartmentalized at apoplast region is responsible for cell wall softening which make oil leakage easy (Figure 2).

Proteome Dynamics and Olive Fruit Development

The oil derived from olive fruit is gaining global importance for its nutritional, anticancer and cardiovascular disease protective properties due to peculiar fatty acids composition (Colomer et al., 2006). The catabolic and anabolic activities during fruit development and ripening mainly determine the quality prospects of olive oil. Remarkable variations take place in composition, flavor, texture, color and accumulating oil in fruit mesocarp, however less dramatic in the seed (Conde et al., 2008). After the inception of ripening the oil content can incline up to 28-30 % of the fresh weight. Owe to significant nutritional value of olive oil, it would be of great concern to exploit the complete comprehension of metabolic profile leading to biosynthesis of compounds determining the quality of both fruit and oil. Fatty acids profile of olive oil reveals its high concentration of monounsaturated FA oleate (18:1) up to 80 percent followed by linoleate (18:2), palmitate (16:0), stearate (18:0) and linolenate (18:3). However, genotypic and environmental interaction determines the fluctuating acyl composition during olive fruit developmental stages. Moreover, other metabolites like polyphenols, terpenoids, carotenoids, sterols, chlorophylls and wide range of other volatile compounds accumulate in mesocarp of olive fruit (Conde et al., 2006) that affects directly or indirectly the oil quality and aroma (Conde et al., 2008). A combination of physiological and biochemical processes under strong genetic control and environmental influence controls the development of olive fruit (Connor et al., 2005). The fruit development process lasts for 4-5 months in olive and is divided into 5 main phases viz fertilization and fruit set (0-30 DAF), seed development (30-60 DAF), pit hardening (60-90 DAF), mesocarp development (90-150 DAF) and ripening (150 DAF) (Connor et al., 2005). The ripening is terminal phase in fruit development where biochemical, physiological and structural...
Table 2: Endogenous enzymes along their candidate genes and compartmentalization in Arabidopsis that serve as base to architect lipid peroxidation pathway in olive by tagging their activities, expression sites and stages.

<table>
<thead>
<tr>
<th>Gene Id</th>
<th>Enzyme</th>
<th>Location</th>
<th>Activity</th>
<th>Expression Sites</th>
<th>Expression Stages</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PG1</td>
<td>Polygalacturonase</td>
<td>Endomembrane system</td>
<td>Loose the cell connection which associated with fruit firmness, flower, petal, pedicel, stamen, root, seed, cotyledon</td>
<td>Meristem, gaurad cell, lamina base, flower, petal, pedicel, stamen, root, seed, cotyledon</td>
<td>MPES, FS, GPS</td>
<td>Li et al., 2013; Park et al., 2015</td>
</tr>
<tr>
<td>AT5G48140</td>
<td>PutativePolygalacturonase or Pectinase</td>
<td>Endomembrane system</td>
<td>Carbohydrate metabolic process, Sepal, flower, stamen, seed, sperm, plant embryo, stamen, carpel, pollen</td>
<td>MPS, FS, PEBS, PEGS</td>
<td></td>
<td>Tabata et al., 2000; Kim et al., 2006</td>
</tr>
<tr>
<td>PG2</td>
<td>Polygalacturonase</td>
<td>Apoplast, cell wall region, Endomembrane system</td>
<td>Biological process, Seed, sepal, root, pollen, sperm, plant embryo, meristem, petal</td>
<td>FS, MPES, PDES, PEBS</td>
<td></td>
<td>Park et al., 2015; Meyer et al., 2012</td>
</tr>
<tr>
<td>AT5G44840</td>
<td>Polygalacturonase</td>
<td>Extracellular region</td>
<td>Carbohydrate metabolic process, cell wall organization, Fruit flesh, seed</td>
<td>PECS, PEGS, VLSS</td>
<td></td>
<td>Keunen et al., 2013; Mabuchi et al. 2016</td>
</tr>
<tr>
<td>LOX1</td>
<td>Lipoygenase 1</td>
<td>Cytoplasm, plastid region</td>
<td>Lipid oxidation, biosynthetic process, membrane disassembly, response to jasmonites, response to abscisic acid.</td>
<td>Carpels, cotyledon, flower, pedicel, seed, trichobil, meristem, stamen, petal, pollen</td>
<td>FS, MPES, PDES, PEBS, PEGS, PECS, PEGS</td>
<td>Marcos et al., 2015; Nalam et al., 2015; Thieme et al., 2015</td>
</tr>
<tr>
<td>LOX2</td>
<td>Lipoygenase 2</td>
<td>Cytoplasm, chloroplast, stroma, thylakoid membrane.</td>
<td>Lipid oxidation, response to jasmonic acid, oxylipin biosynthesis, water deficit response.</td>
<td>Fruit, seed, carpel, pollen, stamen, sperm, petiole, embryo</td>
<td>PDES, PEBS, PEGS, VLSS</td>
<td>Pineda et al., 2015 Thieme et al., 2015 Mira et al., 2016</td>
</tr>
<tr>
<td>LOX3</td>
<td>Lipoygenase 3, Lox3</td>
<td>AT1G17420</td>
<td>Lipoygenase 4</td>
<td>Chloroplast</td>
<td>Anther development, response to ozone, oxylipin biosynthesis, defense response to jasmonic acid biosynthetic process, lipid oxidation,</td>
<td>petal, petiole, pollen, root, sepal, stamen, cotyledon, pedicel, shoot apex</td>
</tr>
<tr>
<td>AT1G72520</td>
<td>Lipoxynase 5</td>
<td>Chloroplast</td>
<td>Regulation of defense response to insects, lipid oxidation, oxylipin biosynthetic process.</td>
<td>Cotyledon, carpel, pollen, stamen, meristem, leaf apex, pollen</td>
<td>FS, PES, PECS, VLSS.</td>
<td>Keunen et al., 2013; Nalam et al., 2015</td>
</tr>
<tr>
<td>LOX5</td>
<td>Lipoxynase 5</td>
<td>Chloroplast, cytoplasm</td>
<td>Jasmonic acid biosynthetic process, lipid oxidation, oxylipin biosynthetic process.</td>
<td>Cotyledon, pedicel, guard cell, stamen, pollen, leaf, pollen, root, guard cell, seed, hypocotyl</td>
<td>FS, MPES, PDES, PEBS, PEGS, PECS, VLSS</td>
<td>De Ollas et al. 2015; Chauvin et al., 2016</td>
</tr>
<tr>
<td>AT1G67560</td>
<td>Lipoxynase 6</td>
<td>Plasma membrane, chloroplast</td>
<td>Jasmonic acid biosynthetic process, lipid oxidation, oxylipin biosynthetic process.</td>
<td>Vascular leaf, meristem, flower, seed, petiole, pollen, sperm, pollen tube cell, seed, petal, sepal, stamen, root, lamina base</td>
<td>MPES, PDES, PEBS, PEGS, PEMS, VLSS</td>
<td>Fun et al., 2015</td>
</tr>
<tr>
<td>SDP1</td>
<td>Triaclglycerol lipase</td>
<td>Integral component of membrane, Monolayer surrounded lipid storage body.</td>
<td>Glycerol metabolic process, triglycerides catabolic process.</td>
<td>Vascular leaf, meristem, flower, seed, petiole, pollen, sperm, pollen tube cell, seed, petal, sepal, stamen, root, lamina base</td>
<td>MPES, PDES, PEBS, PEGS, PEMS, PEGS, PEMS, SMSS</td>
<td>Fun et al., 2015</td>
</tr>
<tr>
<td>GLP1</td>
<td>GLS lipase 1</td>
<td>Extracellular region, extracellular space</td>
<td>Salicylic acid response, lipid catabolic response, ethylene mediated signaling pathway, Jasmonic acid and ethylene-dependent systematic resistance.</td>
<td>Stem</td>
<td>SMS, FRS</td>
<td>Kim et al., 2013; Kim et al., 2014</td>
</tr>
<tr>
<td>AT3G48080</td>
<td>Lipase class 3 family protein</td>
<td>Cytoplasm, nucleus</td>
<td>Lipid metabolic process, hydrolase activity, lipase activity, signal transduction, lipid catabolism.</td>
<td>Flower, guard cells, leaf lamina, petals, Petiole, sepals, stamens, carpels.</td>
<td>FS, MPES, PDES, PEBS, PECS, VLSS</td>
<td>Che et al., 2006; Ascencio-Ibanez et al., 2008</td>
</tr>
<tr>
<td>Gene Symbol</td>
<td>Gene Description</td>
<td>Function</td>
<td>Gene Products</td>
<td>Transcriptomics and Biochemical Profiling</td>
<td>References</td>
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<tr>
<td>EXL4</td>
<td>Extracellular lipase 4</td>
<td>Extracellular matrix, pollen coat, Extracellular region and space</td>
<td>Lipid catabolic process, sexual reproduction,</td>
<td>Flower, petal, sepal, collective leaf structure</td>
<td>FS, PDES</td>
<td></td>
</tr>
<tr>
<td>HPL1</td>
<td>Hydroperoxide Lyase 1.</td>
<td>Chloroplast envelope</td>
<td>Fatty acid metabolic process, oxidation reduction process, metabolism of sterol.</td>
<td>Carpel, cotyledon, flower, pedicel, leaf, seed, meristem, pollen, egg cell, carpel, leaf apex, embryo, lamina base</td>
<td>FS, MPES, PEBS, PECS, VLSS</td>
<td></td>
</tr>
<tr>
<td>BGLU25</td>
<td>Beta-glucosidase 25</td>
<td>Endoplasmic reticulum and extracellular region</td>
<td>Metabolism of carbohydrates, hormonal response, response to salinity, glucosinolate catabolic process.</td>
<td>Leaf apex, lamina, pollen, stamen, carpel, flower, root, sepal, apex, cotyledon, pedicel, guard cell, petiole</td>
<td>PEBS, PECS, PEGS,</td>
<td></td>
</tr>
<tr>
<td>BGLU45</td>
<td>Beta-glucosidase 45</td>
<td>Extracellular region</td>
<td>Metabolism of carbohydrate, glycosyl compound metabolic process, lignin biosynthetic process</td>
<td>Pedicel, flower, sepal, seed, embryo, stamen, carpel, hypocotyl, meristem, petals, egg cell, stamen, root</td>
<td>FS, MPE, PDES, PEBS</td>
<td></td>
</tr>
<tr>
<td>AT5G64120</td>
<td>Peroxidase 71</td>
<td>Plant-type cell wall, apoplast Golgi apparatus, cell wall</td>
<td>Hydrogen peroxide catabolic process, oxidation reduction process, lignin metabolic process, plant-type cell wall organization, respiratory burst, rhythmic process, response to oxidative stress.</td>
<td>Carpel, cotyledon, flower, petiole, vascular leaf, sepal, stamen, plant embryo</td>
<td>FS, PEDS, PEGS,VLSS</td>
<td></td>
</tr>
<tr>
<td>PPO</td>
<td>Polyphenol oxidase</td>
<td></td>
<td>Protein dephosphorylation</td>
<td>Guard cells, pedicel, cotyledons, vascular leaf, apex, seed, pollen, pollen tube cell, stamen, petal, shoot</td>
<td>FS, PEGS, VLSS, PES.</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: MPES, Mature pollen embryo stage; FS, Flowering stage; GPS, Germinating pollen stage; MPS, Mature pollen stage; PEBS, Plant embryo bilateral stage; PEGS, Plant embryo globular stage; PDES, Petal differentiation and expansion stage; PECS, Plant embryo cotyledonary stage, VLSS; Vascular leaf senescence stage, PES, Plant embryo stage; SMS, Seed maturation stage; FRS, Fruit ripening stage; MPE, Mature plant embryo; SDS, Seedling development stage.
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makeup are altered to affect texture, appearance, flavor and aroma. Texture modifies due to change in cell wall structure and cell turgor and metabolism, whereas, colour modifies due to change in composition of carotenoids, chlorophylls and flavonoids, while, nutritional quality, flavor and aroma are affected due to modification of volatiles and sugars (Giovannoni, 2004). Comparative proteomics coupled with mass spectrometry investigate metabolic pathways to assess changes simultaneously at protein level. Currently proteomics is becoming more attractive to plant biologists owe to the availability of sequence information in databases which is opening new horizons in protein identifications. Even for non model plants more practical methods to analyze protein content has been developed due to accumulation of nucleic acids data in databases as a result of revolution in sequencing technologies (Carpentier et al., 2008). Hitherto, limited informations are available about the proteomic profile of olive fruit during developmental phases, although some ESTs have been generated from developing olive fruits recently (Alagna et al., 2009; Galla et al., 2009; Esteve et al., 2012).

Transcriptome Changes in Response to Salinity

Plant productivity is facing serious threat due to increasing olive cultivation in fields irrigated with low quality and saline water. Salinity poses serious threats to fruit production (Cresti et al., 1994) by negatively affecting leaf morphology (Therios, 2009) and photosynthesis (Loreto et al., 2003; Tabatabaei, 2006) as well as shoot growth (Tattini et al., 1992; Klein et al., 1994). The tolerance of olive to NaCl is supposed to be intermediate (Rugini and Fedeli 1990), although, extensive genetic diversity is found in olive germplasm against abiotic stresses (Therios et al., 1988; Chartzoulakis, 2005). Certain physiological mechanisms attribute tolerance through retention of Na⁺ and Cl⁻ by root and stem, Na⁺ exclusion due to influx of K⁺ and by compartmentalization of toxic Na⁺ and Cl⁻ into vacuole.
Tolerant cultivars are able to make exclusion of Na\textsuperscript{+} and Cl\textsuperscript{−} from shoots (Gucci et al., 1997) by restricting their accumulation in actively growing shoots and leaves (Melgar et al., 2009). Although, this tactic increases salt absorption but put a check on its translocation (Benlloch et al., 1991; Demiral, 2005). Comparative transcriptome and microarrays strategies are used for the mining and characterization of genes related to salt stress in relative species or varieties with differential tolerance to abiotic stress (Deyholos, 2010). Although various studies are available but still our information on olive response to salinity is limited at physiological level. Bazakos et al. (2012) have conducted a comparative transcriptome study on tolerant and susceptible olive genotypes to find transcription factors that elicit significant response against salinity by constructing microarrays. Most of the studies on response mechanisms of olive to salinity are limited to ecophysiological level or to the investigation of single metabolic pathway like mannitol metabolism under salinity stress (Conde et al., 2007). However, no comprehensive investigation has been made on molecular logics of salt tolerance in olive. Previously, the unavailability of reference transcriptome sequence has created difficulties in mining and characterization of gene regulatory networks in olive against stress (Salem et al., 2010). For gene expression studies high-throughput transcriptome sequencing is a fast and efficient option to microarrays techniques (Marioni et al., 2008). Limitations imposed by microarrays can be overcome by sequence based approaches, as these are advantageous to trace the dynamicity of transcriptome across various tissues under stress conditions (Mortazavi et al., 2008). Various researchers have noticed that the implementation of 454 Life Sciences sequencing (Margulies et al., 2005) is proved as an efficient method to investigate mRNA expression levels (Weber et al., 2007; Torres et al., 2008; Wang et al., 2010). Comparative deep sequencing analysis of plant transcripomes found the variations in gene expressions in response to genotype, tissue and physiological changes in model and non model plants such as chestnut (Barakat et al., 2009), maize (Ohtsu et al., 2007), grapevine (Al Rwahnih et al., 2011), waterhemp (Riggins et al., 2010), eucalyptus (Novaes et al., 2008) and currently in olive (Muñoz-Mérida et al., 2013; Leyva-Perez et al., 2015). The 454-pyrosequencing technique is advantageous over NGS (Next Generation Sequencing) particularly due to its ability to generate long-read length in non model plants, as these are essential to recognize transcript accurately for which molecular diagnostics are scarce, for example olive. More than thirty families of transcription factors related to abiotic stress including salinity (He et al., 2011) have been reported by comparison between salt tolerant and salt susceptible genotypes of model and non model plants such as Arabidopsis (Taji et al., 2004; Wong et al., 2006), tomato (Sun et al., 2010), potato (Mane et al., 2008), rice (Rabello et al., 2008), poplar (Cohen et al., 2010), and sugarcane (Rodrigues et al., 2009). Learning Module Networks (LeMoNe) algorithm is used to shape the transcriptional regulatory networks during stress response by using reverse-engineering of gene expression (Ihmels et al., 2004; Yu et al., 2006; Michoel et al., 2009).

**Metabolomics of olive fruit under Drought**

Olive is well adapted against extensive drought spans as it is equipped some unique physiological, biochemical and morphological features that make it adaptive in water shortage. Moreover, most crops show permanent wilting at -1. 5 MPa but olive plant has tendency to escape from the severe disaster when leaf water potential decline to -0. 6 MPa (Dichio et al., 2003). Martinelli et al. (2012) found that drought affects fruit development and biochemical makeup that create minor changes in the taste of oil. Now a days, pharmaceutically important nutritional constituents of olive fruit and oil (Martinelli and Tonutti, 2012) such as phenolics have captured great attention due their anticarcinogenic, antiantherogenic and antioxidant characteristics (Llorente-Cortes et al., 2010). Undoubtedly, different cultivars has different levels of phenols, yet, olive fruits with normal supply of water generally has less levels of total (Martinelli et al., 2012). Conversely, there is no staunch evidence supporting the connection between important polyphenol like oleuropein and water availability (Gomez-Rico et al., 2009). Apart from phenols, other major metabolites in olive fruit such as sterols, ter-pens and free fatty acids are also associated with water availability (Gomez-Rico et al., 2007). Currently, Transcriptomics is gaining much importance in elucidating the metabolic profiles of olive fruit particularly under biotic and abiotic stresses (Rizzini et al., 2010; Martinelli et al., 2012). In plant sciences Metabolomics and metabolic profiling techniques...
are used to authenticate the data at proteomics and transcriptomics level (Fernie and Stitt, 2012). As metabolites have direct impact on the phenotype rather than proteins and transcripts, therefore, these are more convincing in indicating the final results of different regulation phases residing in cells and tissues. Recently, non-targeted metabolomics and metabolic profiling techniques have not only made the understanding of metabolic changes easy but also elucidate biomarkers in fruits related to agronomic parameters and abiotic stresses (Tosetti et al., 2012).

**Conclusion**

Current genomic studies aim to understand the molecular basis of stress tolerance in olive. Moreover, how genes modulate plant architecture and qualitative aspects is needed to understand at molecular level. Although, addition of genomic data in databases has accelerated genomic studies but still there is a lot of scope available to researchers for mining the genes related to the breakdown of secondary metabolite in olive fruit. Therefore, integrated transcriptomic, proteomic and metabolomic studies boost the understanding of molecular processes and associated genes which will accelerate olive breeding in future.

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Transcriptomics and Biochemical Profiling of Olive


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