

Meta-analysis of transcriptome reveals key genes relating to oil quality in olive

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Abstract

Background: Olive oil contains monounsaturated oleic acid up to 83% and phenolic compounds, making it an excellent source of fat. Due to its economic importance, the quantity and quality of olive oil should be improved in parallel with the international standards. In this study, we analyzed the raw RNA-seq data with a meta-analysis approach to identify important genes and their metabolic pathways involved in olive oil quality.

Results: A deep search of RNA-seq published data shed light on thirty experiments associated with the olive transcriptome, four of these proved to be ideal for meta-analysis. Meta-analysis confirmed the genes identified in previous studies and released new genes, which were not identified before. According to the IDR index, the meta-analysis had good power to identify new differentially expressed genes. The key genes were investigated in the metabolic pathways and were grouped into four classes based on the biosynthetic cycle of fatty acids and factors that affect oil quality. Galactose metabolism, glycolysis pathway, pyruvate metabolism, fatty acid biosynthesis, glycerolipid metabolism, and terpenoid backbone biosynthesis were the main pathways in olive oil quality. In galactose metabolism, raffinose is a suitable source of carbon along with other available sources for carbon in fruit development. The results showed that the biosynthesis of acetyl-CoA in glycolysis and pyruvate metabolism is a stable pathway to begin the biosynthesis of fatty acids. Key genes in oleic acid production as an indicator of oil quality and critical genes that played an important role in production of triacylglycerols were identified in different developmental stages. In the minor compound, the terpenoid backbone biosynthesis was investigated and important enzymes were identified as an interconnected network that produces important precursors for the synthesis of a monoterpene, diterpene, triterpene, tetraterpene, and sesquiterpene biosynthesis.

Conclusions: The results of the current investigation can produce functional data related to the quality of olive oil and would be a useful step in reducing the time of cultivar screening by developing gene specific markers in olive breeding programs, releasing also new genes that could be applied in the genome editing approach.

Introduction

Olive oil is a particularly important product because of its fatty acids and phenolic compounds, which are mainly responsible for the beneficial health aspects. Among these compound, high oleic acid content and the

presence of minor bioactive compounds are the reason for its attribution as the healthiest among all vegetable oils (Guclu et al., 2020; Lozano-Castellón et al., 2020; Yubero-Serrano et al., 2019). The International Olive Council (IOC) has also stated that oils with the highest levels of oleic acid are the most valuable nutritional products (IOC, 2015). Currently, the cultivation areas and oil production increased in the world but only a few cultivars can yield consistently in the new environmental conditions and often changing negatively their quality profiles (Borges et al., 2017; García-Inza et al., 2016; Torres et al., 2017). Therefore, according to the economic importance of olive oil, the quantity and quality of oil should be improved in parallel, based on international standards.

Several studies have noted that the main factors that influence olive oil quality are genotype, climatic and agronomic conditions, edaphic factors, and the technological method applied for oil extraction. Among these factors, genotype has a preponderant influence (Ambra et al., 2017; Baiano et al., 2013; Beltrán et al., 2016; de la Rosa et al., 2016; Mele et al., 2018; Miho et al., 2021; Mikrou et al., 2020; Pérez et al., 2018; Rugini et al., 2016). Besides studies show that 70% of the observed diversity in terms of fatty acid composition, phenolic compounds, bitterness or taste, and oil stability is genetically influenced (Mousavi et al., 2019, 2022; Parvini et al., 2015; Riachy et al., 2019).

One of the most important goals of RNA-seq experiments is to investigate changes in gene expression profiles under two or more different experimental conditions. The most of the RNA-seq studies performed in olives were related to the study of biotic and abiotic stresses (Grasso et al., 2017; Nissim, Shlosberg, et al., 2020), micro-RNA identification (Yanik et al., 2013), fruit developmental stages (Alagna et al., 2009, 2016; Galla et al., 2009; Guodong et al., 2019), and cold acclimation (De La O Leyva-Pérez et al., 2015; Guerra et al., 2015). Recently, some studies have focused on the role of environmental stresses such as high temperature and the altitude of cultivated areas on oil content and its quality using RNA-seq technique, but a limited number of these studies were directly related to the evaluation of oil quality (Bruno et al., 2019; Nissim, Shlosberg, et al., 2020; Nissim, Shlosberg, et al., 2020). In a study performed by Galla et al. (2009), suppression subtractive hybridization (SSH) was used to isolate and identify a large set of genes that were differentially expressed at three different stages along olive fruit development. In another study conducted by Alagna et al., 2009, differentially expressed genes involved in the metabolism of phenol and fatty acids at different stages of olive fruit development were identified. Moreover, in 2013, the olive cultivars' transcripts were used for *de novo* assembly and functional annotation (Rodríguez, 2013). In a study conducted by Parra et al. (2013), the transcriptional regulation of the ripening process and activation of abscission zone were detected by RNA-seq. In 2016, the genome of Farga was sequenced and its annotation was identified by RNA-seq of leaf, root, and fruit samples (Cruz et al., 2016). Considering the importance of phenols in olive, in 2016, *de novo* transcriptome assembly was reported in olive fruit at different development stages and transcripts involved in flavonoid and anthocyanin pathways were identified (Iaria et al., 2016). In another study (Unver et al., 2017), the wild olive genome was sequenced and transcriptome analysis was performed to identify genes involved in oil biosynthesis. Recently, transcripts of all the enzymes in the biosynthetic pathway of tyrosol, hydroxytyrosol and secologanin, oleuropein's precursor, were identified by the RNA-seq method in Koroneiki cultivar (Mougiou et al., 2018). Furthermore, in 2019, targeted metabolome, Pacbio ISOseq transcriptome, and Illumina RNA-seq transcriptome were combined to investigate the relationship between phenols biosynthesis and differentially expressed genes during olive fruit development (Guodong et al., 2019).

Technical variation in different experiments could affect the reproducibility of the research. Moreover, due to the cost of sequencing, RNA-seq experiments are mainly performed in a limited number of biological replicates, reducing the statistical power and the ability to detect and validate differences in gene expression. Accordingly, one of the most effective ways to improve reproducibility is to use multiple datasets through meta-analysis (Keel et al., 2018). Therefore, re-analyzing existing data derived from several independent experiments can reveal new information and evaluate the most reliable key genes in a certain biosynthetic pathway. Meta-analysis of RNA-seq data can increase the speed of production of functional data related to the quality of olive oil and produce useful information. This study was conducted to analyze RNA-seq data obtained from multiple studies by meta-analysis approach to validate and identify key genes involved in the main metabolic pathways of oil quality.

strategy, to select critical metabolic pathways in oil quality, differential expression genes obtained from the meta-analysis were blasted against 2327 effective genes in oil biosynthesis (Unver et al., 2017). The two steps led to enriching the pathways identified in each strategy. These steps were done separately for each comparison and selected common pathways between overall comparisons, pair comparisons, and specific to each comparison for the up and down-regulated genes (Table 1 and 2). Sixteen metabolic pathways were common between all growth stages for the up-regulated genes (Table 1). The pathways of fatty acids biosynthesis and their elongation were in common between *C1* and *C2*. Furthermore, the most important individuated metabolic pathways were pentose phosphate, pyruvate metabolism, glycolysis/gluconeogenesis, fatty acid biosynthesis, fatty acid elongation, and biotin metabolism. The comparisons' results also showed that the terpenoid compounds biosynthesis pathways were specific to *C2* including limonene and pinene degradation, diterpenoid biosynthesis, and monoterpenoid biosynthesis (Table 1). In the metabolic pathways containing down-regulated genes, 23 metabolic pathways were common between all developmental stages (Table 2).

The identified metabolic pathways were grouped according to the biosynthetic cycle of fatty acids and factors affecting oil quality, to provide more details about the most important identified pathways. In the study of oil biosynthesis, attention should be paid to the three key stages of carbon supply in the skeleton of triacylglycerols including the production of fatty acids, the assembly of fatty acids, and finally the formation of complex lipids (Salas et al., 2013).

1. Pathways related to carbon source: In the first group, there are metabolic pathways that supply carbon for production of fatty acids, and the pathway of galactose metabolism has been identified in this group (Additional file 1). There are several sources of carbon in olive tree (Salas et al., 2013), which are A) Leaves photosynthesis (transfer of photosynthetic products from leaves to other organs) B) Raffinose family oligosaccharides (making of sugar alcohols and oligosaccharides such as mannitol, raffinose, and stachyose along with other photosynthetic materials in separate reactions), and C) Fruit photosynthesis. In olive, biosynthesis of mannitol and sorbitol, which are sugar alcohol, is present, and this pathway was also observed in the *Apiaceae* family (Sánchez & Harwood, 2002). In addition, the biosynthesis of oligosaccharides such as stachyose and raffinose, which are found in the legumes family, was also observed in olives (Salas et al., 2013). Therefore, it is possible to confirm that making sugar alcohols and oligosaccharides, as observed in other plant families, can be a reliable source of carbon also in olives.
2. Pathways related to Acetyl-CoA: In the second group, the presence of acetyl-CoA along with two enzymes acetyl-CoA carboxylase (ACCase) and fatty acid synthase (FAS) is necessary to start the biosynthesis of fatty acids (Salas et al., 2013). It was stated that a stable and rapid pathway for the supply of acetyl-CoA is glycolysis and pentose phosphate pathways using pyruvate by the activity of the enzyme pyruvate dehydrogenase (AID in 2012). In the present study, the key metabolic pathways identified for acetyl-CoA biosynthesis were glycolysis (Additional file 2) and pyruvate metabolism (Additional file 3).
3. Pathways related to fatty acids: In the third group, biosynthesis of the carbon chain of fatty acids, the key metabolic pathways of fatty acid biosynthesis, fatty acid elongation, and biotin metabolism were individuated in the present study. Metabolic pathway glycerolipid metabolism was identified as a key metabolic pathway in the assembling of fatty acids and the formation of complex lipids. In addition, two metabolic pathways alpha-linolenic acid metabolism and linoleic acid metabolism were identified as important pathways in the production of by-products of fatty acid biosynthesis.
4. Pathways related to non-fatty acids components (minor compound): The fourth group was related to metabolic pathways that have a direct impact on the quality of the oil and its taste. In this group, six key metabolic pathways were observed: terpenoid backbone biosynthesis, phenylpropanoid biosynthesis, flavonoid biosynthesis, limonene and pinene degradation, monoterpenoid biosynthesis, and carotenoid biosynthesis.

The detected metabolic pathways affecting the quality of oil in this study have been investigated independently, and the genes affecting each metabolic pathway have been identified and introduced (Additional file

4).

In olive, breeding programs last about thirty years on average (Lavee et al., 2014; Rallo et al., 2018), while the timing for the selection of new cultivars in other fruit crops has been greatly reduced, also by the application of new efficient genomic tools (Biscarini et al., 2017; Cai et al., 2019; Laurens et al., 2018). In cultivated olives (*Olea europaea* subsp. *europaea* var. *europaea*), the crossbreeding activities have been delayed by the particularly long generation time (Santos-Antunes et al., 2005), the extended juvenile phase and the high demanding nursery practices. This work provides the best candidate genes to construct markers for a fast and reliable genotyping of olive cultivars for their oil quality, offering great opportunities to rapidly screen and planning inter-varietal crosses, and reducing the time for seedling selection. Furthermore, the newly detected candidate genes could be applied as a source for interested traits in genome editing approaches.

The IDR index demonstrated that meta-analysis has good power to identify new differentially expressed genes. Therefore, the low value of IDR indicates a subtly increase in the power of meta-analysis compared to the results obtained in the previously published independent studies (Hong & Breitling, 2008). In addition, the decrease in the number of new differentially expressed genes in the meta-analysis compared to the total genes that were significantly different in comparison between the two developmental stages indicated that the meta-analysis was more efficient than independent studies and provided solid results.

Conclusions

According to the economic importance of olive oil, the quantity and quality of oil should be improved in parallel based on international standards in commercial cultivars. Technical variation in different experiments and limited number of biological replicates in RNA-seq researches could affect the reproducibility and the statistical power of the experiments. Meta-analysis of RNA-seq data can increase the speed of production of functional data related to the quality of olive oil and produce useful information. The present work would be an effective and useful step in reducing the time of cultivar screening by developing key genes relating to oil quality in olive breeding programs. The IDR index indicated the meta-analysis had good power to identify new differentially expressed genes and identified 155, 473, and 241 differential expressed genes for the first time respectively in the *C1*, *C2*, and *C3*. The identified key genes were investigated in the metabolic pathways related to oil quality and the identified metabolic pathways were also grouped into four groups according to the pathway of oil biosynthesis and the factors affecting its quality. The metabolic pathways that affecting the quality of oil have been examined independently confirming the earlier published results through meta-analysis and contributing to the reproducibility of the results. The galactose metabolism pathway can be further investigated as a source of carbon supply in olive fruit, and due to the increase in the expression of its genes in the S3, it can help to supply carbon in the final stage of growth when the fruit photosynthesis is reduced. Moreover, the production of acetyl-CoA in various ways in different growth stage shows the high efficiency of olive in the production of fatty acids. In the fatty acid biosynthesis pathway FabF and SAD have a critical role in oil quality and second identified pathway is more suitable for production of high oleic acid. Furthermore, the production of TAG by the PDAT probably occurred in the mesocarp and played an important role in the production of TAG and oil in the mesocarp. Also, the results of present study showed biosynthesis of phenolic compounds in olives in terms of growth stages might be in the opposite of the biosynthesis of fatty acids and there are the highest amount of phenolic compounds after fruit formation, while the biosynthesis of oil is at its lowest. The identified and confirmed genes by meta-analysis approach provided a suitable dataset to draw on for future studies in different scientific sectors. The present research can also play a role in cultivar selection by applying potential molecular markers developed in the here individuated key genes, reducing the duration of breeding programs, and helping the genome editing by introducing key and essential genes for the development of healthy compounds.

Authors' contributions

AA.A. collected data, performed all steps of the meta-analysis of RNA-seq, wrote the main draft, and done

final revision on the manuscript. V.S. was conceived and designed the project, guide and confirmed the data analysis, and revised and approved the final manuscript. S.M and R.M provided critical advices on the manuscript and revised the manuscript. M.HM revised the final manuscript. The research was co-supervised by M.HM. All authors have read and approved the final manuscript.

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Additional files

Additional file 1. Up and down-regulated identified genes of the galactose metabolism pathway in the biosynthesis of raffinose family oligosaccharides.

Additional file 2. Up and down-regulated identified genes of the glycolysis pathway in production of acetyl-CoA.

Additional file 3. Up and down-regulated identified genes of the pyruvate pathway in production of acetyl-CoA.

Additional file 4. Summary of important pathways and identified important genes in the meta-analysis.

Additional file 5. Identification of enriched genes in glycolysis and pentose phosphate pathways.

Additional file 6. Up and down-regulated identified genes in the Fatty acid biosynthesis pathway.

Additional file 7. Up and down-regulated identified genes in the Fatty acid degradation pathway.

Additional file 8. Up and down-regulated identified genes in the glycerolipid metabolism pathway.

Additional file 9. Up and down-regulated identified genes in the terpenoid backbone biosynthesis pathway.

Additional file 10. The results of search strategies and the information of thirty experiments was recorded.