

Olive Tree in the Genomic Era: Focus on Plant Architecture

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Summary

For centuries olive tree is an important crop in many Mediterranean countries because it provides appreciated oil with healthy properties. The lack of genomic tools, such as molecular markers or sequence information, has hindered the development of new cultivars adapted to the challenges that this species faces due to the change in modern cultivation practices, such as the increase in the number of trees per hectare. This tree has an excessive vigour that can be a serious economic limitation for intensive or super-intensive orchards. These and other issues have been recently addressed by a number of scientific efforts. This review will give a broad view over the recent genomic developments in olive tree, and the plant architecture as a complex trait.

Key words

olive tree, OLEAGEN, genomics, SSRs, plant architecture

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Introduction

Early in the history of the Western Civilization Pliny the Elder meticulously described the cultivation of olive tree (*Olea europaea* L.) in its Natural History (Pliny, 1963), gathering previous works on the cultivation of this species. That volume described in detail important aspects of this orchard, as the geographical location, the cultivation practices, oil extraction, and the cultivars at the time. It is worth mentioning that it already described the first mechanically extracted oil as that of the highest quality (Pliny, 1963; González-Plaza, 2013). According to archaeological data, olive tree cultivation dates from 4000 B.C. in the Eastern Mediterranean region, and was probably introduced in other countries during Greek colonisations (Rallo, 2005). This crop gives olive oil and table olives, and besides it has an important contribution to stop desertification in many areas, due to its moderate tolerance to drought in water limited environments (Lo Gullo and Salleo, 1988; Gucci et al., 1997; Chartzoulakis, 2005; Connor and Fereres, 2005). This perennial species is especially relevant for the countries of the Mediterranean basin. Despite its importance, it has lagged behind when joining the genomic era, with scarce sequence data until 2008 (González-Plaza, 2013; Muñoz-Mérida et al., 2013).

Crops in the genomic era

New high-throughput sequencing technologies have unleashed the potential to improve crops in several complex agronomical features, instead of the classical approaches, such as genetic engineering, that could tackle only a handful of genes (Zamir, 2008). Quantitative trait *loci* (QTLs) can be defined as traits with a quantifiable phenotypic variation under complex genetic and/or environmental influence (Abiola et al., 2003). They are defined as genomic regions containing the genes associated to a specific quantitative trait, and they are identified through the use of molecular markers to associate the phenotype and the genotype (Collard et al., 2005). It is relevant because many important agronomic traits such as yield, fruit quality, or pathogen resistance can be under the control of several genes and display a quantitative expression (Kumar, 1999), and that has been described also for olive tree with many agronomic traits being quantitative and showing a continuous distribution (Martín et al., 2005). Very broadly, the knowledge on QTLs can be applied to select for the phenotype of interest through marker-assisted selection (Collard et al., 2005).

These new high-throughput technologies, such as transcriptomics and metabolomics, are much more powerful when used in combination in a multidisciplinary approach (Ferne and Schauer, 2009). However, in order to carry out an integrative approach there is a basic need of genomic knowledge. As it has been already mentioned, sequence information for olive tree was scarce until recent, with few published sequences in NCBI-GenBank until 2008, which were mainly Expressed Sequence Tag (ESTs) (González-Plaza, 2013). One of the first large scale approaches was the OLEAGEN project, in which NGS technologies were used to decipher the first olive tree transcriptome (Muñoz-Mérida et al., 2013). Furthermore, this project has produced a notable amount of scientific achievements, such as the first olive tree microarray (García-López et al., 2014), which has been used to study several features such as juvenility (García-López et al.,

2014), or the plant architecture (González-Plaza, 2013; González Plaza et al., 2016). These two traits are of especial importance for the cultivation of this crop, as they play a major influence in the productivity, or the yield. That is because a long juvenility period delays the entrance into production phase. Regarding the plant architecture, there is a trend towards an increase in the number of trees per unit area (Pastor, 2006; Larbi et al., 2011). An excessive vigour of the growing trees can hinder the production after a few years, due to a competition effect for light between the neighbouring trees. Trimming is applied to limit the competition between trees, although it can damage the tree and decrease the production (Pastor et al., 2007).

Genomics in olive tree

Before discussing plant architecture, it is worth to overview the development of genomics in olive tree. This species has an elevated number of chromosomes ($n=23$), with low size and high morphologic similarity, imposing difficulties in their karyological analysis (Minelli et al., 2000); the estimated nuclear DNA content is 2.2 pg (Rugini et al., 1996), and the genome size is 2,200 Mb (De la Rosa et al., 2003; Doležel et al., 2003). Despite its importance, genomic information prior to 2008 was scarce, in comparison to other crops (24 sequences in “NCBI EST” database, using the search “(olea europaea) AND “Olea europaea”[porgn: __txid4146]”, and published between 1992/1/1 and 2008/12/31; while there were 1,045 sequences in “NCBI Nucleotide” database, using the search “(olea europaea) AND “Olea europaea”[porgn: __txid4146]”, in the period from 1992/01/01 to 2008/12/31; no sequences were found for that period in “NCBI SRA (Short Read Archive)” database, which includes those obtained through massive sequencing platforms). In 2009 there was an increase in the number of published sequences, especially from cDNA libraries that represent the transcriptomic profile of a given tissue, in a specific development period, and with a set of environmental factors. Since then newer sequencing platforms and lower sequencing costs have facilitated the generation of information (Mardis, 2011). Using these technologies, one of the first published approaches was that of Alagna et al. (2009), with generation of reads from cultivars ‘Coratina’ and ‘Tendellone’ in different ripening stages, providing insights of the fruit development in olive tree (NCBI; accession number SRA008270). After assembly they obtained 26,563 contigs, a notable increase regarding the situation in 2008. Another milestone in the development of genomic tools in olive tree is the publication of the chloroplast genome of the cultivar ‘Frantoio’ by Mariotti et al. (2010). The first transcriptome of olive tree was published within the OLEAGEN project, and yielded more than two million reads (Muñoz-Mérida et al., 2013). That publication of the first transcriptome led to the development of the first microarray in olive tree, which was published by García-López et al. (2014), and firstly used to study juvenile to adult transition. Other notable sequencing efforts have been that of Leyva-Pérez et al. (Leyva-Pérez et al., 2015) in a study of the cold acclimation of olive tree, where they reported a new transcriptome assembly.

Molecular markers

One of the most valuable genomic resources for breeders are molecular markers, which are not limited in numbers or depend strongly on the environment in comparison to morphological or biochemical markers (Mohan et al., 1997; Cramer and Havey,

1999; White et al., 2007; Smýkal et al., 2008). The advantage of molecular markers is that they represent an individual variation in the DNA sequence. Although not directly representing a gene of interest, they can be indirect signals of localization of a QTL or gene of interest in the genome for breeding purposes (Collard et al., 2005). Among them, microsatellites (SSR, Short Sequence Repeat) and SNPs (Single Nucleotide Polymorphism) are the most used ones due to their abundance in genome, high reproducibility, automation possibilities, and relative low cost of analysis (Tang, 2008). In olive tree molecular markers have been used for different purposes (Hatzopoulos et al., 2002), such as the identification of cultivars (Bautista et al., 2003), for the traceability of olive oil in order to certify the origin (Agrimonti et al., 2011), the evaluation of germplasm variability (Ganino et al., 2006; Sarri et al., 2006; Belaj et al., 2007; Belaj et al., 2012), in studies aiming to trace the origin of olive tree (Besnard and Berville, 2000; Besnard et al., 2001a; Besnard et al., 2001b), or in breeding efforts, which is the focus of this subchapter.

Before the OLEAGEN project there were only 80 low complexity SSRs described, and not all of them were reproducible between laboratories, and just eight SNPs (Sefc et al., 2000; Carriero et al., 2002; Cipriani et al., 2002; De la Rosa et al., 2002; Díaz et al., 2006a; Díaz et al., 2006b; Díaz et al., 2007). One way to decrease the cost of development is to use bioinformatics approaches. An example of such an approach is the recent development of a new set of SSRs by De la Rosa et al. (2013). The authors used the sequence information generated within the mentioned OLEAGEN project, to find SSRs through a described computational approach (Thiel et al., 2003). The main outcome from this study is the generation of experimentally validated markers with increased complexity, as they consist of repetitions of six nucleotides, and the higher discriminating power compared to the previously available SSRs. This new tools are ready to be used in paternity testing, and could be a great advantage for breeding purposes.

Plant architecture

In a broad sense, plant architecture can be defined as the spatial organization of a plant, being a product of the balance of several environmental and developmental processes. The great matrix of architectures displayed by plants is mainly product of a common growth pattern, and different permutations in a few key growth features (Coen and Carpenter, 1993). Even in the same plant, two apparently different structures as a flower and a shoot are products of the mentioned processes. Furthermore, studies have shown the same through molecular genetics (Coen and Carpenter, 1993). The idea of comparable structures is not new, and was already mentioned in the “Metamorphosis of Plants” by Goethe (Coen and Carpenter, 1993; Mavrodiev, 2009; von Goethe and Miller, 2009). Plants are modular organisms, and their development comprises the repetition of elemental units (Barthélémy and Caraglio, 2007). The understanding of this basic organization of the architecture of a plant is important in order to proceed in downstream analysis or applications. In this sense, plant morphology is relevant for many biological fields, as it deals with fundamental features such as form, structure, or the temporal changes within the plant (Barthélémy and Caraglio, 2007). For a long time it has been one of the few

possible criteria for taxonomic classification, and yet nowadays, it is the most simple way to identify a given species (Reinhardt and Kuhlemeier, 2002; Wang and Li, 2006). It is relevant for agriculture because it influences the plant yield, the efficiency of photosynthesis, and the suitability of the crop for cultivation and mechanization (Reinhardt and Kuhlemeier, 2002; Hanan et al., 2003). Although the work by Goethe dates from 1790 (Coen and Carpenter, 1993), the investigations of plant architecture did not appear as a scientific field until 20th century in the 70's, in pioneering studies dealing with the analysis of aerial vegetative structures in tropical trees (Hallé et al., 1978; Barthélémy et al., 1997; Barthélémy and Caraglio, 2007).

Endogenous determinants of plant architecture

We can understand the architecture of a plant as the expression of a balance between endogenous processes and the environmental limitations (Archibald and Bond, 2003; Barthélémy and Caraglio, 2007), and although plant architecture is influenced by the environment to a certain degree, the genotype is still a very strong determining factor (Busov et al., 2008).

One of the basic units of plant organization are meristems, whose activity during development contribute to the production of great variety of plant shapes (Schmitz and Theres, 2005). The plant has a basic structure composed of two main meristems: the shoot apical meristem (SAM) and the root apical meristem. The main axis of the plant is established among them, occurring early during the plant development (Sussex, 1989; McSteen and Leyser, 2005; Schmitz and Theres, 2005). Several shoot axillary meristems add complexity to the branching pattern and the light distribution, because they can continue their growth or enter in dormancy (McSteen and Leyser, 2005; Schmitz and Theres, 2005). Additionally, axillary meristems are under the control of the SAM, which can suppress their growth through a mechanism known as apical dominance (Davies, 1995; Reinhardt and Kuhlemeier, 2002; Schmitz and Theres, 2005). A deeper control of shoot branching pattern can be determined by the main molecular regulatory mechanism, the WUS-CLV feedback loop (Turnbull, 2005; Wang and Li, 2008), where the product of WUS promotes meristem growth (Kwon et al., 2005).

Endogenous signals as hormones, e.g. auxins, cytokinins, or strigolactones (Gomez-Roldan et al., 2008; Umehara et al., 2008; Vogel et al., 2010) affect the branching pattern, and have many other important roles, e.g. they determine plant height (Strasburger et al., 1994). Gibberelins and brassinosteroids promote growth, while auxin controls the activation and growth of axillary meristems (Strasburger et al., 1994). Cytokinins belong to this group, and they stimulate cell division in axillary meristems (Strasburger et al., 1994). Abscisic acid, on the other hand, inhibits growth and metabolism (Strasburger et al., 1994). The coordinated and fine balance between hormones, which work in very low concentrations, and their activity during key moments of plant development, leads to the final plant architecture. It is then, as already mentioned, necessary to know better all these basic processes in order to be able to improve plant architecture.

Architecture in different crops

Besides the characterization in plant models such as *Arabidopsis thaliana* (Hiraoka et al., 2013) or *Antirrhinum majus* (Bradley et al., 1996), plant architecture has been addressed in

species of agronomic relevance, such as petunia (*Petunia hybrida*) (Dal Santo et al., 2011), pea (*Pisum sativum*) (Beveridge et al., 1994; Fujino and Itoh, 1998), tomato (*Solanum lycopersicum*) (Bettini et al., 2010; Kohlen et al., 2012), and several monocotyledons as maize (*Zea mays*) (Doebley et al., 1997) or rice (*Oryza sativa*) (Jiao et al., 2010). In woody plants architecture has also been studied in fruit trees, as indicated in several works of Hammami and collaborators (Hammami, 2009; Hammami et al., 2011; Hammami et al., 2012), such as apple tree (*Malus domestica*) (Petersen and Krost, 2013; Fanwoua et al., 2014), peach tree (*Prunus armeniaca*) (Socquet-Juglard et al., 2012), eastern black walnut (*Juglans nigra*) (Mourelle et al., 2001), or peach tree (*Prunus persica*) (Mediene et al., 2002; Médiène et al., 2002).

The immediate outcome of crops with modified architectures suitable for intensive exploitations, is that they have higher yield, as previously demonstrated during the “Green Revolution”, by the introduction of semi-dwarf wheat cultivars with higher resistance to damaging effects of wind or rain that led to an increase in the global production (Gale et al., 1985; Evans, 1996; Peng et al., 1999; Wang and Li, 2008). The height of a plant is a trait of agronomic interest, because when trees grow in higher densities, they respond by growing strongly in height due to competition events for available light (Wang and Li, 2006). Plants invest excessively in biomass instead in seeds or fruits, having negative consequences for the yield. Dwarf architectures make plants to use fertilizers more efficiently, as the organic mass is distributed equally, favouring seeds or fruits (Wang and Li, 2008). Especially for fruit tree species, dwarf architectures are desired because they allow higher plantation densities, they require lower maintenance in terms of trimming practices, and permit to apply different mechanical collection approaches (Webster, 2002). Notwithstanding, any process of improvement must attend the limitation of the rule of Leonardo (Richter, 1970). According to that rule, the diameter of all the branches at certain height, equals that of the trunk, being that a consequence of the necessary design to resist mechanical tensions exerted by the wind or other environmental factors (Eloy, 2011). Therefore that mechanical imposition will limit the possible plant architectures that can be obtained through breeding.

The main issue for improvement efforts is that dominant alleles for dwarfism are difficult to find in nature, because dwarf trees are under a strong negative selection exerted in the competition for light with trees of higher vigour (Jennings and Aquino, 1968; Nagano et al., 2005). It could be thought then that the best approach would be to follow the development of modern varieties that have been developed to acquire resistance genes to pests or herbicides. Genetic modification technologies cannot achieve high success rates in plant architecture because this is a complex trait and not a product of a few genes (Schauer et al., 2006; Zamir, 2008). Therefore, the exploration of genetic diversity through recently available high-throughput technologies is an alternative in developing dwarf cultivars, even when those alleles are not common in nature.

Olive tree architecture

Plant architecture is relevant for olive tree due to the new type of plantations. Traditionally, exploitations had a low density of plantation with low yield (Duarte et al., 2008), leading to high costs in fruit harvesting (Yousfi et al., 2012), being one of

the reasons for manual labour requirements (Rufat et al., 2014). On average the number of planted trees per hectare in traditional orchards is lower than 250 (Rugini et al., 2016), while medium density ones range from 250 to 400 trees/ha (Strippoli et al., 2013; Rugini et al., 2016).

The current trend is to increase the number of planted trees (Villalobos et al., 2006; Baptista and Biswas, 2010), allowing a higher mechanization degree, which also decreases costs for the olive oil producers. These type of exploitations are characterized by a high planting density, ranging from 900 to more than 1,200 trees per hectare (Vivaldi et al., 2015; Rugini et al., 2016), and it was indicated that the period from planting the trees to their entrance in the production should be very short (Rufat et al., 2014). The increased density leads to very short distances between trees in the field, and that represents a big issue with olive tree because of the high vigour that characterizes this species. For that reason suitable architectures of the trees are currently achieved through trimming practices (García-Ortiz et al., 2004), which aim is to obtain a balance in the tree between leaf/root, the highest leaf/wood ratio (García-Ortiz et al., 2004), and to lower the shading effect. The last one is especially relevant because prolonged shading could become permanent (Proietti et al., 1988), and have negative effects on the yield, as productivity is a direct outcome of the photosynthetic capacity of leaves (Boardman, 1977; Gregoriou et al., 2007). Shading is translated into a lower or impaired light distribution, potentially leading to a decrease in the number of flowers, and finally the size and quality of the fruit (Jackson and Palmer, 1977; Hampson et al., 1996; Gregoriou et al., 2007).

It has been mentioned previously in this text that the suitable architecture in this type of orchards is achieved through trimming, and that can lead to the reader to wonder why not to choose better suited cultivars. The reason is that currently very few olive tree cultivars display adequate features and behaviour for high density plantations (Rufat et al., 2014; Rugini et al., 2016). The main cultivar used in super-intensive orchards is ‘Arbequina’ (Larbi et al., 2011), which originates from northern Spain (Tous and Romero, 1993; Barranco et al., 2005), with a low-medium vigour and a good agronomic performance (Rallo et al., 2008). However, cultivars like this display an early competition for light (Rallo et al., 2008) that can drive the trees to shade adjacent ones, posing an inconvenience for the super-intensive orchards, and trimming programs have to be applied in order to correct this trend (Larbi et al., 2011). The disadvantage of those practices is the subsequent decreases in productivity (García-Ortiz et al., 2004; Pastor, 2005; Guerrero et al., 2006). Until recently, available cultivars such as ‘Arbequina’ were evaluated for their suitability for super-intensive cultivation (Bernardi et al., 2008; Larbi et al., 2011).

All of these reasons indicated the need for the development of dwarf cultivars in olive tree, with the first successful breeding program initiated at the University of Córdoba. The result was an especially adapted cultivar, registered under the name of ‘Sikitita’ (Rallo et al., 2008). This new cultivar has a high olive oil content, and low vigour, making it an excellent choice for super-intensive orchards (Rallo et al., 2008). The current technologies and development in olive tree genomics make possible to carry out new selection programmes in order to obtain different dwarf

cultivars that are not only suitable for super-intensive cultivation, but also offer increased oil quality for the consumer. It is in this context where genomic and transcriptomic approaches are relevant for breeders, as they can accelerate the selection of suitable cultivars with ideal plant architectures (IPA) (for the concept of IPA view Jiao et al. (2010)). Recently a set of more than 2,000 candidate genes to determine plant architecture was reported in olive tree, and those results could be potentially used for the improvement of cultivars suited for super-intensive cultivation (González Plaza et al., 2016).

Conclusion and future prospects

Olive tree is an important crop for several countries of the Mediterranean basin. Although economically relevant, this species has lagged behind when joining the genomic era, but several recent efforts have been carried out towards the development of new tools aimed to more powerful breeding practices (Alagna et al., 2009; Galla et al., 2009; Donaire et al., 2011; Muñoz-Mérida et al., 2013; Carmona et al., 2015; Leyva-Pérez et al., 2015). The development of tools as new molecular markers becomes an easier task with the aid of bioinformatic analysis, once that a good coverage of the olive tree genome and transcriptome is achieved as demonstrated recently (De la Rosa et al., 2013). Molecular markers have many applications in olive tree (e.g. traceability of olive oil origin, paternity testing, studies on the origin of olive tree, among others), and one of the most important is to aid in the development of new cultivars through Marker-Assisted Selection (MAS) breeding programmes. Plant architecture is a very complex trait that cannot be selected through genetic engineering approaches, and is in the selection of an IPA in olive tree (or other fruit trees) where the new high-throughput technologies can offer an advantage.

Architecture is relevant in agriculture because it affects the production, and the suitability of the cultivar for new type of orchards. Adapted cultivars such as 'Chiquitita' perform better than traditional ones in super-intensive exploitations, but its development was a long effort because there was a lack of available genomic tools. This improvement process can be shortened with the already available knowledge, facilitating the development of new varieties that fulfil the requirements of producers and consumers. It is thrilling to think how these recent advances can be a great resource for breeders in the development of new cultivars with adapted architecture, shorter juvenility, or improved oil features.

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