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Developmental changes of the Olive tree from juvenility to maturity and Genetic basis of vegetative and reproductive traits

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Summary

One of the most challenging questions that fruit growers are facing is to maintain trees in a stable balance between production and vegetative growth from year to year. Fruit trees productivity over years is closely linked to their development. Integrating architectural traits in breeding programs could thus, optimise cultivation management and improve bearing regularity. Here, we investigate the genetic determinism of architectural traits in the olive tree (*Olea europaea L. subsp. europaea*) including vegetative and reproductive behaviour. The olive tree raises high interest because of the importance of olive oil and olives in the human diet. The segregating population under study derived from a cross between 'Olivière' and 'Arbequina' cultivars and was grown on two orchards under contrasting environments. First, we investigated the genetic determinism of juvenile growth and branching traits in one site at three different scales: whole tree, growth unit and internodes. Interaction between tree ontogeny, genetic and environmental factors were considered. Genetic models, including the year of growth, environment, genotype effects and their interactions, were built with variance function and covariance structure of residuals when necessary. After a model selection step, morphogenetic factors impacting significantly tree architecture were identified and heritabilities were estimated. These finding allow us to define when during the olive tree ontogeny and at which observation scale, growth and branching traits are under genetic influence. Moreover, progenies plastic response to contrasted environments was highlighted and traits showing significant genotype-by-environment effect were identified. Changes over time associated to the acquisition of reproductive competence were further observed. Once all progenies attempt adulthood, we studied the genetic basis of reproductive behaviour. Our strategy was based on (i) a decomposition of adult growth units at the crown periphery in quantitative variables related to both flowering and fruiting process in relation to their growth and branching (ii) an annual assessment of individual trees yield. The observation of 'on' or 'off' olive trees in a given year over four years revealed patterns of regular vs. irregular bearing. After developing a new genetic map, a QTL mapping was carried out on reproductive traits, leading to the identification of QTLs with effects from both parents and co-localizations. This study gives an overview of olive tree development during juvenility and maturity periods showing the existence of ontogenic trends, which result in traits heritable mostly at the tree periphery. A phenotyping strategy adapted to its architectural characteristics is proposed. Finally, progenies showing interesting superiority in comparison to their parents were identified and could constitute interesting sources for innovative materials in future selection programs.

Résumé

L'un des défis auxquels sont confrontés les producteurs de fruits est de réussir à maintenir d'année en année un équilibre entre croissance végétative et production. La productivité des arbres fruitiers au cours des années est étroitement liée à leur développement. Intégrer les caractères architecturaux dans les programmes de sélection pourrait donc, améliorer la régularité de production et aider à optimiser la gestion des cultures. Dans cette thèse, nous avons étudié le déterminisme génétique des caractères architecturaux chez l'olivier (*Olea europaea* L. *subsp. Europaea*), en incluant le développement végétatif et reproducteur. L'olivier a un grand intérêt en raison de l'importance de l'huile d'olive et des olives dans l'alimentation humaine. L'étude a porté sur une descendance issue du croisement entre les variétés 'Olivière' et 'Arbequina' qui a été cultivée en verger dans deux environnements contrastés. En premier lieu, nous avons étudié le déterminisme génétique de la croissance et ramification des arbres durant la phase juvénile sur un site en considérant trois échelles d'observation: arbre, unité de croissance et entrenœuds. Les interactions entre les facteurs liés à l'ontogénie de l'arbre ainsi que les facteurs génotype et environnement ont été prises en compte. Des modèles génétiques, incluant les effets année de croissance et/ou ordre de ramification, environnement, génotype et leurs interactions respectives, ont été construits en intégrant une fonction de variance et une structure de covariance lorsque cela était nécessaire. Après une étape de sélection de modèle, les facteurs impactant significativement l'architecture de l'arbre ont été identifiés et les valeurs d'héritabilité au sens large ont été estimées. Ces résultats nous ont permis de définir à quel moment au cours de l'ontogenèse de l'olivier et à quelle échelle d'observation, les caractères de croissance et ramification sont déterminés génétiquement. De plus, cette étude a permis d'investiguer la plasticité des caractères architecturaux et leur stabilité entre environnements contrastés. Enfin, les changements associés à l'acquisition des facultés reproductives ont été observés. Quand tous les descendants ont atteint l'âge adulte, j'ai étudié les bases génétiques du développement reproducteur. La stratégie était basée sur (i) une décomposition d'un sous échantillon d'unités de croissance localisées à la périphérie de la couronne de l'arbre en variables quantitatives liées à la fois aux processus de floraison et fructification en relation avec leur croissance et ramification (ii) une évaluation annuelle du rendement des arbres durant quatre années. L'observation d'arbres 'ON' ou 'OFF' pour une année donnée a révélé des tendances de production régulière vs irrégulière au sein de la descendance. Après avoir développé une nouvelle carte génétique, une recherche QTLs associés aux caractères reproductifs a été réalisée. Des QTLs présentant les effets des deux parents et des co-localisations ont été identifiés. Cette étude a mis en évidence le schéma de développement de l'olivier pendant les phases juvénile et mature mettant en évidence l'existence de gradients ontogéniques se traduisant par des caractères héritables qu'à la périphérie de l'arbre. Une stratégie de phénotypage adaptée aux caractéristiques architecturales de l'olivier a été proposée. Enfin, les descendants montrant une supériorité intéressante par rapport à leurs parents ont été identifiés. Ces génotypes pourraient être valorisés dans les programmes de sélection futurs pour la création de variétés innovantes.

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INTRODUCTION AND PROBLEMATIC

After the seminal work of Hallé and collaborators in the 1970s, that have introduced plant architecture as a scientific discipline, plant scientists have increasingly focused on the mechanisms involved on the edification of plants structure and morphology. Architectural analysis initially developed on tropical trees (Hallé et Oldeman, 1970; Hallé et al. 1978) have been expanded to temperate forest (Edelin, 1981 ; Caraglio et Edelin, 1990 ; Nicolini, 1998; Sabatier et Barthélémy, 1999) and fruit species (Lauri, 1991 ; Costes, 1993; Lauri et Térouanne, 1995 ; Costes et al. 2003 ; Solar et Stampar, 2003).

In fruit trees, the architecture spark particular interest because it influences traits closely linked to production (Willaume et al. 2004). Trees internal organisation affects light interception capture necessary to obtain high biomass production and thus fruit quality (Jackson 1980). The canopy needs to be highly porous to light in order to ensure efficiently its distribution between fruiting structures and minimize fruit quality fluctuations (Wünsche and Lakso, 2000; Costes et al. 2006). Lastly, the internal organisation of trees influences the production and its regularity. In fact, an excess of fruit at the expense of vegetative growth leads to alternate bearing phenomenon i.e. irregular production of a tree over consecutive years which have dramatic economical impacts (Costes et al. 2006; Guitton et al. 2012).

Besides the initial choice of orchard components (i.e. soil, rootstock, cultivar, density), commercial fruit crops performances are inherent to the training and management systems at both orchard and tree scale. With the rise of intensive cultural systems, the control of tree height and volume on orchard will enable mechanized harvesting (e.g. on olive) and facilitate fruits harvest from the ground (e.g. on apple). Tree shape and fruit load management require considerable manual care at both early and adult stage including pruning, bending and thinning which is chemically done for species as apple, pear and peach. It is therefore of major interest to take advantage of the natural growth and fruiting habits of cultivars in order to reduce the economic and environmental costs and, enhance agronomic performances (e.g. reducing unproductive period of fruit trees). For that purpose, a precise knowledge of tree architecture including vegetative (i.e. growth and branching) and reproductive (i.e. flowering and fruiting) development is essential.

There is a also clear issue in several countries, including France, to integrate architectural traits in fruit tree breeding and selection programs whose main goals are disease resistance and fruit quality (Laurens, 2000). In order to propose appropriate selection criteria to fruit tree breeders, it is necessary to access the inheritance of architectural traits which are complex quantitative characters. An approach of quantitative genetics is thus required to determinate the genetic fundament of traits of interest. As they present a relatively manageable architectural development in comparison to forest species, temperate fruit tree species allow detailed morphological description on a large number of individuals, required for a quantitative genetics study. However, the perennial character of trees implies particular methodological developments because architectural traits evolve throughout tree lifespan and depending to their position within tree structure (Costes et al. 2003; Renton et al. 2006). Some traits are of cumulative nature (i.e. primary and secondary growth), whereas others are transient i.e. decrease of long annual shoots over time. In this latter case, traits are expressed principally during the juvenile or unproductive period of trees and decrease afterwards. These morphogenetic gradients affect considerably the phenotyping strategy because successive years of growth are not independent and can not be considered as repetitions. Moreover, the comparison of successive years integrates the effect of both different climatic conditions and ontogeny which are not easy to distinguish. This makes phenotyping architectural traits difficult and time consuming but, their quantitative analysis could provide valuable methodological and cognitive insights into the genetic determinism of complex horticultural traits.

The case study of my thesis is the olive tree (*Olea europaea* L.) which is one of the most ancient domesticated fruit trees and the most extensively cultivated fruit crop in the world, covering an area of about 7.5 million hectares (Fabbri et al. 2009). The continuous trend in the modernisation of its industry, the high cost of harvesting and pruning and the expansion of its cultivation area outside the Mediterranean basin has deeply increased the demand for improved cultivars by olive growers. Programs of clonal selection and cross-breeding have been started in the main olive growing countries, aiming at selecting genotypes characterised by early bearing, resistance to pests and to abiotic stresses (such as frost and drought), limited alternate bearing, suitability to intensive culture and to mechanical harvesting, as well as high-quality productions, in terms of both organoleptic characteristics of fruits and oils, and

high contents in substances useful for human health (Bellini et al. 2008; Fabbri et al. 2009). In spite of the great efforts implemented, results from such programs are still modest. This is due to high heterozygosity and chromosomes number (2n=46) and the lack of knowledge on the inheritance of the characters in the olive tree, especially those related to morphology and fruiting. To date, the significance of the genetic component has been reported only on elementary quantitative traits related to the early growth habit and vigour of young seedlings (Hammami et al. 2011). The olive tree form has been also qualitatively studied again on seedlings (Hammami et al. 2012) and evidence of architectural variability among olive cultivars has been shown on the basis of the description of vegetative and reproductive organs distribution on branches as well as fruit production (Moutier et al. 2004, 2008). Knowing that, on perennial species, the traits of agronomic interest occur after several years i.e. at the end of the juvenile period (Hackett, 1985), it appears essential to study such traits over several years of measurements on a segregating population grown in field conditions.

In this context, my thesis aims at investigating the architectural development of the olive tree including its vegetative and reproductive development and identifying its genetic determinants. A first step consists on analysing the changes of the architectural traits expression over years (i.e. from juvenility until the first occurrence of flowering) and dependencies on the position within the tree. A second step was to estimating the respective effects of the genotype, ontogenetic gradients and environment and their possible interactions. Another target of my thesis was the investigation of the major factors impacting the relations between inflorescences localisation on shoots and fruit set in olive progenies. This relied on a detailed phenotyping of floral annual shoots at both flowering and fruiting periods. Ultimately, my thesis aimed to initiating the study of bearing behaviour on the studied olive population. In particular, the bearing regularity habit of progenies. To reach these targets, a segregating population derived from a cross between two contrasted olive cultivars was developed. Trees were grown on field condition and were unpruned.

During my research, I thus investigated the following questions:

1. *Are the architectural concepts and methods developed on deciduous species transferable to the olive tree?*

The olive tree is an evergreen Mediterranean species whose growth is rhythmic in temperate climate but without winter buds. Such specificities make difficult the detection of growth cessation. The complexity of the olive tree architecture was dissected in a number of quantitative variables related either to the tree constituent's organisation (i.e. the plant topology) or to the dimensions and spatial location of organs (i.e. the plant geometry; Godin, 2000). Variables were classified in growth and branching traits, including reproductive organs organization, and were collected on different shoot types that resulted from different growth and elongation characteristics leading to axes polymorphism often observed in fruit trees. Shoots were classified with respect to lengths, differentiation states i.e. vegetative and floriferous and branching mode (Zimmerman and Brown, 1971; McManus and Veit 2002; Barthélémy and Caraglio 2007). This phenotyping methodology has been proposed in apple by Segura et al. (2006) and was adapted to the specificities of the olive tree in my thesis.

2. *Is the olive tree structure and architectural features depends on 'morphogenetic gradients'? If yes, what are they? On which traits they impacts significantly? How they interact with the genetic determinism?*

Architectural analyses of several plant species revealed that taking into account the intrinsic organization rules of tree structure helps explaining the resulting architecture and its evolution during the ontogeny (Caraglio and Edelin, 1990; Heuret et al., 2000; Nicolini et al., 2000; Barthélémy and Caraglio, 2007). In order to assess efficiently the genetic effect on the olive tree, it is essential to investigate the morphogenetic gradients and their effects on architectural traits. For the analysis of the genetic parameters i.e. broad sense heritability, the ontogenetic, climatic and genetic effects have been distinguished in a mixed linear model. The presence of significant morphogenetic gradients implies the development of a complex statistical modelling of the residual variances to account for their potential heterogeneity. Within-tree dependencies must be taken into account in the modelling by integrating structures of correlations.

3. *Is the genetic determinism of architectural traits stable over contrasted environments? Is there a significant genotype-by- environment interaction effect on the traits?*

Genotype-by-environment interaction has never been investigated in tree segregating population. This is probably due to the long generation time and to the investment required to install and maintain such trial. In this thesis, I had the opportunity to access to two different

sites (Southern France and Southern Spain) where architectural traits have been recorded during the first years of growth. The interpretation of the genotype-by-environment interaction could allow the identification of the most stable genotypes regarding to the traits of interest.

4. *Can we propose a methodological framework to simplify the architectural phenotyping strategy that account for the architectural variability of the olive tree?*

The phenotyping of architectural traits on large number of individuals, required for quantitative genetics analysis, remains expensive considering the long generation time of an olive progeny, the time of notation and the maintenance of the field experiment. The expected results of previous questions, will allow the optimization of phenotyping strategy focusing on the most heritable variables measured at the most suitable observation scale and ontogenetic stage for capturing the genetic determinism. Thus it can be easily applicable to a high number of individuals in quantitative genetics studies.

5. *What is the main bearing behaviour of the studied progeny? Is the alternate bearing habit segregate in an olive progeny? There is a genetic determinism of the traits related to the reproductive development? How many loci are involved in flowering and fruiting traits and what are their effects?*

The olive tree is known to be highly alternating in its fruit production and studying the involved processes has been a target for many years. However, alternate bearing has never been studied in an olive segregating population. Here, a first bearing behaviour study has been initiated on an olive progeny. Several bearing pattern have been identified among progenies and biennial bearing has been quantified in terms of mass of harvested fruits.

The fruit production is a complex process integrating the flowering habit, the number of fruit per inflorescence, the rate of fruitset. Studying reproductive traits in an olive progeny present some constrains related to the variability of juvenility phase length among progenies. Once all trees had entered maturity phase, the relations between inflorescences localisation on shoots and fruit set have been investigated. Then, the genetic component of flowering, fruitset and production has been determined. Expected results could give key information about the relationship between reproductive organs and the genetic control of production traits.

The latest genetic developments i.e. an initial genetic map constructed on the progeny under study and the high heritability of flowering and fruiting traits led us to initiate a first linkage analysis on these traits. QTL mapping has been exhibited on a new linkage map integrating a set of new microsatellite markers. Hence, the initial linkage map saturation has been greatly improved and will then benefit for future linkage studies in olive.

6. What is the breeding potential of the studied progeny?

The identification of progenies showing promising characters with respect to the breeding targets e.g. low vigour, stable production could constitute valuable innovative genetic material for further genetic studies or breeding programs. Hence, heterosis has been investigated for architectural traits in the studied progeny.

My Thesis is organised in three sections. First the scientific and methodological background part details the basic concepts necessary to the comprehension of the problematic and objectives of my thesis. The methods used to fulfil my research are also presented in this first section: architectural analysis, quantitative genetic and linkage mapping. The main results generated in this study are presented in the second section under the format of three scientific articles. Finally, the manuscript ends with the general discussion section.

SCIENTIFIC AND METHODOLOGICAL BACKGROUND

I. OLIVE TREE

Historical biogeography

Olive tree species (*Olea europea* L.) is one of the most ancient crops of the Mediterranean region where it has occupied since prehistorical time, a major place in the Mediterranean culture. Morphological and genetic diversity analysis has demonstrated an east-west segregation of wild olive population which reflects the bio-geographical division of the Mediterranean basin along which eastern and western areas are separated by a line running Adriatic Sea and the Libyan Desert (Blondel and Aronson et al. 1995; Besnard et al. 2000, 2002a,b; Lumaret et al. 2004).

Evidence of an indigenous and contemporaneous beginning of the domestication process has been demonstrated at both ends of the Mediterranean. In the near East, the introduction of olive cultivation has been dated to the Early Bronze Age by the discovery of ancient olive oil presses, pollen grains, stones and wood remains (Zohary and Spiegel-Roy, 1975; Liphschitz et al. 1991). In the north-west area, domestication process including the emergence of farming and selective practices has been recognized since the Chalcolithic/Bronze Age and that by the analysis of archaeological and modern olive stones (Terral, 2000, Terral et al. 2004). From the 6th century BC, cultivated olive spreading reached Tunisia in North Africa and Sicily and later Northern Italy (Doveri and Baldoni, 2007). Nowadays, *Olea europea* L. complex diffused largely beyond its native area by human-mediated dispersal; it has been repeatedly introduced in the New World and has become naturalized in Australia, New Zealand and the Pacific islands (Green, 2002; Besnard et al., 2007).

Botanical Description

The olive tree belongs to the *Oleaceae* family, sub-family *Oleideae*. The *Oleaceae* family comprises about 24 genera and 600 species (Johnson, 1957; Wallander and Albert, 2000; Green, 2004) including ornamental shrub species like lilac (*Syringa vulgaris* L.) jasmine (*Jasminum fruticans* L.) and tree species such as ash (*Fraxinus excelsior* L.). The genus *Olea* which includes 33 species and 9 subspecies is classified into three subgenera *Ole*a, *Paniculatae* and *Tetrapilus* (Green, 2002; Besnard et al. 2009). The subgenus *Ole*a is divided in two sections: *Ole*a, which is formed exclusively by the olive complex (*Olea europaea*) and *Ligustroides*. Among *Olea europaea*, six sub-species have been recognized, spread from South Africa to China, across the Saharan mountains, Macaronesia and the Mediterranean

basin (Vargas et al., 2001; Green, 2002; Doveri and Baldoni, 2007; Besnard et al. 2009): (1) Subsp. *europaea*, including two botanical varieties *europaea* (cultivated olive) and *sylvestris* (wild olive): present in the Mediterranean basin ; (2) Subsp. *cuspidate*: distributed from south eastern Asia to South west china, and from the Arabian peninsula through East and South Africa; (3) Subsp. *laperrinei*: present only in the Sahara region; (4) Subsp. *maroccana*: present only in Morocco; (5) Subsp. *cerasiformis*: present only in the island of Madeira; (6) Subsp. *guanchica*: present only in the Canary Islands. The study of ploidy level among these 6 subspecies revealed that all species are diploid except Subsp. *cerasiformis* and Subsp. *maroccana* which showed tetraploid and hexaploid levels, respectively (Besnard et al. 2008).

The cultivated olive is an evergreen subtropical, vegetatively propagated long-living tree with a high overall genetic variability. The olive life span exceeds 500 years with exceptional trees older than 2,000 years have been recorded. At least 1,250 varieties conserved in over 100 collections, have been described and listed in the FAO olive germplasm database, but many other local varieties and ecotypes contribute to the richness of the olive genetic patrimony (Bartolini et al. 2008; Cantini et al. 1999). Several cultivars may have the same name (homonyms), or the same can be called different names (synonyms) in different areas (Barranco et al. 2000; Besnard et al. 2000). This can create some issues for olive growers and researchers. The nuclear content analysis for olive genetic resources characterisation revealed an intra-specific variation in genome size. In fact, The DNA content in several Italian cultivars is 2.2pg per haploid nucleus (1C), while in Portuguese cultivars it was ranging between 2.9 and 3.07pg/2C (Rugini et al. 1996).

Economic and Social Importance

The olive tree is known around the world for it's symbolism of peace and tranquillity. The expression "to hold out an olive branch" means to seek harmony and peace. Olive cultivation is still the basic tree cultivation in the Mediterranean and dominates its rural landscape. As it grows successfully in marginal and harsh areas (inclining, shallow and barren soils), olive plays a major role as a feature of the landscape keeping these area productive (Bonazzi, 1997; Spennemann and Allen, 2000). This cultivation is completely adapted in the climatic and territorial conditions of the Mediterranean (Mazoyer and Roudart, 1998). Semi-intensive systems present low requirements in chemical inputs and machinery in comparison to other agricultural crops (Loumou, 1994). Olive cultivation even in intensive farming can be

described as having low inputs requirements, in particular in comparison to other fruit crops. So the olive cultivator constitutes a real model of a farmer who is not only a producer of goods but also a preserver of the environment (Loumou et al. 2003).

Olive is one of the major crops as a source of healthy foods like olive oil and table olives. Although 90% of olive production is used for oil extraction (Colmagro et al. 2001), the consumption of table olives is increasing worldwide. Olives contain antioxidants in abundance (up to 16g/kg) and olive oil contains compounds considered as anticancer agents e.g. terpenoids and squalene (Owen et al. 2003, 2004).

Olive production is concentrated in Southern Europe (79.5% of the world production) with Spain and Italy as leaders, followed by Greece, Portugal and France (Figure 1). In volume terms, Tunisia is the world's second producer and exporter, behind the European Union taken as a whole, and it lies fourth when the individual countries of the European Union are considered, coming after Spain, Italy and Greece. Tunisia provides 19% of the world olive oil exportations (Figure 2) exportation forecasts 2011/2012, IOC). The olive sector provides a livelihood directly or indirectly for over 1 million people in Tunisia and it generates 34 million workdays a year, equivalent to over 20% of the employment in agriculture. Olive oil exportations are important somehow because it is a relatively expensive vegetable oil. This is due to its high cultivation costs and limited production (2t/ha and 15-20kg/tree on average) in comparison with other oil crops. Yield is fluctuant from year to year and oil extractability which depends on the variety, agro-climatic conditions and extraction method, barely exceeds 24% of fresh weight (Harwood and Sanchez, 2000; Doveri and Baldoni, 2007).

Among oil-producing crops (i.e. including palm oil), olive oil production occupy 22% of the international trade which exceeds rapeseed and sunflower representing yet 16 and 21% of world production respectively against only 4.5% for olive oil (Touzani et al., 2004).

Morphological characteristics and growth habit

The olive tree is an evergreen perennial species whose growth is rhythmic in temperate climate but without winter buds. Gucci and Cantini (2000) reported a clear tendency toward bushy growth in mature unpruned olive, with a canopy often formed by multiple stems originating directly from the trunk. This could also reflect a 'basitony' observed even in seedlings i.e. the development of vigorous basal shoots that progressively overgrow the central stem (Hammami et al. 2012).

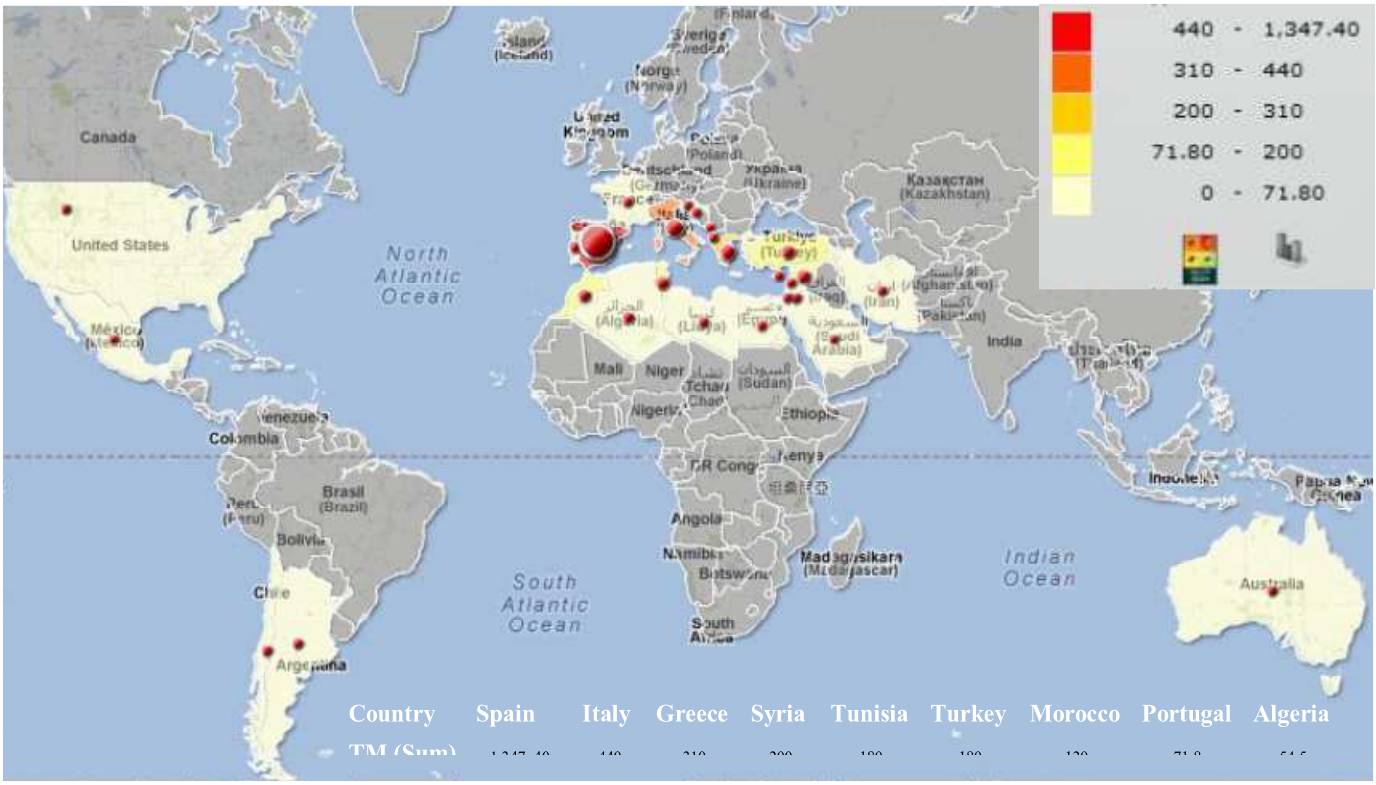


Figure 1. World olive oil production: estimation for 2011/2012 season (data from the International Olive Oil Council)

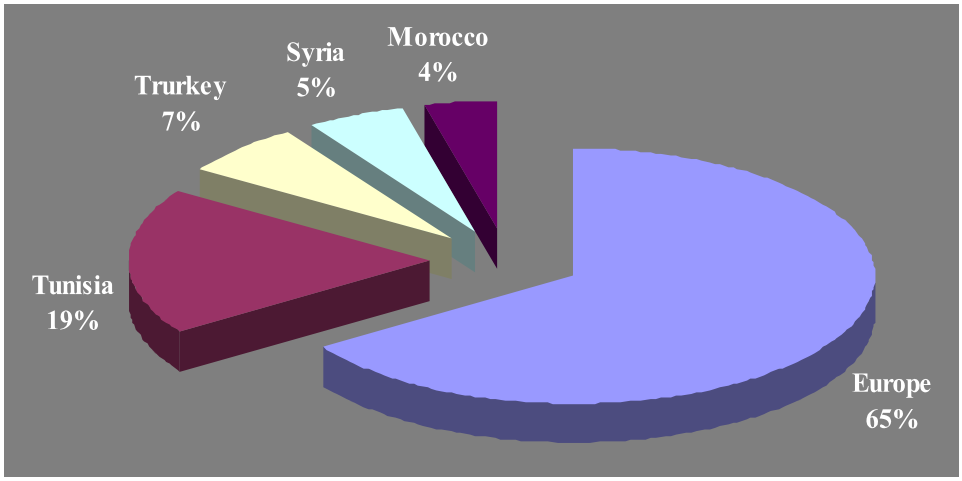


Figure 2. Exportations Forecasts for 2011-2012 (Based on IOC estimations)

If left free to grow for few years, the canopy tends to expand laterally, more than in height. In unpruned plants, the canopy form is spherical, hemispherical or ellipsoidal. Olive tree shape results not only from the strong basitonic growth habit of the shoots, but also from the production of many stems from adventitious buds. The lateral shoots on the proximal part naturally grow more than those in the distal part (Gucci and Cantini, 2000). Likewise, shoots on the lower side of a branch are less vigorous than those on the upper side (Figure 3). Four main growth habits have been identified in olive: upright or erect; open or round and pendulous or semi-pendulous (Gucci and Cantini, 2000). Most of the cultivars have an intermediate growth e.g. 'Arbequina'; 'Picqual' and 'Manzanilla' canopy have been described as 'open' which is intermediate between the erect and pendulous canopy.

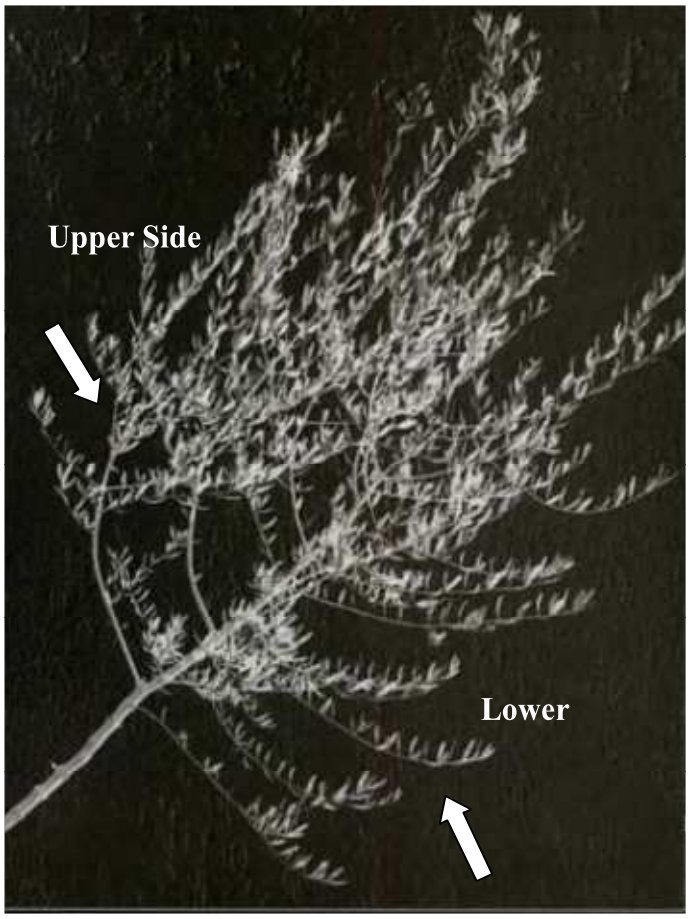


Figure 3. Growth pattern of lateral shoots inserted on an inclined branch: basal shoots on the upper side are more vigorous than those on the lower side or those on the apical part of the main branch. (adapted from Gucci and Cantini, 2000)

The olive foliage can persistent for two or three years in optimal conditions where trees are not shaded. As observed for most evergreen and semi-deciduous species, the olive leaf is more costly in energy in comparison to other deciduous species i.e. it presents higher costs of tissues construction (CC) expressed per unit dry mass: Olea europeae: 1.653g glucose g^{-1} vs Mean leaf CC of 49 deciduous species: 1.46 g glucose g^{-1} (Villar and Merino, 2001, Villar et al. 2006). This is due to the evergreen character of the leaf and to the abundance of protective

compounds against abiotic and biotic stress: waxes, celluloses, carbohydrates, lignins, phenolic substances, and oil in the cuticle; cell wall and cytoplasm of cells (Villar and Merino, 2001). The cost of the evergreen character is partially compensated by the high annual photosynthetic gain and the longevity of the leaf. The olive thick cuticle is rich in lipid compounds, which may be involved in reducing water loss (Larcher, 1995). Owing to their position, the leaves on a well lighted shoot (1,600 μmoles of photons $m^{-2}s^{-1}$) receive an average light exposure of 900-1,000 μmole photons $m^{-2}s^{-1}$ (Tombesi and Tombesi, 2007). Mature leaves are elliptic, green-gray in color with trichomes (star hairs) that gives a protection from the high Mediterranean UV radiation. They are small and rigid which confer an efficient balance of heat in summer (Grammatikopoulos et al. 1994).

The phyllotaxy (leaf arrangement) is opposite decussate. Leaves are arranged in pairs growing out of the opposite sides of the branch and each opposite pairs is arranged at right angles to those above or below (Figure 4).



Figure 4. Opposite-decussate olive leaf arrangement

Olive Reproduction

Flower induction and differentiation

Prior to the elaboration of inflorescence structures, plants must make the transition from the vegetative to the reproductive phase. During the vegetative phase of growth, the shoot apical meristem (SAM) has vegetative identity, and cells on the periphery of the SAM give rise to leaf primordia that bear secondary (axillary) meristems, which may be dormant or develop into side shoots. In response to appropriate environmental stimuli and developmental signals, the plant then switches from its vegetative phase to the reproductive phase of development,

and the meristem acquires an inflorescence identity (Bhatt, 2005). The transition of a vegetative meristem to a reproductive one occurs after a long juvenility phase during which perennial species are unable to flower and bear fruit. Fernandez-Escobar et al. (1992) reported that olive flower induction occurs around the time of endocarp sclerification. During winter happen the differentiation phase involving series of anatomical changes during which the tissues of flower organs are formed reaching completion just before anthesis (Fabbri and Benelli, 2000; Kitsaki, 2010). Environmental conditions, hormonal balance, nutrition and other factors in both steps are determinant in the final set of flower buds. It is assumed that winter chilling will release olive flower buds from dormancy but its effect as pre-requisite for floral induction and differentiation is still in question (Lavee, 1996; Fabbri and Benelli, 2000).

Reproductive structures

Inflorescence

Flower bud inflorescences (a maximum of two per node) are born at leaf axils. Flower buds are induced on shoots developing the year before anthesis (i.e. opening of flowers with anthers and ovary exposed), (Fabbri and Benelli, 2000). Olive flowers are grouped into inflorescences called panicles. The olive inflorescence is a branched raceme that is opposite-decussate as observed for leaves arrangement. Pairs of secondary peduncles branch out in opposing direction from the main stalk of the inflorescence. Each ones are offset 90° from those above or below (Figure 5).

(a)



(b)



Figure 5. Opposite and decussate floral structure of olive: (a) Inflorescence (b) flower

(Original picture from the tree species BlogSpot: http://tree-species.blogspot.fr)

The inflorescence development begins in early spring and takes usually 4 to 6 weeks from inflorescences bud opening to anthesis (period during which the flower is fully open and functional). Inflorescences start to elongate rapidly when they are 2cm long. The flower buds begin to expand when inflorescences attempt the 2/3 of their final length (Lavee, 1985; 1996). The inflorescences length depends on the cultivar and range from 3 to 8 cm.

Flowers and Flowering

An adult olive tree produces about 500,000 flowers a year (Martin et al. 2005). The number of flowers per inflorescences ranges normally between 15 and 30 depending on the cultivar (Martin and Sibbett, 2005). It may also vary between inflorescences, shoots, trees and years (Lavee and Datt, 1978; Lavee, 1985; Cuevas et al. 1999; Lavee et al. 2002; Reale et al. 2006).

Olive flowers are composed of four fused green sepals, four creamy-white petals fused at their base, two stamens, and two carpels (Figure 6). Petals drop as a unit often with the stamens attached. The style is thick and short with a large stigma (Fabbri et al. 2004; Martin et al. 2005).



Figure 6. Olive flowers at full bloom.

The olive flowers are generally hermaphroditic (perfect), but certain cultivars are male-sterile, while others are purely staminate (Besnard et al. 2000; Doveri and Baldoni, 2007). Pistil is functional in perfect flowers while it is degenerated in staminate flowers. Pistil degeneration can happen at any flower developmental stage, and nutrient and water stress can lead to abortion (Lavee, 1985; Fabbri et al. 2004; Martin and Sibbett, 2005). The number of

staminate flowers is environmentally and genetically controlled (Lavee et al. 2002; Fabbri et al. 2004; Martin and Sibbett, 2005). However, the amount of staminate flowers doesn't affect significantly the yield (Lavee et al. 1996; Lavee et al. 2002).
The flower position on the inflorescence can affect its gender as more perfect than staminate flowers are located in the tip and middle (Griggs et al. 1975; Bouranis et al. 1999; Dimassi et al. 1999; Atteyyeh et al. 2000; Cuevas and Polito, 2004; Martin et al. 2005). Its also affects its opening e.g in 79% of cases, the first flowers to open in 'Mission' cultivar are those located on the primary branches while the last are on the secondary branches (Cuevas and Polito, 2004)

Anthesis and pollination

Full bloom occurs in full spring, but differences can be observed among cultivars. Anthesis takes typically 2-3 days in one inflorescence and 5-6 days in one tree. It may occur in more than a flush when the temperature is instable, but it is usually the first one that sets the fruits that reach maturity (Lavee, 1985). In cool environments, anthesis can take 10 to 15 days (Fabbri et al. 2004).

Olive flowers bear large quantities of pollen grains (up to 200,000 pollen grains per flower), but they don't produce any nectar as they don't possess nectaries (Martin, 1994). The abundant pollen production shows the flowers adaptation to be wind-pollinated. However, insects like honeybees can assist pollination (Lavee, 1985; Free, 1993). Cuevas et al. (2001) showed that olive flowers are naturally adapted to cross pollination considering the presence of staminate flowers (with male parts only) acting as pollen donors. Pollen grains can be distributed by wind over 12km but in normal conditions; the effective pollination distance has been reported to be 30m (Fabbri et al. 2004; Ayerza and Coates, 2004; Sibett and Osgood, 2005). A satisfactory yield can be ensured with only 10% of pollenisers along the effective pollination distance (Lavee et al. 1996).

Fertilization success is very sensitive to extreme temperature (high or cool) since it inhibits pollen germination and slow down or stop the pollen tube (Bartoloni and Guerriero, 1995). Although critical environmental conditions affecting pollination may reduce the fruit set, in normal conditions, the viability of the olive pollen is high and not correlated to fruit set (Lavee et al. 2002). The receptivity of the stigma can also be shortened by dry winds and hot environmental conditions (Atteyyeh et al. 2000).

Sterility

Most olive cultivars are self-incompatible or partially self-compatible and needs compatible pollenisers to ensure fertilization and yield (Besnard et al. 1999; Dimassi et al. 1999; Moutier, 2000; Conner and Fereres, 2005). Diaz et al. (2006) showed the self-incompatibility of 'Arbequina' and 'Piqual' cultivars by testing seeds using paternity analysis with data obtained from four microsatellites markers. The compatibility relationships have been studied in several olive cultivars showing that most of them needed cross-pollination for adequate fruit set (Moutier, 2000; Wu et al. 2002). It has been found also that compatibility relationships change depending on the environmental conditions (El-Kholy, 2001). This may explain the conflicting results in the literature for some cultivars e.g. 'Frantoio' was characterised as self-compatible in some studies (Fontanazza and Baldoni, 1990; Fabbri et al. 2004) while self-incompatible in others (Wu et al. 2002, Mookerjee et al. 2005). These variations may also be due to contamination during pollen collection, annual changes in the amount of flowering and the use of homonyms and synonyms (Bartolini et al. 1994; Lanza et al. 1996; Mekuria et al. 1999; Lavee et al. 2002). Moreover, some cultivars are cross-incompatible and can not fertilize each other (Cuevas and Polito, 1997; Martin et al. 2005).

The self-incompatibility in olive cultivars require the plantation of three to four cultivars, with an overlapping anthesis, in a commercial orchard to ensure a good fruit set (Fabbri et al. 2004).

Fruitset

The olive fruit develops on inflorescences developing from buds borne on the previous year's strong and well lignified shoots (Lavee, 2007). In the absence of sterility barriers, pollen grains germinate on the flower stigma to produce pollen tubes. Most pollen tubes are inhibited in the stigma before entering through the style and normally one or two pollen tubes grow toward the ovary, one reaches the carpels and the other penetrates one of the four ovules. Following the fertilization, the flowers shed petals and stamens, the ovary grows in size and many pistils shrink and drop. Within a month from full bloom, only 7-8% of flowers are maintained on the tree as fruitlets; of them, 25 % will be present by late summer (Fabbri and Benelli, 2000).

Fruit Growth

At early summer, the initial growth is characterized by an intense cell division that leads one month later to the identification of the olive fruit parts: exocarp; mesocarp and endocarp (Tombesi, 1994). The vacuole of mesocarp cells increases in size and the endocarp accumulates lignin and begins to harden. At mid-summer, the rate of fruit growth slows down, the embryo and endocarp reach maximum size and the endocarp hardens forming the stone. At late summer, oil starts to accumulate in the cells of the mesocarp. This stage continues through to autumn, where the olive changes from dark green to a yellow-green straw colour ripe stage due to a reduction in chlorophyll. By the end of autumn most varieties achieve maximum oil accumulation. Colouration starts in the skin mostly at the tip of the olive and proceeds to the base. When the skin colour reaches the stalk (petiole), the flesh begins to colour.

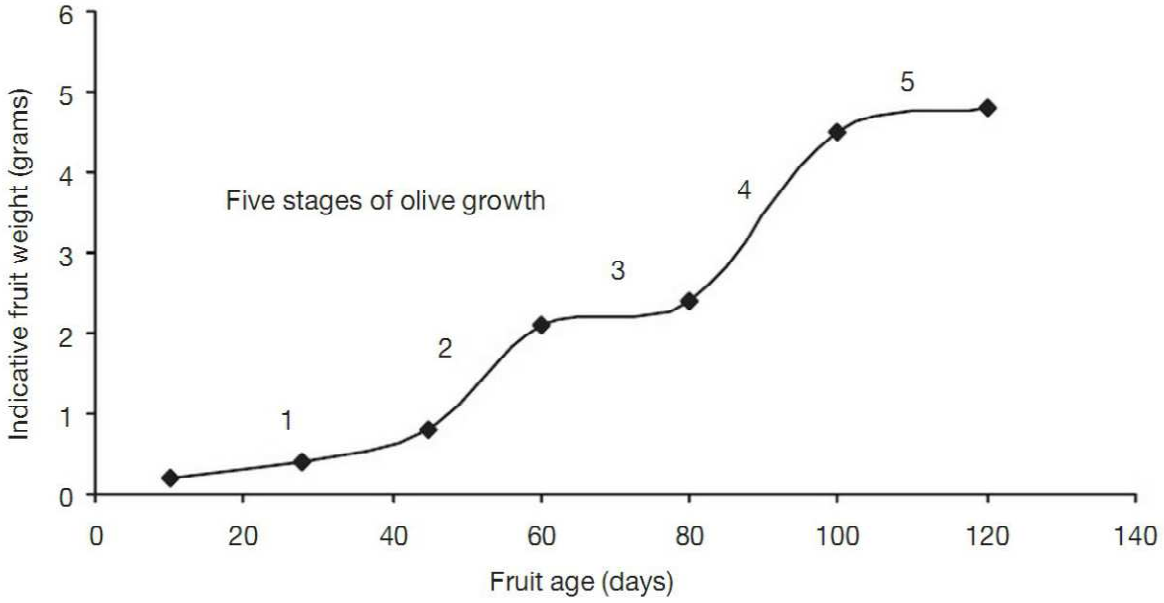


Figure 7. The five olive growth stages: (1) intense cell division at fruitset, (2) Embryo and Endocap (stone) development, (3) Endocarp hardening, (4) Mesocarp development and oil accumulation, (5) olive fruit ripening. (Source: World Olive Encyclopaedia, 1996)

The fleshy oil-accumulating mesocarp contains oil (22%), water (50%), carbohydrates (19.1%), cellulose (5.8%), proteins (1.6%) and minerals (1.5%), (Doveri and Baldoni, 2007). Fruit growth usually follows three stages: (1) growth as a result of cell division (2) endocarp hardening when mesocarp stops growing (3) growth as a result of cell enlargement (Lavee,

1986; Fabbri et al. 2004). The fruit shape, pit size and surface morphology are the most reliable morphological traits to distinguish between cultivars (Fabbri and Benelli, 2000; Moutier, 2000).

Horticultural characteristics and objectives

The olive tree is usually propagated by cuttings or grafting onto seedling rootstocks. It grows in semi-arid to temperate climates, on well drained soil with a pH < 8.5. It is tolerant to mild soil salinity and temperatures as low as -12°C. Although, productivity can be compromised for several years after such severe cold period, olive has the resources to initiate vegetative growth from the trunk basis. The olive tree is sensitive to two specific pathogens *Spilocaea oleagina* Cast., causing olive leaf spot and *Bactrocera oleae* commonly known as the olive fruit fly which attacks the fruit mesocarp inducing an early fruit fall or a total disruption of the pulp (Doveri and Baldoni, 2007). Non-specific pathogens cause olive knot (*Pseudomonas syringae* subsp. *savastanoi*) and *Verticillium* wilt (*Verticillium dahliae* Kleb.).

Traditional olive oil production is limited by its high cost, mainly due to labour expenses for harvesting and pruning. In order to set an orchard that is efficient, competitive in terms of management costs, olive growing has to aim for quality product and a drastic reduction of labour by mechanizing cultural care, especially harvesting. The orchards have to be planted at a suitable density on a land not too sloping. Soil cultural care such as tillage, fertilization and irrigation must also be easy, and pruning, which accounts for 10-20% of cultural care, must be cost-effective, i.e. it must be simple, quick and cheap (Tombesi and Tombesi, 2007). Most of the efforts of olive growers and breeders are focused towards overcoming the limiting factors for production i.e. regular and commercially valuable production of fruits but also shortening juvenility period, increasing oil content and quality, improvement resistance to biotic and abiotic stress and reducing the reliance on pollinators by the promotion of self-pollination.

As olive is an industry dependent crop, the problems rising from alternate bearing behaviour are economically alarming and the information about the endogenous pathways and genes involved in vegetative and reproductive bud transformation is very limited. Alternate bearing can be limited by cultural techniques like pruning, thinning and girdling only in regions of favourable and stable climate and such manipulation generates costs that can be disabling for the growers. Tree shape and architecture control is thus essential to facilitate mechanisation.

For a better understanding and control of the vegetative vs reproductive stages balance wide scale observations and genetic investigation are necessary. Natural olive tree growth habit needs to be investigated from the juvenility and until maturity phase.

Factors affecting fruit production

The final number of fruits on limbs is mainly governed by three 3 physiological processes:

- ✓ Flower induction which determines the amount of axillary inflorescences
- ✓ Pistil abortion, a developmental process that occurs between six and four weeks before full bloom (Uriu, 1959), regulates the fruiting potential of inflorescences at anthesis.
- ✓ Fruit set which is modulated by fertilization of ovaries and by competition among fruits (Fernandez- Escobar, 1985), determines the final fruit population

As mentioned above, a high level of flower and fruit abscission is observed in olive (Cuevas et al. 2001; Ghrisi et al. 1999). Martin, (1990) observed that 98% of the flowers abscise in olive, but in normal conditions with a normal flowering, 1 to 2 % of fruit set is sufficient for a commercial yield (Lavee, 1996; Fabbri et al. 2004; Martin et al. 2005). However, before abscission, the large amount of flowers produced spends the resources of the tree which explain that the highest fruit drop is observed following the highest fruit set rates (Martin, 1990). Fruit set increases when level of flowering and rate of perfect inflorescences decreases between years. Ait Radi et al. (1990) suggested that competition among fruits for the nutritional resources leads to obtaining a heavy crop of small fruits in a year and a light crop of large fruits in another. This problem affects severely the commercial olive production and is called: alternate bearing. In ‘on’ years, fruits represent more than 65% of the seasonal dry matter accumulated in the fruiting shoots. This reduces vegetative growth in the ‘on’ years, contributing to the alternate bearing of the olive trees (Rallo and Suarez, 1989).
In the following section, I will focus on alternate bearing among all the problems affecting olive production described above.

Alternate or biennial bearing in olive

Although alternate or biennial bearing is commonly observed in various fruit species, the processes involved are far from universal and rather different in the various tree species (Goldschmit, 2005). The cultivated olive is highly alternating in its fruit production; however, environmental conditions influence its development by acting directly on the flowers and

indirectly by impacting endogenous metabolic changes induced by specific gene activation or repression. The gap between 'on' and off' years may vary between 5 to 30 t/ha without special intervention.

Under high fruiting condition, the few lateral buds induced to developed vegetative terminal buds and extension growth are inhibited and develop rather fragile shoots. Thus, the number of shoots, their length and the number of buds available for fruiting in the following year as well as their potential to differentiate into reproductive buds will be low (Lavee, 2007). Cuevas et al. (1994) and Kitsaki et al. (1995) reported that this is due to endogenous metabolic changes inhibiting both flowering bud differentiation and viable flowers. The 'off' year that follows a highly fruiting one is characterised by strong vigorous vegetative growth. Shoots bear numerous buds that could potentially develop into reproductive ones and reconstitutes the large inflorescences production characterizing the 'on' year coming next. It is thus clear that a balance between the amount of developing fruits and the vegetative growth impact and condition the potential fruit production for the following season (Lavee, 2007).

For various fruit tree species, developing fruits are a major competing sink for metabolites with vegetative growth (Monselise and Goldschmit, 1982). In olive, the existence of a relation between the organic metabolites levels and alternate bearing phenomenon appears to be not significant and still is not clear (Suttle and Martin, 1986a). These authors showed that seedless fruits (developed after destroying embryos before stone hardening) had only a small effect on the returned bloom and fruit development in the following year (Suttle and Martin, 1986b). It therefore seems that alternate bearing in olive is not a result of nutrients depletion but it is of regulatory nature via signals produced from the developing embryos. Significant changes in phenolic metabolites such as chlorogenic acid (whose synthesis and accumulation is initiation in the embryos of developing fruits) were found in the leaves of olive trees between 'on' and 'off' years. Likewise, significant differences in proteins content have been found in both leaves and young shoots during 'on' and 'off' years with specific and different proteins induction. Changes in mineral content of leaves between 'on' and 'off' years were also reported as linked to growth regulating systems activity (Fernandez-Escobar et al. 1999, 2004). The N and K content in leaves were drained at the end of the 'on' year while it increased at the end the 'off' year. This shows that mineral content retrieval is necessary to allow flower bud differentiation (Erel, 2008). Hence, besides competing with the vegetative growth, the developing fruits in the current year, affect directly the metabolism leading to reproductive induction and differentiation of the buds for the following year production. These relations are conditioned by the environmental conditions (Figure 8). Unfavourable

temperature regimes especially in the winter are predominent in inducing alternate bearing and particularly in the orchard and regional synchronization of this phenomenon (Morettini, 1950; Hartamn, 1951; Lavee, 2007).



Figure 8. Timing and nature of involvement of both environmental and endogenous factors during the olive developmental cycle in consecutive 'on' and 'off" years (biannual cycle represented by a circle) starting from the spring in the 'on' year (upper half) till the following spring from were events on the 'off' year (lower half) are described: Original Figure from (Lavee, 2007).

Breeding, Heritability and Genetic Maps in Olive tree

Breeding in Olive

Despite the pressing need to improve the agronomic performances, there has been sparsely olive breeding so far (Doveri and Baldoni, 2007). Clonal selection has been the most practiced breeding method in Olive. This relied on the assumption that in a long-living perennial species such as Olive, natural mutations generating positive alteration in a trait of

agronomic interest can be maintained by vegetative propagation (Rallo, 1995; Belaj et al. 2004). Clonal selection have been used for identifying virus-free material (Loreti et al., 1994; Leitao et al., 1999; Sebastiani and Vitagliano, 1999) and have led to the identification of valuable clones within various cultivars (Suarez et al. 1990; Lavee et al. 1995; Bartolini et al. 2002; Grati-Kamoun et al. 2002). Overall, clonal selection in olive has improved the knowledge on olive germplasm but, produced only few clones with remarkable performance (Loussert et berrichi, 1995; Tous et al. 1998; Bellini et al. 2008).

Little cross-breeding programs have been developed in olive owing to the following biological and morphological characteristics (Bellini et al. 2008):

- ✓ *The long generation time*: for seedlings *juvenility phase* can last up to (10-20 years), but it is variable depending on the progeny.
- ✓ *Flower morphology (small flowers, closed anthers, delicate pistils)*: hindering the emasculation and controlled cross-pollination.
- ✓ *Self-incompatibility*: prevents selfing, important for developing homozygous progenies, and makes the paternity of progenies doubtful to some extent.
- ✓ *Low fruit set*: of most cultivars
- ✓ *Germination rates*: very low for some breeding combinations (Bini and Bellini, 1975; Pritsa et al. 1999).
- ✓ *Instability of traits of interest*: Production productive and qualitative traits (oil or table olives) are not stable in time, space or with processing methods (Pannelli et al. 2006). Thus a multi-site evaluation is necessary.
- ✓ *High heterozygosity and chromosomes number*: *Olea europaea* has a high number of chromosomes (2n = 46), and their size is so small that even the position of the centromere cannot always be recognized with certainty (Minelli et al. 2000). Nevertheless, most of the chromosome pairs have been distinguished (Falistocco and Tosti, 1996), and structural heterozygosity was observed in three chromosome pairs (I, V and VII of cv. Coratina; Minelli et al. 2000). Most olive cultivars are diploid (2n = 46), but some triploid, tetraploid and polysomatic (2n = 55) genotypes have been observed (Wu et al. 2004).

The main breeding programs are currently being carried out in Tunisia (Jardak, 2006), Egypt (Laz, 2006), Israel (Lavee, 2007), Syria (Al Ibrahem, 2006), Turkey (Zafer Can and Isfendiyaroglu, 2006), Spain (Diaz, 2006) and Iran (Zeinanloo, 2006) where new genotypes from cross-breeding programs are being evaluated.

Olive breeding programs aim at selecting genotypes characterised by early bearing, limited alternate bearing, resistance to pests and to abiotic stresses (such as frost and drought), suitability to intensive culture and to mechanical harvesting, high-organoleptic quality productions (fruits and oils), and high contents in substances useful for human health (Fabbri et al. 2009). Their efficiency appears similar to that found for other species, with a ratio of selected genotypes/seedlings of about 0.4% (Bellini et al. 2000).

Heritability Studies

Very little genetic knowledge is available on the hereditary behaviour of the characters in the olive (De la Rosa, 2003; Fabbri et al. 2009; Zeinanloo et al. 2009). The reasons for this scarceness have to be traced to the difficulties described above.

Traits related to the vegetative growth

The first traits for which heritability has been studied in olive are plant height and shoot diameter (Martins et al., 1998), and limited to clonal selection of 'Cobrançosa' cultivar. More recently, Hassani and Tombesi (2008) evaluated the broad sense heritability h^2_B and narrow sense heritability h^2_N (see chapter 3 for definition and formula) for three vegetative growth traits (i.e. internode length, growth speed and plant height measured during 2001-2002), obtained from a complete diallel cross between nine olive cultivars. For both h^2_B and h^2_N values exceeding 0.75 were reported for all traits.

Traits related to the fruits

The broad sense heritability vs. environmental effects on olive acidic composition and phenolic content (Ripa et al., 2008) and on fruit and oil yield, fruit size and oil content (Padula et al. 2008) have been recently studied on 134 genotypes (selected from 5000 seedlings derived from 127 crossings) grown in three locations of southern and central Italy (Padula et al. 2006). Both environment and genotype had significant effects on all parameters tested. The environment had quantitatively more impact than the genotype on fruit weight and oil content on a fresh matter basis while pulp to pit ratio and oil content on a dry matter basis were predominantly under genetic control and shows higher heritability values (Padula et al. 2008).

Another analysis of diallel cross involving six parents showed that expression of majority of the fruit quantitative traits in olive (i.e. fruit weight, stone weight, percentage of dry matter, fruit width, stone width, fruit length, stone length, flesh and stone ratio and oil percentage) is regulated predominantly by gene action that is dominant in nature (Zeinanloo et al. 2009).

Yet, highly significant variances due to both general and specific combining ability effect (GCA and SCA, respectively) reveal the importance of both additive and non-additive gene for all fruit traits (see chapter 3 for GCA and SCA definition and formula). Traits showed h^2_B and h^2_N values varying from 0.31 to 0.86 and 0.17 and 0.36, respectively (Zeinanloo et al. 2009).

In spite of the development of different cross progeny populations for genome mapping purposes, there is almost no information available so far on segregation analysis of genes or molecular makers in an olive cross population (Bellini et al. 1993; Parlati et al. 1994; De la Rosa, 2003). Toward quantitative genetics studies in olive, developing saturated genetic maps and evaluating (over years and contrasting environments) the traits of interest on large segregating populations grown on field conditions is required.

II. Tree Architecture: General concepts and phenotyping methods

Plant morphology, introduced by von Goethe (1790), deals with temporal and topological changes of plant form and structure during ontogeny. Plants were assumed to develop by the repetition of elementary botanical entities which morphological, dimensional, functional and anatomical traits change over life stages (Robbins, 1957; Wareing, 1961; White, 1979; Nozeran, 1984; Bell, 1991; Greenwood, 1995; Jones, 1999, 2001; Claßen-Bockoff, 2001; Kaplan, 2001).

The nature and the relative arrangement of plants constituents i.e. plants architecture result from the interaction between endogenous growth processes and environmental conditions. Architectural analysis aims to distinguishing these processes through macroscopic measurements and sometimes microscopic observations. Architectural studies have been initiated with Hallé and Oldeman works on the aerial vegetative structure of tropical trees (Hallé and Oldeman, 1970; Oldeman, 1974; Hallé et al. 1978). These authors identified a framework of invariant morphological characteristics with respect to the environment on which tree architecture is established. The identification of the architectural model of a given tree (i.e. its basic growth strategy defining how is built its form and structure and categorises its architecture) is based on the observation of the following features (Hallé and Oldmann, 1970):

- ✓ *Growth pattern*: determinate or indeterminate and rhythmic or continuous.

- ✓ *Branching pattern*: terminal, lateral branching or no branching; monopodial or sympodial; rhythmic, continuous or diffuse (i.e. irregular laterals distribution along the parent); immediate or delayed.
- ✓ *The morphological differentiation of axes*: orthotropic (i.e. general vertical orientation and radial symmetry with lateral branches arranged in all directions), plagiotropic (i.e. general horizontal to sloping orientation and bilateral symmetry with lateral branches arranged in one plane) or mixed axes with plagiotropic and orthotropic portions.
- ✓ *The position of reproductive organs*: (lateral or terminal)

Architectural concepts have been widely used on temperate species (Edelin, 1981; Caraglio and Edelin, 1990; Nicolini, 1998; Sabatier and Barthélémy, 1999; Stecconi et al., 2000; Lauri, 2002, 2010; Costes et al. 2003, 2006). These studies showed that depending on the structural complexity of trees, qualitative and quantitative morphological or even anatomical data can be collected on varying numbers of individuals and on several scales e.g. whole tree, shoots or buds. In the following section, I will focus on the main morphological traits categories used in plant architectural analysis and addressed in my thesis.

Primary growth

The primary growth of a plant result from two morphogenetic events: *organogenesis* and *extension* (Champagnat et al. 1986). The *organogenesis* refers to the onset of new organs resulting from apical meristem undifferentiated cells activity (Figure 8). Cells in the meristematic zone will develop into embryonic leaves, then leaves into stems. Leafs insertion points on the stem are called nodes and the stem section separating two successive nodes is called internode (Nougarède, 2001; Caraglio and Barthélémy, 2007). The basic structural entity of leafy axis (the *metamer or phytomer*) appears to be composed by an internode and a node associated with its leaf (or leaves) and axillary bud(s) (Figure 9), (Hallé et al. 1978; White, 1979).

Regarding *extension* process of the leafy axis, several growth patterns have been reported among plant species. Shoots growth can have *determinate* (i.e. axes have a definite extension resulting from apex abscission or transformation into a specialised structure) or *indeterminate* growth (i.e. extension is maintained indefinitely by the apical meristem), (Hallé et al. 1978; Bell, 1991).

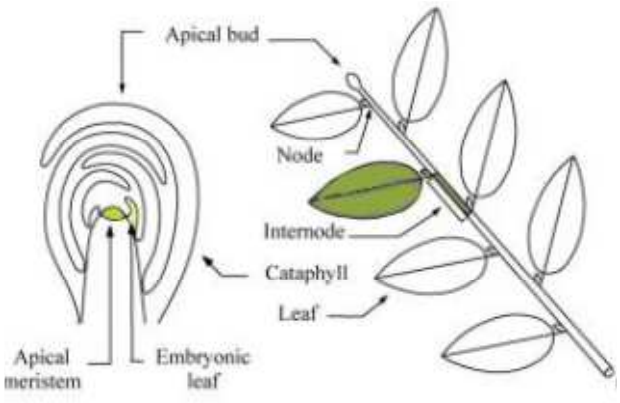


Figure 9. Representation of stem organization: Each stem comprises a succession of metamers ending in an apical meristem frequently protected in an apical bud. Each metamer (colored in green) is composed by one internode, the node located at its tip (i.e. insertion point of the leaves on a stem) and the corresponding leaves and associated lateral buds (Caraglio and Barthélémy, 2007).

Shoots extension can be *continuous* (i.e. no marked endogenous growth cessation and homogenous production of leaves and shoots through the year) or more frequently *rhythmic* (i.e. alternation of periods of rest in meristem activity or dormancy and periods of active extension). The portion of shoot developing during an uninterrupted period of extension is called 'growth unit' (Hallé and Martin, 1968). The limit between two growth units is usually marked by a zone of short internodes and/or cataphylls (i.e. scale leaves) corresponding to the protective organs of the bud from which it derives. However, as previously mentioned, growth cessation mark can be not obvious in some species such as olive where no winter bud with scars are formed. In some temperate species, shoot extension may occur in one, two or more successive events in a growing season giving rise to an 'annual shoot' made up of one or a succession of several growth units (Caraglio and Barthélémy, 1997).

During the period of rest, metamers are present at an embryonic stage in the buds from which a '*preformed*' shoot will elongate. The *preformed* portion of shoot can be followed by a '*neoformed*' one (i.e. corresponding to meristem activity recovery) giving rise to a mixed shoot (Rivals, 1965; Hallé and Martin, 1968; Nitta and Ohsawa, 1998; Souza et al., 2000; Seleznyova et al., 2002; Costes et al., 2003; Gordon et al., 2006). In fruit tree species, 'neoformed' shoots elongated during the meristematic activity show clear length polymorphism (i.e. long (*auxiblasts*), medium (*mesoblasts*) and short (*brachyblasts*) shoots) resulting from differences in meristems activity (Champagnat, 1965).

Branching process

The most common branching process consists in developing several lateral axes repetitively from axillary meristems. A lateral branch may elongate immediately after lateral meristem

initiation (i.e. *sylleptic branch:* showing generally long internodes without proximal cataphylls) or after a rest period as observed during winter for most temperate species (i.e. *proleptic branch:* showing short internodes and one or several cataphylls near the insertion point) (Hallé et al. 1978; Bell, 1991; Wu and Hinckley, 2001). Crabbé (1987) reported that *sylleptic* and *proleptic* branches of temperate trees, show *mesotonic* and *acrotonic* organization, respectively (Crabbé, 1987). Sylleptic shoots have been reported to be naturally positioned in the median portion of the main stem in apple (Costes and Lauri, 1995; Costes and Guédon, 1997) and peach trees (Lauri, 1991; Fournier, 1994).

Branching patterns may be *monopodial* or *sympodial* (i.e. one or more branches may develop after the apex death, abscission or transformation), depending on the indeterminate or determinate growth pattern of shoots, respectively (Emberger, 1960). Besides its mode (*monopodial* or *sympodial*) and dynamics (*sylleptic* or *proleptic*), branching can be characterized by its position on the parent shoot (Hallé et al. 1978; Caraglio and Barthélémy, 1997; Costes et al. 2006). The topological arrangement of lateral branches along the parent shoot is often related with their increasing or decreasing length and/or vigour. Generally, lateral branches present three main preferential repartitions on the parent shoot: *acrotonic*, *mesotonic* or *basitonic* when the lateral branches are located on the distal, median and the proximal part of the main vertical parent shoot, respectively (Figure 10; Bell, 1991; Caraglio and Barthélémy, 2007).

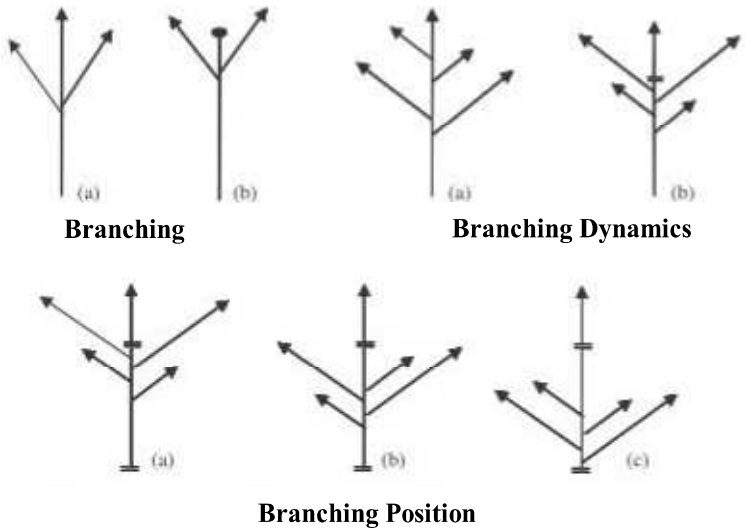


Figure 10. Branching Features: *Mode* (a) monopodial, (b) sympodial; *Dynamics* (a) sylleptic (b) proleptic; *Position* (a) acrotonic, (b) mesotonic, (c) basitonic (adapted from Caraglio and Barthélémy, 1997)

Branching systems description implies the use of a precise topological terminology. It is thus usual to consider the main stem developing from seed (the trunk for trees) as the order 1 axis whereas the axes it gives rise to are referred to order 2 axes and so on (Hallé, 1978; Barthélémy et al., 1989, 1991).

Branching control

The apical dominance (i.e. the leading shoot dominate the growth of the axillary buds in the leaves below it) has been pointed as controlling the inhibition of the axillary meristems outgrowth. The mechanism of apical dominance has been a matter of debate since the classical experiments of Thimann and Skoog (1934) who pointed out the regulatory role of phytohormones: The plant hormone 'auxin' (i.e. indole acetic acid: IAA) synthesized in the apex passes basipetally through the stem and inhibits the growth of lateral buds. Knowing that 'auxin' does not travel upward into the inhibited bud or shoot, it acts indirectly through a second messenger. Two candidates that could act as upward-moving second messenger were reported: the plant hormone 'cytokinins' which is a bud growth activator (Pillay and Railton, 1983; Bangerth, 1994; Li et al. 1995; Beveridge et al. 1997) and a novel-branch-inhibiting hormone not yet chemically defined requiring the activation of MAX1, MAX3 and MAX4 genes in *Arabidopsis* (McSteen and Leyser, 2005; Booker et al. 2005). However, an additional theory has been suggested whereby 'auxin' transport in the main stem can regulate the establishment of 'auxin' transport out of the lateral buds, repressing their outgrowth (Morris and Johnson, 1990; Li and Bangerth, 1999). This model depends not only on the amount of 'auxin' in the stem but also its distribution between intracellular and extracellular compartments and its capacity to move between them (Leyser, 2005). Despite all these advances in annual species, the underlying physiplogical mechanisms are still not fully understood in perennial ones (Crabbé, 1987; Cline, 1997).

Shoots morphological differentiation and reproductive organs position

According to their growth direction associated with phyllotaxy, shoots can be *plagiotropic* or *orthotropic*. Shoots characterized by a horizontal to oblique growth direction with *alternate* or *distichous phyllotaxy* and a plane symmetry are *plagiotropic*, while *orthotropic* shoots combine erect growth orientation with a spiral phyllotaxy and axial symmetry (Costes et al. 2006). Sometimes a single meristem may generate a mixed shoot with an *orthotropic* proximal portion and a *plagiotropic* distal end or the reciprocal configuration. For most

woody plants, *orthotropy* is associated with the exploration of the vertical space and the plant skeleton building, whereas *plagiotropy* is generally related to the exploitation of the horizontal space and reproductive functions (photosynthesis, flowering), (Caraglio and Barthélémy, 2007). Because of their impact on growth and branching processes, reproductive structures (e.g. inflorescences) are considered as a whole. Their position can be either lateral or terminal whether rising from a lateral or terminal meristem transformation (Bell, 1991).

Architectural unit and reiteration concepts

The concepts described above showed that whatever its complexity, the architecture of a branched system, can be summarized in terms of a limited number of developmental patterns, defined at the whole tree and axis scales (Edelin 1981; Caraglio and Edelin, 1990; Thorp and Sedgley, 1993; Costes et al. 2006). Architectural units (i.e. the detailed expression of plants architectural model, Edelin, 1977; Barthélémy et al., 1989, 1991) are often replicated within a tree. This morphogenetic process has been named '*reiteration*' by Oldeman, (1974). It can happen late in ontogeny, or under particular conditions. Both *immediate* (from apical buds) and *delayed* (from dormant, suppressed or adventitious buds) *reiteration* can be seen in many tree species where architectural units ranging from clusters of shoots to entire branches and stems are replicated. The *reiteration* can be '*complete*' or '*partial*' whether it involves the total or a part of the architectural unit, respectively (Oldeman, 1974; Caraglio and Barthélémy, 2007). This process can rise as a response to an increase in resource levels i.e. *adaptive reiteration* or structural damage i.e. '*traumatic reiteration'* or it can happen automatically during the tree development after a threshold of differentiation i.e. '*automatic or sequential reiteration'* (Edelin,1984; Nicolini, 1997; Ishii et al. 2007). In order to take into account the intrinsic organisation rules of the whole plant structure, the concept of 'morphogenetic gradients' has been introduced (Barthélémy et al. 1997). These morphogenetic gradients reflect the various processes of differentiation related to morphogenetic repetition phenomena.

Quantification of tree architecture

Trees develop by the repetition of nested entities corresponding in increasing order of integration to: metamer, growth unit, sympodial unit, annual shoot, axis, architectural unit, and whole reiterated complex (Barthélémy, 1991; Caraglio and Barthélémy, 1997, 2007). Since the initiation of the stochastic modelling of meristem activity on coffee trees (Reffye,

1981), tree structure has been widely explored in several fruit species on the basis of primary growth patterns and branching; flowering locations and the probability of meristem and shoot mortality (Costes et al. 1992; Costes et al. 1999; Costes and Guédon, 2002; Costes, 2003; Fournier et al. 1994; Fournier et al. 1998; Seleznyova et al. 2002; Lauri et al. 1997, 2002). Godin et al. (1999) classified the quantitative architectural features into to categories:

- ✓ Topological traits accounting for organs relative connection and number.
- ✓ Geometrical traits accounting for the form or spatial orientation of organs (angles, lengths and diameters).

The topological description integrates all the nested entities described above with respect to their level of organisation using a Multi-scale Tree Graph (MTG) as a formalization model (Godin and Caraglio, 1998). This formalisation is based on the decomposition of tree architectural into several quantitative measurements at several observation scales: tree, growth unit, sympode and node. Different tree shoots categories are distinguished according to their level of differentiation. In MTGs, tree organs connections are formalized by topological relations of succession (symbol '<') or branching (symbol '+') and geometrical measures are integrated as attributes of the described entities (Figure 11), (Godin, 2000). Such formalization leads to the constitution of detailed architectural data bases by specific tool such as VPlants (a successor of AMAPmode software, Godin et al. 1997) which can be analysed by appropriate statistical tools.

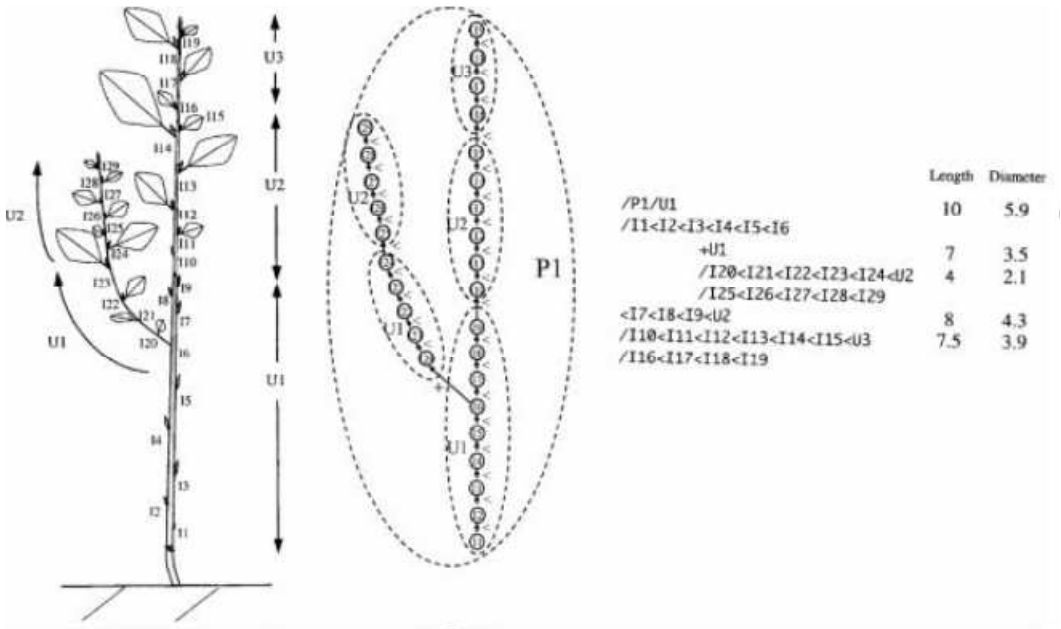


Figure 11. Plant structure formalisation in a Multi-scale Tree Graph (MTG): Nested entities: P (Plant); U (Growth Unit); I (Internode); Geometrical traits: Length; Diameter (Source: Godin, 2000).

Genetic control of plant architecture

The architectural models have been recognized as genetically controlled, but their expression can be affected by the environment (Hallé, 1978; Barthélémy et al. 1995, 1999; Grosfeld and Barthélémy, 2004; Stecconi, 2006). The genetic control of plant architecture has been investigated on the basis of segregation population derived from crosses between progenitor and evolutionary derivatives (teosinte/maize model, Doebley and Wang, 1997). Such dispositive allowed the identification and cloning of the *teosinte-branched 1 (tb1)* gene and the production of a *teosinte-branched mutant of maize* that show strong morphological resemblance to teosinte in its branching and reproductive characteristics (Cubas et al. 1999). Thus, it should be possible to generate plants with desired traits by altering the expression pattern of genes identified in target species as affecting architecture (Sussex and Kerk, 2001). Information on genes useful to engineer desired architectural traits has been reported among which genes required for differential internode elongation (*Arabidopsis*, Grbic and Bleeker, 1996); genes required for the axillary meristem initiation (*Tomato*, Shumacher et al. 1999; Schmitz and Theres, 1999 and *rice* (Zou et al. 2006); genes controlling the seasonal onset of bud dormancy and bud regrowth (*Poplar*, Frewen et al. 2000); and genes required for fate change in apical meristems (*Arabidopsis*, Ferrandiz et al. 2000).

Studies on the model plants *Arabidopsis*, *Antirrhinum* and petunia and on the crop plants pea, tomato, maize, wheat and rice have provided key informations towards the molecular regulatory mechanisms of plant architecture comprehension. The identification of genes encoding for biosynthetic pathways of plant height major regulators i.e. gibberillic acid (GA) and brassinosteroid (BR) has been done using dwarf mutant of *Arabidopsis,* pea, tomato, maize, wheat and rice (Peng et al. 1999; Hong et al. 2003; Ueguchi-Tanaka et al. 2005; Tanabe et al. 2005; Zhu et al. 2006; Wang and Li, 2006). Likewise, key genes related to inflorescences architecture have been identified in *Arabidopsis,* maize and rice (Li and Chory, 1997; Clark et al. 1997; Chuck et al. 2002).

Accessing the molecular basis of fruit trees architecture is time consuming and requires the collection of architectural quantitative data on a high number of related individuals. Several genes have been localized in apple tree related to the dwarfing character (Decourtye, 1967; Alston, 1976) and columnar habit (Hemmat et al. 1997; Kim et al. 2003). In my study, I aimed at investigating the genetic control of the architectural traits in a segregating olive population with the intention of locating the first QTL related to agronomical traits of interest such as flowering and production.

III. Quantitative Genetics and QTL mapping

All quantitative characters present variations both within and between populations. Quantitative genetics is concerned with discerning the foundation of these differences and the relative importance of genetic versus environmental factors which can be estimated from the phenotypic resemblance between relatives for the considered traits. The quantitative genetics provides thus the theoretical basis for plant and animal breeding (Gallais, 1989; Walsh, 2001; Collard et al. 2005). Historically, the basic statistical tools (such as regression and correlation) used in quantitative genetics have been developed by Galton and Pearson in 1880–1900.

R. A. Fisher's 1918 treatment of the inheritance of quantitative characters has marked the outset of the field introducing the analysis of variance method and has shown how Mendelian genetic models of inheritance explain the resemblance between relatives. Nowadays, molecular tools are being exploited to locate quantitative trait loci (QTLs) at which segregation contributes to the observed character variance (Walsh, 2001). Such identification is obviously relying deeply on an efficient phenotypic assessment of traits.

The phenotype of an organism is the way that it seems at a given observation time moment. It is the result of the expression of its inherited genetic information i.e. genotype in a given developmental stage and a given environment (Gallais, 1989).

The quantitative genetics is based on the phenotypic plasticity of the studied traits. Phenotypic values can be affected by genotype, ontogeny and environment mutual interactions. This is particularly true for all perennial species for which traits of agronomical interest can vary with tree age and their position within the tree canopy e.g. flowering traits (Renton et al. 2006; Costes et al. 2003).

Genetic and Environmental values

One of the foundations of quantitative genetics is that the phenotypic value P of an individual is the sum of its genotypic value G plus its environmental value E:

$$P = G + E \quad (1)$$

Case of one segregating population

In the case of the study of a population in segregation in field conditions, decomposing the phenotype into an additive linear model as a function of the genotype and micro-environment factors (i.e. uncontrolled environmental factors result from local micro-phenomena applying

differently for each individual, inducing small variations) assumes that the progeny satisfy the Hardy-Weinberg equilibrium, i.e. both allele and genotype frequencies in a population remain constant from generation to generation; and that genotypic and environmental factors effects are uncorrelated (Gallais, 1989). In order to comply with these conditions, the population must be with a large size, replicated and distributed randomly on orchard. The additive linear model is formalised as follows:

$$P_{ij} = \mu + G_i + e_{ij} \quad (2)$$

where P_{ij} is the phenotypic value of the repetition j of the genotype i, μ is the phenotypic mean value of the progeny, G_i is the value of the genotype i, and e_{ij} is the effect of the micro-environment on the repetition j of the genotype i.

The phenotypic and genotypic values are considered as random variables and the effect of micro-environment as a random residual whose distribution is the same for all genotypes. As mentioned in (4), the model (7) can be extended to an additional model of variances:

$$\sigma^2_P = \sigma^2_G + \sigma^2_e \quad (3)$$

where σ^2_P is the variance of phenotypic value, σ^2_G is the variance of genotypic value, and σ^2_e is the residual error variance.

When macro-environmental conditions are considered i.e. controlled environmental factor common to all progeny, the additive linear model will be formalized as:

$$P_{ijk} = \mu + G_i + E_k + (GxE)_{ik} + e_{ijk} \quad (4)$$

where P_{ijk} is the phenotypic value of the repetition j in the environment k of the genotype i, μ is the phenotypic mean value of the progeny, G_i is the random value of the genotype i, E_k is the fixed effect of the environment k, $(GxE)_{ik}$ is the random interaction between the genotype i and the environment k and e_{ijk} is the random effect of the micro-environment on the repetition j in the environment k of the genotype i.

In quantitative genetics, the most broadly used statistic is the fraction of the total phenotypic variance due to genetic effects, called broad sense heritability h_b^2 (Hanson, 1963; Comstock et Moll, 1963; Gallais, 1989):

$$h_b^2 = \sigma^2_G / \sigma^2_P = \sigma^2_G / \sigma^2_G + \sigma^2_e \quad (5)$$

In the case of repetitions in the same micro-environment, broad-sense heritability of phenotype means can be expressed as follows:

$H = \sigma^2_G / \sigma^2_P = \sigma^2_G / \sigma^2_G + \sigma^2_e/n;$ where n is the number of replicates per genotype.

When based on model (4), the broad sense heritability of phenotypic means h_b^2 is formalized as follows:

$$H = \sigma^2_G / \sigma^2_P = \sigma^2_G / \sigma^2_G + \sigma^2_{GxE}/l + \sigma^2_e/nl \quad (6)$$

where n is the number of replicates per genotype and l is the number of environments considered (Gallais, 1989).

According to Knapp et al. (1985), broad-sense heritability of phenotypic means can be expressed in all cases as follow:

$$H = 1\text{-}1/F \quad (7)$$

Where, F is the Fisher statistic for the genotypic effect of the considered model in the variance analysis.

The significance of the heritability values can be estimated by the calculation of a confidence interval based on the theoretical distribution of the statistic F (Knapp et al. 1985). Generally, a variable is considered heritable when the correspondent low limit for the confidence interval is greater than zero and when the heritability value is greater than 0.2 (Gallais, 1989).

Case of mating designs

Mating designs (e.g. diallels) make it possible to link full-sib families and allows the decomposition of the genetic variance into additive, dominance and epistasis components as follows:

$$G = A + D + I \quad (8)$$

where A accounts for the average effects of individual alleles (additivity), D for the interaction between alleles at each locus (dominance), and I for the interaction between genotypes at different loci (epistasis). The G values from different types of relatives share different amounts of these components, allowing inferences about the amount of variation contributed by each of these components (Walsh, 2001).

Such experimental device provides access to the genetic parameters: general and specific combining abilities (GCA and SCA, respectively), heritability values and coefficients of genetic correlation between traits and allows selecting effectively parents or families for further studies (Cilas, 1991, 2003).

Ignoring epistasis, heritability in the strict sense can be estimated as the ratio between the variance due to additive genetic effects and the phenotypic variance (Gallais, 1989) as follows:

$$h_N^2 = \sigma^2_A / \sigma^2_P = \sigma^2_A / \sigma^2_A + \sigma^2_D + \sigma^2_e \qquad (9)$$

Following the diallel approach, Griffin (1956) suggested partitioning the variance due to diallel progenies into two components: an additive variance component σ^2_A due to GCA and a dominance variance component σ^2_D due to SCA. σ^2_A and σ^2_D can be expressed as follows (Singh and Chaudhary, 1985; Zeinanloo et al. 2009):

$$\sigma^2_A = 2\,\sigma^2_{GCA}\ ;\ \ \sigma^2_D = \sigma^2_{SCA} \qquad (10)$$

Based on (10), (9) can be expressed as:

$$h_N^2 = 2\,\sigma^2_{GCA} / 2\,\sigma^2_{GCA} + \sigma^2_{SCA} + \sigma^2_e$$

Prediction of the genetic values

In this section I will focus on the statistical methodology used in this thesis for the genetic analysis. The architectural measurements were repeated over years and grouped according to several observation units including genotypes, branching orders, years of growth and environments. Thereby, mixed-effects models were built in order to access properly the genetic determinism of the studied traits. Mixed-effects models are used to data where observations are grouped according to one or more levels of experimental units and incorporate both fixed and random effects terms (Pinheiro and Bates, 2000). One key difference between fixed and random effects is in the kind of inference we want from the analysis of the effects. In the case of a fixed factor, our interest is comparing the scores of the dependent variable among the levels of the factor; based on differences between means. In the case of a random factor, we are not really interested in the specific differences in means from one level of the factor to another; but we are interested in the extent to which the random factor accounts for variance of the dependent variable. So, rather than being interested in the

individual means across the levels of the fixed factor, we are interested in the variance of means across the levels of a random factor (Littell et al. 2002).

Although, linear mixed effects models allow considerable flexibility in the specification of the random-effects, they restrict the within-group errors (residuals) to be independent, identically normally distributed with mean zero and constant variance and they are independent from random effects. For some of the variables studied in this thesis the within group errors were heteroscedastic i.e. showing unequal variances or/and correlated. It is thus essential to extend to basic linear mixed-effects model formulation in order to decompose the variance-covariance structure of the within group errors into two independent components: a *variance* function and/or a *correlation* structure. Among the various methods of parameter estimation used for linear mixed- effects models, I applied the restricted maximum likelihood (REML) criterion (Patterson and Thompson, 1971; Harville, 1977).

In the case of significant heteroscedasticity, which is estimated using Levene's test to assess the equality of variances (Levene, 1960), I tested several variances functions (varFunc classes) provided in the *nlme* library. I quote from them, varIdent (representing a variance model with different variances for each level of a stratification variable); varPower (representing a variance model with a power of the absolute value of the variance covariate); varExp (representing a variance model with an exponential function of the variance covariate) and varConstPower (which is a constant plus power of the absolute value of the variance covariate), (Pinheiro and Bates, 2000). To model dependence among the within-group errors, correlation structures also available in the *nlme* library were used (corStruct classes). Among them, the compound symmetry correlation structure (corCompSymm), the autoregressive correlation structure of order 1 (corAR1) and the spatial exponential correlation structure (corExp) were tested in our data analysis.

In order to examining the fitted model, plots of the residuals, the fitted values and the estimated random effects can be useful. Models were compared using the *Akaike Information Criterion* (*AIC*) (Sakamoto, Ishiguro and Kitagawa, 1986) and the *Bayesian Information Criterion* (*BIC*) (Schwarz, 1978) which is called also *Schwarz's Bayesian Criterion* (*SBC*). Models minimizing these criteria are selected afterwards. Predicted values of random effects of mixed linear models can be extracted allowing a precise estimation of the genotypic value. These predicted values of genotypic effect and its interactions when selected are called *Best*

Linear Unbiased Predictors (BLUP). Genotype BLUP values are independent of the fixed effects considered in the linear mixed model. These BLUPs will be used for detecting quantitative trait loci associated with the most heritable traits in which they improve the power of the analysis (Bernardo, 1996a and 1996b).

Genetic correlation

Phenotypic values of different traits in the same trees are often correlated, such as height and diameter. This is due to both environmental factors and genetic effects. Thus, we can decompose phenotypic correlation into its genetic and environmental components in the same to phenotypic variance partitioning (Isik, 2009). Genetic correlation is the proportion of variance that two traits share due to genetic effect (Naele and Maes, 1996). A genetic correlation between two characters can result of different molecular phenomena: (1) pleiotropy, i.e. the same gene or group of genes acting on these two characters; (2) linkage, i.e. combinations of genes representing a selective advantage tend to be grouped in the same chromosomal region; and the linkage disequilibrium, the non-random association of alleles at two or more loci, not necessarily on the same chromosome (Gallais, 1989).

In practice, it is assumed that the genetic correlation is close to the correlation between genotypic means which is why the genotypic means or BLUPs are commonly used for correlation calculation (Hill, 1971; Gallais, 1989). According to the intensity of correlation, it is possible to predict the value of a trait by measuring another correlated trait.

Quantitative trait loci (QTL) Mapping and resources available in olive tree

The identification of the genomic regions associated with quantitative traits (QTLs) depends on markers segregation via chromosome crossing-over during meiosis (i.e. recombination). Mapping quantitative traits is based on the detection of a significant association between a measured phenotype and the genotype of markers. It requires the phenotypic evaluation of a population derived from sexual reproduction, a set of polymorphic DNA markers and a linkage map. Each of these components will be described in the following sections (Jones et al. 1997; Collard et al. 2003).

Mapping Population

For QTL Studies, mapping plant population must be generated by crossing two parental varieties showing sufficient polymorphism. Parents that have high polymorphism are selected on the basis of the level of genetic diversity (Collard et al. 2003). Generally, cross pollinating species have high levels of DNA polymorphism in comparison to inbreeding species. However, most linkage maps in plants have been constructed using segregating population issued from crossing inbred lines because they are easy to construct and not time consuming: (F2) populations derived from F1 hybrids or back cross populations (BC) generated by crossing an F1 hybrid to one parent. For cross pollinating species, among forest and fruit species, which mostly do not tolerate inbreeding, obtaining such population is difficult (Venkateswarlu et al. 2006). For heterozygous woody perennial species like Eucalyptus (*E. grandis* W. Hill ex Maiden and *E. urophylla* S.T. Blake), Apple (*Malus Domestica*), Maritime Pine (*Pinus pinaster* Aiton) and Olive (*Olea europaea* L. *ssp. europaea*) F1 full sib mapping population could be compared to an F2 or BC generation mapping population of inbred (Scott et al. 2005; Dondini et al. 2004; Chagné et al. 2002). The strategy commonly used to map such population is the two-way pseudo- test cross (Grattapaglia and Sederoff, 1994). For quantitative genetics studies, plant populations sizes ranges from 50 to 250 individuals (Collard et al. 2005).

DNA Markers

Complex trait analysis was greatly enhanced by the establishment of large collections of genetic markers. Their use was effective not only for genetic maps construction but also for assessing the genetic diversity level of both wild and domesticated species (Doerge, 2002; Collard et al. 2005). DNA markers are the most widely used type of genetic markers. They are useful if they discriminate individuals of the same or different species by DNA polymorphism detection. Unlike morphological and biochemical markers i.e. phenotypic trait and Allozymes, DNA markers are stable, numerous and detectable in all tissues regardless of the developmental state of the cell. In addition, they are not influenced by environmental, pleiotropic and epistatic factors effects (Winter and Kahl, 1995; Jones et al. 1997; Collard et al. 2005; Mondini et al. 2009). Molecular markers reveal virtually, neutral sites of variation at the DNA sequence level. These variations arise from DNA mutations; rearrangements or errors in replication of tandem DNA repeats (Paterson, 1996). Molecular markers do not

affect the phenotype, they can be anonymous or of known sequence; coding or non-coding, based on their length or on variations in their sequence and they can be dominant or co-dominant. Co-dominant markers can discriminate between homozygous and heterozygous individuals whereas dominant markers do not. These latter markers have only two alleles and are either present or absent while co-dominant markers shows differences in size and could have several alleles. In general, an ideal molecular marker is characterized by the following properties (Mackay, 2001; Rungis et al. 2005; Mondini et al. 2009):

- ✓ Highly polymorphic nature
- ✓ Co-dominant inheritance
- ✓ Frequent occurrence in genome
- ✓ Require no prior information about the genome of an organism
- ✓ Selective neutral behaviour
- ✓ Generate multiple and independent markers
- ✓ Easy access (availability)
- ✓ Easy and fast assay with small tissue and DNA quantity required
- ✓ High reproducibility
- ✓ Transferability of data between laboratories, populations, pedigrees and species

Molecular markers can be classified into three groups according to method of analysis: (1) Hybridization-based; (2) Polymerase chain reaction (PCR)-based markers and (3) DNA sequence-based markers (Jahufer et al. 2003; Semagn et al, 2006; Mondini et al. 2009). Markers belonging to each of these groups as well as new generation systems like genome scanning for expressed genes are compared in Table 1. Among them, Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSR) and Amplified Fragment Length Polymorphism (AFLP) are the most commonly used for QTL analysis in olive (See Box 1 for definitions). In the following section, I will focus on the type of markers developed in the context of this thesis:

Simple Sequence Repeats (SSR) or microsatellites

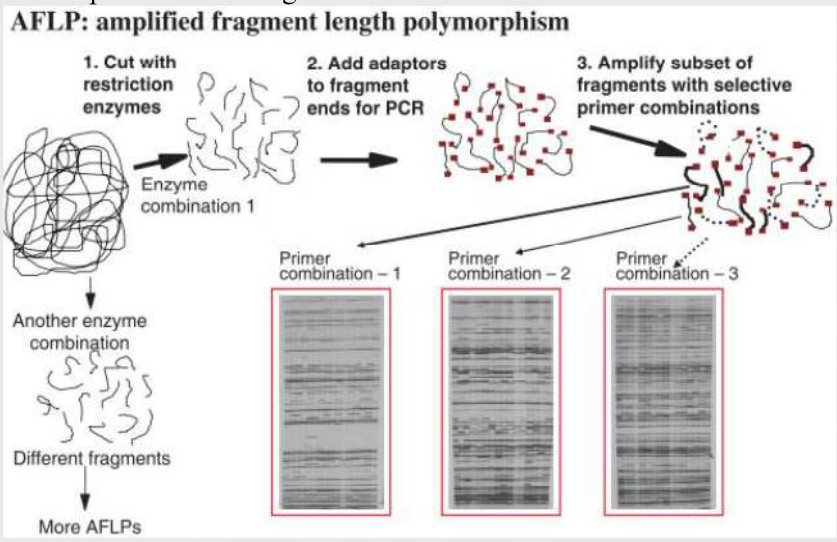
The great value of microsatellites arises from their multi-allelic nature, co-dominant inheritance, relative abundance, extensive genome coverage and simple detection by PCR using two unique primers that flank the microsatellite and hence define the microsatellite locus (Powell et al. 1996). They are relatively rare in protein-coding regions but constitute a large fraction of non-coding DNA (Li et al. 2002). The high polymorphism found in SSR markers is due to their high mutation rate.

Box 1: Molecular markers technologies used for mapping: Vos et al. 1995; Vignal et al. 2002 ; Semagn et al. 2006 ; Meudt & Clarke, 2007; Jones et al. 2009; Mondini et al. 2009.

Restriction fragment length polymorphism (RFLP): are restriction-hybridisation-based markers Based on DNA fragmentation by bacterial enzymes called 'Restriction endonucleases'. The resulting restriction fragments are separated according to their length by gel electrophoresis.

Random Amplified Polymorphic DNA (RAPD): are the first PCR-based markers used in genetic variation analysis (Williams et al. 1990). They are generated through random amplification of genomic DNA using a short primer (10 arbitrary nucleotides). A set of DNA fragments of various sizes will be generated during PCR reaction.

Amplified fragment length polymorphism (AFLP): developed by Vos et al. (1995), this technique is based on detecting polymorphism among genomic restriction fragments. Fragment length polymorphisms are detected combining DNA digestion through restriction endonuclease enzymes and PCR amplification of fragments methods:



Simple sequence repeat (SSR or microsatellite): They consist on repeated sequences of 2-6 base-pairs long found within eukaryotic genomes. They may be dinucleotides (AC)n, (AG)n, (AT)n; trinucleotides (TCT)n, (TTG)n; tetranucleotides (TATG)n, etc, where n is the number of repeating units within the microsatellite locus. Polymorphism associated with a specific locus is due to the variation in length of the microsatellite:

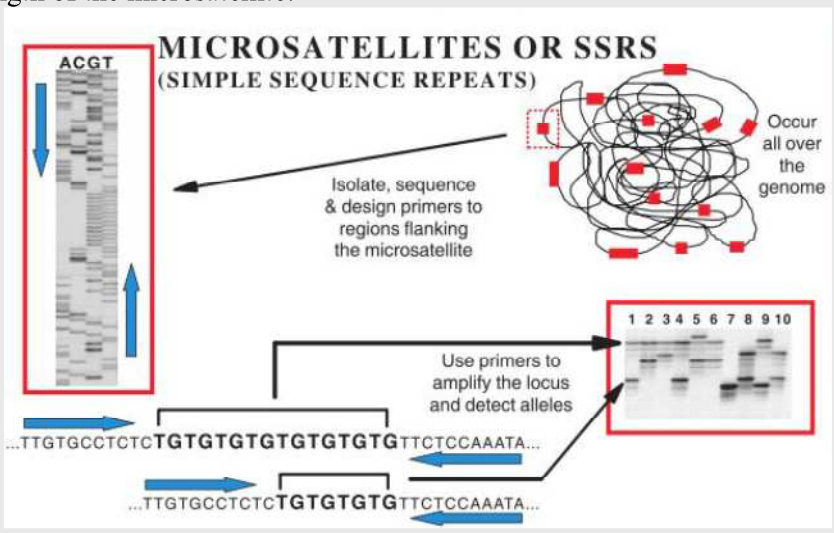


Table 1. Advantages and disadvantages of molecular markers classified according to the method of their detection (Jones et al. 2009)

Marker system	Advantages	Disadvantages
First-generation markers based on restriction fragment detection		
Restriction fragment length polymorphism (RFLP)	Co-dominant; highly reproducible.	Low multiplex ratio*; high on time/labour.
Second-generation markers based on PCR		
Cleavage amplification polymorphism (CAP)	Insensitive to DNA methylation; no requirement for radioactivity.	Produces informative PCR products.
Random amplified polymorphic DNA (RAPD)	Low on time/labour; medium multiplex ratio*.	Dominant; low reproducibility.
Amplified fragment length polymorphism (AFLP)	High reproducibility; high muliplex ratio*.	Dominant; moderate time/labour.
Sequence-specific amplification polymorphism (S-SAP)	Applicable for targeting any gene, transposon or sequence of interest.	Sequence must be known to enable design of element-specific PCR primers.
Simple sequence repeat (microsatellite) (SSR)	Co-dominant; highly reproducible; low on time and labour.	High cost of development; low multiplex ratio*.
Inter-simple sequence repeat (ISSR)	Technically simple; no prior genomic information needed to reveal both inter- and intraspecific variation.	Dominant markers; band staining can be weak.
Variable number tandem repeat (minisatellite) (VNTR)	Numerous multiallelic loci.	Low-resolution fingerprints in plants.
Sequence tagged sites (STS)	Co-dominant; useful for mapping.	Reproducibility; based on some degree of sequence knowledge.
Sequence characterised amplification region (SCAR)	May be dominant or co-dominant; better reproducibility than RAPDs.	More difficult to reproduce than RAPDs.
Sequence amplification of microsatellite polymorphic loci (SAMPL)	High multiplexing*; co-dominant markers; extensive polymorphism.	Some blurred banding; stutter bands.
Third-generation markers based on DNA sequencing		
Single nucleotide polymorphism (SNP)	Common; evenly distributed; detection easily automated; high throughput; low assay cost; useful for association studies; potentially high multiplex ratio*.	Usually only two alleles present.
Genome scanning for expressed genes		
Expressed sequence tag (EST)	Easy to collect and sequence; reveals novel transcripts; good representation of transcripts.	Error-prone; isolation of mRNA may be difficult.
Sequence-related amplified polymorphism (SRAP)	Simplicity; high throughput; numerous co-dominant markers; high reproducibility; targets coding sequences; detects multiple loci without previous knowledge of sequence information; PCR products directly sequenced.	Detects co-dominant and dominant markers, which can lead to complexity; null alleles detected directly.
Target recognition amplification protocol (TRAP)	Simple to use; highly informative; produces numerous markers by using existing public EST databases; uses markers targeted to a specific gene.	Requires cDNA or EST sequence information for primer development.
Markers using array technology		
Microarrays (arrangements of small spots of DNA fixed to glass slides)	Whole-genome scanning; high-throughput technology; genotype–phenotype relationship; expression analysis of large numbers of genes.	Expensive; needs gene sequence data; technically demanding.
Diversity array technology (DArT)	No sequence data required; high throughput; detects single base changes and indels; rapid germplasm characterization.	Dominant markers; technically demanding.
Other marker systems		
Single-strand conformational polymorphism (SSCP)	Detects DNA polymorphisms and mutations at multiple sites in DNA fragments.	Temperature-dependent; sensitivity affected by pH.
Denaturing gradient gel electrophoresis (DGGE)	Separates individual sequences from a complex mixture of microbes based on sequence differences.	PCR fragment size limited to about 500 bp; difficult to resolve fragments that differ by only one or two bases.
Temperature gradient gel electrophoresis (TGGE)	Almost identical to DGGE; more reliable; uses temperature gradient.	Technically demanding; little used in plants.
Methylation-sensitive PCR	Detects sites of methylated DNA.	

*The multiplex ratio is the number of independent loci detected in the assay.

In Olive, as observed in many plant species (48- 67%), the majority of SSRs found so far are di-nucleotide repeats, due to their high frequency in genomes (Rallo et al. 2000, 2003; Sefc et al. 2000; Carriero et al. 2002; Cipriani et al. 2002; De La Rosa et al. 2002; Diaz et al. 2006; Sabino Gil et al. 2006). The most frequently observed motif in plants are (AT)/(TA) and (AG)/(CT) (Wang et al. 1994). In Olive, they are mainly di-nucleotide (AC)/(GT) repeat SSRs (Morgante and Olivieri, 1993). Di-nucleotide microsatellites raise some genotyping difficulties as they require precise protocols to identify properly neighbouring alleles and attribute their lengths. When same loci were compared, 83% of allele size assignment differences among laboratories were reported (Weeks et al. 2002; Amos et al. 2007; Rekik et al. 2008; Baldoni et al. 2009).

SSR markers have proved valuable in Olive for framework map construction and cultivar fingerprinting. In fact, the discrimination power of a few SSR markers was sufficiently high to distinguish more than 100 olive genotypes (Sarri et al. 2006; Muzzalupo et al. 2008; Baldoni et al. 2009). In the following paragraph, SSR development methods are detailed:

Methods for obtaining SSR flanking primers

The traditional method for developing SSR-markers involves the creation of a small-insert genomic library, the subsequent screening with tandemly repeated oligonucleotides and the sequencing of positive clones. The majority of SSRs developed in Olive were identified using this method (Rallo et al. 2000; De La Rosa et al. 2002; Diaz et al. 2006). However, this process has a low rate of SSRs recovery and is expensive, time consuming and laborious (Rassmann et al. 1991; Zane et al. 2002; Thiel et al. 2003).

With the increasing information available in DNA sequence databases, polymorphisms associated with these sequences have been detected, including SSR markers. An alternative method for SSRs development thus arised from Expressed Sequence Tags (EST) sequences exploitation. ESTs are sequenced portions of complementary DNA copies of mRNA. In dicotyledonous species, DNA sequence analyses revealed a range from 2.65 to 16.82% of ESTs containing SSR motifs (Kumpatla and Mukhopadhyay, 2005). Up to now, 7883 Olive EST sequences are publicly available on NCBI database (July 2012: http://www.ncbi.nlm.nih.gov/nucest).

In this study, I relied on a data base of EST sequences to develop a set of new SSR markers that will be used in linkage mapping construction.

In silico data mining for the development of EST-derived SSR-markers starts with the removal of polyA and polyT stretches corresponding to polyA-tail in eukaryotic mRNA (Figure 12). EST sequences of less than 100 bp are not considered and redundant ones are clustered using CD-HIT software (Li and Godzik, 2006). The screening of microsatellites can be accomplished by a microsatellite MISA PERL 5 Script (available at http://pgrc.ipk-gatersleben.de/misa/) or can be done using SAT software (Dereeper et al., 2007). The pipeline for SSR detection was developed using the script SSRIT developed in Perl by Temnykh (http://www.gramene.org/db/searches/ssrtool). Once SSR-containing ESTs have been identified, primer design can be performed using Primer3plus (Rozen and Skalestsky, 2000) software (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi/).

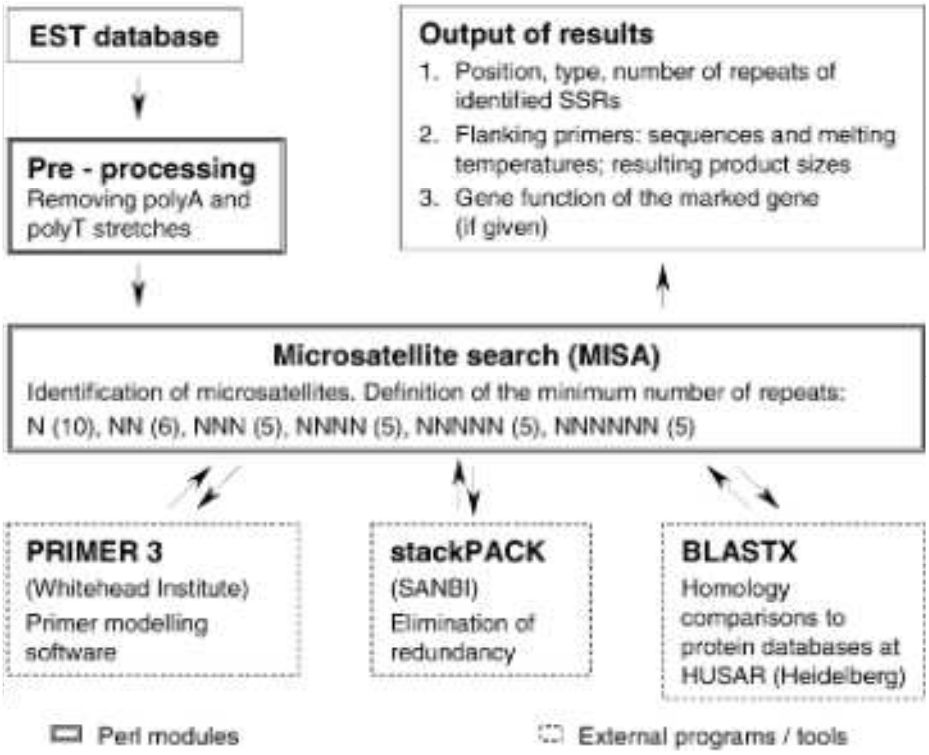


Figure 12. Development of EST-derived SSR markers. Primer 3, stackPACK and BlastX are complementary Softwares supporting primer sequences design the search for a non-redundant set of SSRs and data about putative functions (Thiel et al. 2003).

Polymerase chain reaction amplification and visualisation of microsatellites

The developed primers pairs will be screened using the cross parents for their effectiveness to amplify SSR fragments of the expected size and to detect allele polymorphism. Then, the effective primer pairs from the screening will be genotyped on the entire progeny. SSRs can be analysed by gel electrophoresis coupled with staining silver or as used in this study, they can be analysed through a capillary sequencer with an economic labelling method of PCR fragments (Schulke, 2000).

Linkage Map Construction

Linkage maps show the position and the relative genetic distances between markers along chromosomes (Collard et al., 2005). A major step in linkage maps construction is the identification of polymorphic DNA markers i.e. showing polymorphism between the parents of the mapping population (Young et al., 1994). These markers will be genotyped across all the progeny afterwards (Figure 13). Markers data will be coded on each progenies in order to analyse their linkage using software programs like: JoinMap (Stam, 1993); Map Manager QTX (Manly et al., 2001) and Mapmaker EXP (Lander et al., 1987; Lincoln et al., 1993). Markers usually segregate in a Mendalian fashion, however, distorted ratios can be found. The significance of these deviations can be determined using a chi-square analysis (Sayed et al., 2002; Collard et al., 2005). Linkage between markers is estimated using logarithm of odds ratios (linkage vs no linkage), (Rish, 1992):

$$\text{LOD} = \log_{10} \frac{\textit{Likelihood of presence of genetic linkage}}{\textit{Likelihood of absence genetic linkage}}$$

LOD values >3 are usually used. A LODScore of 3 indicates that linkage is 1000 times more likely than no Linkage; a LOD of 4: 10000 times.

Markers will partition the mapping population into linkage groups representing a chromosome or a chromosomal segment. The adequacy of genetic distance and marker order estimation depends on the size of the mapping population (Collard et al., 2005). Linkage map calculation can be done by a regression algorithm or a maximum likelihood algorithm (See Box 2 for details).

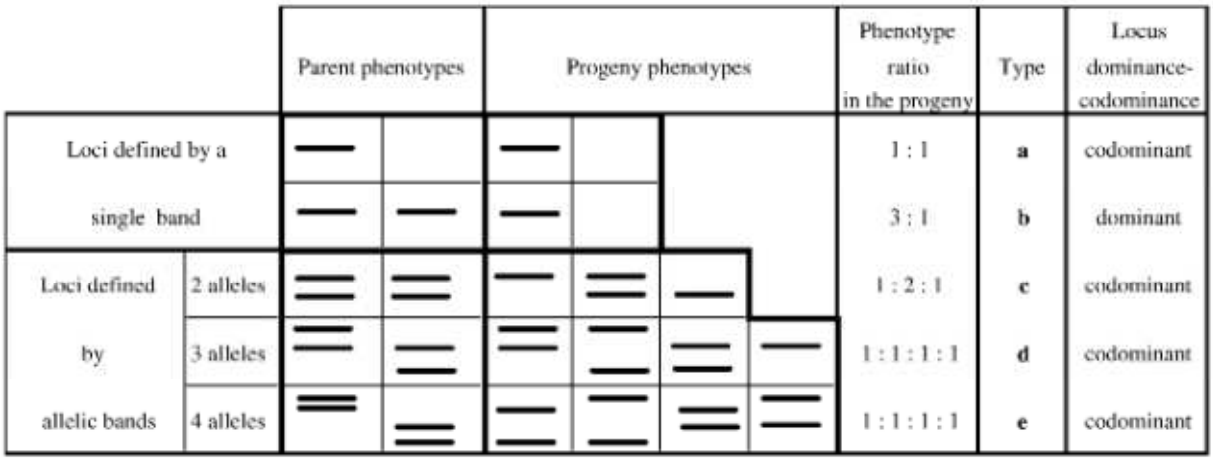


Figure 13. Representation of the five segregating loci configurations than can be found in a F1 progeny as defined by Ritter et al. (1990). Two classes of loci are presented: loci defined by a single band for which one segregating allele is found and loci defined by different segregating alleles up to 4 different parental alleles (Lespinasse et al. 2000).

Genetic distances along linkage groups are inferred from the frequency of recombination between markers. The higher the frequency of recombination between two markers, the further away they are situated on a linkage group. Genetic distances between markers on a chromosome are expressed in Morgan or more commonly in centiMorgans (cM). This unit is defined as the distance along which one recombination event is expected per gamete and per genotype (Doerge, 2002). Map distances <10 cM, is considered equal to the recombination frequency. However, this not supported for distances greater than 10 cM (Paterson, 1996; Hartl and Jones, 2001). The mapping functions used for converting recombination fractions into centiMorgans (cM) are the Kosambi and Haldane mapping functions. The Haldane function, assumes no interference between recombination events while Kosambi function assumes that recombination events influence the occurrence of adjacent crossovers (Collard et al., 2005). The frequency of recombination is not equal along chromosomes, there are recombination 'hot spots' and 'cold spots' i.e. chromosomal regions with more or less frequent recombination events (Yao et al. 2002; Ma et al. 2001; Hartl and Jones, 2001; Young, 1994). Moreover, markers can be clustered in some chromosomes regions and be absent in others. These factors make obtaining an equal number of linkage groups and chromosomes difficult (Paterson, 1996).

Box 2: Linkage map calculation methods:

For genotype data populations, linkage map calculation can be done using a regression or a maximum likelihood algorithm, both available in JoinMap 4, (Stam, 1993). With a regression mapping procedure, maps are built by adding loci one by one starting from the most informative pair of loci according to LOD and recombination frequency thresholds. The method of calculation is a linear regression (weighted least squares procedure) with squares of the independence LODs as weights. For each pair of loci, two distances measures are estimated as recombination frequencies (1) the direct recombination frequency estimated from the genotype data of the two loci considered (2) the derived recombination frequency estimated from the map with an inverse mapping function. Best loci positions were found by comparing all direct recombination frequencies with the map derived recombination frequencies. This ratio is called goodness-of-fit measure which is a G^2 likelihood ratio statistic expressed as a chi-square value. A large jump in goodness-of-fit i.e. normalized difference in the goodness-of-fit chi-square estimated before and after adding loci indicates poor fit of added markers expressed by a large chi-square value (Van Ooijen et al. 2006). A threshold value for the jump ranging from 3.0 to 5.0 is used to maintain or not a locus during map building process.

As the speed of regression mapping algorithm become worsen when more than 50 loci are mapped on a linkage group, a more efficient multipoint maximum likelihood based algorithm was developed. It combines Gibbs sampling, spatial sampling and simulated annealing procedures to compute loci order and mutual distances (Jansen et al. 2001).

Gibbs sampling: is a Monte Carlo Expectation Maximization method used to estimate the maximum likelihood multipoint recombination frequencies (Dempster et al. 1977).

Spatial sampling: in case of missing information or genotyping errors, this technique allows reaching the global optimum solution.

Simulated annealing: is a general Monte Carlo optimization method used to find the map order that has the maximum likelihood. The optimization criterion is the sum of recombination frequencies in adjacent map segments (Van Ooijen et al. 2006).

According to the two-way pseudo- test cross mapping strategy, parental linkage maps are first constructed on the basis of the markers that are polymorphic in each parent. Then, a third linkage map is an integrated linkage maps or consensus (Figure 14) in which all polymorphic

markers are used: markers segregating only in one parent and markers segregating in both parents (Grattapaglia and Sederoff, 1994).

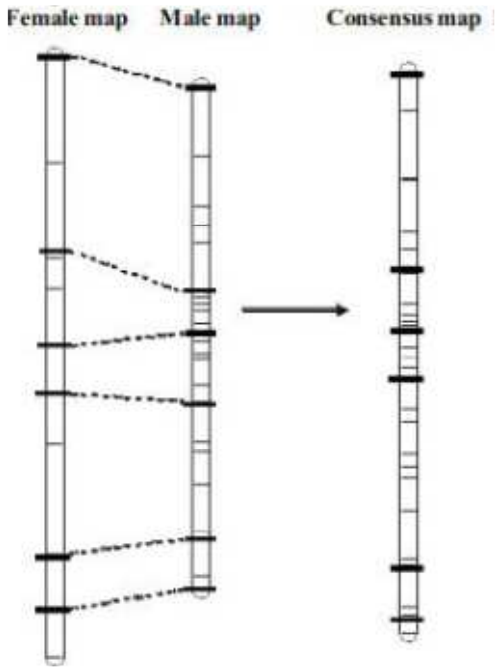


Figure 14. Representation Construction of a consensus map: Male and female maps have been aligned using markers in common. Common markers among the maps are indicated by a bold line ▬ and markers specific to each map are indicated by a simple line ⎯ (courtesy from JM. Celton).

Three linkage maps of the olive genome have been developed so far (De la Rosa et al. 2003; Wu et al. 2004 and Khadari et al. 2010) based on 'Leccino' x 'Dolce Agogia' ; 'Frantoio' x 'Kalamata' and 'Olivière' x 'Arbequina' segregating population respectively. The characteristics of each map are presented in Table 2. Wu et al. (2004) estimated the genomic sizes of 'Frantoio' and 'Kalamata' as 3427 and 2614 cM, respectively, based on their partial linkage data. Therefore, for the olive species, the estimated genomic size should be around 3000 cM.

Table 2. Comparison between the available olive genetic maps (Source: Khadari et al. 2010)

Crossed cultivars	Population size	LOD threshold[a]	Maximum recombination fraction	Linkage group (no.)	Map length (cM)	Mapped loci (no.)	Mapped SSR (no.)	Mean distance between loci (cM)	Mean size of linkage group (cM)
Frantoio	104	3	0.49	27	798	92	3	12.3	29.5
Kalamata	104	3	0.49	23	759	89	1	11.5	33.0
Integrated map	104	3	0.49	15	879	101	5	10.2	58.6
Leccino	100	4	0.30	39	2765	249	3	13.2	70.9
Dolce agogia	100	4	0.30	30	2445	236	4	11.9	81.5
Integrated map	—	—	—	—	—	—	—	—	—
Olivière	147	6	0.40	34	2210.20	197	34	11.2	61.3
Arabequina	147	6	0.40	31	1966.20	191	30	10.3	63.4
Integrated map	147	6	0.40	42	3823.20	436	26	8.7	91.0

QTL detection method

It is essential for QTL analysis to access reliable estimates of the quantitative traits of interest for each individual in the mapping population (or progeny). This involves the establishment of a randomized experimental design in which multiple replications of each individual among the population is measured. Growing conditions and records assessment must be as uniform as possible across the progeny especially when measurements are repeated over several scales, years and environmental conditions (George et al. 2003; Hittalmani et al. 2002). Once phenotypic data sets are collected, the question is to find whether or not these traits are governed by genes.

Several programs have been developed for detecting QTL including MAPMAKER/QTL (Lincoln et al. 1992), QTL Cartographer (Basten et al. 1997; Wang et al. 2011), Map Manager QT (Manly, 1997; Manly and Elliott 1991), RQTL (Broman et al. 2003), MCQTL (Jourjon et al. 2005) and MapQTL (Van Ooijen et al. 2006).

I will focus here on the three methods I used in my QTL analysis with MapQTL® 6, namely: single-marker analysis, simple interval mapping and multiple-QTL model mapping (Liu, 1998; Tanksley, 1993; Van Ooijen et al. 2006).

Single-marker analysis

Single-marker analysis is appropriate for non normally distributed data. This test investigates the segregation of a phenotype with respect to marker genotype and point to the existence of an association. Each marker locus is tested independently without requiring their positioning in genetic map order. The null hypothesis is that the mean value of the tested trait is independent of the genotype at a particular marker. This hypothesis is rejected when significant differences ($P<0.05$) are detected between mean trait values with respect to the marker locus tested.

Single-marker analysis can be performed with standard statistical analysis softwares by *t*-tests, analysis of variance (ANOVA) and linear regression. Linear regression method is widely employed as it provides an estimation of the phenotypic variation explained by the QTL linked to the marker by an R^2 coefficient of determination.

Association between marker and QTL can also be detected using a nonparametric test. It is a rank sum test of Kruskal-Wallis performed on each locus separately without any use of the linkage map other than for sorting the order of loci on the linkage groups. No assumptions are

made for the probability distribution of the quantitative trait so that, traits showing a discrete distribution can be analyzed. The test ranks all the individuals according to the quantitative trait, while it classifies them according to their marker genotype. A statistic test K* is calculated afterwards based on the ranks in the genotype classes (Lehmann, 1975). A segregating QTL tightly linked to the tested marker will result in large differences in average rank of the marker genotype classes and corresponding linkage group will have a significant K* test (Van Ooijen, 2006).

Detecting QTL with single-marker analysis implies to consider a large number of individuals in order to observe recombinant events and estimate reliable statistics. An issue with this method is that the further a QTL is from a marker, the less likely it will be. This disadvantage can be managed using a large number of markers covering the entire genome detected (Tanksley, 1993; Doerge, 2002).

All of these methods do not provide an estimation of QTL position or recombination frequency between the QTL effect and the marker. That's because the QTL location and effect are confounded. They are considered as efficient means to screen rapidly large populations for specific traits addressing primarily individual markers instead of genomic regions. In order to overcome this limitation, is fixing incrementally the QTL location and estimate the QTL effect between intervals of markers. This method leads to the estimation of both QTL position and effect and is called: interval mapping.

Simple Interval mapping

The interval mapping (IM) method, developed by Eric Lander and David Botstein (1989) uses an estimated genetic map as the framework for the location of QTL (QTL likelihood map). The intervals between adjacent pairs of linked markers along chromosomes are searched in increments of 1 cM for example i.e. it tests statistically for a single QTL at each increment across the ordered markers in the genome in a linear way. The use of linked markers gives more power to the statistical test compared to single point analysis as it compensate for recombination between the markers and the QTL (Lander and Botstein, 1989; Liu, 1998; Doerge, 2002; Collard et al. 2005). The test compares the estimation of the likelihood function under the null hypothesis (no QTL) with the alternative hypothesis (presence of a segregating QTL at the tested position) in order to locate a QTL. Results are expressed as LOD (logarithm of the odds) score. A significance threshold of the LOD score is calculated

by a resampling method called 'permutation test' in order to appreciate the significance of the QTL detected (Churchill and Doerge, 1994). In each of permutation, the trait values are permuted i.e. sampled without replacement over the progenies while the marker loci remain fixed. This destroys the relationship between trait and markers. Over 1000 to 10000 permutations, an estimate of the frequency distribution of the maximum LOD score under the null hypothesis (no QTL) is given per linkage group and over the whole set of linkage groups (genome wide). To determine the significance threshold, a P-value is determined for each linkage group (generally 0.05) and the LOD corresponding to this value is taken as the threshold (Van Ooijen, 2006). It depends on our strategy to choose between using individual linkage group or genome wide threshold. When the LOD score exceeds the significance threshold it reflects the presence of segregating QTL on the corresponding linkage group. The estimated position of the QTL on the map corresponds to the position with the largest LOD on the linkage group.

Although interval mapping method is more powerful than single-marker mapping, it has some limits regarding its one dimensional screening that does not take into account interactions between multiple QTLs. As it is a single QTL model, the power of detection as well as the estimates of QTL location and effects can be biased with the possible interfering effect of QTL located else where in the genome and ghost QTL i.e. non existing QTL appearing (Lander and Botstein, 1989; Knapp, 1991, Haley and Knott, 1992; Jansen, 1993; Martinez and Curnow, 1992).

MQM mapping: Multiple-QTL model mapping

Using true multiple-QTL model to identify and map QTL implies multidimensional search over linkage groups which is computationally not really feasible. The theoretical framework of MQM or Composite Interval Mapping (CIM) was introduced and explored by Ritsert Jansen (Jansen, 1993; Jansen, 1994) and Jansen and Stam (1994). It has several advantages of which (1) higher power to detect linked as well as unlinked QTL, as long as the QTL explain a reasonable amount of variation (2) detection of negative QTL (QTL in repulsion phase), (Jansen, 1994).

Although the definition of MQM mapping is very large, I will focus here in the implementation in MapQTL® 6. Markers close to detected QTL (by interval mapping) are selected as cofactors to take over the role of the nearby QTL in the approximate multiple-QTL model used in the subsequent MQM mapping with additive and dominant gene actions only.

With this MQM mapping, a search over the genome is performed by testing for a single segregating QTL, while simultaneously fitting the selected cofactors, both under H0 and H1. The use of linked markers as cofactors will reduce the residual variance of the QTL already detected and will enhance the power in the search for other segregating QTL if a QTL explains a large proportion of the total variance (Jansen, 1993; Zeng, 1993 and 1994). Several rounds of MQM mapping may be necessary to obtain the best possible final solution, and cofactor markers can be added or dropped according to the latest results (Van Ooijen, 2006). As for interval mapping, the results of this test are presented using LOD score.

QTL complementary analysis

Considering that interactions among multiple gene loci are frequent in most genetic systems testing the QTL detected for epistasis (i.e. interaction between two or more different gene loci controlling a single phenotype) is essential. For that purpose, a regression model is employed to test the significance (P-value) of interactions between significant genetic markers. The lower is the P-value, the higher is the probability that a QTL truly exists in the region of the marker. The global phenotype variability explained by the QTLs (global R^2) is then estimated from the model minimizing the AIC and BIC criterion. The proportion of total phenotypic variation explained (R^2) at the marker enlightens the QTL importance in influencing the trait.

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RESULTS

In this section, results are presented under the format of three scientific articles:

1- **Genetic determinism of the vegetative and reproductive traits in an F1 olive tree progeny: Evidence of tree ontogeny effect**

- Identification of the most heritable growth and branching traits (i.e. topological and geometrical) :
  - Investigation of the changes in primary growth and branching variables during tree ontogeny: analysis of the first five years of tree growth.
  - Study of the genetic effects on each studied variable.
- Identification of the most suitable observation scale (i.e. whole tree; growth unit; sympode) and ontogenetic phase for capturing the genetic effect in order to develop an optimized phenotyping methodology adapted to the specificities of the olive tree.

2- **Studying genotype-by-environment interactions affecting the vegetative growth in an F1 olive tree progeny during its juvenile phase**

- Analysis of the three first years of growth and branching traits recorded in two contrasted environments: Melgueil (France) and Cordoba (Spain).
- Evaluation of the traits covariation within and across environments.
- Description of tree ontogeny considering the two environments studied separately and jointly.
- Study of the genetic and genotype-by-environment effects on each trait and estimation of broad sense heritability values.
- Evaluation of the progenies stability between environments and identifications of interesting traits combinations.

3- **QTL mapping of flowering and fruiting traits**

- Investigation of the changes in flowering and fruiting traits according to their localisation within the floral GUs and the years:
  - Analysis of the first 3 years of the mature phase of the progeny.
- Identification of different production pattern within the progeny:
  - Analysis of the Fruit weight/tree within the progeny over four years.
- Study of the genetic effects on each trait and estimation of broad sense heritability values.
- Mapping and QTLs Detection:
  - Development of new microsatellites markers (SSRs) in order to saturate the initial linkage map (Khadari et al. 2010).
  - QTL mapping on the new genetic map.

Chapter 1

Genetic determinism of the vegetative and reproductive traits in an F1 olive tree progeny: Evidence of tree ontogeny effect

This chapter present the article first published in Tree Genetics and Genomes Journal.

Investigated questions:

- ***Is the olive tree structure and architectural features depends on 'morphogenetic gradients'? If yes:***
  - ***What are they?***
  - ***On which traits they impacts significantly?***
  - ***How they interact with the genetic determinism?***
- ***Can we propose a methodological framework to simplify the architectural phenotyping strategy that account for the architectural variability of the olive tree?***
- ***Is the traits related to the vegetative development are heritable?***
- ***Are there sources of transgressive forms for innovative material within the progeny?***

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ORIGINAL PAPER

Genetic determinism of the vegetative and reproductive traits in an F1 olive tree progeny

Evidence of tree ontogeny effect

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Abstract The agronomic performance of fruit trees is significantly influenced by tree internal organization. Introducing architectural traits in breeding programs could thus lead to select new varieties with a regular bearing and lower input demand in order to reduce training and environmental costs. However, an interaction between tree ontogeny and genetic factors is expected. In this study, we investigated the genetic determinism of architectural traits in the olive tree, accounting for tree development over 5 years until first flowering occurrence. We studied an F1 progeny issued from a cross between two contrasted genotypes, 'Olivière' and 'Arbequina'. Tree architecture was decomposed in quantitative traits, related to (1) growth and branching, (2) first flowering and fruiting. Models, including the year of growth, branching order and genotype effects, were built with variance function and covariance structure when necessary. After a model selection, broad sense heritabilities were calculated. During the first 3 years, both the mean values of vegetative traits and genetic factor significance depended on the shoot within-tree position. Dependencies between consecutive years were revealed for traits related to whole tree form. Whole tree form variables showed medium to high broad sense heritability values, whereas reproductive traits were highly heritable. This study demonstrates the existence of ontogenic trends in the olive tree, which result in traits heritable only at the tree periphery. A phenotyping strategy adapted to its architectural characteristics and a list of relevant traits, such as maximal internode length, is proposed. Transgressive effects suggest that genetic progress could be performed in future selection programs.

Keywords Heritability · Phenotyping · Tree architecture · Growth · Branching · Flowering · Fruiting

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Introduction

Plant architecture is of major importance in cultivation management and productivity enhancement. This is particularly true in fruit trees species, for which productivity is closely linked to tree development over years (Costes et al. 2006). Trees internal organization and volume in orchard have been shown to influence their light interception efficiency, fruit quality and yield regularity (Jackson 1980; Lauri et al. 1997; Maguylo and Lauri 2004). The integration of architectural traits in breeding programs is thus a promising way to improve

the control of vegetative development and bearing regularity (Laurens et al. 2000; Costes et al. 2004). Several studies have been performed on the genetic determinism of fruit tree growth traits on young seedlings (de Wit et al. 2004; Hammami et al. 2011). However, on perennial species, the traits of agronomic interest occur after several years, usually at the end of the juvenile period (Hackett 1985), when the trees have reached a high level of structural complexity. Due to the high number of individuals that must be described for studying trait heritability and performing quantitative trait loci (QTL) analysis, an efficient phenotyping strategy is required to perform quantitative genetic studies on such species. A first strategy has been proposed in apple tree (Segura et al. 2006, 2007), in which plant architectural analysis, as initially defined by Hallé et al. (1978) and further developed in a range of forest and fruit tree species — oak (Heuret et al. 2003), peach (Fournier et al. 1998), apple (Lauri et al. 1997; Costes et al. 2003; Renton et al. 2006), walnut (Sabatier and Barthélémy 2001) — provided the conceptual framework. The proposed strategy was to dissect tree architecture in a number of quantitative variables related either to the tree constituent's organization (i.e., the plant topology) or to the dimensions and spatial location of organs (i.e., the plant geometry; Godin 2000). Variables were classified in growth and branching traits, including reproductive organs organization, and were collected on different shoot types that resulted from different growth and elongation characteristics leading to axes polymorphism often observed in fruit trees (Champagnat 1965; Zimmerman and Brown 1971). More generally, different shoot types can be considered with respect to lengths, differentiation states, i.e., vegetative and floriferous and branching which can be terminal or lateral, monopodial or sympodial depending on the species (McManus and Veit 2002; Barthélémy and Caraglio 2007).

Studying the genetic determinism in a perennial crop requires the estimation of the different effects that interact during tree architectural development. Indeed, plant architecture results from a balance between endogenous growth processes and environmental constraints (Hallé et al. 1978), and architectural traits change over time due to tree ontogeny (Barthélémy and Caraglio 2007). In fact, the structure of axes depends on their topological and ontogenetic position within the tree structure: annual shoots having a same differentiation stage can be observed at different tree ages and the most differentiated ones are usually located at the highest rank and branching order at the whole tree level (Nicolini 1998; Heuret et al. 2006). In the apple tree, Segura et al. (2008) have proposed to distinguish ontogenetic, climatic and genetic effects in a mixed linear model for repeated data applied after a staggered start experimental design. Nevertheless, the application of a similar strategy based on (1) architectural analysis for identifying shoot types and differentiation stages, (2) decomposition of tree architecture in quantitative variables at different scales and observed over years, and (3) mixed linear modelling for estimating the genetic effects, to other species with different architectural pattern is still challenging. The olive tree (*Olea europaea* L. subsp. *europaea*) constitutes an interesting case study in this prospect. The olive tree is an evergreen perennial species, mainly present around the Mediterranean Basin, where it is one of the oldest and most important fruit trees (Doveri et al. 2008). Its growth is rhythmic in temperate climate, but without winter buds. Its phyllotaxy is opposite decussate. Branching is most often sympodial after terminal growth cessation. Olive flowers either staminate (male) or perfect (hermaphrodite) are born on inflorescences called panicle mostly developed at leaf axils. Their proportion is variable among different inflorescences, branches, cultivars and years of growth (Fabbri et al. 2004; Martin and Sibbett 2005). Moreover, olive trees are predominantly allogamous (Díaz et al. 2006) and have a high genetic variability with more than 2,600 cultivars described (Rugini and Lavee 1992). The lack of genetic knowledge, the natural long juvenile phase (up to 15 years), the difficulties in tree manipulation and the genetic complexity ($2n=46$) for a genome size of about 3,120 Mbp (Loureiro et al. 2007; Khadari et al. 2010), makes olive breeding difficult (Omrani-Sabbaghi et al. 2007).

We assumed that, even though olive tree exhibits additional difficulties than those previously mentioned for the apple tree, in particular the absence of morphological markers to detect the growth cessation, it represents an interesting model on which the previously described strategy could be tested. In the present study, we investigated the genetic determinism of architectural traits in an F1 olive tree progeny derived from a cross between two olive cultivars with contrasted architectural traits. Because the two parents are highly polymorphic and the number of progenies is high, the observed phenotypic variability in this population is assumed to represent a large range of the species phenotypic plasticity. The knowledge acquired on this phenotypic plasticity should help olive cultivation as clonal varieties are the traditional types for olive products (Loussert and Brousse 1978). Giving an overview of olive tree development during the first years of growth, and until first flowering, we investigated major factors impacting tree architecture and the relations between inflorescences localization on shoots and fruit set. This led us to define a strategy based on architectural analysis which takes into account the degree of differentiation of growth units during tree ontogeny. Finally, we carried out a heritability estimation which highlights the most promising traits for further genetic analysis, and provides the foundation for QTL detection in this progeny.

Materials and methods

Genetic material

The progeny under study derived from 'Olivière' × 'Arbequina' cross and counts 160 genotypes. 'Olivière' is a vigorous French cultivar with a fast growth rate, a large fruit and a rapid entrance in production (Moutier et al. 2004b, 2008). 'Arbequina' is a very productive Spanish cultivar with low vigor, adapted for high density, and a small fruit (Rallo et al. 2004). After having optimized the conditions for cutting success in a range of cultivars, i.e., considering the period in the year, the shoot type to be sampled, the cutting method including soil type and humidification techniques (Garcia et al. 2005), a cutting campaign was performed on the whole population and the two parents, in order to obtain at least six replications per genotype. In 2005, six cuttings were chosen as homogeneous as possible for each genotype and the two parents. Progenies and parents were planted at INRA-Montpellier experimental station, according to a six complete block design, where the genotypes were randomly distributed in each block (6×2 m; N/S rows orientation). After plantation, trunks were cut back to 50 cm to homogenize the plantation and trees were not pruned afterwards. Standard irrigation and phytosanitary treatments were carried out all over the experiment.

Architectural description

Phenotyping strategy

Tree description was performed on the first two blocks of the experimental plot because of high phenotyping costs. On a total of 324 trees (two replicates/genotype), tree topology was formalized in a multiscale tree graph (MTG) according to the methodology defined by Godin et al. (1999). The data base contained four different scales: tree, sympode, growth unit (GU) and internode. In the first year of growth (2005), all GUs, whatever the branching order, were observed. Branching orders were incremented from axes developed after cutting, which were considered as order 0. GUs were classified into three types according to their length: long (≥20 cm); medium (5 cm≤20 cm) and short (<5 cm). Topological traits such as the number of internodes (Nb_IN) and the number of sylleptic axillary shoots per GU (i.e., shoots developed immediately without bud resting period, Nb_AS_s) were measured for long and medium GUs. Two geometrical variables were linked to each GU, their length (L) and basal diameter (Bdiam), whereas the length of the longest internode (IN_Max) was noted for each annual shoot. The mean internodes length was deduced from the GU length and number of internodes (Mean IN_L = L/Nb_IN). At the whole tree scale, trunk height (H) and projection of the longest branch on the tree (Proj) were used to calculate tree basis area (B_area = Π Proj²) and volume (V=1/3 * B_area * H).

In the following 2 years (2006 and 2007), a subsample corresponding to the main path of GUs in the tree and along the longest lateral branch was selected within each tree. The same observations than in the first year, i.e., including topology and geometry, were performed on the GUs belonging to the path and at whole tree scale (Fig. 1a). In the fifth year (2009), a last description of the trees was done, collecting at once data on 2008 and 2009 GUs, following the main path of GUs in the tree. Branching records included both sylleptic and proleptic axillary shoots (i.e., developed immediately or after a resting period, respectively). These sylleptic and proleptic axillary shoots were classified depending on their length (Nb_L_s, Nb_M_s, Nb_S_s and Nb_L_p, Nb_M_p, Nb_S_p, respectively). It must be noticed that incremental variables such as basal diameters were not available for 2008 year of growth.

In 2009, the trees had entered into their reproductive period allowing us to initiate the study of their flowering and fruiting behaviour. For this, two 1-year-old GUs (long or medium) were selected at the tree periphery. Each GU was described at both flowering and fruit set periods (Fig. 1b). The number of inflorescences and fruits born along these GUs or along their sylleptic laterals were noted Inflo (or Fruit)_direct and Inflo (or Fruit)_AS_s, respectively. These axillary GUs were classified, as previously, depending on their length (Nb_L_{sF}, Nb_M_{sF}, Nb_S_{sF}), and their total number of internodes was noted. The total number of axillary GUs (Nb_AS_{sF}) and the percentage of fruiting (% Fruiting, % Fruitset_direct, % Fruitset_AS_s) were calculated afterwards. The basal diameter of each floral GU ($Bdiam_GU_F$) was recorded.

All data extraction from MTG files was performed using VPlants software (http://openalea.gforge.inria.fr). Both measured and derived variables (Table 1) were classified according to the observation scale: whole tree and growth units.

Data analysis

First, changes in primary growth and branching variables during tree ontogeny were examined. Two main factors were considered, the year of growth and the branching order. Since the studied variables were measured on the same trees, their values on consecutive years of growth or branching orders may be not independent. Moreover, diameters were measured repeatedly at the basis of trunks or GUs and consisted in an incremental variable. Thus, variances homogeneity and covariance between consecutive years or orders were considered. Due to the lack of the basal diameter measurements for 2008, genetic analysis of trunk basal diameter measurements did not consider this year. However, the analysis was possible at GU scale, using the basal diameter

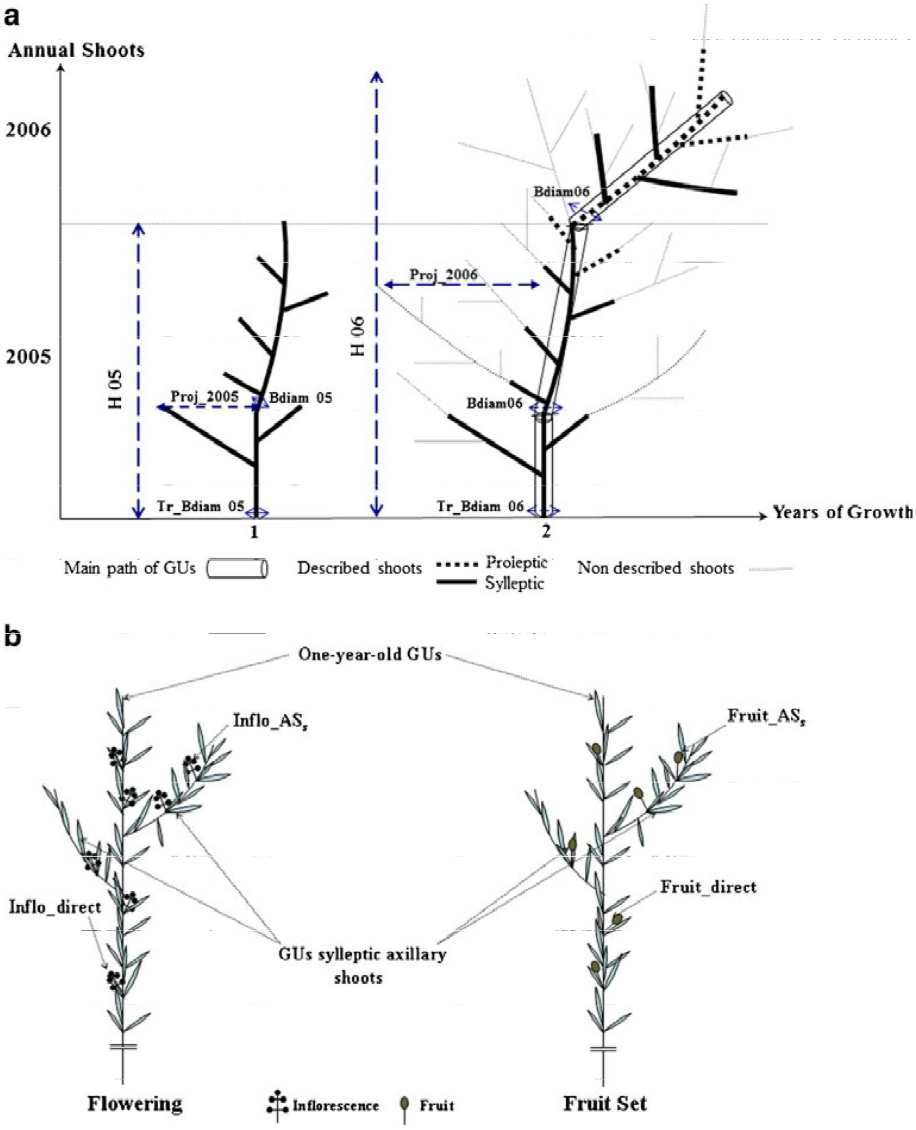

Fig. 1 Schematic representation of a 2-year-old phenotyped olive tree: **a** architectural traits (whole tree form, primary growth and sylleptic vs. proleptic branching traits) collected along the trunk and main path of growth units (GUs) over 5 years; **b** flowering and fruiting traits collected on 1-year-old GUs at flowering and fruit set periods; the number of inflorescences and fruits born along the floral GUs (Inflo(Fruit)_direct) or along a sylleptic lateral (Inflo(Fruit)_AS$_s$) were counted

of 1-year-old floral 2008 GUs measurements. In addition, due to the high number of variables under study and their possible redundancy, correlations between variables were also considered. Non parametric Kendall's tau correlation coefficient was used because it is more convenient for not normally distributed variables (Becker et al. 1988).

Second, we analyzed the genetic effect on each studied variable. Depending on the variable and the experimental unit considered in the tree, different mixed linear models were built. The normality of each variable distribution was checked. When not verified, the corresponding variable was transformed with a square root transformation (distinguished by the index sqrt).

For 1-year-old GUs considered to characterize the flowering and fruiting behaviour in a single year, the following mixed linear model was used:

$$P_{il} = \mu + G_i + \varepsilon_{il} \qquad \text{(model1)}$$

where P_{il} is the phenotypic value of genotype i on the l measured GUs, μ is the overall mean of the progeny, G_i is the random effect of the genotype i, and ε_{il} is the random residual error effect for the l measured growth units.

Flowering and fruiting variables were not normally distributed and were thus transformed for the analysis.

For variables collected at the whole tree level over consecutive years, the effect of genotype and year of growth,

Table 1 List of quantitative variables collected on olive tree topology, geometry, flowering and fruiting traits with detailed formula for calculated variables

Observation scale	Measured and derived Variables	Abbreviations	Formula
Whole tree	Height (m)	H	
	Projection (m)	Proj	
	Basis area (m²)	B_area	$\Pi(Proj)^2$
	Tree Volume (m3)	V	1/3 × B_area × H
	Trunk basal diameter (mm)	Tr_Bdiam	
Growth units	Topology		
	Nb of internode	Nb_IN	
	Nb of sylleptic axillary shoots (from 2005 to 2009)	Nb_AS_s	
	Nb of long, medium, short sylleptic axillary shoots/GU	Nb_L_s; Nb_M_s; Nb_S_s	
	Nb of proleptic axillary shoots (in 2008)	Nb_AS_p	
	Nb of long, medium, short proleptic axillary shoots/GU	Nb_L_p; Nb_M_p; Nb_S_p	
	Geometry		
	Length (cm)	L	
	Mean Internode length (cm)	Mean IN_L	L/Nb_IN
	Length of the longest internode (cm)	IN_Max	
	Basal diameter (mm)	Bdiam	
One-year-old floral growth units	Topology		
	Nb of internodes	Nb_IN_F	
	Nb of sylleptic axillary shoots	Nb_AS_{sF}	
	Nb of long, medium, short sylleptic axillary shoots	Nb_L_{sF}; Nb_M_{sF}; Nb_S_{sF}	
	Flowering and Fruiting		
	Nb of inflorescences (or Fruit)	Inflo (or Fruit) _tot	Inflo(or Fruit) _direct + Inflo(or Fruit) $_AS_s$
	Nb of direct inflorescences (or Fruit)	Inflo(or Fruit) _direct	
	Nb of inflorescences(or Fruit) on sylleptic axillary shoots	Inflo(or Fruit) $_AS_s$	
	id. on long, medium, short sylleptic axillary shoots	$Inflo_L_s$; $Inflo_M_s$ $Inflo_S_s$	
	Percentage of fruiting	% Fruiting	Fruit_tot/Inflo_tot
	Percentage of direct fruit set	% Fruitset_direct	Fruit direct/Inflo_direct
	Percentage of laterals fruit set	% Fruitset_AS_s	Fruit_AS_s/Inflo_AS_s

The index (sqrt) refers to root square transformation of the corresponding flowering and fruiting variables

and their interactions were estimated according to the following model:

$$P_{ijl} = \mu + G_i + Y_j + (G \times Y)_{ij} + \varepsilon_{ijl} \qquad \text{(model2)}$$

where P_{ij} is the phenotypic value of genotype i, at the year of growth j, μ is the overall mean of the progeny, G_i is the random effect of genotype i, Y_j is the fixed effect of the year of growth, $(G \times Y)_{ij}$ is their random interaction and ε_{ijl} is the random residual error effect for the l measured trees.

For architectural variables collected at the GU level over several years, the dataset was unbalanced, because the GUs developed at a given year of growth were located at different branching orders and the number of GUs developed per year and order depended on each tree. Thus, we were not able to test both year and branching order factors and their interaction jointly. As a consequence, different models were considered, including either the year or the branching order effect and their interaction with the genotype. These models

were compared on the basis of Bayes Schwarz information criteria (BIC). Because all selected models included a year effect, only model 2 is presented in the following results.

Variance homogeneity between levels was examined for all studied traits. For traits showing heterogeneous variances, a function of variance was introduced into the model. Different variances functions, as described by Pinheiro and Bates (2000), were compared: varIdent, with different variances per level of the fixed factor; varPower, with variance increasing as a power function; varExp, with variance increasing as an exponential function; varConstPower, combines a constant value with a power function. In the three latter cases, the function parameters were estimated during model fitting. Moreover, because correlations between consecutive years or orders may exist, a covariance structure was taken into account in the residual term of the corresponding mixed linear model. Different covariance structures were tested, i.e., compound symmetry (corComSymm), autoregressive of order 1 (corAR1) and exponential (corExp). For each variable, a model selection was performed on the basis of BIC minimization, which allowed us to select the significant factors, the variance function and the covariance structure to be considered. After this model selection step, the normality of residual distribution was checked.

An additional analysis of the genetic effect was performed at the GU level for the number of internodes, and the length gathering measurements on high branching orders at the tree periphery: from order 5 to order 7 recorded from 2007 until 2009 years of growth. The model used included the year of growth and genotype effects.

Broad-sense heritability of branching, flowering and fruiting traits was estimated as the ratio between the genotypic and the phenotypic variances: $h_b^2 = \sigma_G^2/\sigma_P^2$. Because no significant interaction between the genotype and the year factor was selected whatever the variable, heritability calculation was: $h_b^2 = \sigma_G^2/[\sigma_G^2 + (\sigma_\varepsilon^2/n)]$, where σ_G^2 is the genotypic variance, σ_ε^2 is the residual error variance estimated from the selected model, and n is the number of replicates per genotype (Gallais 1989; Knapp et al. 1985). All statistical analyses were performed using R software v.2.9.2, with REML estimation method, under lme4 and nlme packages (R Development Core Team, 2009).

Lastly, a first evaluation of the population performance was performed in comparison to the average values of the two parents (mid-parent) and the best parent (high-parent). Transgressive effects were estimated for all studied traits using the following equations, respectively (Fehr 1987; Barbosa-Neto et al. 1996).

$$\text{Mid} - \text{parent heterosis}(\%) = (\text{F1} - \text{MP})/\text{MP} \times 100 \quad (1)$$

$$\text{High} - \text{parent heterosis}(\%) = (\text{F1} - \text{HP})/\text{HP} \times 100 \quad (2)$$

where F1 is the average trait value in the population, MP is the average trait value for the parents and HP is the average trait value for the best parent. In the following, 'Arbequina' was considered as the best parent because of its high productivity combined to a low vigour.

Results

From juvenility to maturity: tree ontogeny analysis

Vegetative development

Every year after plantation, the olive trees showed a constant gain in height and volume and the trunks increased in basis diameter over years of growth (Tr_Bdiam) with mean values ranging from 0.83 cm in the first year of growth to 7.72 cm in the fifth (Table 2). For all these whole tree traits, Kendall's tau (τ) coefficients between consecutive years were significant. The highest correlation values were identified between 2 consecutive years and decreased when the lag between years increased, e.g., for the trunk basal diameter (τ=0.12 (2005–2006); τ=0.07 (2005–2009); τ=0.28 (2006–2007). Similar correlation tendency was found for the tree volume, basal area (B_area), trunk height (H) and projection (Proj; data not shown). The variances of all these variables increased over years and were thus heterogeneous (data not shown).

The average length of GUs (L), positively correlated to their number of internodes (Nb_IN), was maximal at orders 0 and 1, whereas lower values were observed at order 2 during the first and second year of growth (Fig. 2). Subsequently, the number of internodes (Nb_IN) and GU length (L) decreased in average in the third year of growth and until the fifth year. The mean number of internodes ranged from 19.4 in the first year of growth (in 2005) to 13.4 in the fifth year of growth (in

Table 2 Mean values and standard deviations of whole tree variables as a function of years (2005–2009) in 'Olivière' × 'Arbequina' progeny: Trunk Basal diameter (Tr_Bdiam), tree height (H), Projection (Proj), Basis area (B_area) and volume (V)

	2005	2006	2007	2009
Tr_Bdiam (cm)	0.83 (0.19)	2.24 (0.52)	4.50 (0.93)	7.72 (1.15)
H (m)		1.25 (0.25)	1.69 (0.29)	2.58 (0.30)
Proj (m)		0.60 (0.16)	1.00 (0.15)	1.45 (0.22)
B_area (m^2)		1.25 (0.64)	3.22 (0.99)	6.83 (1.93)
V (m^3)		0.53 (0.33)	1.85 (0.7)	5.96 (2.07)

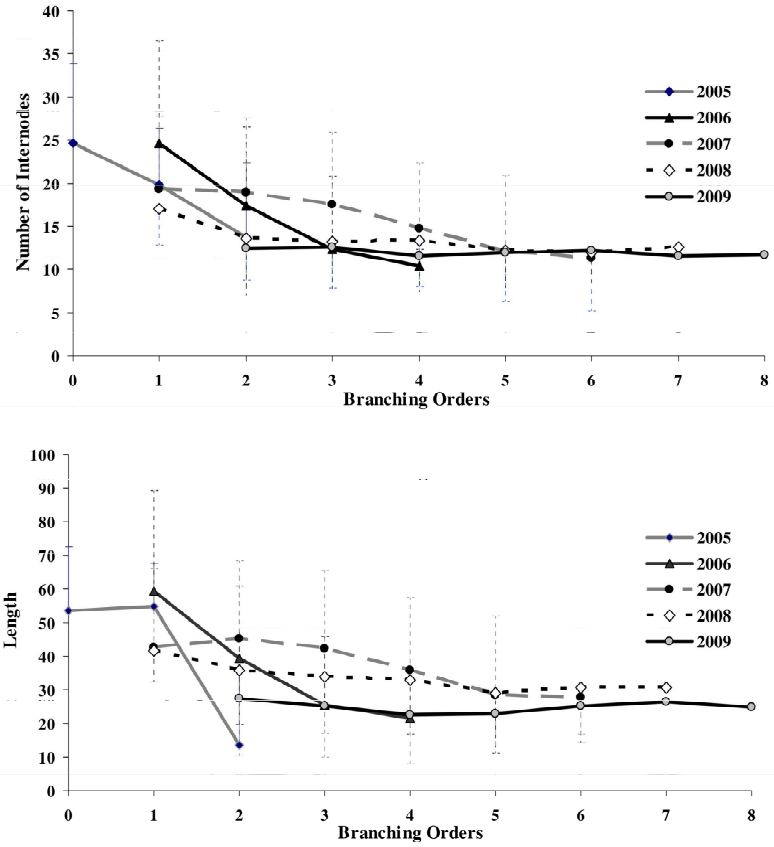

Fig. 2 Mean values and standard deviations of the number of nodes and length (cm) per growth unit (*GU*) as a function of years and branching orders in 'Olivière' × 'Arbequina' progeny: illustration of the ontogenic trend and occurrence of a stable phase from order 5 in the third year of growth

2009). A stable phase was reached from order 5 in the third year of growth (2007: Nb_IN=12 and L=28 cm; Fig. 2) and at all observed orders in the fourth and fifth year of growth (2008: Nb_IN=12 and L=30 cm; 2009: Nb_IN=12 and L=25 cm; Fig. 2). Neither the GU length nor the number of internodes was significantly correlated over consecutive years with τ values not significantly different from zero (τ=0.07 is the highest coefficient value). Similarly, these traits were not correlated between successive branching orders of the GUs (data not shown).

The mean number of sylleptic lateral GUs per parent GU decreased with years and branching orders, in a similar way to that observed for GU length and number of nodes (data not shown). The three types of GUs (long, medium and short) were observed as sylleptic laterals whatever the year and branching order (Fig. 3). However, sylleptic lateral GU types changed depending on the parent GU age: the older the parent GU the higher the proportion of long GUs (Fig. 3). The percentage of long GUs decreased during the first 3 years whereas short GUs increased from the third year of growth becoming the majority in the fifth year of growth. The proportion of GUs of medium length was almost stable during the first 5 years of growth. No significant correlations were found for the number of sylleptic axillary shoots per GU neither between consecutive years nor between branching orders (data not shown). On 2008 GUs, proleptic laterals were also observed. The mean number of sylleptic and proleptic laterals per parent GU was of 4.9 and 3.15, respectively. As observed for the sylleptic axillary shoots, the three types of GUs (long, medium and short) were observed as proleptic laterals along 2008 GUs (Table 3). At that developmental stage, medium and short sylleptic lateral GUs were prevalent whereas the number of long, medium and short proleptic lateral GUs were almost equal in average (Table 3).

Reproductive development

The number of inflorescences born on the 1-year-old floral GUs was 39 in average. Among them, a large proportion (75 %) was born on sylleptic axillary shoots (Inflo_AS) whereas a lower proportion was born directly along the GU (Inflo_direct; Fig. 4a). Likewise, inflorescences on sylleptic laterals were mainly born on short and medium types (Fig. 4b). The fruit set observed in 2009 on all flower locations, was of 53 % in average. Fruit set on direct inflorescences (Fruit_direct) was greater than that on inflorescences born on laterals (Fruit_AS; 62 % and 35 %, respectively).

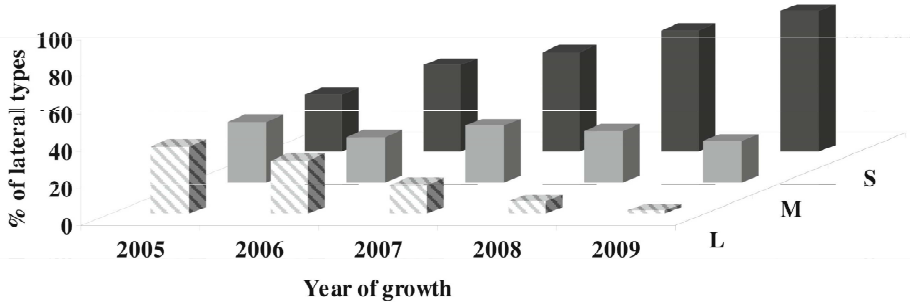

Fig. 3 Percentage of sylleptic lateral growth units (*GUs*) types (long, medium or short) depending on parent GU age in 'Olivière' × 'Arbequina' progeny

Correlation between variables

Phenotypic correlations were investigated between variables measured at a same scale through bi-plot examination (Fig. 5). Whole-tree form traits were highly correlated: for instance, tree projection and height were positively correlated to trunk basal diameter with a Kendall's τ coefficient of 0.62 and 0.66, respectively (Fig. 5a1, a2). At GUs level, the number of internodes was highly correlated to the GU length (τ=0.78; Fig. 5b). Significant correlation was observed between traits related to internodes lengthening at local scale, i.e., between IN_Max and Mean_IN_L (τ=0.55; Fig. 5c), even though the relationships is not perfectly linear but tends to be logarithmic. High correlation coefficients were found between most flowering and fruiting traits. The total number of inflorescences or fruits, Inflo (or Fruits)_tot, was highly correlated to the number of inflorescences born on axillary shoots, Inflo (or Fruits)_AS, (τ=0.82; Fig. 5d) and mostly to those born on medium laterals, Inflo_M_s, (τ=0.59 to be compared with 0.27 and 0.40 for Inflo_L_s and Inflo_S_s, respectively, data not shown). It is noticeable that a negative correlation was found between the number of direct inflorescences (or Fruits) and the number of the floral GU laterals, Nb_AS_{sF} per node, (τ=−0.30; Fig. 5e), even though a large dispersion of values was observed.

Genetic analysis

Since significant correlations were identified for the variables related to overall tree form measured over years, covariance structures were taken into account into the modelling approach (model 2, Table 4). The model selected according to the BIC criteria included an auto-regressive structure (corAR1) for all tree form traits except for the basal diameter of the trunk (Tr_Bdiam) where the compound symmetry (corCompSymm) structure was the most suitable. Heterogeneous variances were found for the tree basis area (B_area) and volume (V) as well as the basal diameter of the trunk (Tr_Bdiam). Thus, a variance function, power or constant power of covariate, was selected in the selected models (Table 4). Significant effect of both factors genotype and year of growth was found for all studied variables except trunk basal diameter where the genotype effect was not significant. The interaction effect ($G \times Y$) was not significant for all tree form traits (Table 4).

For traits related to primary and secondary growth measured on growth units, no significant correlation was observed over consecutive years or branching orders. However, because heterogeneous variances were found, the models included a variance function. The genotype effect was significant for two topological variables only (Nb_AS_s and Nb_S_s). For all the other variables, the genotype had no significant effect whereas the year of growth had a highly significant effect. For all variables, the interaction $G \times Y$ was not significant (Table 4).

For geometrical traits, the models selected according to BIC criteria also included the genotype and year effects. Because variances were heterogeneous for the GU length and internode maximal length, a power of covariate variance function was selected in the corresponding model (L; IN_Max, Table 4). The effect of the year of growth was highly significant for all variables without significant interaction with the genotype effect. Genotype effect was significant for variables measured on internodes (IN_Max; Mean IN_L), whereas no significant effect was found on GU length (L; Table 4).

When GUs at high branching orders only were considered during growth stability phase (2007 to 2009 year of growth), significant genotype effect was found for both topological and geometrical traits (Nb_IN and L,

Table 3 Mean values and standard deviations of the number of sylleptic and proleptic lateral growth units (GUs) depending on their types (long, medium or short) in 'Olivière' × 'Arbequina' progeny observed along 2008 GUs, at the tree periphery: whatever the type, the mean number of proleptic axillary GUs was almost stable

Laterals	*L*	*M*	*S*
Proleptic	1.13 (2.10)	1.08 (1.88)	0.93 (1.78)
Sylleptic	0.33 (1.05)	1.71 (3.01)	2.86 (3.63)

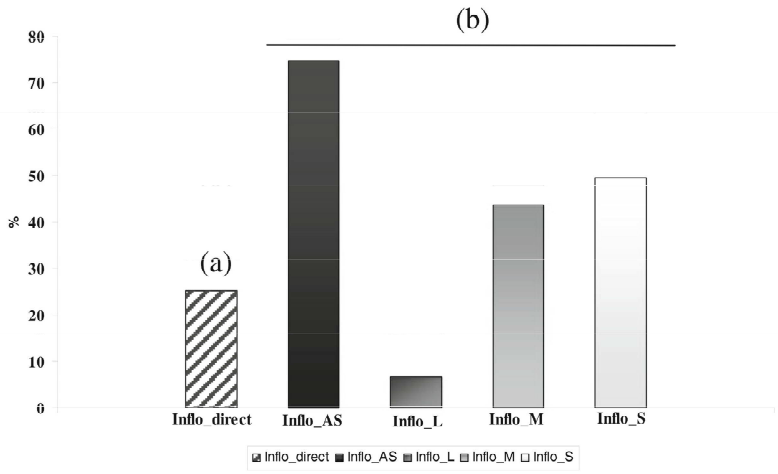

Fig. 4 Percentage of inflorescences along floral growth units (*GUs*) observed at the tree periphery in 'Olivière' × 'Arbequina' progeny: **a** direct inflorescences; **b** inflorescences born on laterals and per lateral types (long, medium or short)

Table 4). For all variables, the branching order effect was not significant whereas the effect of the year of growth was still significant, even though less markedly than in the previous years.

Looking for the number of sylleptic and proleptic axillary GUs, the selected model included genotype effect only, since the branching order effect was not significant for all the variables. Genotype effect was highly significant for the proleptic axillary shoots (Nb_AS_p), whereas it was barely significant for the sylleptic axillary shoots (Nb_AS_s, Table 5).

All traits related to flowering and fruiting measured on 1-year-old GUs at the tree periphery displayed highly significant genotypic effects (Table 4). Similarly, genotypic effect was significant for the basal diameter of these floral GUs.

Broad-sense heritability

Consistently with the genetic analysis results, traits related to tree form showed moderate to high heritability values ranging from 0.45 and 0.47 for the tree longest branch projection (Proj) and tree volume (*V*) to 0.58 for tree height (*H*) and 0.68 tree basis area (B_area, Table 4).

Among traits related to tree topology, moderate heritability values were found for two sylleptic branching traits: Nb_AS_s (h_b^2=0.25), Nb_S_s (h_b^2=0.45). Moderate heritability values were also estimated for the number of proleptic axillary shoots along 2008 GUs: Nb_AS_p (h_b^2=0.26), as well as for all the three types of proleptic axillary shoots: Nb_L_p (h_b^2=0.29), Nb_M_p (h_b^2=0.23), Nb_S_p (h_b^2=0.37). Summing long sylleptic and proleptic GUs, the total number of long axillary GUs became heritable: Nb_L (h_b^2=0.41, data not shown).

Considering geometrical traits, low to high heritability values were estimated for variables related to internodes lengthening: Mean IN_L (h_b^2=0.20), IN_Max (h_b^2=0.56), whereas the basal diameter of floral 2008 GUs, showed a moderate h_b^2 value of 0.29. Most traits related to their flowering and fruiting behaviour had high heritability values (Table 4), especially $Inflo_tot_{(sqrt)}$ (h_b^2=0.55) and $Fruit_tot_{(sqrt)}$ (h_b^2=0.69).

Heterosis

All traits related to tree vegetative and reproductive development showed high variation among progenies with values exceeding the best parental form (Fig. 6). Negative transgressive effects were observed for trunk basal diameter and tree height when compared to both mid-parent and best parental form, whereas the tree volume showed a similar transgressive effect only when compared to the mid-parent. This indicates that some progenies were less vigorous than the low vigor parent 'Arbequina' (Table 6). Consistently, the number of internodes, the length of GUs and their total number of axillary shoots also showed negative transgressive effects. More local variables, such as the mean and maximal internode length, exhibited negative transgressive effects only when compared to the mid-parent but did not transgress the best parental form (Mean IN_L and IN_Max, Table 6).

Most of the variables measured on the floral GUs had a negative transgressive effect when compared to either the mid or the best parental form. However, positive transgressive effect was observed for the number of direct inflorescences (Inflo_direct, Table 6).

Discussion

The present study aimed at decomposing the variance of architectural traits observed within an olive segregating population into morphogenetic factors, i.e., genetic,

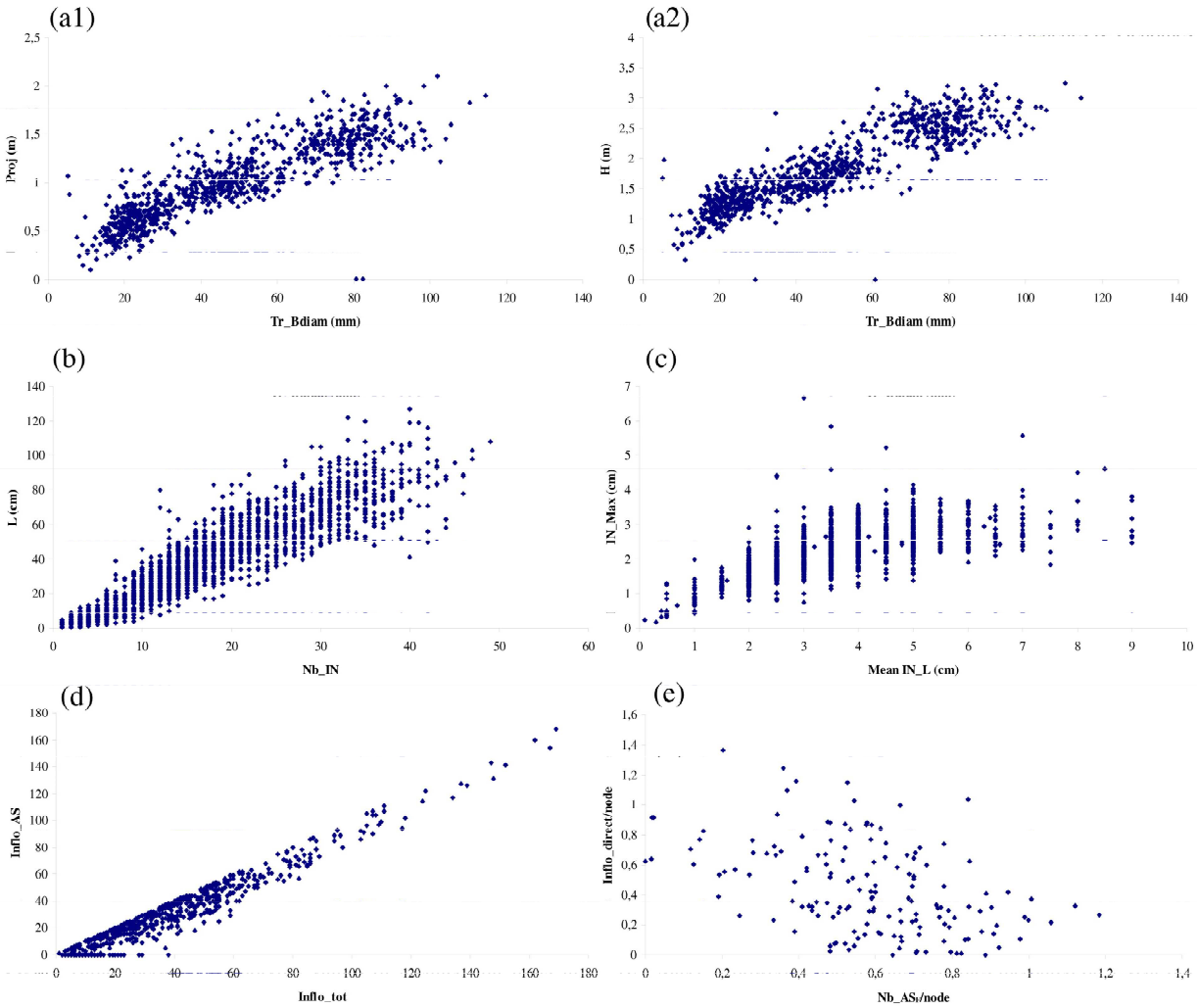

Fig. 5 Biplot between phenotypic values of architectural traits in 'Olivière' × 'Arbequina' progeny: **a1** whole tree variables: Trunk basal diameter (Tr_Bdiam) and tree longest branch projection (Proj); **a2** Trunk basal diameter (Tr_Bdiam) and Tree height (*H*); **b** topological and geometrical variables measured on GUs: Number of internodes (Nb_IN). and GU length (*L*); **c** internodes lengthening variables: mean internode length (Mean_IN_L) and maximal internode length (IN_Max); **d** flowering and fruiting variables measured on 1-year-old floral GUs: total number of inflorescences (Inflo_tot) and number of axillary inflorescences (Inflo_AS); **e** flowering and branching variables measured on 1-year-old floral GUs: mean number of direct inflorescences per node (Inflo_direct/node) and mean number of axillary floral short GU per node (Nb_AS_F/node). See text for the corresponding Kendall's tau (τ) correlation coefficients

branching order and year of growth. Since the experiment was performed in field, environmental conditions may have generated uncontrolled effects due to micro environmental and climatic year variability (Alía et al. 1997; Li and Wu 1997). However, low variability in the minimum, maximum and mean temperatures was observed between the 5 consecutive years of observation (Fig. S1). Moreover, as trees were irrigated, their growth was not limited by water demand for transpiration. We can thus hypothesize that tree ontogeny and genetic factors were the main sources of variations between years, without being able to formally separate climatic effects from true ontogenetic effects, as previously proposed by Segura et al. (2006). As another source of variation may result from spatial heterogeneity within the orchard, a first spatial analysis was performed that showed not significant effect of tree rows and blocks (data not shown).

Another source of heterogeneity may result from the multiplication method, since the trees originated from cuttings which are likely to generate more heterogeneous root growth than after grafting on a single rootstock genotype (in addition, rootstocks are usually chosen as homogeneous as possible before grafting). The heterogeneity may also concern the scion growth due to the uncertainty of the cutting technique success. For the 'Olivière' × 'Arbequina' progenies, the

Table 4 Models selected according to Bayes Schwarz information criterion (BIC) for architectural traits related to whole tree form, growth units (GUs) and 1-year-old floral GUs in 'Olivière' × 'Arbequina' progeny

Variables	Modelling factors			Variance function	Covariance structure	Variance estimates		H^2
	G	*Y*	*G* × *Y*			V_G	V_P	
Whole tree								
H	**	**	NS	–	corAR1	0.03	0.06	0.58
Proj	**	**	NS	–	corAR1	0.01	0.02	0.45
B_area	**	**	NS	varPower	corAR1	0.16	0.23	0.68
V	**	**	NS	varConstPower	corAR1	0.04	0.08	0.47
Tr_Bdiam	NS	**	NS	varPower	corCompSymm	8.51×10^{-8}	0.08	NS
Growth Units								
Geometry								
L	NS	**	NS	varPower	–	24.31	320.03	0.07
L (07 to 09)	*	*	NS	–	–	34.14	151.29	0.23
Mean IN_L	*	**	NS	–	–	2.78×10^{3}	1.36×10^{4}	0.20
IN_Max	**	**	NS	varPower	–	1.50	2.65	0.56
Topology								
Nb_IN	NS	**	NS	varPower	–	1.27×10^{-5}	2.76×10^{-3}	NS
Nb_IN (07 to 09)	*	*	NS	varConstPower	–	1.38×10^{-11}	8.87×10^{-10}	0.16
Nb_AS_s	**	**	NS	varExp	–	2.03	12.25	0.25
Nb_L_s	NS	**	NS	varExp	–	3.15×10^{-8}	0.65	NS
Nb_M_s	NS	**	NS	varPower	–	5.29×10^{-11}	4.91	NS
Nb_S_s	**	**	NS	varConstPower	–	0.09	2.15	0.45
One-year-old floral GU								
Geometry								
$Bdiam_GU_F$	**	–	–	–	–	0.57	1.91	0.29
Flowering and Fruiting								
$Inflo_tot_{(sqrt)}$	**	–	–	–	–	1.75	3.15	0.55
$Inflo_direct_{(sqrt)}$	**	–	–	–	–	0.77	1.49	0.52
$Inflo_AS_{s\ (sqrt)}$	**	–	–	–	–	2.39	4.61	0.52
$Inflo_M_{s\ (sqrt)}$	**	–	–	–	–	1.05	3.46	0.30
$Inflo_S_{s\ (sqrt)}$	**	–	–	–	–	0.97	2.16	0.44
$Fruit_tot_{(sqrt)}$	**	–	–	–	–	3.00	4.29	0.69
$Fruit_direct_{(sqrt)}$	**	–	–	–	–	0.85	1.82	0.46
$Fruit_AS_{s(sqrt)}$	**	–	–	–	–	2.30	3.88	0.59

Variance function and correlation structure when selected are indicated. Broad sense heritability (H^2) was calculated as the ratio between genotypic and phenotypic variances estimates

Significance of effects: *NS* not significant; *significant ($0.01<p\leq0.05$); **highly significant ($p\leq0.01$)

initial percentage of success — corresponding to root growth resumption — was about 40 % and depended on cutting periods, i.e., autumn or spring (G. Garcia, personal communication). However, after optimization of cutting technique (Garcia et al. 2005), no bias was introduced in the population and cuttings were obtained for each genotype before transplantation.

A main limitation for estimating the genetic effects also results from the low number of replicates per genotype since only two trees per genotype were observed. However, it would have been difficult to phenotype a larger number of trees due to the complexity of tree architecture (we presently phenotyped 324 trees in total). Therefore, we attempted to found a balance between a large number of genotypes necessary to increase the power of QTL detection (Bernardo 2004) and the number of replicates per genotype which improves the estimation of the genotypic effect.

Table 5 Models selected according to Bayes Schwarz information criterion (BIC) for sylleptic and proleptic branching traits along 2008 growth units (GUs) in 'Olivière' × 'Arbequina' progeny

	Variables	Factor	Variance function[a]	Variance estimates		H^2
		G		V_G	V_P	
2008 Growth Units	Sylleptic branching					
	Nb_AS_s	*	–	4.22×10^{-13}	17.61	NS
	Nb_L_s	*	varExp	5.37×10^{-9}	0.09	NS
	Nb_M_s	*		0.41	4.74	0.09
	Nb_S_s	*		1.48×10^{-13}	6.59	NS
	Proleptic branching					
	Nb_AS_p	**	–	2.52	9.66	0.26
	Nb_L_p	**	–	0.75	2.60	0.29
	Nb_M_p	**	–	0.46	2.02	0.23
	Nb_S_p	**	–	0.72	1.96	0.37

Variance function when selected is indicated. Broad-sense heritability (H^2) is calculated from genotypic and phenotypic variances estimates

Significance of effects: *NS* not significant; *significant ($0.01<p\leq 0.05$); **highly significant ($p\leq0.01$)

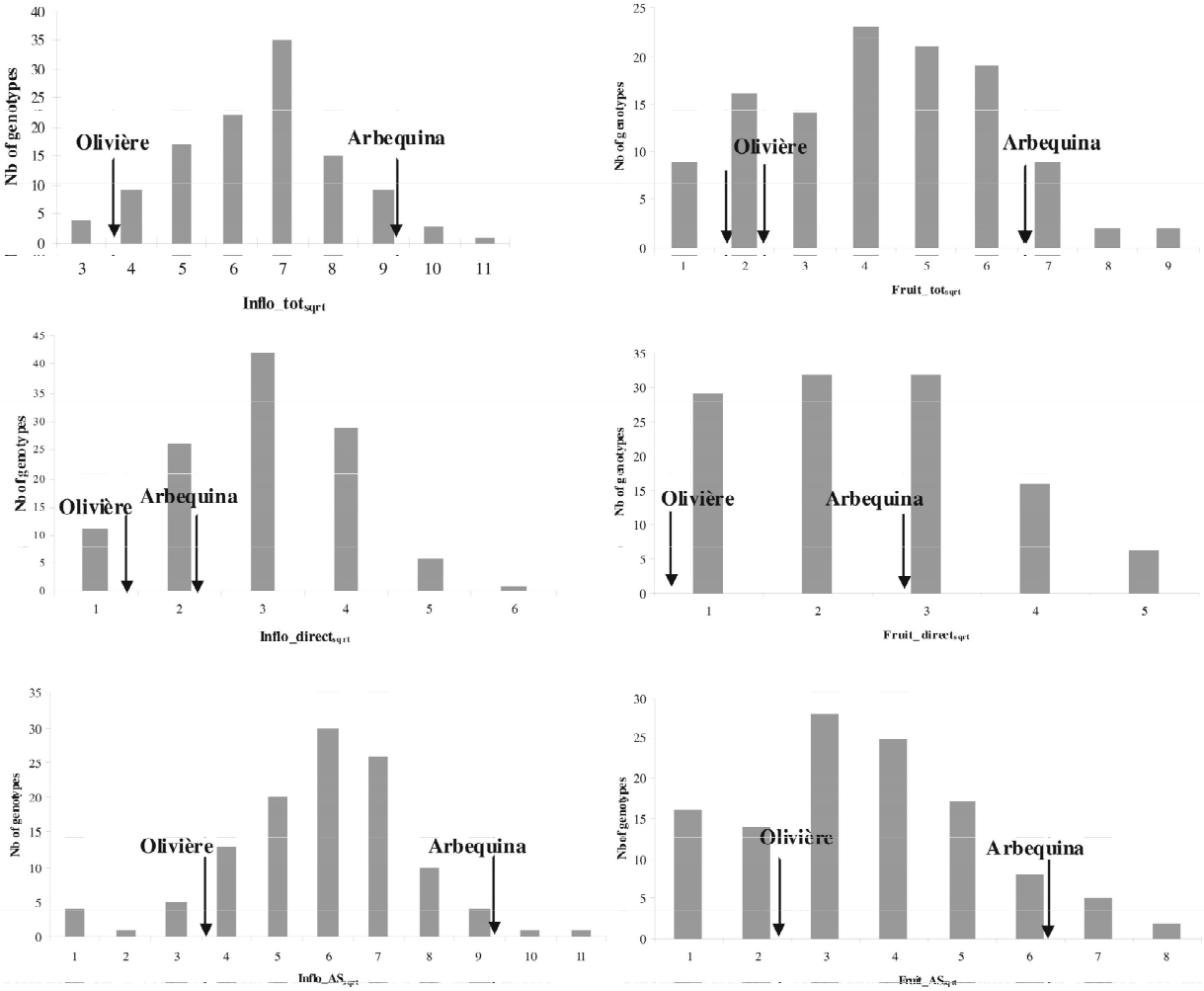

Fig. 6 Histograms of flowering and fruiting traits, measured at the tree periphery on 1-year-old floral growth units (GUs) in 'Olivière' × 'Arbequine' progeny based on genotypic means. Mean values of the parents are indicated by arrows. Transgressive effects were observed among the progeny, with some progenies values more extreme than parental values. A root square transformation was applied for all variables. See Table 2 for variable abbreviations

Table 6 Estimation of progenies performance for the variables related to vegetative and reproductive development

Variables	'O × A'	'A'(HP)	'O'	MP Heterosis (%)	HP Heterosis (%)
Whole tree					
Tr_Bdiam (cm)	47.25	49.90	53.12	**−8.27**	**-5.31**
H (m)	1.87	2.00	2.27	**−12.51**	**−6.50**
B_area (m^2)	3.78	3.40	3.95	2.77	11.17
V (m^3)	2.92	2.64	3.64	**−7.10**	10.60
GUs					
Nb_IN	16.97	18.07	17.10	**−3.49**	**−6.08**
IN_Max (cm)	3.55	3.33	4.03	**−3.53**	6.60
Mean IN_L (cm)	2.15	2.12	2.62	**−9.28**	1.41
L (cm)	38.27	39.81	45.62	**−10.40**	**−3.86**
Nb_AS_s	11.58	15.53	10.26	**−10.19**	**−25.43**
Nb_L_s	3.44	6.76	4.10	**−36.64**	**−49.11**
Nb_M_s	3.26	3.38	1.73	27.59	−3.55
Nb_S_s	4.92	5.23	4.42	1.968	−5.92
Flowering GU					
Inflo_tot	39.39	86.75	14.5	−22.19	−54.59
Inflo_direct	7.12	4.75	2.00	**110.96**	**49.89**
$Inflo_AS_s$	32.26	82.00	12.5	−31.72	−60.65
Fruit_tot	16.89	45.50	5.50	−33.76	−62.86
Fruit_direct	4.96	8.50	0.25	**13.37**	−41.53
$Fruit_AS_s$	11.92	37.00	5.25	−43.57	−67.75

Trait mean values were presented for the parents 'Arbequina' ('A', male parent) and 'Olivière' ('O', female parent) and the progeny 'O × A'. The heterosis was expressed as percentage of F1 progeny trait mean value over the best parental form, here Arbequina (High-Parent, HP) and over the average of both parental forms (Mid-Parent, MP). Values showing transgressive effect and corresponding to a potential improvement of tree shape and flowering are indicated in bold

Olive tree ontogeny

Architectural traits of the studied olive progenies showed major changes during the first years of growth, which can be interpreted with respect to tree ontogeny. Primary growth and branching decreased during the first 3 years, consistently with studies previously carried out on several, either fruit or forest, perennial species (Costes et al. 2003; Sabatier and Barthélémy 2001; Solar et al. 2006; Suzuki 2002). For most topological traits measured on GUs, the observed variance was not explained by the genetic factor and the measurements on consecutive years of growth and branching orders were independent. These results are unlike those found on the same characters observed on apple over the first 3 years of growth (Segura et al. 2008). By contrast, significant effect of the year of growth was found on all primary growth and branching variables during the same period. The relative low genetic control in this progeny found for topological traits of GUs during the first years of tree development, suggests that the young olive trees were in constant adaptation to environmental conditions. This may result from the multiplication method and interactions with climatic conditions (see above). Among topological traits observed over the first 5 years of growth, only the total number of sylleptic axillary shoots and those of short length were under significant genetic control.

Regarding geometrical traits, such as GU length and diameter, similar changes over years were found, with similar conclusions with respect to absence of genetic control. The absence of significant interaction ($G \times Y$) effect whatever the variable considered shows that the ontogenetic gradient is common to all the genotypes. However, the variables which represented shoot geometry more locally, such as the maximal internode length, remained stable over the years of growth, and showed a significant genetic effect. This suggests that internode lengthening is under genetic control whereas metamer appearance is a process sensitive to the environment leading to architectural plasticity. A similar finding was proposed on apple tree by Segura et al. (2006). Significant genetic effect was also found on other geometrical traits that were related to whole tree form, cumulated over years, and h^2 estimations for tree volume and trunk height are in the same range as those found in previous studies on apple (Watkins and Spangelo 1970; Durel et al. 1998; Liebhard et al. 2003). Moreover, high correlations between consecutive years were found with an auto-regressive structure. This indicates that the tree form is progressively built with a 'memory', which intensity decreases with years. It may be noticed that no significant genetic factor was found on the same variables, e.g., for trunk height, when less years were cumulated: not significant after the second year of growth (2006), the genetic effects became significant from the third year (2007). Similarly, genetic effect was not significant on branching angles that correspond to a more local tree form descriptor (data not shown).

The decrease in growth and sylleptic branching with increasing tree age reaches constant values in the fourth and fifth years of growth (2008 and 2009), at all described orders. These observations on olive trees are consistent with previous studies on several fruit and forest species such as apple, peach, birch and alder (Crabbé 1987; Génard et al. 1995; Lauri and Costes 1994; Champagnat 1954). However, syllepsis did not entirely disappear along olive tree GUs when tree age increased. On the contrary, GUs observed at the tree periphery at adult stage bear both sylleptic and proleptic shoots. Such mixture of sylleptic and proleptic laterals along shoots has been reported on species grown in tropical environment (Bell 1991). In the population, the stable phase coincided with the transition to adult state, since progenies have started to produce inflorescences and fruits in 2008. Along floral GUs, the presence of sylleptic laterals is likely to increase the tree production potential since these laterals bear inflorescences which largely contribute to the total shoot flowering potential. However, because sylleptic laterals may also compete with the inflorescences born directly along the GUs, branching organization and its relationship with fruiting behaviour must be further investigated.

The present study confirms that ontogeny, climatic years and observation scales must be taken into account for studying the genetic determinism of architectural traits in trees. These finding allow us to define when during the olive tree ontogeny and at which observation scale, growth and branching traits are under genetic influence. Unlike previous results on apple trees (Segura et al. 2007, 2008), the crown periphery is a suitable observation area for studying genetic determinism of olive tree architectural traits. Local and stable variables such as internode length and global variables collected at the whole tree scale were revealed appropriate to capture genetic effects contrary to intermediate scales such as growth units, at least during the exclusively vegetative phase of the trees. This allows us to propose a short list of the most relevant traits: whole tree form (tree height and longest branch projection), internode lengthening (IN_Max), and a detailed description focused on floral GUs at the crown periphery giving access to both branching and flowering–fruiting variables.

Reproductive growth

The 'Olivière' × 'Arbequina' progenies entered into flowering in 2008, which corresponds to the fourth year of growth. The majority of trees (74 % of genotypes) started to produce fruits on 3-year-old shoots. This indicates a precocious entering into the adult stage, in a perennial known for having a slow entrance into production, up to 6 years for cv. Santa Caterina (Tous et al. 2005). This earliness is more likely to be inherited by 'Arbequina', which was characterized as precocious being reported to reach production stage on the second year after plantation in Catalonia (Tous et al. 2005).

In 2009, all progenies reached maturity and produced 39 inflorescences per GUs on average which is on the range of values observed in cultivars considered as highly flowering, e.g., Manzanillo and Barnea: 34 to 40 inflorescences originated from 17 to 21 nodes (Lavee et al. 1999). Inflorescences were mainly located on lateral axes born along medium and short shoots (75 % of the total number of inflorescences observed in our study). Since 'Arbequina' has been described as the cultivar producing the most abundant axillary inflorescences among a range of French and Spanish olive cultivars (Moutier et al. 2004a), the high percentage of inflorescences in axillary position observed in the progeny may be inherited from that parent (male).

Total fruit set calculated on the basis of number of inflorescences was high (e.g., 52 % of inflorescences had at least one fruit) in comparison to low values usually reported for olive tree (Hartmann 1950). For instance, the fruit set percentage based on inflorescences number observed for Kadesh, Manzanillo, Maelia and Barnea cultivars was of 22.4 %, 28.9 %, 38.8 % and 44.8 %, respectively (Lavee et al. 1996). Generally, among 500,000 flowers that a mature olive tree can produce, only 1–2 % develop into a fruit able to reach maturity (Rallo and Fernandez-Escobar 1985; Martin 1990; Lavee et al. 1996, 1999). For 'Arbequina', only 4 % fruit reaches maturity from 60 % of hermaphrodite (perfect) flowers (Cuevas et al. 1995). The reasons of the low final fruit set on olive include genetic mechanisms as well as environmental and agronomic conditions. Firstly, the flower and drupes abscission may be caused by nitrogen deficiency, a lack of soil moisture and olive fly early damage (Pansiot and Rebour 1960). Secondly, low fruit set may result from dysfunctions during the development of reproductive organs leading for instance to pistil abortion. Lastly, both male sterility and incompatibility between cultivars is likely to hamper ovule fertilization (Cuevas et al. 1999). In olive, non-fertilized perfect flowers start to abscise through 3 weeks after anthesis while well fertilized ones abscises partially through 6 weeks after anthesis (Ateyyeh et al. 2000). In our studied progeny, 'Olivière' parent is a male sterile (Bellini et al. 2003), self-incompatible cultivar, whereas 'Arbequine' is self-compatible (Moutier et al. 2004b). The high fruit set observed in the population suggests that enough quantity of pollen were delivered and parental genotypes were compatible. Because male sterility has been shown to be encoded by mitochondrial variants and maternally transmitted (Besnard et al. 2000), we suspect that the success of fruit set in the progeny may be due to open pollination conditions and to the presence of a diversity of genotypes surrounding our progeny, i.e., a collection of about twenty French and foreign olive cultivars. However, compatibility between cultivars has been shown to be under a

complex genetic control which involves numerous alleles (Mookerjee et al. 2005; Saumitou-Laprade et al. 2010). Further studies are thus required to decipher which alleles are present in the 'Olivière' × 'Arbequina' progeny and to distinguish between self and cross pollinations.

In 'Olivière' × 'Arbequina' progeny, the higher number of inflorescences leading to a lower fruit set on lateral GUs than on direct inflorescences suggests higher competition for nutrients on lateral GUs than in direct positions. Competition between developing buds may also drive the axillary bud fate, as we observed negative correlation between the number of sylleptic axillary shoots and the number of inflorescences at a given node. This is consistent with the results of Rugini and Pannelli (1993), who found that vegetative shoot removal or growth regulators applications at bloom reduce the competition between vegetative and reproductive activities at the shoot scale. Moreover, competition for nutrients among fruitlets and between fruitlets and flowers has been shown to be a major factor for post-anthesis flower and fruit abscission (Rallo and Fernandez-Escobar 1985; Rapoport and Rallo 1991; Lavee et al. 1996, 1999). These authors reported that an artificial reduction in the number of flowers increases proportionally the fruit set, and have suggested that higher distance between inflorescences and fruits is likely to increase fruit set (Uriu 1959; Lavee et al. 1999). The relationship between inflorescences or fruit positions along the shoots and fruit set has also been shown to differ depending on the cultivar. In particular, flowering density differs between the two parents, with very dense flowering on 'Arbequina' shoots in comparison to 'Olivière' (Moutier et al. 2004a).

Conclusion

Our study confirms the importance of taking into account the morphogenetic factors in a quantitative genetics approach since results found in a fruit species may be partially transferable to another species. Our finding led us to propose an efficient phenotyping method for a quantitative genetics approach in olive tree, focusing on particular developmental stages and on particular variables such as the maximal internode length that may be interesting for selecting new cultivars with compact crown. The presence within the F1 full-sib population of genotypes showing interesting superiority in comparison to their parents, suggests that genetic progress can be performed in future selection programs. This heterosis probably results from the high level of heterozygosis of the species and allele recombination within the population we studied. In particular, progenies exhibiting higher values than their parents for flowering and fruiting traits, or less vigorous than 'Arbequina', could constitute sources of transgressive forms for innovative material. A step forward in this direction will be performed in the next future, by performing QTL detection on the traits investigated in the present study. This step will be possible, thanks to the high level of polymorphism found within the progeny that allowed us to develop a genetic map mainly based on simple sequence repeat markers (Khadari et al. 2010). Trait combination between vegetative development, fruiting precocity and regularity must be further investigated since irregular bearing is often observed in olive trees and constitute a major agronomic limitation (Martin and Sibbett 2005).

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Chapter 2

Studying genotype-by-environment interactions affecting the vegetative growth in an F1 olive tree progeny during juvenility phase

This chapter is adapted from an article in preparation.

Investigated questions:

- ***What are the morphogenetic gradients impacting the architectural traits over contrasted environments?***
- ***Is the genetic determinism of architectural traits stable over two environments?***
- ***Does the covariations between architectural traits sensitive to environmental variations? How their internal grouping structure evolves within and across environements?***
- ***Is there a significant genotype-by- environment interaction effect on the traits?***
- ***I there some progenies exhibiting interesting traits combinations and less impacted by the environmental variation?***

Studying genotype-by-environment interactions affecting the vegetative growth in an F1 olive tree progeny during its juvenile phase

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INTRODUCTION

Plant architecture is determined by a genetic component and evolves depending on environmental conditions (Hallé et al. 1978). Considering the particular case of perennial species, trees structure is established by a succession of growth units which change during ontogeny (Barthélémy et al. 1997). Tree architecture complexity is thus determined by genetic, environmental and ontogenetic factors and their interactions.
The concepts of the architectural analysis (Hallé et al. 1978) have been applied in several forest and fruit species highlighting the foundation of tree form establishment (Barthélémy and Caraglio, 2007). Despite apparent variation in many fruit tree architectural characters, little attention has been given to genetic and environmental sources of this phenotypic plasticity, in spite of their potential effects on bearing regularity and fruit quality as well as orchards management efficiency (Maguylo and Lauri 2004, Costes et al. 2006). Investigating the inheritance of traits related to the vegetative growth over contrasted environments could permit an early selection of stable architectural traits for fruit tree breeding (Laurens et al., 2000) and could shed light on the basis of their variability.

The interaction between the genetic structure of a tree and the environmental factor is called genotype by environment interaction (i.e. GxE). Breeders make use of GxE interaction for developing cultivars which are suitable for various environment conditions and least affected by environmental variations (Demir and Tosun, 1991). GxE interaction of individual traits have been assessed in numerous experiments with annual crops like wheat (Nachit et al. 1992; Ahmad et al. 1996), rice (Nassir and Ariyo, 2011), oat (Holland et al. 2002) and soybean (Fehr et al. 2003; Vinay et al. 2006; Arsalanoglu and Aytac, 2010); fibres such as cotton (Swan et al. 2003); forest trees: poplar (Noh et al. 1997; Koo et al. 2007), pine (Barnes et al. 1984, Baltunis et al. 2010), spruce (Isik et al. 1995). Comparatively, fewer studies have been performed on fruit trees: apple (King et al. 2000), wild cherry (Curnei et al. 2003), blueberry (Finn et al. 2003). Above all studies, the considered traits were mainly related to fruit production and quality, whereas growth traits were poorly represented i.e. limited to stem diameter, height and volume. These latter traits appeared to be significantly impacted by the GxE effect in forest trees (Carson, 1991; McKeand et al. 1997; Matheson and Wu, 2005). Likewise, genotype, environment and GxE interaction effects were found to be significant in

soybean (*Glycine max* (L.) Merrill) in terms of plant height (Joshi et al. 2005; Mondal et al. 2005) and number of branches (Bhandarkar et al. 2001).
Evaluating quantitative architectural traits across environments is challenging for perennial species considering the long juvenile period, the high level of structural complexity and the cost of such an experiment implying a large population survey in contrasted locations. This is particularly true for the olive tree (*Olea europaea* L. *subsp. europaea*), grown since the antiquity around the Mediterranean Bassin (Besnard et al. 1998) with a remarkable economic and symbolic power among the different cultures and nations.

A first study of the genetic basis of olive growth and branching traits has been proposed by Ben Sadok et al. (2013). The increasing structural complexity of olive trees was described over several years giving evidence of the importance of accounting for the ontogenetic and climatic year factors effect to better capture the genetic basis of traits related to the vegetative development. A phenotyping methodology adapted to the olive characteristics was proposed for quantitative genetics approaches. Due to the presence of ontogenetic trends, growth and branching traits measured at growth unit scale were heritable only at the tree periphery. Nevertheless, local and stable variables such as maximal internode length and global variables describing the overall tree form (e.g. tree height and volume) were revealed appropriate for capturing the genetic effects. However, this experiment did not allow the distinction between ontogenetic and climatic effects and no information was provided about the environmental effect and its interaction with the genetic factor.

The present study aimed at evaluating the effect of the environment and its eventual interaction with the genotype factor on growth and branching traits in olive while accounting for effects related to tree ontogeny. Field experiments were conducted at two contrasted locations and analysis were carried out using data collected on an F1 olive tree progeny derived from a cross between two highly polymorphic olive cultivars. A mixed linear modelling of the genotype, ontogenetic and environmental factors and their respective interactions was performed and heritability values were estimated for each studied trait. This led us to give an overview of the plasticity of the architectural traits and to define the variables that are the less impacted by the environmental change. The internal grouping structure among variables and among progenies was also evaluated. This work also provides a foundation for QTL detection in this progeny based on Best Linear Unbiased predictors

(BLUPs) of genotype effect (G) and interactions with the environment (GxE) when significant.

MATERIALS AND METHODS

Experimental design

The progeny under study derived from 'Olivière' x 'Arbequina' cross and counts 140 genotypes. Parents were chosen for their contrasting architecture and bearing habit. The 'Olivière' maternal parent is a vigorous male sterile cultivar displaying an alternate bearing habit (Moutier et al. 2004). By contrast, the 'Arbequina' male parent is a relatively small self-fertile cultivar, suitable for dense planting and characterized by a small alternating production. The progeny was grown at two locations: the experimental station of INRA Montpellier 'Domaine de Melgueil' in Mauguio (3º 58' 45.47" E, 43º 36' 35.01" N, 10m. a.s.l) and the experimental farm of IFAPA 'Alameda del Obispo' in Córdoba (04º 48' 00" W, 37º 51' 42" N, 117 m.a.s.l.), noted E1 and E2, respectively. The trees, originated from cuttings, were planted in a randomised complete block design (E1 in 2005: 6m x 2m; E2 in 2009: 4m x 2m; N/S rows orientation) in a clay-loam soil at both sites. Architectural traits previously studied in Melgueil (E1) over the first 5 years of growth were recorded in Cordoba (E2) over the first three years of growth following the same phenotyping strategy as described in Ben Sadok et al. (2013). The present study is thus based on the analysis of the first three years of growth of the progenies in both E1 and E2, which correspond to different climatic years (see Figure S1 for meteorological records in the corresponding years in each environment). In E1, trunks were cut back to 50 cm to homogenize the plantation and trees were not pruned. In E2, trees were trained as single trunk and were not pruned afterwards. Standard irrigation and treatments were carried out all over the experiments in both sites.

Phenotyping methods

Tree topology was formalized in a multiscale tree graph (MTG) according to the methodology defined by Godin et al. (1999). The data base contained four different scales: tree, sympode, growth unit (GU) and internode. All GUs were described in the first year of growth i.e. 2005 (E1) and 2009 (E2), whereas a subsample corresponding to the main path of GUs in the tree was selected in the second and third years. Branching orders were incremented from axes developed after cutting, which were considered as order 0. GUs were classified into three types according to their length: long (≥ 20 cm); medium (5 cm ≤ 20 cm) and short (<5 cm). Growth and branching traits such as the number of internodes (Nb_IN) and the number of sylleptic axillary shoots per GU (i.e., shoots developed immediately without bud resting period, Nb_AS) were measured for long and medium GUs. Regarding

geometrical traits, the length (L) was measured of each GU, whereas the length of the longest internode (IN_Max) was noted for each annual shoot. The mean internodes length was deduced from the GU length and number of internodes (Mean IN_L=L/Nb_IN). At the overall tree scale, variables related to the global form of the tree were collected in (E2) at the end of 2010 and 2011 years of growth. Trunk height (H) and projection of the longest branch of the tree (Proj) were used to calculate tree basis area (B_area= Π*Proj^2) and volume (V=1/3 * B_area * H). Then, the basal diameter was assessed for each trunk (Tr_Bdiam). All data extraction from MTG files was performed using VPlants software (http://openalea.gforge.inria.fr). Both measured and derived variables (Table 1) were classified according to the observation scale: whole tree and growth units.

Data analysis

On a total of 480 trees (two replicates/genotype and 120 genotypes/location), changes in primary growth and branching variables during tree ontogeny were examined considering the year of growth and the branching order in both E1 and E2 jointly and separately. The normality of each variable distribution was checked. When not verified, the corresponding variable was transformed with a square root transformation (distinguished by the index sqrt). As dependencies were expected between repeated measurements on the same trees, variances homogeneity was checked using the Levene's test (Levene, 1960) and correlations between consecutive years or orders were examined.

Depending on the variable and the experimental unit considered in the tree, different mixed linear models were built in order to access the effect of the genetic, ontogeny (i.e. the year of growth and/or the branching order), environment and their interactions on the studied traits. For variables collected at the whole tree level over consecutive years, the effect of genotype, year of growth, environment and their interactions were estimated according to the following global mixed linear model:

$$P_{ijkl} = \mu + G_i + Yj + E_k + (G\times Y)_{ij} + (G\times E)_{ik} + \varepsilon_{ijkl} \qquad (1)$$

where P_{ijkl} is the phenotypic value of genotype i at the year of growth j and the environment k, μ is the overall mean of the progeny, G_i is the random effect of the genotype i, Yj is the fixed effect of the year of growth, E_k, is the fixed effect of the environment k, $(G\times Y)_{ij}$ and $(G\times E)_{ik}$ are their random interaction and ε_{ijkl} is the random residual error effect for the l measured trees.

Because the GUs developed in a given year of growth were located at different branching orders and the number of GUs developed per year and order depended on each tree and environment, the dataset of growth and branching variables recorded at the GU level over three consecutive years was unbalanced. Thus, the random interactions between the genotype factor and the year of growth and branching order (i.e. G×Y and G×O, respectively) could not be tested jointly. According to the Bayes Schwarz information criteria (BIC) minimization, the following global mixed linear model including the G×O and G×E interactions was selected for GUs traits analysis:

$$P_{ijnkl} = \mu + G_i + Yj + O_n + E_k + (G\times O)_{in} + (G\times E)_{ik} + \varepsilon_{ijnkl} \qquad (2)$$

where P_{ijnkl} is the phenotypic value of genotype i at the year of growth j, branching order n and the environment k, μ is the overall mean of the progeny, G_i is the random effect of the genotype i, Yj is the fixed effect of the year of growth, O_n is the fixed effect of the branching order, E_k, is the fixed effect of the environment k, $(G\times O)_{in}$ and $(G\times E)_{ik}$ are their random interaction and ε_{ijnkl} is the random residual error effect for the l measured trees.

For traits showing heterogeneous variances, different variance functions, as described by Pinheiro and Bates (2000) were tested i.e. varIdent, with different variances per level of the fixed factor; varPower, with variance increasing as a power function; varExp, with variance increasing as an exponential function; varConstPower, combines a constant value with a power function. Moreover, when significant correlations between consecutive years or orders were found, several covariance structures were compared i.e. compound symmetry (corComSymm), autoregressive of order 1 (corAR1) and exponential (corExp).

For each variable, a model comparison was performed on the basis of BIC minimization, which allowed us to select the significant factors and when necessary, the variance function and the covariance structure to be taken into account in the residual term of the corresponding mixed linear model. After this model fitting on data, the normality of residual distribution was checked. All model estimation and selection were performed using R software v.2.9.2, with REML estimation method, under lme4 and nlme packages.

Broad-sense heritability of all studied traits was estimated as the ratio between the genotypic and the phenotypic variances: $h_b^2 = \sigma^2_G / \sigma^2_P$. When significant interaction between the genotype and the environment factor was selected, the heritability calculation was: $h_b^2 = \sigma^2_G / [\sigma^2_G + \sigma^2_{GxE}/a$

+ σ^2_ε/na] where σ^2_G is the genotypic variance, σ^2_{GxE} is the variance of genotype and environment interaction, σ^2_ε is the residual error variance estimated from the selected model, n the number of replicates per genotype, and a the number of environments (i.e. n= 2; a= 2).
When significant interaction between genotype and branching order factors was selected, the heritability calculation was: $h_b^2 = \sigma^2_G / [\sigma^2_G + \sigma^2_{GxO}/b + \sigma^2_\varepsilon/nb]$ where σ^2_G is the genotypic variance, σ^2_{GxO} is the variance of genotype and order interaction, σ^2_ε is the residual error variance estimated from the selected model, n the number of replicates per genotype, and b the number of orders considered (i.e. b= 6). When no significant interaction was selected, heritability was calculated as: $h_b^2 = \sigma^2_G / [\sigma^2_G + (\sigma^2_\varepsilon/n)]$, where σ^2_G is the genotypic variance, σ^2_ε is the residual error variance estimated from the selected model, and n the number of replicate per genotype (Knapp et al. 1985; Gallais, 1989).

Giving that univariate analysis cannot account for changes in the relationship among traits between environements; we performed a multivariate analysis to investigate covariations between architectural traits. The phenotype represented by a number of observable traits could be better illustrated as a multidimensional space interacting with the environment (Pigliucci and Preston, 2004; Wagner and Zhang, 2011). In order to analyze jointly the phenotypic space across and within environments, a multiple factor analysis (MFA) was preferred to a classical principal component analysis because it takes into account the internal grouping structure among variables or among individuals. The contribution of a data point to the inertia of an axis is the quotient between the inertia of its projection and the inertia of the whole scatterplot's projections on this axis (Pages, 2002). Principal components (PCs) will represent major axis of covariation between sets of phenotypic traits. The MFA analysis was performed on the basis of genotypic mean values using the *dual multiple factor analysis* function under FactoMineR package (R Development Core Team, 2009). Euclidean distances between variables were also calculated on a PCA coordinate matrix for all variable pairs considering separately each environment and Ward's minimum variance algorithm was used to construct the corresponding dendrogram (Ward, 1963). The principle of this algorithm is to cluster variables, genotypes or groups at each step by maximizing intergroup sum of squares/total sum of squares ratio (Saporta, 1990). The clustering was performed using the program clustering calculator, developed by John Brzustowski: http://www.biology.ualberta.ca/jbrzusto/cluster.ph. Unrooted trees were drawn by the TreeView Software (Page, 1996).

RESULTS

Genetic analysis

Whole tree form variables showed heterogeneous variances over consecutive years. Nonetheless, models selected did not include a variance function, except for tree volume for which an exponential variance function was taken into account in the residual term of the model (V; Table 2).

All traits related to the whole tree form showed significant differences among years and environments (i.e. highly significant Y and E factor effects; Table 2). The highest mean values of the projection of the longest lateral branch of the tree (Proj), tree basis area (B_area) and volume (V) and trunk basal diameter (Tr_Bdiam) were observed in E1, whereas the trunk height (H) was higher in E2 (Table 3). The genotype effect was significant for the projection of the longest lateral branch of the tree (Proj), tree basis area (B_area) and volume (V), (Table 2). The G×E interaction effect was highly significant for the trunk height (H), whereas it was barely significant to non significant for all other tree form variables (Tr_Bdiam, Proj, B_area, V; Table 2).

At GUs scale, the genotype by environment interaction G×E was not significant for all studied variables (i.e. geometrical and topological variables) and was thus excluded according to BIC criteria (Table 2). No significant correlation was observed over consecutive years or branching orders for all GUs variables. Even though variances were heterogeneous, the models selected did not include a variance function, except for the maximal internode length for which a power of covariate variance function was taken into account in the residual term of the model (IN_Max; Table 2). The GUs length (L) was significantly impacted by the effect of both environment and branching order factors. The effect of genotype was not significant for this trait, whereas the interaction G×O effect was barely significant (L; Table 2). For internode lengthening variables, a highly significant genotype effect was found for both mean and maximal internode length (IN_Max, Mean_INL; Table 2). The maximal internode length (IN_Max) was also significantly impacted by the effect of the year factor, whereas the mean internode length (Mean_INL) was weakly impacted by the interaction G×O effect (Table 2). The difference in means between E1 and E2 environments was not significant for IN_Max and barely significant for Mean IN_L (Table 2 and 3).

For traits related to primary growth and branching measured on growth units, the year and branching order factors effect was highly significant except for the number of internodes per

GU and the number of sylleptic long laterals (Nb_IN and Nb_L), for which the year effect was not significant (Table 2). These traits showed significant differences between environments, with the highest mean values found for trees grown in E1 (Table 3). The genotype effect was weakly significant for two topological variables only (Nb_AS and Nb_S), whereas the interaction G×O was weakly significant to non significant for most growth and branching traits, except for the number of sylleptic laterals of short length (Nb_S, Table 2).

Broad-sense heritability

Consistently with the genetic analysis results, traits related to overall tree form showed moderate heritability values ranging from 0.31 for the tree longest branch projection (Proj) and basis area (Proj, B_area; Table 2) to 0.52 for tree volume (V; Table 2). Considering geometrical traits, moderate to high heritability values were estimated for variables related to internodes lengthening: Mean_INL (h_b^2= 0.28), IN_Max (h_b^2= 0.74). Among topological traits, only two branching traits showed low to moderate heritability values: Nb_AS (h_b^2= 0.11), Nb_S (h_b^2= 0.25).

Between-Environments ontogenetic changes

Considering that the interaction between ontogenetic and environment factors i.e YxE and OxE was not significant for all architectural traits (data not shown), these factors were excluded from the mixed linear models. However, we described qualitatively the observed architectural changes due to tree ontogeny in each environment.

Progenies showed similar ontogenetic trends in both E1 and E2 environments. Trees have grown in height and volume and trunks gained in basal diameter from the first to the second studied year.

During the first and second year of growth, the average length of GUs (L), positively correlated to their number of internodes (Nb_IN) was maximal at orders 0 and 1, whereas lower values were observed at order 2 during (Figure 1). Afterwards, the number of internodes (Nb_IN) and GU length (L) decreased in average in the third year of growth. The mean number of internodes ranged from 22.4 (E1) and 18.14 (E2) in the first year of growth to 15.76 (E1) and 13.37 (E2) in the third year. The number of internodes (Nb_IN) showed almost similar values in both environments at order 5 and 6 in the third year of growth (e.g. at order 6: Nb_IN= 9.8-10.6 in E1 and E2 respectively; Figure 1). The mean number of sylleptic lateral GUs per parent GU decreased with years and branching orders, in a similar way to that

observed for GU length and number of nodes (data not shown). The three types of GUs (long, medium and short) were observed as sylleptic laterals whatever the year and environment (Figure 2). Sylleptic lateral GU types changed depending on the parent GU age in a similar way in both E1 and E2 environments (i.e. the youngest the parent GU the higher the proportion of short GUs). The percentage of long GUs decreased during the first 3 years of growth whereas short GUs increased (E1, E2; Figure 2). Even though the proportion of long laterals was on average the highest in E1 during the three years studied, it was more abundant in E2 than in E1 during the first year of growth. Accordingly, its decrease observed in the second and third year was greater in E2 than in E1 (Figure 2). Therefore, the proportion of GUs of medium length increased widely in E2 during that period whereas it was almost stable over years in E1.

Structure of the phenotypic space

The multidimensional phenotypic space was explored using a multivariate factor analysis (MFA) considering jointly the genotypic means of all studied traits in order to compare the general pattern of trait covariations *across* environment to the patterns of trait covariations *within* each environment (Page, 2002).

Across environments, the first three principal components (PCs) explained together 66% of the total variance in the phenotypic data (Table S1.a). The set of variables that contributed the most to PC1 was composed by the whole tree form traits which were positively correlated to each other (Table S1.b and c). Growth and branching variables contributed the most to the variability along PC2, whereas geometrical traits (i.e. L, IN_Max and Mean_INL) contribution was the highest along PC3 (Table S1.b and c). Giving that each PC was defined by a group of variables corresponding to the three scales considered: tree, GU and internode, it was possible to identify traits combination defining progenies architectural phenotypes (Figure 3). Examining the internal grouping structure among progenies revealed the presence of transgressive forms showing interesting trait combinations: medium global volume and high primary growth and branching (progenies girded in green, Figure 3); low volume, GU length and internode length, but medium branching and internode number (progenies girded in purple and brown, Figure 3); High volume and GU length, low internode length and branching (progenies girded in black and pink, Figure 3) and low global volume, primary growth, branching and internode length (progenies girded in blue, Figure 3).

The within-environment MFA show the internal structure of trait covariations within each environment (Figure 4). Variables contributions to the principal components under E1 and E2 environments were remarkably similar (Table S2.a). However, some minor differences could be revealed with traits inversely correlated between E1 and E2 in the PC1-PC2 and the PC1-PC3 planes (Figure 4). The total number of laterals (Nb_AS) contributed negatively to PC3 under E1 and positively under E2. Growth and branching variables recorded either in E1 or E2 were clustered in two groups i.e. cluster 1 and cluster 2, respectively (Figure 5.a). A comparable closeness was found between Nb_AS, Nb_M and Nb_S in both cluster 1 and 2. Likewise, proximity between Nb_IN and Nb_L was found in both clusters. However, within cluster 1, the lowest distance was found between Nb_AS1 and Nb_M1, whereas within cluster 2 it was found between Nb_IN2 and Nb_L2 (Table S3.b).
The GU length (L) contributed positively to PC2 under E1 and negatively under E2 (Figure 4a,d; Table S2.a). A high positive correlation was observed between GU length and number of internodes in E1. This correlation was weaker under E2 (Figure 4; Table S2.b). The GU length recorded in E2 was instead strongly correlated to the mean internode length in E2 and was grouped with internode lengthening variables (i.e. IN_Max and Mean_INL) recorded in both environments (cluster 3; Figure 5.b). Among the variables belonging to this cluster, the lowest distance was found between Mean_INL2 and L2 (Table S3.b).

Whole tree form traits showed similar correlations tendency under each of the two environments considered, with higher correlation coefficients found in E2 than in E1 (Table S2.b). In fact, most variables recorded in a same environment were grouped together (i.e. cluster 1 for E1 and cluster 2 for E2; Figure 5.a). The trunk basal diameter (Tr_Bdiam) and the number of laterals of medium length (Nb_M) contributed positively to PC3 under E1 and negatively under E2 (Figure 4b,e; Table S2.a). In fact, a strong correlation value was revealed between trunk height and basal diameter in E1 but in E2 trunk height was more correlated to tree volume (Figure 4; Table S2.b). The trunk height recorded in E1 was clustered with the trunk basal diameter variable recorded in both environments (cluster 3; Figure 5.a), whereas that recorded in E2 was grouped under cluster 2 (Figure 5.a). Overall, the lowest distance was found between Proj and B_area in both environments (Table S3.a).

DISCUSSION

In the present study, our objective was to determine the relative contributions of genotype, environment and G x E interaction to variation in architectural traits in a segregating olive population. As previously recommended by Ben Sadok et al. (2013), ontogenetic factors i.e. year of growth and or branching orders were taken into account in the quantitative genetics analysis. Observing only two replicates per genotype in each environment could be considered as a limiting factor in estimating the genetic effect. However, the balanced experimental design that was used (i.e. an equal number of replicate was considered in the two-site analysis) give unbiased estimator of population mean and avoid the loss of variance estimation efficiency by a sampling or design factor which could increase the error terms (Pavlidis and Noble, 2001; Thomson, 2007). Despite that mixed modelling is particularly recommended for unbalanced data, missing data are still difficult to handle for complex models including several fixed effects and random interaction effects. Considering the increasing structural complexity of the olive tree over years, describing a larger number of genotypes in the two-site experiment would have been hard to manage (a total of 480 trees were phenotyped in this study).

Tree Ontogeny and Plastic response to contrasted environments

Architectural components of the studied olive progeny showed similar ontogenetic variation in both environments during the three first years of growth, but most of these vegetative growth traits exhibited plastic responses to environmental variation. In fact, almost all traits related to whole tree form trait and growth unit topology and geometry were significantly impacted by environmental and ontogenetic effects (i.e. significant effects of the year of growth and/or branching order). These findings confirm that at early ontogenetic stage, young trees structure establishment follow intrinsic organization rules which appear to be generic (Prusinkiewicz et al. 2001; Barthélémy and Caraglio, 2007). In spite of the predictability of the observed ontogenetic decrease in primary growth and branching traits at growth units scale as well as the global increase in trees volume, trees were able to tolerate the variation in the environmental through growth plasticity. The internal structure of trait covariation was not affected by environmental variation. This suggests a pleiotropic structure of the architectural phenotypic space (Wagner et al. 2011).

According to De Lucia et al. (2000), growth plasticity might be particularly important for adaptation to increased temperatures and drought. In the present study, trees were irrigated in

both Melgueil (E1) and Cordoba (E2) sites in order to not penalize their growth by water demand for transpiration. Some rainfall differences were recorded between the two environments over the first two years of growth during the active vegetative period (e.g. February, Figure S1a). Nevertheless, the highest amount of precipitation was recorded during the period of vegetative rest of the olive progeny (i.e. December-January) at both sites. Regarding humidity records, low within-environment variability was observed between the consecutive studied years. Likewise, low variability was observed across environments with a higher mean humidity percentage recorded in E1 during the first two years only (Figure S1a). By contrast, the minimal, maximal and mean temperatures observed during the three consecutive years of study were higher in E2 than in E1 (Figure S1b, c). Even though mean temperature variability varied only slightly between the consecutive years observed within each environment, the second year observed in E1 corresponded to a heat wave year (i.e. 2006). This could explain that primary growth observed in 2006 was lower than that observed in 2007 (Order 2 to 4, Figure 1). Above all, we can presume that the major source of environmental variability in our study is temperature. Our experimental design still does not allow us to separate tree age from climatic year effect.

Genotypic effects

Considering whole tree form traits, the highest heritability value was found for the tree volume which is consistent with previous observations, showing that the global variables are suitable for capturing the genetic effect (Ben Sadok et al. 2013). The observed variance of most primary growth and branching traits was not explained by the genetic factor and measurements on consecutive years and branching orders were independent. In fact, only the total number of axillary shoots and those of short length appeared under a weak genetic control. The number of short GU laterals was also significantly impacted by genotype by branching order interaction effect. This suggests a higher variance between orders than that between years. These results confirm that metamers appearance is a process sensitive to environmental variation leading to architectural plasticity in young olive trees. It also supports that considering topological and geometrical variables at intermediate scales (i.e. growth units and annual shoot) is not appropriate for capturing the genetic foundation of growth and branching habit during first years of growth (Ben Sadok et al. 2013).

In spite of the major environmental effect revealed on almost all architectural traits in this study, two geometrical traits were interestingly not affected by exogenous conditions. In fact, internode lengthening variables (i.e. IN_Max and Mean_INL) were mainly genetically

determined and showed stability across environments. These results confirm the genetic control of internode lengthening process as previously proposed by Ben Sadok et al. (2013) and strengthens the relevance of such traits for breeding programs. In fact, one of the olive breeding objectives is the control of canopy shape and density which affects mechanical harvesting and depends on the interaction among the length of the internodes, the number and vigour of the shoots and the size of the leaves (Tombesi et al. 2002; Ferguson et al. 2010). The importance of internode length for breeding has been underlined in various woody and annual species. Taking radiate pine for example, the internode length has been reported to play an important role in clearwood stiffness as a plank, as long internode trees have fewer larger branches (Shelbourne, 1997). In alfalfa plants, the ideal stem morphology for yield and quality should be constituted by a high number of short and regular internodes, thus optimising leaf to stem ratio (Rotili et al. 1999; Lumb et al. 2006).

Genotype-by-environment interaction effect on olive architecture

It is remarkable that almost all studied architectural traits were not affected by GxE interaction effect. This result shows that progenies reaction to different environments was uniform i.e. there is no genetic variability for adaptability to specific environments. For instance, a general decrease in trees vegetative growth was observed in Cordoba conditions (i.e. E2). The environment effect is thus cumulative. Only trunk height trait showed large GxE interaction effect which could indicates differential response of olive progenies to changing environmental conditions. These results are consistent with those observed on forest trees (McKeand et al. 1997; Matheson and Wu, 2005). However, trees were trained as single trunk after plantation in E2 where plantation density was the highest. This could have generated the observed differential height plasticity among progenies. In fact, a response to training could particularly explain that only trunk height was higher in E2 than in E1 among all tree form variables. Moreover, benefiting of less space to grow in width could have lead trees in E2 to grow more in height. The trunk height appeared to be not significantly impacted by the genotype effect. This result is unlike that found on the same character observed over the first five years of growth in E1 location, revealing a highly significant effect of both genotype and year of growth (Ben Sadok et al. 2013).

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Tables

Table 1. List of quantitative variables collected on olive tree global form, topology and geometry traits with detailed formula for calculated variables.

Observation scale	Mesured and calculated Variables	Abbreviations	Formula
Whole Tree	Height (m)	H	
	Projection (m)	Proj	
	Basis area (m^2)	B_area	$\Pi(Proj)^2$
	Tree Volume (m3)	V	1/3* B_area*H
	Trunk basal diameter (mm)	Tr_Bdiam	
Sympodes and Growth Units	**Topology**		
	Nb of internodes	Nb_IN	
	Nb of axillary shoots	Nb_AS	
Growth Units	Nb of long, medium, short axillary.shoots/ GU	Nb_L; Nb_M; Nb_S	
	Geometry		
	Length (cm)	L	
	Mean Internode length (cm)	Mean INL	L/Nb_IN
	Length of the longest internode (cm)	IN_Max	

Table 2. Models selected according to Bayes Schwarz information criterion (BIC) for architectural traits related to whole tree form and growth units (GUs) in 'Olivière' × 'Arbequina' progeny.

Variables	**Factors**						**Variance Function**	**Correlation Structure**	**Variance estimates**				***H²***
Whole tree	*G*	*E*	*Y*	*O*	*GxE*	*GxO*			V_G	V_{GxE}	V_{GxO}	*Vr*	
Tr_Bdiam_{sqrt}	-	***	***		.					0,171		0,296	NS
Proj	*	***	***		.				0,003	0,004		0,018	0,31
B_area_{sqrt}	*	***	***		.				0,010	0,014		0,057	0,31
H	-	**	***		***					0,023		0,045	NS
V_{sqrt}	**	***	***		-		VarExp		0,010			0,019	0,52
Growth Units													
Geometry													
L_{sqrt}	-	***	-	***	-	.					1,91E-10	4,518	NS
IN_Max	***	-	***	-	-	-	VarPower		0,148			0,100	0,74
$Mean_INL_{sqrt}$	**	-	-	-	-	*			0,002		0,0032	0,054	0,28
Topology													
Nb_IN_{sqrt}	-	***	-	***	-	.					5,22E-11	1,568	NS
Nb_AS_{sqrt}	*	***	**	**	-	*			0,029		0,125	2,397	0,11
Nb_L_{sqrt}	-	***	-	***	-	.					1,96E-10	1,089	NS
Nb_M_{sqrt}	.	***	***	***	-	-			0,0173			1,232	NS
Nb_S_{sqrt}	*	***	***	***	-	***			0,0485		0,0869	1,525	0,25

P.value : '***' <0.0001 '**' <0.001'*' <0.01 '.' <0.1 '-' ns

Table 3. Mean values and standard deviations of architectural traits as a function of environments (E1: Melgueil; E2: Cordoba) in 'Olivière' × 'Arbequina' progeny. (see Table 1 for variables definitions).

Variables	E1 (Melgueil)	E2 (Cordoba)
Whole tree		
Tr_Bdiam (mm)	**34,62 (13,97)**	24,59 (11,82)
Proj(m)	**0,80 (0,25)**	0,71(0,25)
B_area (m^2)	**2,22 (1,29)**	1,76 (1,13)
H(m)	1,49 (0,36)	**1,60 (0,36)**
V(m^3)	**1,22 (0,85)**	1,03 (0,80)
Geometry		
L (cm)	**43,62 (27,17)**	35,42 (25,01)
IN_Max (cm)	4,12 (1,33)	4,10 (1,34)
Mean_INL (cm)	2,22(0,82)	2,15 (0,78)
Topology		
Nb_IN	**18,56 (10,39)**	16,07(10,13)
Nb_AS	**13,66 (13,33)**	7,48 (8,72)
Nb_L	**4,47 (4,86)**	2,32 (3,41)
Nb_M	**3,70 (4,59)**	2,11 (3,24)
Nb_S	**5,57 (7,48)**	3,01 (4,35)

Figures

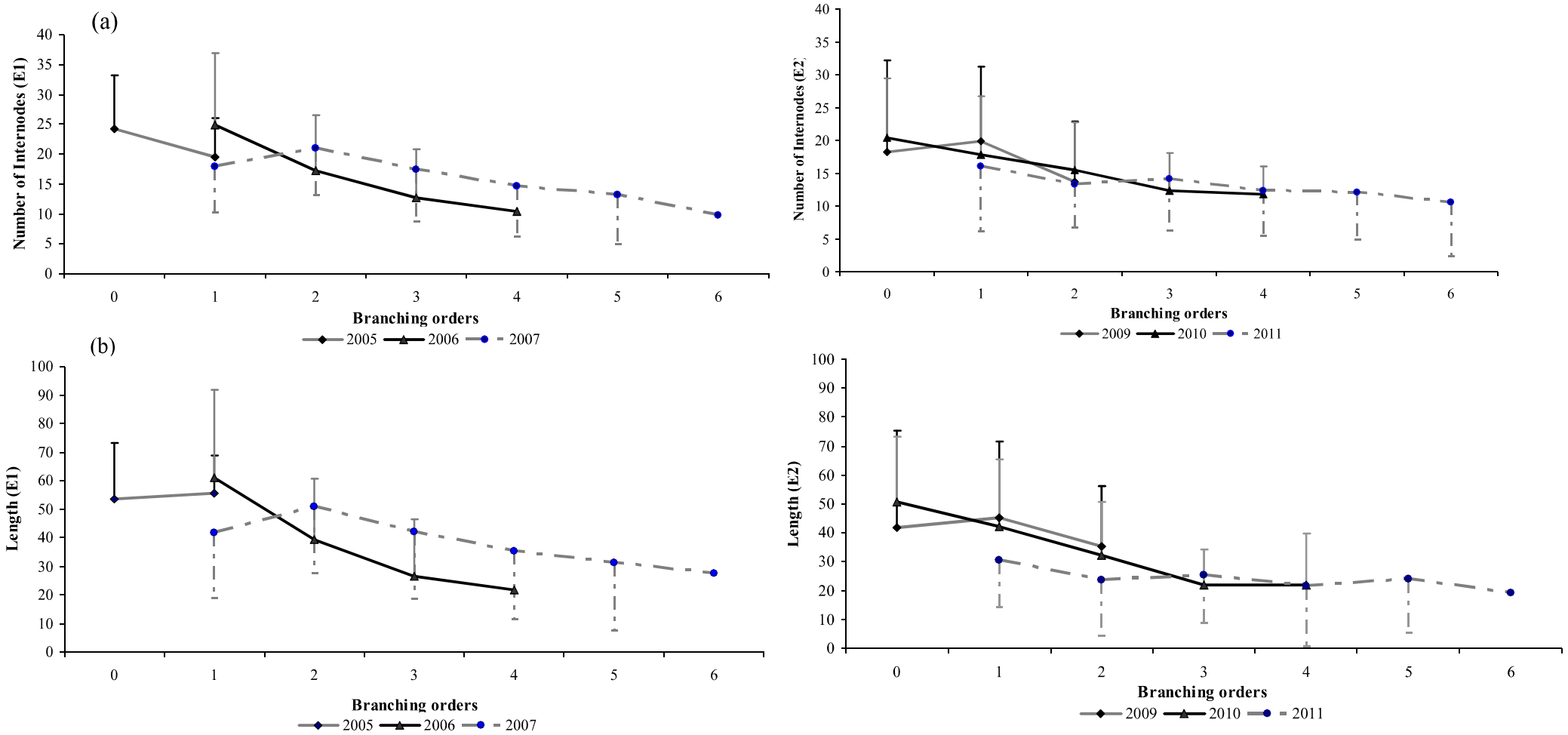


Figure 1. Mean values and standard deviation of the number of nodes per growth unit (GU) and GU length (cm) as a function of years and branching orders, calcula ted on 'Olivière' x 'Arbequina' progeny: illustration of the ontogenic trend.

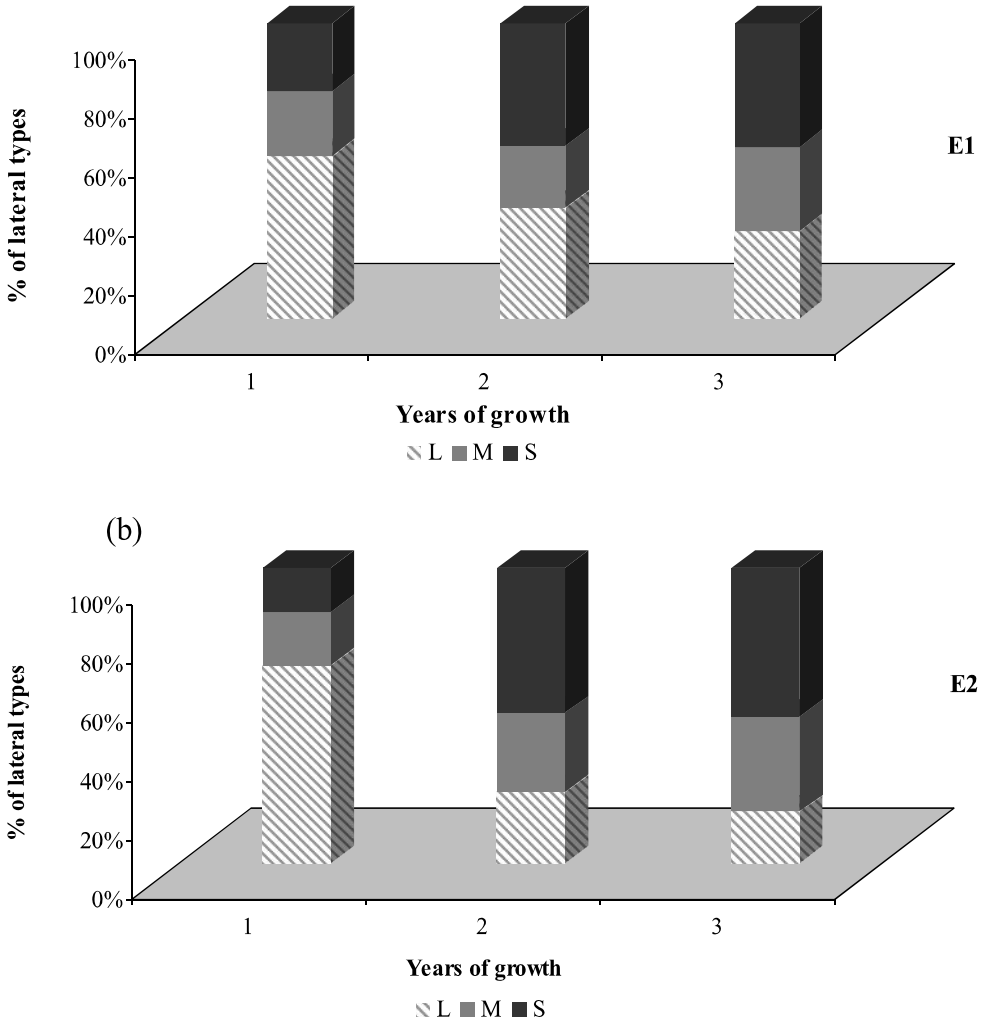


Figure 2. Percentage of sylleptic lateral growth units (GUs) types (long, medium or short) depending on parent GU age in 'Olivière' x 'Arbequina' progeny: (a) traits recorded in Melgueil experimental station (E1); (b) traits recorded in Cordoba experimental station (E2).

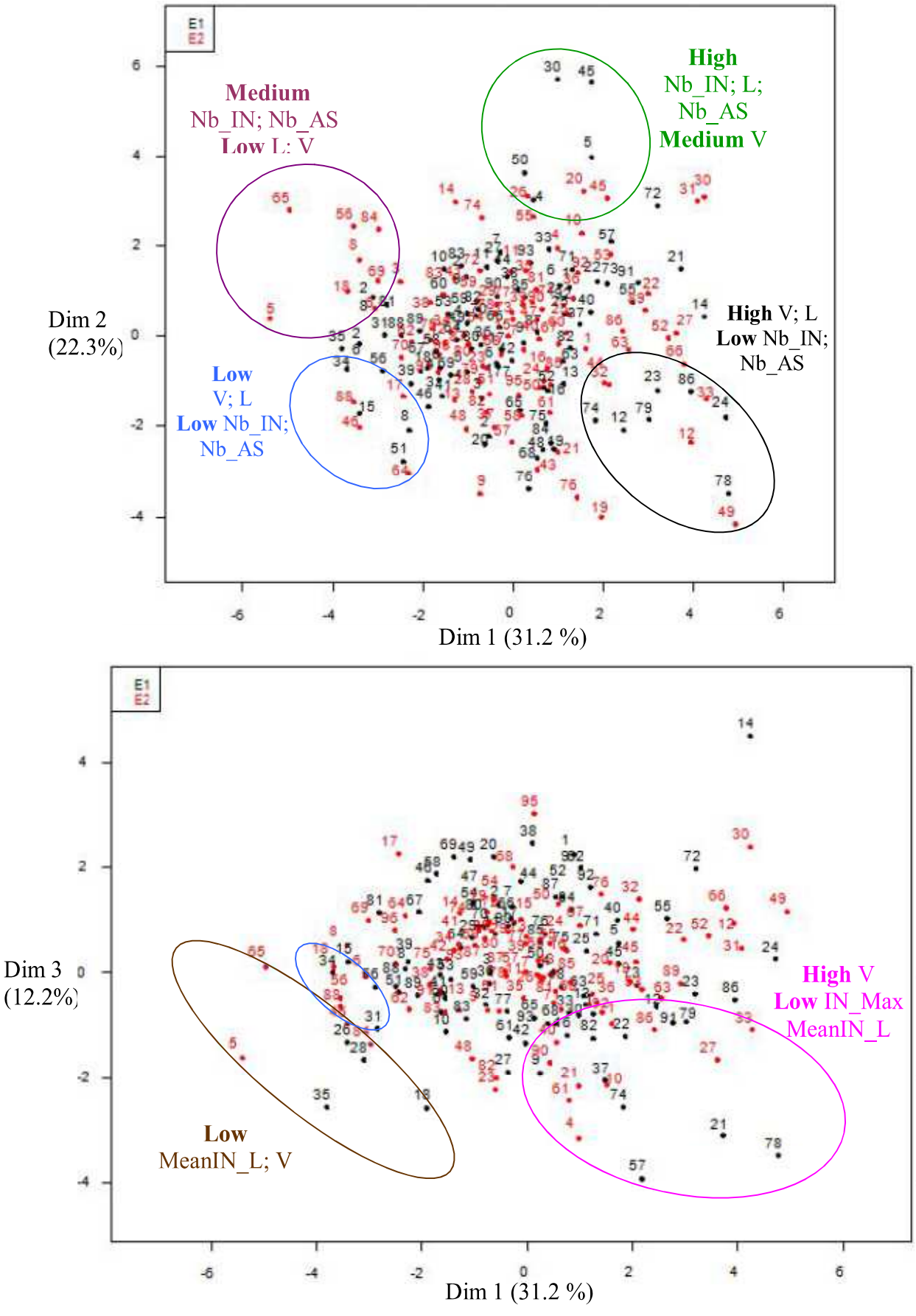


Figure 3. Progenies factor map (PCA): Dim 1: whole tree form variables; Dim 2: Primary growth and branching variables; Dim 3: Internode lengthening variables.

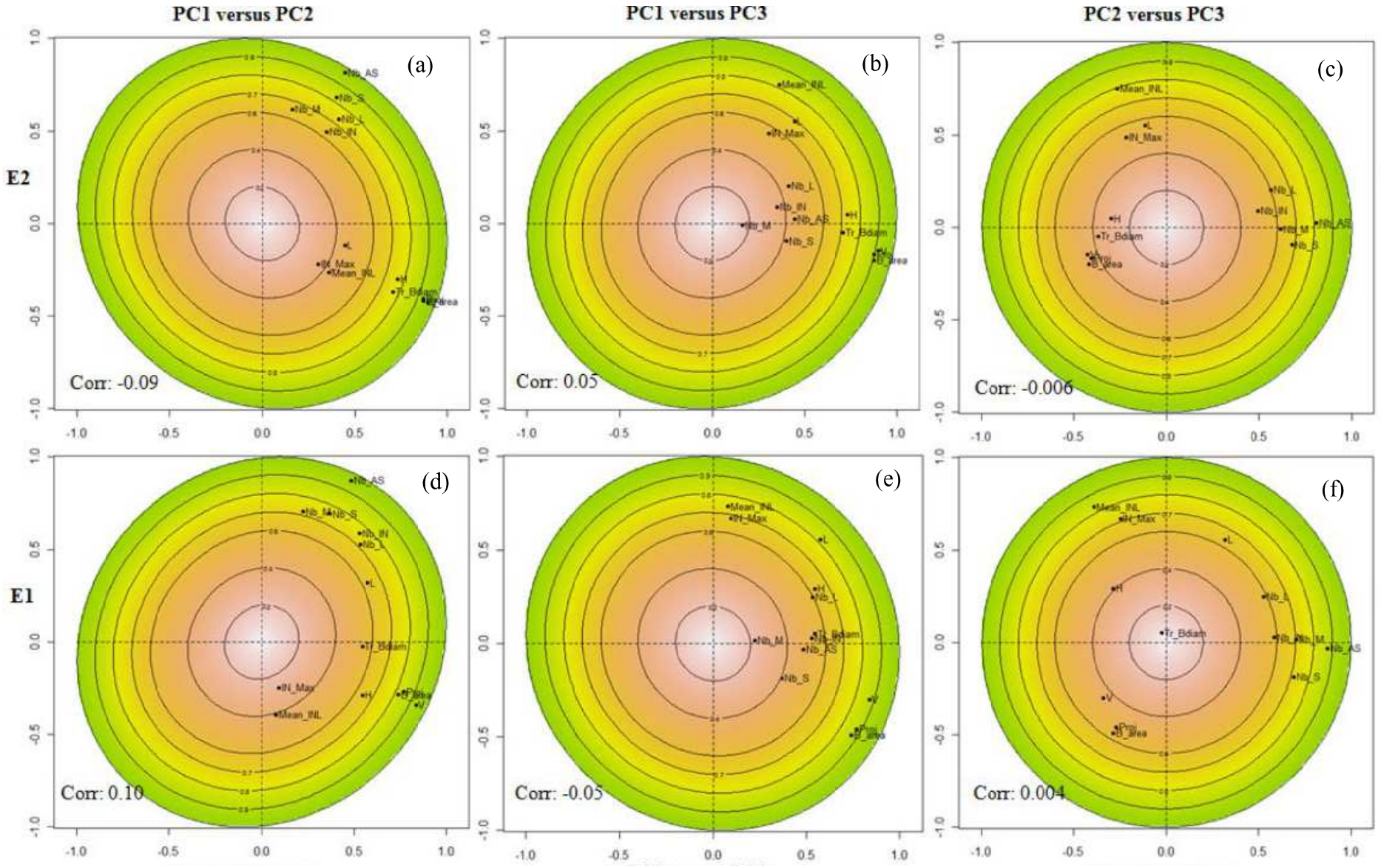


Figure 4. Duale Multiple factor analysis (DMFA): within-environment structure. Points display the covariation between phenotypic trait and PCs. Ellipses represent the quality of the variable projection on the PCs plane. 'Corr' is the coefficient of correlation between PCs. (a-c) Structure of the phenotypic space in E2. (d-f) Structure of the phenotypic space in E1. (See Table 1 for variable definitions)

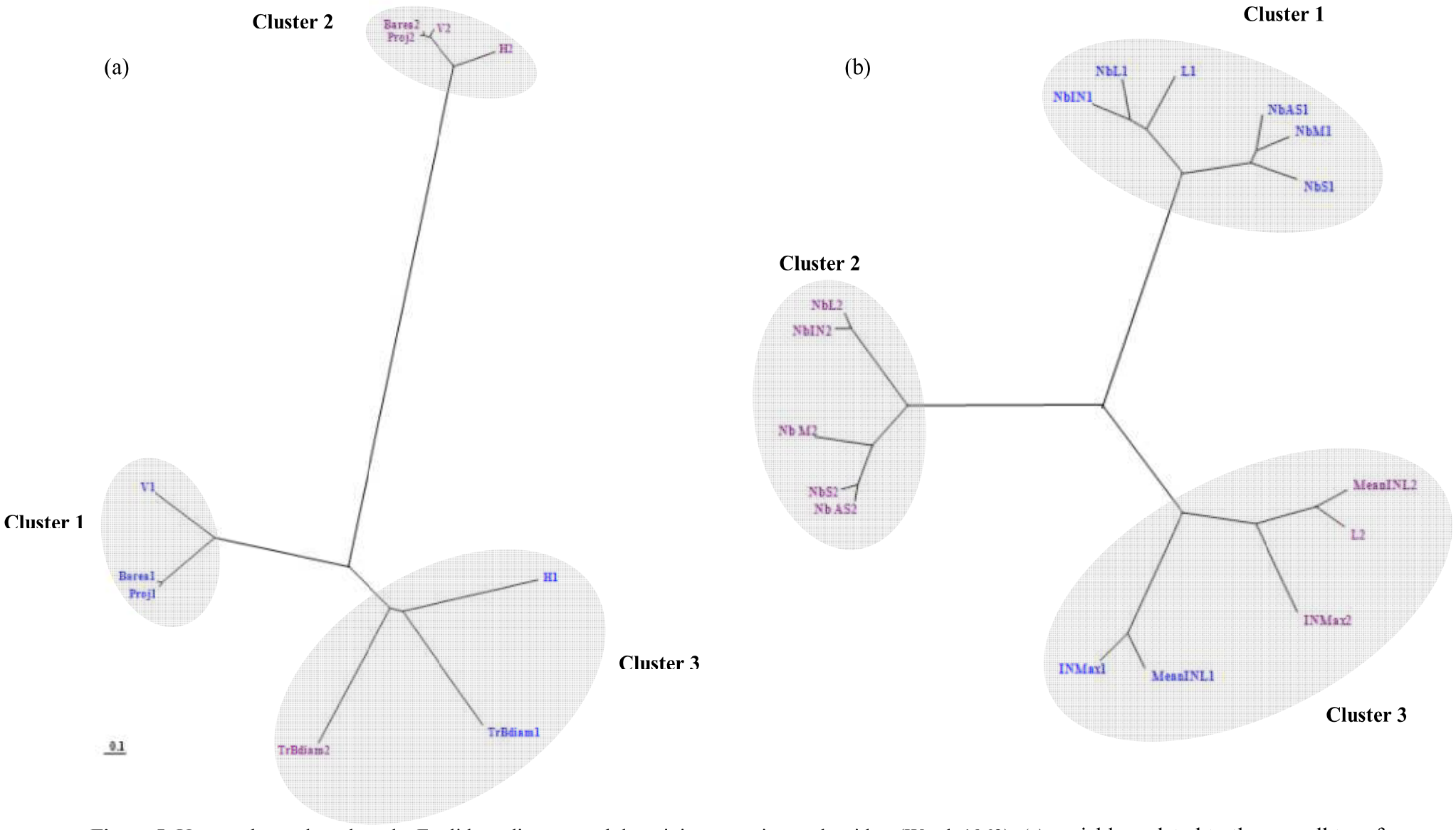


Figure 5. Unrooted trees based on the Euclidean distance and the minimum variance algorithm (Ward, 1963): (a) variables related to the overall tree form (b) variables related to the GUs growth and branching and internode lengthening variables.Variables in blue were recorded in E1 (Melgueil) and those in purple were recorded in E2 (Cordoba).

SUPPLEMENTARY DATA FILE

(a)

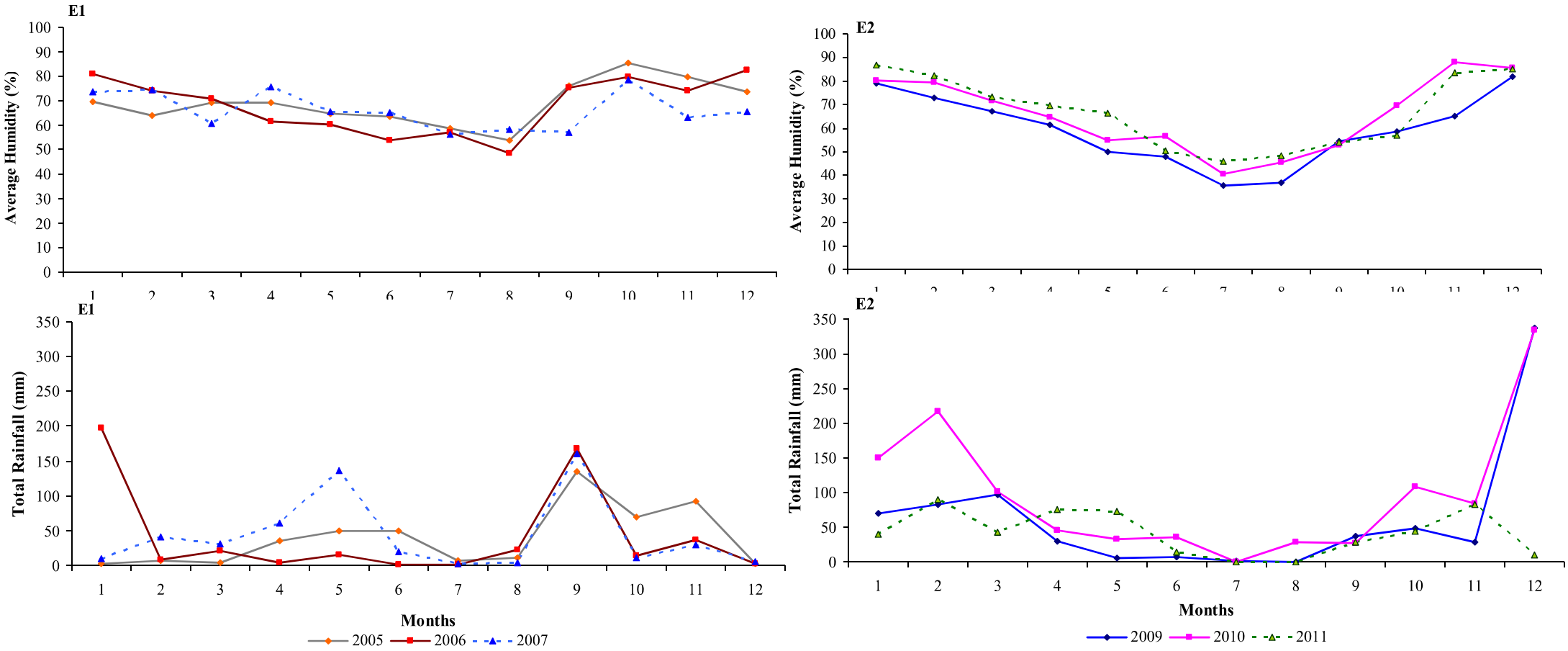
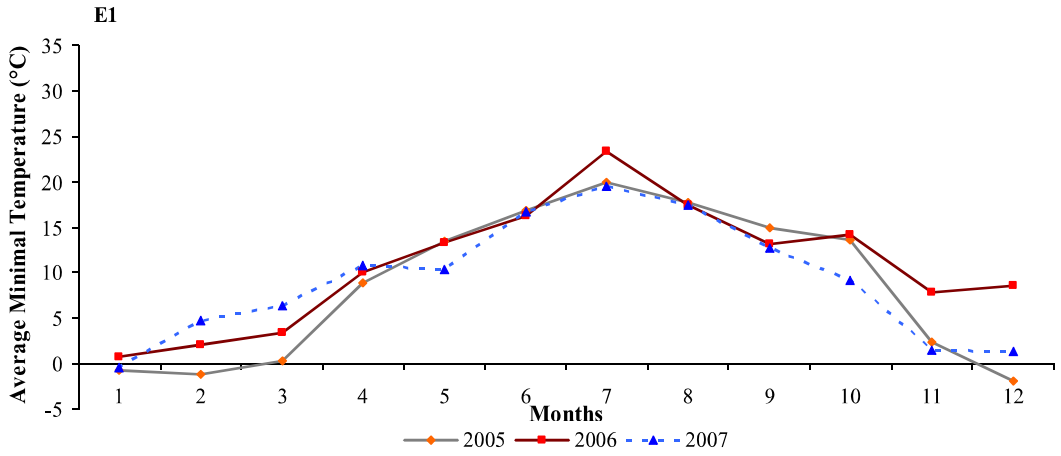
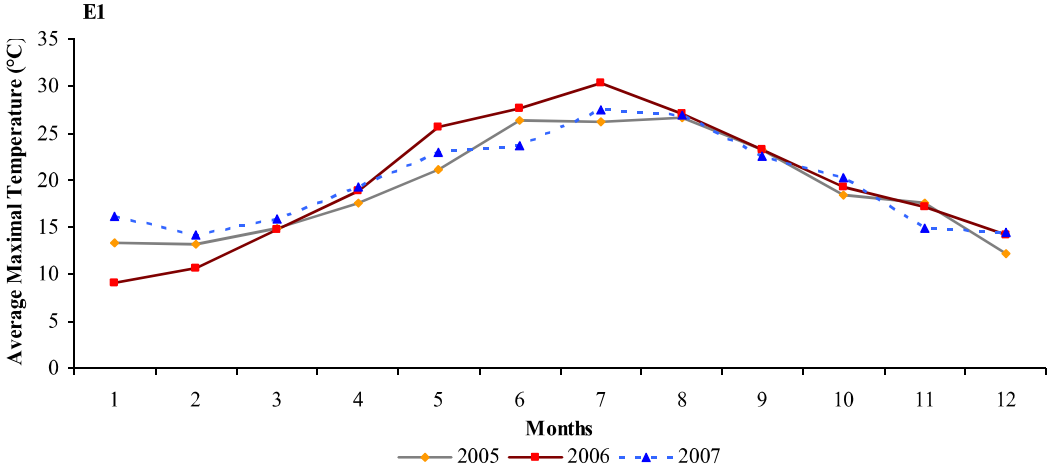
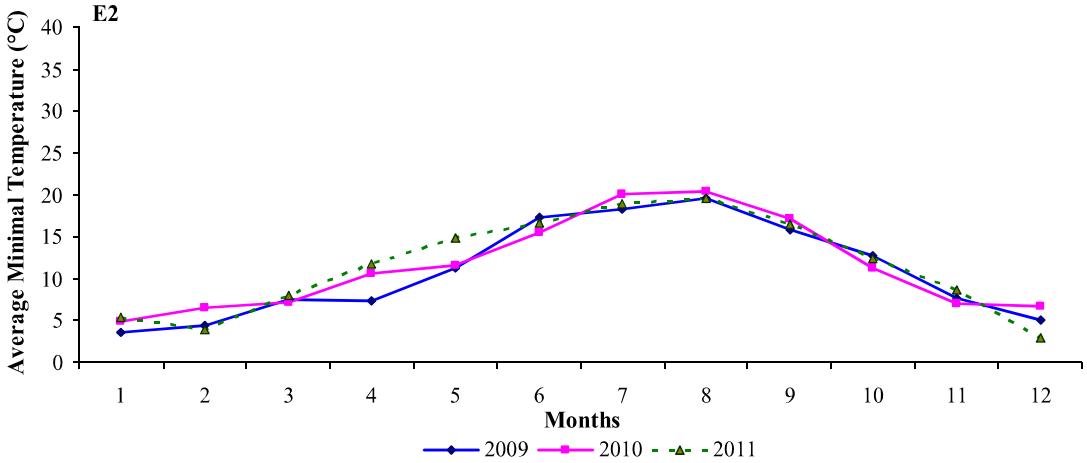


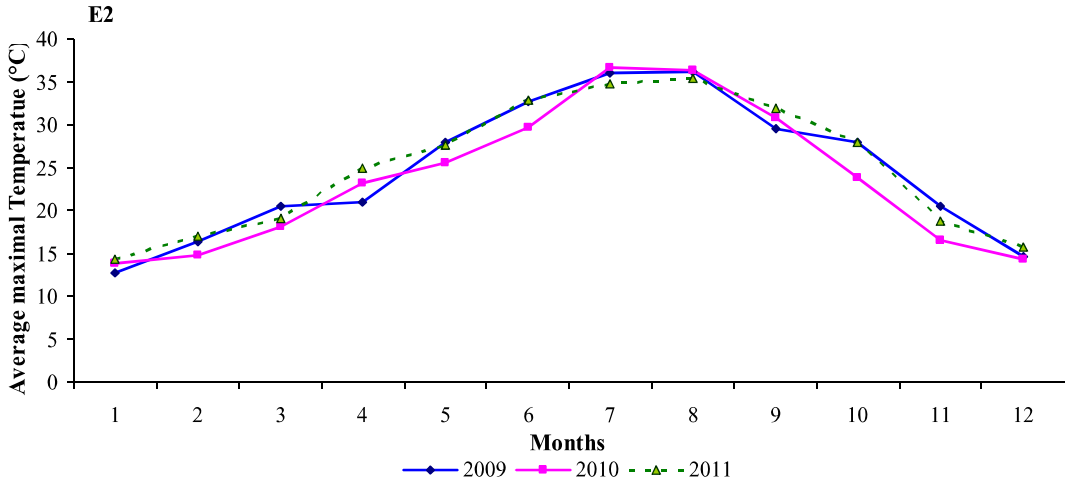
Figure S1. Meteorological records during 2005-2007 in Melgueil (E1) and 2009-2011 in Cordoba (E2): (a) Humidity and Rainfall (b) Minimal and maximal Temperature (c) Average temperature: Data were from meteorological stations at Melgueil-INRA and IFAPA experimental stations

(b)

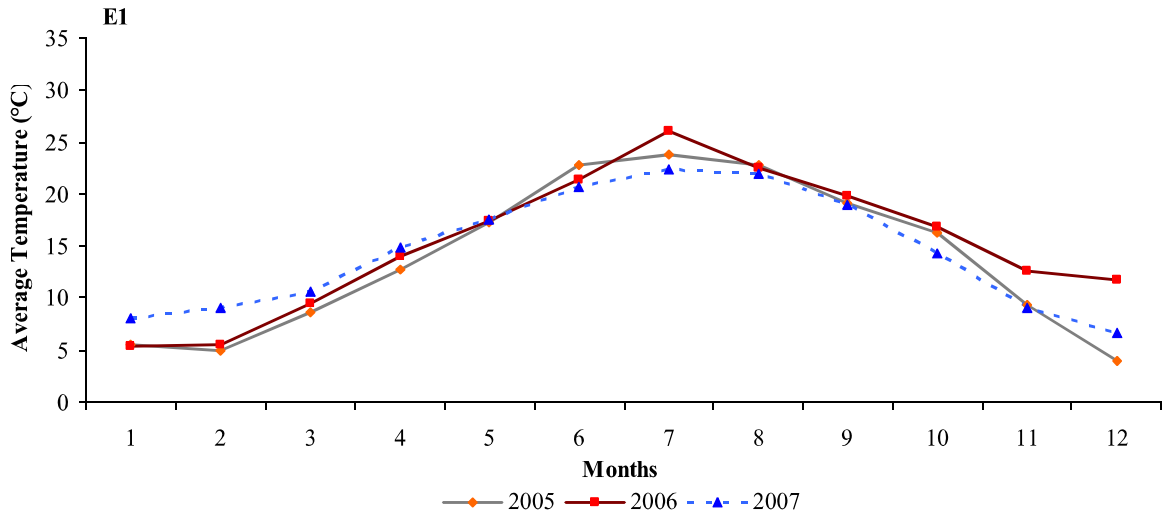

E1
Average Minimal Temperature (°C)
35
30
25
20
15
10
5
0
-5
1 2 3 4 5 6 7 8 9 10 11 12
Months
2005 2006 2007


E1
Average Maximal Temperature (°C)
35
30
25
20
15
10
5
0
1 2 3 4 5 6 7 8 9 10 11 12
Months
2005 2006 2007


E2
Average Minimal Temperature (°C)
40
35
30
25
20
15
10
5
0
1 2 3 4 5 6 7 8 9 10 11 12
Months
2009 2010 2011


E2
Average maximal Temperatue (°C)
40
35
30
25
20
15
10
5
0
1 2 3 4 5 6 7 8 9 10 11 12
Months
2009 2010 2011

(c)


E1
Average Temperature (°C)
35
30
25
20
15
10
5
0
1
2
3
4
5
6
7
8
9
10
11
12
Months
2005
2006
2007

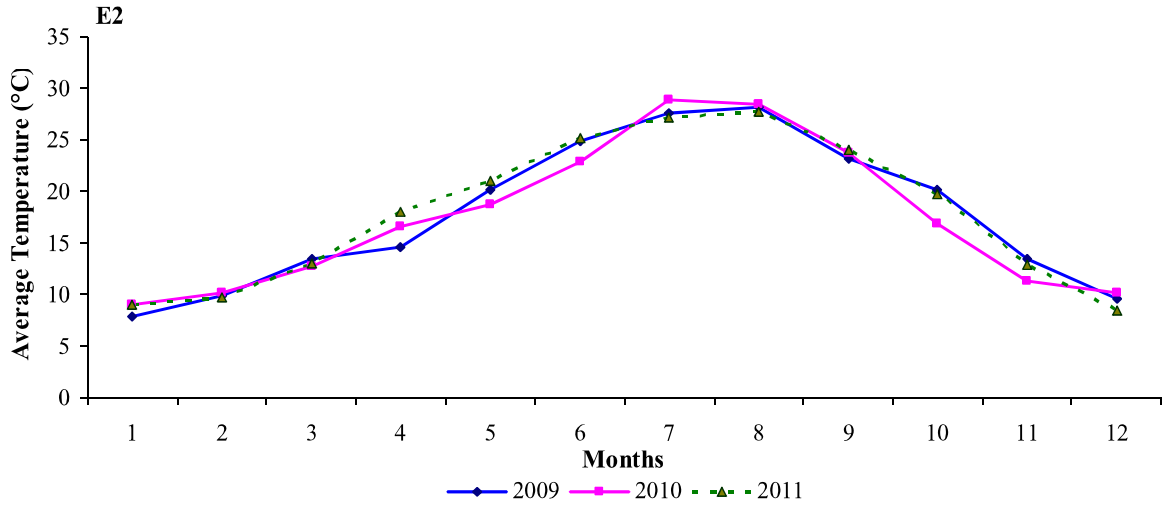

E2
Average Temperature (°C)
35
30
25
20
15
10
5
0
1
2
3
4
5
6
7
8
9
10
11
12
Months
2009
2010
2011

Table S1. Across environment MFA analysis: (a) Eigenvalues and Percentage of Explained Inertia by the first five components (b) correlations between variables and PCs (c) variables contribution to each PC.

(a)

	eigenvalue	%variance	% cumulative variance
PC 1	4,05	31,19	31,19
PC 2	2,89	22,26	53,45
PC 3	1,59	12,20	65,65
PC 4	1,00	7,70	73,35
PC 5	0,85	6,51	79,86

(b)

	PC1	PC2	PC3	PC4	PC5
V	0,87	-0,38	-0,23		
Proj	0,82	-0,34	-0,33		0,26
B_area	0,81	-0,35	-0,36		0,26
H	0,64	-0,29	0,18		-0,43
Tr_Bdiam	0,63	-0,19			-0,60
L	0,51		0,55	-0,44	
Nb_L	0,47	0,55	0,23	-0,17	
Nb_AS	0,47	0,84		0,21	
Nb_IN	0,44	0,54		-0,47	0,22
Nb_S	0,39	0,69	-0,14	0,15	
Mean_INL	0,23	-0,33	0,73		
IN_Max	0,21	-0,23	0,58	0,49	0,33
Nb_M	0,19	0,66		0,48	

(c)

	PC1	PC2	PC3	PC4	PC5
Nb_IN	4,67	**10,13**	0,19	21,96	5,81
L	6,32	0,36	**19,15**	19,13	0,48
Nb_AS	5,34	**24,56**	0,00	4,38	0,08
Nb_L	5,47	**10,30**	3,23	2,74	0,31
Nb_M	0,91	**15,13**	0,00	23,46	0,90
Nb_S	3,70	**16,28**	1,32	2,14	0,25
IN_Max	1,05	1,88	**21,56**	24,07	12,62
Mean_INL	1,28	3,75	**34,03**	1,22	0,07
Tr_Bdiam	**9,76**	1,31	0,00	0,04	41,92
H	**10,17**	2,98	2,14	0,05	21,55
V	**18,51**	5,09	3,38	0,22	0,41
B_area	**16,19**	4,28	8,20	0,38	7,84
Proj	**16,64**	3,95	6,79	0,20	7,74

Table S2. Within environment MFA analysis: (a) Variables contribution to each PC within E1 and E2 environments (b) Correlation between variables.

(a)

Variables	E1			E2		
	PC1	**PC2**	**PC3**	**PC1**	**PC2**	**PC3**
Nb_IN	0,53	0,59	0,03	0,35	0,50	0,09
L	0,57	0,32	0,56	0,45	-0,12	0,55
Nb_AS	0,49	0,87	-0,03	0,45	0,81	0,02
Nb_L	0,53	0,53	0,25	0,41	0,56	0,20
Nb_M	0,23	0,70	0,02	0,16	0,62	-0,01
Nb_S	0,37	0,69	-0,19	0,40	0,68	-0,09
IN_Max	0,10	-0,25	0,67	0,31	-0,22	0,49
Mean_INL	0,08	-0,39	0,73	0,36	-0,27	0,75
Tr_Bdiam	0,54	-0,02	0,05	0,71	-0,37	-0,05
H	0,55	-0,29	0,29	0,73	-0,30	0,05
V	0,84	-0,34	-0,30	0,90	-0,43	-0,15
B_area	0,74	-0,29	-0,49	0,87	-0,42	-0,20
Proj	0,77	-0,27	-0,46	0,87	-0,41	-0,17

(b)

E1	Nb_IN	L	Nb_AS	Nb_L	Nb_M	Nb_S	INMax	MeanINL	TrBdiam	H	V	B_area	Proj
Nb_IN	-												
L	0,67	-											
Nb_AS	0,55	0,41	-										
Nb_L	0,48	0,47	0,63	-									
Nb_M	0,32	0,18	0,76	0,37	-								
Nb_S	0,45	0,31	0,76	0,28	0,31	-							
INMax	-0,06	0,28	-0,14	0,05	-0,07	-0,21	-						
MeanINL	-0,29	0,30	-0,23	0,03	-0,15	-0,31	0,47	-					
Tr_Bdiam	0,18	0,26	0,19	0,29	0,04	0,15	-0,09	0,13	-				
H	0,06	0,35	-0,03	0,20	-0,07	-0,07	0,25	0,29	0,44	-			
V	0,18	0,22	0,07	0,15	-0,07	0,07	0,04	0,05	0,37	0,54	-		
B_area	0,18	0,08	0,09	0,11	-0,05	0,09	-0,04	-0,07	0,21	0,21	0,90	-	
Proj	0,21	0,11	0,10	0,15	-0,04	0,09	-0,03	-0,06	0,23	0,24	0,89	0,99	-
E2	**Nb_IN**	**L**	**Nb_AS**	**Nb_L**	**Nb_M**	**Nb_S**	**INMax**	**MeanINL**	**TrBdiam**	**H**	**V**	**B_area**	**Proj**
Nb_IN	-												
L	0,18	-											
Nb_AS	0,45	0,05	-										
Nb_L	0,51	0,18	0,70	-									
Nb_M	0,22	-0,11	0,65	0,18	-								
Nb_S	0,31	0,03	0,89	0,43	0,46	-							
INMax	-0,09	0,07	0,06	0,04	0,09	0,03	-						
MeanINL	0,03	0,46	0,00	0,12	-0,07	-0,04	0,35	-					
Tr_Bdiam	-0,04	0,23	0,08	0,12	-0,05	0,08	0,21	0,22	-				
H	0,16	0,30	0,12	0,03	0,07	0,14	0,20	0,24	0,60	-			
V	0,12	0,31	0,11	0,10	-0,03	0,13	0,27	0,27	0,69	0,76	-		
B_area	0,10	0,29	0,11	0,13	-0,06	0,13	0,27	0,25	0,66	0,60	0,96	-	
Proj	0,11	0,30	0,12	0,15	-0,06	0,14	0,27	0,29	0,66	0,58	0,92	0,98	-

Table S3. Similarity measures with the Euclidean distance using Ward's minimum variance.

(a)

cluster 1		
V1	B_area1	0,54
V1	Proj1	0,52
B_area1	Proj1	**0,04**
cluster 2		
B_area2	H2	0,31
Proj2	H2	0,30
V2	H2	0,28
V2	Proj2	0,07
V2	B_area2	0,06
B_area2	Proj2	**0,03**
cluster 3		
Tr_Bdiam2	H1	1,47
Tr_Bdiam2	Tr_Bdiam1	1,35
Tr_Bdiam1	H1	**1,31**

(b)

cluster 1		
Nb_AS1	L1	0,85
Nb_M1	Nb_IN1	0,72
Nb_S1	Nb_L1	0,72
Nb_M1	Nb_L1	0,64
Nb_IN1	L1	0,60
Nb_L1	Nb_AS1	0,57
Nb_S1	Nb_M1	0,54
Nb_AS1	Nb_IN1	0,51
Nb_L1	L1	0,50
Nb_S1	Nb_IN1	0,49
Nb_L1	Nb_IN1	0,41
Nb_S1	Nb_AS1	0,39
Nb_M1	Nb_AS1	**0,36**
cluster 2		
Nb_M2	Nb_L2	0,87
Nb_M2	Nb_IN2	0,82
Nb_AS2	Nb_IN2	0,55
Nb_M2	Nb_AS2	0,53
Nb_S2	Nb_IN2	0,53
Nb_S2	Nb_L2	0,52
Nb_L2	Nb_AS2	0,51
Nb_S2	Nb_M2	0,43
Nb_S2	Nb_AS2	0,17
Nb_L2	Nb_IN2	**0,16**
cluster 3		
L2	M_INL1	1,25
IN_Max1	L2	1,15
M_INL2	M_INL1	1,10
IN_Max2	M_INL1	0,98
IN_Max1	M_INL2	0,97
IN_Max2	L2	0,97
IN_Max2	IN_Max1	0,76
IN_Max2	M_INL2	0,68
IN_Max1	M_INL1	0,39
L2	M_INL2	**0,35**

Chapter 3

QTL mapping of flowering and fruiting traits in olive

This chapter is adapted from an article published in PlosOne Journal.

Investigated questions:

- ***What is the main bearing behaviour of the studied progeny?***
- ***Is the alternate bearing habit segregate in an olive progeny?***
- ***There is a genetic determinism of the traits related to the reproductive development?***
- ***How many loci are involved in flowering and fruiting traits and what are their***

QTL mapping of flowering and fruiting traits in olive

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ABSTRACT

One of the challenge fruit growers are facing is to balance between tree production and vegetative growth from year to year. To investigate the existence of genetic determinism for reproductive behaviour in olive tree, we studied an olive segregating population derived from a cross between 'Olivière' and 'Arbequina' cultivars. Our strategy was based on (i) an annual assessment of individual trees yield, and (ii) a decomposition of adult growth units at the crown periphery into quantitative variables related to both flowering and fruiting process in relation to their growth and branching. Genetic models, including the year, genotype effects and their interactions, were built with variance function and correlation structure of residuals when necessary. Among the progeny, trees were either 'ON' or 'OFF' for a given year and patterns of regular vs. irregular bearing were revealed. Genotype effect was significant on yield but not for flowering traits at growth unit (GU) scale, whereas the interaction between genotype and year was significant for both traits. A strong genetic effect was found for all fruiting traits without interaction with the year. Based on the new constructed genetic map, QTLs with small effects were detected, revealing multigenic control of the studied traits. Many were associated to alleles from 'Arbequina'. Genetic correlations were found between Yield and Fruit set at GU scale suggesting a common genetic control, even though QTL co-localisations were in specific years only. Most QTLs were associated to flowering traits in specific years, even though reproductive traits at GU scale did not capture the bearing status of the trees in a given year. Results were also interpreted with respect to ontogenetic changes of growth and branching, and an alternative sampling strategy was proposed for capturing tree fruiting behaviour. Regular bearing progenies were identified and could constitute innovative material for selection programs.

Keywords: Flowering, Fruit set, Regular bearing, Ontogeny, Heritability, Blups, Genetic map, QTLs

INTRODUCTION

Once they reach adulthood, perennial species undergo several morphological and physiological changes from vegetative to reproductive phases. The process of flowering involves converting into flowers or inflorescences a portion or sometimes all meristems that would otherwise produce vegetative shoots [1]. However, a minimum of vegetative development is needed to reach the adult phase and the ability to develop flowers [2,3]. The transition from a vegetative meristem to a reproductive one is usually divided into two phases: i) the induction phase, that involves biochemical modifications leading the apical bud to the formation of a reproductive structure, and ii) the differentiation phase during which the tissues of flower organs are formed [4]. Flower initiation is usually limited by concomitant heavy cropping which can lead to alternate bearing [5].

Alternate bearing, described as a sequence of heavy yields followed by light ones over several years, affects several temperate, tropical and sub-tropical fruit tree species such as apple, pear, apricot, coffee, and mangos [5]. The inhibition of floral induction by developing fruits has been pointed as playing a central role in alternate or irregular bearing but the physiological causes of alternation are still under investigation [5,6]. The hormonal control of floral induction has been reported in perennial fruit species. Gibberellins (GA) and auxins could potentially act as inhibiting signals, whereas cytokinins are likely to enhance floral induction [7]. Using apple as a model, Guitton et al. [8] demonstrated the genetic determinism of fruiting behaviour and identified several genes related to metabolism, degradation and transport of GA and auxins, that co-localized with QTLs for biennial bearing. These authors have showed that the control of fruit bearing is a complex multigenic process, influenced by both tree age and climatic year. In addition, the development of new shoots plays a central role in the complex interactions determining vegetative and reproductive growth in woody plants. In fact, in the fruiting year, resources are principally oriented to flowering and fruiting at the expense of vegetative growth [5,9] leading to a reduced vegetative growth and possibly a reduced floral induction in the following year.

Quantifying the regularity of fruit tree production usually relies on evaluation over consecutive years and can be summarized through indices such as the biennial bearing index (BBI). BBI initially proposed by Hoblyn et al. [10] and further renamed by Wilcox [11] has

been used in several species such as apple [8], citrus [12] and pistachio [13]. Its calculation accounts for the intensity of deviation of tree yield, over successive years whatever its sign.

In the olive tree (*Olea europaea L. subsp. europaea*), cyclical changes in crop production are particularly severe and cause drastic economical issues [14]. Similar hypothesis about the inhibition of the floral induction by developing fruits as well as the role of vegetative growth under bearing condition are privileged [15,16]. Fruit production has a competitive effect on new vegetative development, and thus on the formation of potential flowering sites as olive inflorescences are usually developed from the buds formed in the leaf axils of current growing season's shoots [17-19]. Fruits representing more than 65% of the seasonal dry matter accumulated in the fruiting shoots, reduce shoot growth in bearing years and affects fruit ripening [20-21]. Both reproductive shoot percentage and flowering have been reported to be substantially higher in previously non-bearing olive trees [18]. The relationship between the reproductive behaviour and new shoot development has been studied on the olive cultivar 'Hojiblanca', showing that even though new shoots development was higher for non-bearing years, shoots were predominantly of short length during both bearing and non-bearing years [22].

Besides biennial bearing habit, olive fruit production is characterized by a massive abscission of young fruits in post-bloom period. The capacity to set fruit results from an equilibrium between flowers within the inflorescence and is modulated by fertilization of ovaries and by competition among fruitlets [15,23]. Moreover, fruit set is not only controlled by competition within each inflorescence but is dependent on the global fruiting potential of the tree [24]. Another potential factor affecting olive tree cropping is the abundance of staminate flowers relative to hermaphroditic ones, and/or their irregular distribution in the canopy [25,26]. Staminate flowers are incompetent to set fruits due to their inability to complete pistil development. Moreover, environmental and nutritional stress between bud burst and 6 weeks before anthesis reduce the number of flowers per inflorescences and increases pistil abortion [26,27].

To date, most reproductive behaviour studies have focused on mature olive cultivars, giving insights on the physiological processes and environmental constrains affecting flowering and fruiting habits. Very little is known about the segregation of reproductive development traits and the morphogenetic factors impacting fruiting behaviour in the olive tree. Knowledge on olive genetic remains scarce, due to the long generation time (juvenility phase can last up to

15 years). This is also owed to the olive high heterozygosity and chromosome number (2n=46) for a genome size of about 3,120Mbp [28].

In the present study, we investigated the genetic basis of the reproductive development in a F1 olive tree progeny derived from a cross between 'Olivière' and 'Arbequina' cultivars, with contrasted architectural traits and bearing behaviour. Our strategy was based on the quantification of the regularity of fruit tree production among progenies and the monitoring of the flowering and fruiting habit changes on adult growth units selected at the crown periphery with respect to trees ontogeny. We investigated major factors impacting reproductive traits and the relations between tree bearing status i.e. 'ON' or 'OFF' and the number of inflorescences and fruits developed on growth units. After building a new genetic map, QTLs mapping was finally carried out on the heritable traits.

MATERIALS AND METHODS

Plant Material

The studied progeny derived from 'Olivière' x 'Arbequina' cross and counts 160 genotypes, each replicated twice [29]. Parental cultivars were chosen for their contrasting architecture as well as their different flowering and fruiting potential. 'Olivière' is a vigorous male sterile French cultivar with a fast growth rate and a rapid entrance in production [30]. 'Arbequina' is a very productive Spanish cultivar adapted for high density [31]. In 2005, progenies and parents were planted at INRA-Montpellier experimental station using a random experimental design. After plantation, trunks were cut back to 50 cm to homogenize the plantation and trees were not pruned afterwards. Trees were regularly irrigated and phytosanitary condition was controlled by conventional practices all over the experiment.

Phenotyping

In 2008, 74% of progenies have started to produce inflorescences and fruits. Once all trees had entered into reproductive period in 2009, we initiated the study of their flowering and fruiting behavior. For studying 'Olivière' x 'Arbequina' progenies bearing behavior at the whole tree scale, fruits were harvested and weighed for each individual. Four harvesting campaigns were performed from 2008 and until 2011, i.e. from the forth to the seventh year of growth. Since, fruit ripening was relatively late in time among the progeny, the harvesting period was in December of each year. The mass of 100 fruits was determined for each tree in order to estimate the mass of individual fruits. This information was also used to estimate

fruits dropped on the ground. The total fruit weight per tree (Yield) was calculated as the sum of the weight of harvested fruits on the tree and the weight of the fruits on the ground.

To quantify growth and branching as well as flowering at the growth unit (GU) scale, two one-year-old GUs (long or medium) were selected per tree at the tree periphery [29]. Each GU was described twice per year, at flowering and fruit set periods during 3 consecutive years (2009-2011), (Figure S1). The number of inflorescences and fruits born at the leaf axils along these GUs or along their laterals were noted Inflo (or Fruit)_direct and Inflo (or Fruit)_AS, respectively. We considered the capacity for an inflorescence to develop at least one fruit as a measure of fruit set. The percentage of fruit set was calculated afterwards as the ratio between the number of inflorescences with at least one fruit and the total number of inflorescences along the GUs (Total_Fruitset), or the direct inflorescences (Fruitset_direct), or inflorescences along GUs sylleptic laterals (Fruitset_AS). All measured and calculated descriptors of bearing behaviour were classified according to the observation scale: whole tree and growth unit (Table 1).

Qualitative classification of progenies bearing behaviour and BBI calculation

Considering the mean Yield value per genotype, the sequence of the four years of production (2008-2011), was graphically drawn. Only genotypes having started producing in 2008 were considered i.e. a total of 141 progenies. Genotypes were then classified according to their yield evolution from a year *n* to *n*+1, as proposed by Guitton et al. [8]. For each pair of years, the direction of yield variation was coded by '+' or '-' symbols. Genotypes following the same pattern were clustered manually, and the mean Yield value of all genotypes per year was calculated for each cluster. Genotypes exhibiting lower or higher production in years *n*-1 and *n*+1 than in year n were considered as irregular. When production was increasing stably over years, genotypes were qualified as regular bearing.

Alternate bearing behavior of each genotype was quantified by the BBI calculation according to the formula below [10,11]:

$$\textit{Biennial bearing index (BBI)} = \frac{\sum_{i=2008}^{2011} \left(\left| y_{i+1} - y_i \right| \right) / \left(y_i + y_{i+1} \right)}{n-1}$$

Where y_i refers to the yield for year i, and n the number of studied years of production (in our case, n=4).

Statistical analysis

At tree scale, yields were analyzed on 282 trees (141 progenies repeated twice), previously used for manual classification. At GU scale, the analysis of flowering and fruiting variables was restricted to 240 trees (120 progenies repeated twice), on which data were available over the 3 years (2009 to 2011). Even though GUs phenotyped from a year to another were different, the studied variables may be correlated as the same trees were considered for the three consecutive years. Thus, variances homogeneity and covariance between successive years were examined by Levene's test [32]. Because distribution of flowering and fruiting variables was not Gaussian, all the variables were transformed with a square root transformation.

At both the GU scale and whole tree scale, the effect of genotype and year factors and their interactions on the reproductive behaviour variables were estimated according to the following mixed model:

$$P_{ijl} = \mu + G_i + Y_j + (GxY)_{ij} + \varepsilon_{ijl}$$

where P_{ij} is the phenotypic value of genotype i for year j, μ is the overall mean of the progeny for all years and genotypes, G_i is the random effect of genotype i, Y_j is the fixed effect of the year j, $(GxY)_{ij}$ is their random interaction and, ε_{ijl} is the random residual error effect for the l measured GUs per tree or trees per genotype.

For traits showing heterogeneous variances between years, a function of variance was introduced into the model. Different variances functions, as described by Pinheiro and Bates [33] were compared: i.e. varIdent, with different variances per level of the fixed factor; varPower, with variance increasing as a power function; varExp, with variance increasing as an exponential function; varConstPower, combines a constant value with a power function.

Moreover, because correlations between successive years may exist due to irregular and/or biennial bearing, a covariance structure was taken into account in the residual term of the corresponding mixed linear model. Different covariance structures were tested i.e. compound symmetry (corComSymm), autoregressive of order 1 (corAR1) and exponential (corExp). The minimization of Akaike and Bayes Schwarz information criteria (AIC and BIC respectively), allowed us to select the significant factors, variance function and covariance structure to be considered in the model. Therefore, the normality of residual distribution and model predictions adequacy with phenotypic values were checked.

All statistical analyses were performed using R software v.2.9.2, with REML estimation method, under lme4 and nlme packages [34].

Finally, heritability of flowering and fruiting genotypic means was estimated as the ratio between the genotypic and the phenotypic variances: $H^2 = \sigma^2_G / \sigma^2_P$. When significant interaction between the genotype and the year factor was selected, the heritability calculation was: $H^2 = \sigma^2_G / [\sigma^2_G + \sigma^2_{GxY}/a + \sigma^2_\varepsilon/na]$ where σ^2_G is the genotypic variance, σ^2_{GxY} is the variance of genotype and year interaction, σ^2_ε is the residual error variance estimated from the selected model, *n* the number of replicates per genotype, and *a* the number of studied years.

When no significant interaction between the genotype and the year factor was selected, heritability was calculated as: $H^2 = \sigma^2_G / [\sigma^2_G + (\sigma^2_\varepsilon/n)]$, where σ^2_G is the genotypic variance, σ^2_ε is the residual error variance estimated from the selected model, and *n* the number of replicate per genotype [35,36].

Linkage map construction

New genomic and expressed sequence tag (EST)-derived microsatellite (simple sequence repeat: SSRs) markers have been developed in olive and used to screen 147 genotypes from the 'Olivière' and 'Arbequina' progeny and the parents [Essalouh et al. unpublished data]. Polymorphic SSRs were integrated together with amplified fragment length polymorphism (AFLP), intersimple sequence repeat (ISSR) and SSR markers previously used in Khadari et al. [37], to build a new genetic map.

The Map construction was carried out using JoinMap version 4.0 [38]. Chi square values were calculated for each marker to detect segregation deviations ($P \leq 0.05$) from the expected Mendelian ratio. Highly distorted markers were discarded. Linkage groups (LG) were determined by a regression mapping procedure using an independence test of the logarithm of the odds (LOD score) with a minimum threshold of 5.0 [39]. Map construction was performed using the Kosambi mapping function [40] and the regression mapping algorithm following the JoinMap parameters: Rec=0.40, LOD=1.0, Jump=5. The obtained LGs were drawn using the MapChart software [41]. New connections were established between the original LGs, in particular the smallest one. Likewise, the new intercross markers allowed connecting the parental LGs. Thus, LG numbering was redefined in the present maps and was defined as OA, O and A for the integrated map 'Olivière'x'Arbequina', the 'Olivière' female parent map and 'Arbequina' male parent map, respectively.

To estimate observed genome coverage, the expected genome length in centiMorgans was estimated for each LG using the following method proposed by [42]: LG *expected length* = LG *observed length* * $[(m+1)/(m-1)]$ where *m* is the number of markers of each linkage group.

Observed genome coverage was assessed by dividing the observed genome length by the expected genome length.

Quantitative trait loci (QTL) detection

For each trait, the "Best linear unbiased predictions" (BLUPs) of random effects were extracted from the selected mixed linear models i.e. the genotype effect and used for QTL detection. The BLUP for the genotype effect was noted by the trait name. When significant, the BLUPs for the GxY factor were computed for each studied year of growth. These BLUPs were noted by the name of the trait followed by the considered year of growth.

Both new parental and integrated maps were used to identify QTL controlling flowering and fruiting traits at GUs scale as well as whole tree scale. QTL analyses were carried out using MapQTL version 6.0 [43]. A first step was the Kruskal-Wallis analysis in which, the effect of the marker significance level on a specific trait was estimated by simple linear regression between the marker and the trait [44]. Then, QTL mapping was performed using interval mapping (IM) which estimates the likelihood score of a putative QTL placed in any position within an interval flanked by two adjacent markers [45]. The LOD score threshold (P-value of 0.05) at which a QTL was declared significant, was determined using a permutation test for each LG as well as for the genome wide. Over 1000 permutations the frequency distribution of the maximum LOD score under the null hypothesis (no QTL) was determined [46]. When several markers displayed a LOD score equals or superior to the LOD score determined by the permutation test, they were declared as cofactors for a multiple QTL mapping (MQM) analysis with a step size of 1 cM [45].

Significant QTLs were characterized by their closest associated marker cofactors, LOD score, percentage of explained phenotypic variation, and confidence interval in centimorgans (cM) estimated on the basis of a LOD score drop of 1 or 2 on both sides of the likelihood peak. Genomic positions of the QTLs detected on the linkage groups were drawn using MapChart software [42]. Allelic effects were estimated as $A_f = [(\mu_{ac} + \mu_{ad}) - (\mu_{bc} + \mu_{bd})]/4$ for female additivity; $A_m = [(\mu_{ac} + \mu_{bc}) - (\mu_{ad} + \mu_{bd})]/4$ for male additivity and $D = [(\mu_{ac} + \mu_{bd}) - (\mu_{ad} + \mu_{bc})]/4$ for dominance where μ_{ac}, μ_{ad}, μ_{bc} and μ_{bd} are estimated phenotypic means associated to each of the 4 possible genotypic classes *ac*, *bc*, *ad* and *bd*, deriving for an *ab* x *cd* cross [47,48].

For traits showing multiple QTLs, a global model was built including all cofactors and their interactions in order to test epistatic effects between QTLs. A model selection was performed according to the Akaike's information criterion (AIC) and Schwarz's Bayesian criterion (BIC) minimization. The analysis was performed by a step model (i.e. iterative removal of

non significant cofactors) using R software v.2.9.3, with REML estimation method, under nlme package [34].

RESULTS

In our study, the two parents 'Olivière' and 'Arbequina', differed significantly for flowering and fruiting traits and transgressive segregation was observed in the progeny (Table S1). The examination of production averages over four years showed that 'Arbequina' production was stable during the two first years then decreased in the following years whereas 'Olivière' showed an alternate bearing habit (Figure S2). The average production of the progenies also showed an alternate bearing with heavy productions in 2009 and 2011 (i.e. 'ON' year) whereas a lower production was observed in 2010 ("OFF" year", Figure S1).

Identifying types of Bearing Behaviours in 'Olivière' x 'Arbequina' progeny

The examination of the sequences of the mean yield value per genotype over the four production years revealed seven bearing behaviours that were graphically identified in the population (Figure 1). Only 22 % of the progenies exhibited a regular bearing behaviour (R1 and R2; Figure 1) among which 16% increased regularly their production during the four consecutive years and showed a BBI of 0.25 (R1; Figure 1). About 6% of these regular bearing progenies showed late production behavior since their yield did not exceed 1Kg on average during the first 3 years of production and then increased in 2011 up to 2.7Kg (R2; Figure 1). Considering these deviation, the R2 class showed a BBI of 0.6 (Figure 1). The remaining 78% of the progenies exhibited irregular bearing behavior (classes I1 to I5; Figure 1). Among this fraction, 43% were typically biennial bearing, but with two subclasses exhibiting opposite phases (33% and 10% for I1 and I2, respectively; Figure 1). Indeed, 2009 and 2011 were 'ON' years in I1 type whereas they were 'OFF' in I2. The BBI value was of 0.6 and 0.42 for I1 and I2, respectively (Figure 1). The last three types corresponded to progenies with irregular bearing. In I3 (21%), progenies were characterized by only one 'ON' year during the 4 studied years, that coincided with the 'ON' year of I1 (2009). In I4 (4%), progenies were characterized by a weak production in 2009 (as progenies in I2), followed by a constant increase in production reaching a yield of 6 Kg on average in 2011. In I5 (10%), progenies showed an increasing production from 2008 to 2010, as in R1, but this was followed by a drastic diminution in 2011, reaching a lower yield value than in 2008. The BBI values were of 0.4, 0.33, and 0.39 for I3, I4 and I5, respectively (Figure 1).

Changes over time in the number and position of inflorescences and fruits along GU

An important variability in both the number of inflorescences and their repartition along the one year-old GUs was found among the three studied years. In 2009, 42 inflorescences on average were observed, this number including inflorescences born along the GUs and their sylleptic laterals. In 2010 and 2011, the mean values were lower, 15 and 18 inflorescences respectively (Figure 2; a). This decrease was also observed for the number of fruits produced per GU which mean values ranged from 17.89 in 2009 to 6.88 in 2010 and 8 in 2011 (Figure 2; b). The number of inflorescences born directly along GUs (i.e. Inflo_direct) showed an increase from the first to the third year whereas a drastic reduction of the number of those born on GUs sylleptic laterals was observed. This was concomitant with the decrease in the number of GUs laterals (i.e. Nb_AS). It may be noticed that the number of internodes of the floral GUs remained almost stable over the same years (Nb_IN, Figure 3). Consistently, a negative correlation between Inflo_direct and Nb_AS was observed during the three reproductive years (r= -0.42, data not shown). Changes were also found in the proportion of inflorescences born on each type of lateral GUs (Figure 4). The proportion of inflorescences on long sylleptic laterals (Inflo_L) ranged from 9.47% in the first year (2009) and decreased to 2.18 % and 0 % in the second (2010) and third year (2011), respectively. The proportion of inflorescences born on medium laterals (Inflo_M) also decreased whereas those on short laterals increased (Inflo_M and Inflo_S, respectively). Finally, in all studied years, a majority of inflorescences were born on short and medium types.

Changes over years were also observed on both Fruit_direct and Fruit_AS (Figure 2; b), with a progressive increase of the Fruit_direct and a decrease in the mean number of Fruit_AS. By contrast, fruit set (i.e. Total_Fruitset), observed whatever the inflorescences location, was relatively high (53 %, 62% and 48% in 2009, 2010 and 2011, respectively). Fruit set was greater on direct inflorescences than on inflorescences born on sylleptic laterals whatever the year (Figure 2; c).

Genetic analysis

Significance and variance estimation of genotype and year factors

The total yield per tree showed heterogeneous variances over years, whereas a weak correlation was found between consecutive years, for the whole progeny, according to Kendall's tau coefficients (Table S2). A strong genetic determinism was identified with highly significant genotype and interaction between the genotype and year (GxY) effects (Table 2). Highly significant effect of the year of production was also identified.

No significant correlation was observed between successive years for all flowering traits at GU scale, and models selected according to BIC criteria did not include any variance function (Table 2). The year factor was significant for all flowering traits whereas the genotype factor was significant only for two of them: Inflo_direct and Inflo_M. Highly significant interaction was found between the genotype and year factors for all these traits (Table 2).

Correlations between years were significant for all fruiting traits at GU scale, except Fruit_AS and Fruitset_AS, and an exponential spatial correlation structure corExp was selected in the corresponding models (Table 2). Heterogeneous variances were also found for all fruiting traits, again with the exception Fruit_AS, and were modeled by a power of covariate variance function (i.e. varPower; Table 2). Significant effects of both the genotype and year factors were found without significant interaction for all fruiting traits, except Fruit_AS. For this trait, genotype factor was not significant whereas interaction effect (GxY) was highly significant (Table 2).

Heritability of genotype mean

Heritability of genotype mean was estimated for all flowering and fruiting traits that showed significant genetic effect. Moderate heritability values were found for flowering traits, with H^2 values ranging from 0.20 to 0.52 for Inflo_tot and Inflo_direct, respectively (Table 2). Considering fruiting traits, moderate to high heritability values were estimated with values exceeding 0.7 for Total_Fruitset and Fruitset_direct (Table 2). Likewise, a high heritability value was found for tree yield ($H^2 = 0.6$).

Correlation between variables

Correlations between reproductive traits were investigated on the basis of the mean phenotypic values per genotype and year (Table S3). High Pearson correlation coefficients (r) were found between flowering and fruiting traits measured at GU scale: the total number of inflorescences (i.e. Inflo_tot) was highly correlated to the number of inflorescences born at the leaf axil along the GU (i.e. Inflo_direct) as well as the number of inflorescences born on GU laterals (i.e. Inflo_AS) in the three studied years (r values in italic; Table S.3a). The correlation between Inflo_tot and Inflo_AS decreased over years (from 0.96 in 2009 to 0.7 in 2011) whereas that with Inflo_direct increased (from 0.42 in 2009 to 0.74 in 2011), consistently with the respective changes in these latter variables over years. Total fruit set was highly correlated to Fruitset_direct whatever the year (underlined r values; Table S.3a). A slight negative correlation was found between the total number of inflorescences and the total

fruit set in the three years with (-0.31 and -0.38 framed values; Table S.3a). The tree production was not or weakly correlated to flowering and fruiting trait at GU scale (Table S.3b).

Map construction

In the present study, 94 new olive SSR were developed, of which 40 were from olive EST and 54 were from olive genomic library. SSRs were named 'Zit' referring to the Arabic word for 'Oil'. EST-SSRs were designated with indexes from 001 to 113 following the name 'Zit' i.e. Zit001 while genomic-SSRs were designated with indexes from 302 to 505 i.e. Zit302. Among these 94 SSRs, 55 were polymorphic in both parents (Table 3a). The remaining 39 SSRs were polymorphic in one parent only: 30 SSRs were polymorphic in 'Olivière' and 9 SSRs were polymorphic in 'Arbequina' (Table 3a). Among these new SSRs, 53 (56%) were mapped on 'Olivière' female parent, 48 (51%) on 'Arbequina' male parent, and 72 (76 %) on the integrated map 'Olivière' x 'Arbequina' (Table 3b).

The linkage analysis in 'Olivière' female parent was based on 362 markers including 257 AFLPs, 102 SSRs and 4 ISSRs. Among them, 212 markers including 68 SSRs were mapped in a total of 25 LGs (Table 3c; Figure S3a). The 'Olivière" map length was 1745.3 cM, representing an observed genome coverage of 77.9%. The average marker spacing was 8.23 cM, with a maximum gap of 32.5 cM between adjacent markers observed in the LG O9 (Table 3c, Figure S2a).

The linkage analysis in 'Arbequina' male parent was based on 352 markers including 256 AFLPs, 89 SSRs and 7 ISSRs. Among them, 252 markers including 75 SSRs were mapped in a total of 21 LGs (Table 3c; Figure S3b). The 'Arbequina' map length was 1597.6 cM, representing an observed genome coverage of 83.8%. The average marker spacing was of 6.34 cM with a maximum gap of 30.8 cM between adjacent markers observed in the LG A14 (Table 3c, Figure S3b).

Among the 535 segregating markers (407 AFLPs, 117SSRs and 11 ISSRs), 450 were mapped in the 'Olivière' x 'Arbequina' integrated map including 103 SSRs (Table 3c). The markers were assigned to 26 linkage groups (Table 3c; Figure 5). The integrated linkage map was 2148.4 cM, representing an observed genome coverage of 86.9%. The average marker spacing was of 4.77 cM with a maximum gap of 30.5 cM between adjacent markers observed in the LG OA14 (Table 3c).

QTL detection and Mapping

Results of QTL detection are detailed for each reproductive trait measured at both tree and GU scales. QTLs detected on the integrated map 'Olivière'x'Arbequina' are presented in Table 4 and Figure 5. Those that were also detected on parental maps are indicated in bold in Table 4. QTLs detected on parental maps are detailed in the supporting information file (Table S.4). In the following sections, QTLs for G effect BLUPs are described first. Second, results of QTL analysis for year-specific BLUPs are provided. In all cases, QTLs with the highest percentage of explained variability are detailed first, and co-localisations between QTLs are mentioned

Reproductive traits measured at tree scale

A QTL explaining 15.1% of the variability was detected on OA12 for yield per tree (Table 4; Figure 5). Seven others QTLs were detected for year-specific BLUPs of Yield. Two were detected on OA2 and OA6 for yield in 2008 and explained 13.6% and 9.6% of the variability, respectively (Yield_08; Table 4, Figure 5). Both QTLs were mainly due to a female additive effect. A global model built for these QTLs showed no significant interaction between cofactors and explained 16.4% of the trait variability (Table 5).

One QTL was detected on OA3 for the yield in 2009 (Yield_09; Table 4, Figure 5). This QTL explained 16.8% of the variability and resulted from both female and male additive effects. Three QTLs were mapped on OA4, OA9 and OA3 for the yield in 2010 (Yield_10; Table 4, Figure 5). These QTLs explained 13.4%, 11.1% and 10.8% of the variability, respectively. The QTL mapped on the median part of OA3 resulted from both male additive and dominance effects. The QTL detected on OA4 resulted from female and male additive as well as dominance effects while the QTL detected on OA9 resulted from dominance effect (Table 4). Together, these QTLs explained 18.8% of the variability (Table 5). One QTL was detected on the distal part of OA25 for the yield in 2011 (Yield_11; Table 4, Figure 5). This QTL explained 20.2% of the variability and resulted from both female and male additive as well as dominance effects.

Reproductive traits measured at GU scale

The total number of inflorescences

A QTL was detected on the median part of OA25 for the total number of inflorescences per GU (Inflo_tot; Table 4, Figure 5). This QTL explained 11.6% of the variability and resulted from male additive effect (Table 4). Five QTLs were found for year-specific BLUPs for this

variable. The first QTL detected for 2009 co-localized with the QTL previously detected for Inflo_tot on OA25 (Inflotot_09; Figure 5). This QTL explained 14.7% of the variability and resulted from a dominance effect (Inflotot_09; Table 4). A second 2009 specific QTL was found on OA3 and co-localized with the QTL previously found for Yield_09 (Figure 5). This QTL explained 12.1% of the variability and resulted mainly from a male additive effect (Table 4). The global model did not include any interaction between QTLs and explained 24.4% of the variability (Table 5).

Considering the 2010 BLUPs, a QTL was identified on the distal part of OA12 and explained 16.6% of the variability. This QTL resulted from male additive effect (Inflotot_10; Table 4). Two 2011 specific QTLs were detected on OA1 and OA7. The QTL mapped on the median part of OA1 explained 18.0% of the variability and resulted from both male additive and dominance effects (Table 4). The QTL on the distal part of OA7 explained 14.2% of the variability and resulted from both female and male additive effects (Table 4). The global model did not include any interaction and explained 19.0% of the variability (Table 5).

The number of direct inflorescences born at the leaf axils along GUs

A QTL was detected on the median part of OA8 for the number of direct inflorescences per GU (Inflo_direct; Table 4, Figure 5). It explained 12.1% of the variability and resulted from male additive effect (Table 4).

Seven other QTLs were detected for year-specific BLUPs of Inflo_direct. One QTL detected on OA16 for Inflo_direct in 2009 (Inflodirect_09; Table 4, Figure 5) explained 15.0% of the variability and resulted from female additive effect. Three 2010 specific QTLs were detected on OA3, OA9 and OA2 (Inflodirect_10, Table 4). The first QTL on the distal part of OA2 explained 13.9% of the variability and resulted from both female and male additive effects (Table 4). The second QTL on OA9 co-localized with a QTL previously detected for yield in 2010 (Figure 5). This QTL explained 9.5% of the variability and resulted from both female additive and dominance effects (Table 4). The third QTL detected on the distal part of OA2 explained 13.0% of the variability and resulted from both female and male additive as well as dominance effects (Table 4). Together the three QTLs explained 14.3% of the Inflo_direct variability (Inflodirect_10; Table 5).

Three QTLs were detected on OA1, OA9 and OA16 for 2011 BLUPs of Inflo_direct (Inflodirect_11; Table 4, Figure 5). The first on OA1 co-localized with a QTL previously detected for the 2011 BLUP of Inflo_tot (Figure 5). It explained 15.0% of the variability and resulted from male additive and dominance effects (Table 4). The QTLs detected on OA9 and

OA16 explained 12% and 9.8% of the variability and resulted from female additive effect (Table 4). Altogether these QTLs explained 22.5% of the variability (Inflodirect_11; Table 5).

The number of inflorescences born on medium and short lateral GUs

QTLs were detected for genotype effect BLUP of the number of inflorescences per short laterals only (Inflo_S). Three QTLs were detected on OA8, OA10 and OA9 (Table 4, Figure 5), and explained 19.1%, 14.1% and 8.4% of the variability, respectively. The QTL mapped on OA8 co-localized with the QTL previously detected for Inflo_direct whereas that mapped on OA9 co-localized with both the previously detected QTLs for yield in 2010 and Inflo_direct (Figure 5). Both QTLs resulted mainly from female and dominance effects. The third QTL mapped on the distal part of OA10 resulted from female additive effect (Table 4). The global model did not include any interaction and QTLs explained 25.6% of the variability (Inflo_S; Table 5).

Year-specific QTLs were detected for both BLUPs of Inflo_M and Inflo_S. Four QTLs were detected for the year-specific BLUPs of Inflo_M. One was detected on OA2 for Inflo_M in 2009 (Inflo_M_09; Table 4, Figure 5), which explained 13.2% of the variability and was mainly due to female additive effect. A QTL was detected on OA3 for the 2010 BLUPs. This QTL explained 11.1% of the variability and resulted mainly from male additive effect (Inflo_M_10; Table 4, Figure 5). Two QTLs were detected on the distal part of OA20 and OA7 for the 2011 BLUPs, and explained 13.3% and 12.4% of the variability, respectively (Inflo_M_11; Table 4, Figure 5). The QTL identified on OA7 co-localized with the QTL previously detected for Inflo_tot in the same year (Figure 5) and resulted from both female and male additive as well as dominance effects whereas that on OA20 resulted from male additive effect (Table 4). Together, these QTLs explained 23.7% of the variability (Inflo_M_11, Table 5).

Three 2009 specific QTLs were detected on OA20, OA11 and OA21 for BLUP of Inflo_S (Inflo_S_09; Table 4, Figure 5). The QTL mapped on the medium part of OA20 explained 12.9% of the variability and resulted from both male additive and dominance effects. The QTL on OA11 explained 8.3% of the variance and was mainly due to dominance effect. The QTL on OA21 explained 8.3% of the variance and resulted from both male additive and dominance effects. Together, the three QTLs explained 40.2% of the variability (Inflo_S_09, Table 5). One 2011 specific QTL detected on OA7 (Inflo_S_11; Table 4, Figure 5) explained 10.1% of the variability and resulted from male additive effect (Table 4).

The number of fruits born at the leaf axils along GUs and fruit set on GUs sylleptic laterals

A QTL was detected on OA20 for the number of direct fruits per GU (Fruit_direct; Table 4, Figure 5). This QTL explained 13.1% of the variability and mainly resulted from male additive effect (Table 4). Another QTL was detected on OA2 for the percentage of axillary fruit set (Fruitset_AS; Table 4, Figure 5). It explained 15.3% of the variability and resulted from female additive and dominance effects (Table 4).

DISCUSSION

Fruiting behaviour of progenies, quantification and scale of description

For most alternate bearing species, alternate bearing phenomenon is linked to a lack of flower bud differentiation, commonly observed following a heavy fruiting year [5,8]. Considering the large population size and the intense flowering and abscission characteristic of olive, it was not realistic to quantify the total number of inflorescences per tree, as previously proposed by Guitton et al. [8]. Rather we chose to quantify the total yield per tree and year, and to describe a sub-sample of one-year old GUs, at flowering and fruiting stages. Based on yield per year, genotypes could be classified according to their bearing pattern. The evaluation of 'Olivière' x 'Arbequina' population over the first four production years showed the segregation of fruiting behaviour among progenies with approximately 3/4 of irregular or alternate bearing progenies (78%) and 1/4 of regular progenies (22%). We thus consider that yield and its variation depending on the years is suitable for characterising the fruiting behaviour in an olive tree progeny. Each class of trees with contrasted patterns of fruiting behaviour was characterised by its mean biennial bearing index (BBI) [11]. However, since BBI calculation takes into account the intensity of deviation in yield during successive years whatever its sign (i.e. absolute value), its values did not correctly captured the differences between trees categories as identified in Figure 1. These results are consistent with those observed on apple [8]. In fact, BBI has been reported as being efficient for characterising a strictly biennial pattern but to a less extent a triennial one [49]. The same authors also recommended to evaluating BBI with series of at least 10 years, and when the production is stabilised. Indeed, during the first years of tree maturity, BBI is sensitive to the tree's yield variation due to the ontogenetic increase in tree production in addition of the annual variation [50]. Because it may be of interest for selection schemes to be able to distinguish between regular and irregular bearing genotypes as soon as possible during tree ontogeny, these authors have proposed to remove trend due to the increase in fruit production in the first production years and accounting for year's variations exclusively i.e. the between years residuals of y. To this end, an index based on deviation around yield trend over years and accounting for the dependencies between consecutive years was developed by Durand et al. [unpublished data]. This approach could notably improve biennial bearing estimation and could be further explored in olive progenies. Besides estimating the bearing irregularity through BBI, other indices could be considered as for instance the quantification of synchronism of flowering occurrences within branches or trees as previously proposed by

Lauri et al. [51,52]. In the present study, because the number of years was still low, we did not used BBI at genotype level, as a variable for genetic study and QTL detection.

By contrast, the variables characterising the flowering and fruiting at GU scale, did not capture the 'ON' or 'OFF' status of the tree in a given year. This can be exemplified by comparing the two 'ON' years: in spite of the lower amount of flowering in 2011 at GU scale, the final fruit production was almost similar to that found in 2009 when the flowering was the highest along GUs. Likewise, Fruit set variables were not impacted by the bearing status of the trees, e.g. the final fruit production in 2010, was low despite high fruit set (see Figure S2). This suggests that it is neither the amount of flowering nor the fruit set percentage along GUs determine the final fruit production of trees. Similar finding was reported in olive tree by Lavee [53] who underlined the lack of direct relationship between the production and the initial abundance in flowers at the tree scale. The lack of relationship between yield at tree scale and flowering and fruiting variables at GU indicate that our GU sampling strategy must be reconsidered. Indeed, we selected each year GUs that were similar in length and with at least some flowers. This choice may have introduced a bias since 'OFF' trees are likely to have a large number of shoots without any flowers which were avoided. Alternatively to our choice, sampling branched systems could be more appropriate in the objective to capture the tree bearing status in a given year and evaluate the synchronism of flowering occurrence within branches (see below).

Effect of climatic years and tree ontogeny

The climatic year significantly impacted all studied traits. Considering that trees were regularly irrigated in our experiment and that inflorescences initiation is known to be insensitive to photoperiod in olive [54,55], temperature constrains could be the main climatic effect that could affect flowering in our progeny (Figure S.4). Regulation of flowering by temperature has been widely studied in olive tree [54-58]. Moreover, fertilization success is very sensitive to extreme temperature since it inhibits pollen germination and slow down or stop the pollen tube [59]. The receptivity of the stigma can also be shortened by dry winds and hot environmental conditions [60]. In addition, the co-existence in a given year of both 'ON' and 'OFF' trees (as it was the case in 2009 and 2011 which were 'ON' in average in the progeny, but with 16% and 41% of 'OFF', respectively), led us considering that climatic conditions in a given year are not directly responsible for tree bearing status. However, our experimental design did not allow us to distinguish between the ontogenetic and climatic

effect within the year effect as proposed by Segura et al. [61]. Nevertheless, changes in flowering and fruiting potential at the GU scale could be interpreted in relation with their growth and branching, giving an insight into tree ontogeny. After a plentiful flowering and fruiting in 2009, the total number of inflorescences and fruits per GU decreased in the two following years (2010 and 2011). Because, in olive tree, inflorescences develop from the axillary buds of well lignified shoots induced the previous year [14], this decrease is due to that of sylleptic branching whereas the number of inflorescences and fruits located at the GUs leaf axil increased regularly. This is also supported by the negative correlation between the number of GUs laterals and the number of inflorescences born at GUs leaf axils. Here we show that this negative correlation, previously reported in Ben Sadok et al. [29], is maintained over the three studied years which correspond to the tree mature phase. Consistently with Rugini and Panelli [62], these results show an antagonism in the development of axillary meristem which can be either vegetative laterals or inflorescences born at the leaf axils along GUs. Likewise, the number of internodes per GU decreased slightly in the same period but this low reduction in the potential number of flowering sites was compensated by the reduction of sylleptic laterals, since the number of flowers born at GU leaf axils (or Inflo_direct) increased regularly overs the years. These observations can also be interpreted with respect to tree ontogeny. Indeed, we have shown a significant decrease in primary growth and sylleptic branching in the progeny during the first years of growth, reaching a stable phase at first flowering occurrence [29]. Here, we complement our analysis showing that this stable phase is maintained so far until 2010 and is characterised by a clear shift towards short shoots. This is consistent with studies previously carried out on fruit [63-66] and forest trees [67-69]. The predominance of short length shoots in mature trees has also been already reported in Hojiblanca olive cultivar in both bearing and non-bearing years [22]. However, it must be noticed that the 'reiteration' phenomenon which can give rise to long and vigorous branched systems, even late in the tree ontogeny [69-71], is frequent in olive tree. Indeed, this morphogenetic process, which was not investigated in the present study, is at the basis of "production unit" [19].

A new olive genetic map as an efficient tool for QTL mapping

One of the targets of this study was to use the 94 new developed SSRs in olive [Essalouh et al. unpublished data] to enrich a genetic map previously constructed with AFLP, ISSR and few SSR markers [37]. Map saturation and LGs in the first maps were considerably improved by the addition of SSRs. As a result, 25 LGs covering a total of 1745.3 cM and 21 LGs

covering a total of 1597.6 cM were obtained for the female 'Olivière' and male 'Arbequina' parental maps, respectively. Khadari et al. [37] reported 2210.2 cM for 'Olivière' and 1966.2 cM for 'Arbequina'. Differences in genome length with the initial maps result mainly from the differences in the average distance between adjacent markers. The higher length of the female linkage map could indicate a higher recombination rate for the 'Olivière' female parent during meiosis as previously reported in various species such as Arabidopsis thaliana [72], tomato [73] and apple [74]. Using strict grouping parameters, we obtained a number of LGs close to the number of haploid chromosomes (i.e. n= 23) in which markers were well distributed. Similar results were obtained by Wu et al. [75] for 'Frantoio' and 'Kalamata' cultivars. Even though, these maps were based on a limited number of markers (i.e. 104), and were constructed with a more relaxed grouping parameters that those used in our study (i.e. recombination fraction= 0.49, LOD threshold= 3).

The integrated linkage map developed in the present study cover 2148.4 cM and is saturated at 86.9%. In comparison to previously developed maps in olive i.e. 'Leccino' and 'Dolce agogia' [76]; 'Frantoio' x 'Kalamata' [75]; 'Picholine marocaine' x 'Picholine du Languedoc' [77] and 'Olivière' x 'Arbequina' [37], the present integrated map 'Olivière' x 'Arbequina' includes the largest number of molecular markers i.e. 450 markers of which 103 are SSRs. The density of markers in the present map (i.e. one marker every 4.77 cM) is higher than that observed in the previous olive genetic maps (i.e. one marker every 10.2~8.1cM [75, 77]). Marker order in the integrated map was consistent with marker order in each parental map reflecting its precision.

QTL detection and BLUP correlation

In the present study, a two-stage approach has been chosen whereby the best linear unbiased predictors (BLUPs) of the genotypic effect and its interaction with the year factor were obtained first from the mixed linear models of repeated data and then were used for interval mapping (IM) analysis and multiple QTL mapping (MQM) which allows reducing type I error (a QTL is indicated at a location where there is no QTL present) and type II error (a QTL is not detected) for QTL detection [45]. BLUPs of genotypic effect were thus considered as independent of ontogenetic and climatic effects, while BLUPs of interaction effects between genotype and year were assumed to be specific of a year. This method has been used in apple, optimizing the statistical power to detect significant QTL [48]. Nevertheless, such approach could lead to a higher false positive rate and inferences about the magnitude of QTL effects could be biased [78,79]. One-step modelling of phenotypes in which genotype effect

would be replaced by molecular marker effects could be considered to improve this method. In addition to the application of such new mapping procedure that requires high computational time, QTL analysis resolution could be improved through increases in sample size and marker number.

A strong genetic control was highlighted for most flowering and fruiting traits with relatively high heritability values, yet specific to the progeny under study and the climatic years analysed [80]. Most QTL were not significant at genome wide LOD threshold. This may be due to high LOD threshold value for some LG. Most QTLs showed small effects, suggesting that traits related to the reproductive development have a multigenic control as previously reported on apple tree [8]. The majority of QTL was associated to flowering traits, most of them being Year-specific QTL. Their higher number in comparison to those based on genotype effects BLUPs is consistent with the significant GxY effect. In addition, the QTLs detected for BLUP of genotypic effect for either yield or flowering and fruiting variables at GU scale were mainly under allelic effects from Arbequina parent. This is consistent with the high production potential [30,81].

Two QTL were found for BLUP of genotype effect on Yield and Inflo_tot, but without co-localisations between them. The QTL for Yield (on OA12) had a main and positive effect coming from 'Arbequina', and associated to the allele of *Zit053* locus of size 150bp. This QTL having a large effect needs to be further confirmed. Indeed, we cannot exclude the risk this QTL being a false positive because of the relative low size of our population. The significant statistic correlation between BLUPs of genotype effect on tree yield and the number of fruits and fruit set at GU scale suggests that these variables could have a common genetic control. However, no co-localization was found between the associated QTLs. The QTL found for BLUP of genotype effect on Inflo_tot (on OA25) co-localized with that detected for Inflo_tot_2009, consistently with the significant correlation found between these BLUPs (r=0.54, see Table S.5). This co-localisation can be interpreted as the result of the high variance for Inflo_tot observed in 2009 which was the first year of high flowering and was considered as 'ON' year.

Numerous QTLS were also detected for all variables either in a specific year or born on a specific position along GUs. No co-localization was found between the year-specific QTL for yield, this revealing the absence of a common genetic control. By contrast, co-localisations were found between year-specific QTLs for yields and either QTLs of total number of

inflorescences or fruit set at GU scale. The QTL for yield in 2008 (on OA2) co-localized with that found for Fruitset_AS. For yield in 2009, the QTL detected on 'Arbequina' parental map only (on A23) co-localised with QTL associated with the three Fruitset variables (Total_Fruitset, Fruitset_direct and Fruitset_AS). It is noticeable that (i) these co-localisations do not rely on significant correlations between these variables (see Table S.5) and (ii) no co-localisations were found in the last two years, 2010 and 2011, even though QTLs were detected for yield in both these years. Although revealed in the two first production years only, these co-localizations confirm the common genetic control between Fruit set and Yield. As the number of lateral flowering GUs was the highest during these two first years, we could assume that the tree production for a giving year is likely to rely more on branching, and therefore on the number of flowering GUs present within the trees, rather than on the number of inflorescences per GU, particularly when the trees are young. This highlight the interaction between the vegetative growth and branching with the reproductive behaviour reported at the whole tree in olive [19, 22].

In 2009 and 2010, the QTLs for Yield co-localized with QTLs detected for Inflo_tot_2009 (OA3) and inflo_direct_2010 (OA9), respectively. The co-localization in 2009 is consistent with the relatively high correlation between the two variables and can be interpreted as previously with respect to the high flowering observed in 2009. It is noticeable that the co-localisation in 2010 concerns the number of inflorescences born directly along GU. This may indicate that vegetative growth could have a higher impact on yield, when tree is ageing and its branching decreases.

Several co-locations were also highlighted between QTLs for the number of inflorescences in a specific year or born on a specific lateral type. Regarding genotypic BLUP of number of inflorescences in specific positions, three non-epistatic QTLs were mapped for Inflo_S. One of them overlapped the genomic region associated to the genotypic BLUP of Inflo_direct (on OA8). This could not be interpreted by their correlation, but rather by a common genetic control of flowering at local scale, probably at meristem scale. Another QTL for Inflo_S co-localized with Inflo_direct in 2010. In addition to co-localisations previously commented, QTLs for Inflo_tot, Inflo_M and Inflo_S in 2011 overlapped in a specific region of OA7. Another QTL found for Inflo_tot in 2011 was in the same genomic region as that for Inflo_direct in the same year (on OA1). These co-localizations are consistent with significant correlations between these traits. All together they indicate that flowers born either on short and medium laterals or directly along GUs, both contribute to the total number of flowers, but

may involve different genomic regions depending on the year. Moreover, among all QTLs, the three QTLs for Inflo_S in 2009 explained the highest proportion of phenotypic variance. All QTLs detected for Inflo_S either for the genotypic BLUP or specific to 2009 confirm the large variation of the number of inflorescence born on short shoots in the studied olive tree progeny, and reveal a complex genetic determinism for this trait, associated to the most abundant branching type in mature olive trees [22].

Conclusion

Our study of the olive reproductive behaviour has provided new knowledge about its complex genetic control. Progenies showing an increasing production over the first four years of mature phase and a regular bearing pattern constitute a promising material for olive breeding programs for regularity improvement. However, additional production years need to be further investigated in order to validate their stability over time. Two comprehensive maps for the cultivars 'Olivière' and 'Arbequina' and one integrated map 'Olivière'x'Arbequina' were developed, including the largest number of SSRs so far. Additional SSR markers will be added in the future in order to expand the genome coverage and obtain more bridge markers that will be useful not only in saturating our maps but also for comparative mapping studies.

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Tables

Table 1. Variables related to olive flowering and fruiting habit collected at tree and GUs scale.

Observation scale	Measured and calculated Variables	Abbreviations	Formula
Whole tree	Mass of fruits at harvest	Yield	
One year- old floral Growth Units	Nb of inflorescences (or Fruit)	Inflo(or Fruit) _tot	Inflo(or Fruit) _direct + Inflo(or Fruit) _AS
	Nb of direct inflorescences(or Fruit)	Inflo(or Fruit) _direct	
	Nb of inflorescences(or Fruit) on axillary.shoots	Inflo(or Fruit) _AS	
	id. on long, medium, short axillary. shoots	Inflo_L ; Inflo_M Inflo_S	
	Percentage of fruiting	Total_Fruitset	Fruit_tot/Inflo_tot
	Percentage of direct fruit set	Fruitset_direct	Fruit_direct/Inflo_direct
	Percentage of laterals fruit set	Fruitset_AS	Fruit_AS/Inflo_AS

Table 2. Effects in selected models for traits related to reproductive development in 'Olivière' x 'Arbequina' progeny. Significance of effects: NS, not significant; *, significant ($0.01<p \leq 0.05$); **, highly significant ($p \leq 0.01$).

Variables	Factors			Variance Function[a]	Covariance structure[b]	Variance estimates			H^2
Tree scale	G	Y	GxY			V_G	$V_{G:Y}$	V_R	
Yield	**	**	**	-	-	239,58	242,66	476,78	0.60
GUs scale									
Inflo_tot	.	**	*	-	-	0,04	0,13	0,30	0.30
Inflo_direct	*	**	**	-	-	0,22	0,23	0,81	0.52
Inflo_AS	NS	**	**	-	-	-	1,18	2,93	NS
Inflo_L	NS	*	*	-	-	-	0,07	0,93	NS
Inflo_M	*	**	**	-	-	0,09	0,57	2,43	0.32
Inflo_S	.	**	**	-	-	0,03	0,47	1,65	0.36
Fruit_tot	**	**	NS	varExp	corExp	0.79	-	0.50	0.75
Fruit_direct	**	**	NS	varPower	corExp	0.67	-	1.00	0.57
Fruit_AS	NS	*	**	-	-	-	0,79	1,48	NS
Total_Fruitset	**	**	NS	varPower	corExp	6.81	-	2.28	0.85
Fruitset_direct	**	*	NS	varExp	corExp	6.50	-	5.57	0.70
Fruitset_AS	*	*	NS	varExp	-	0.49	-	3.27	0.23

Table 3. Characteristics of new microsatellites and genetic maps developed in 'Olivière' x'Arbequina' progeny: (a) percentages of mapped SSRs 'Zit' in parental maps and integrated map (b) Segregation types identified among genomic and EST SSRs 'Zit' and their mapping percentage on integrated map (c) mapping characteristics of 'Olivière' female parent map, 'Arbequina' male parent map and 'Olivière' x 'Arbequina' integrated map: correspondence with the first genetic map characteristics are indicated between brackets.

(a)

Total Polymorphic SSRs	**94**		
Maps	*Zit*	*Mapped Zit*	*% total Zit*
'Oliviere'	85	53	56%
'Arbequina'	64	48	51%
Integrated	94	72	76%

(b)

Number of SSRs ZIT	**Segegation**	**Genomic (*% mapped**)**	**EST (*% mapped**)**
20	<abxcd>	11 (*90,9%*)	9 (*100%*)
35	<efxeg>	18 (*66,66%*)	17 (*70,58%*)
9	<nnxnp>	4 (*50%*)	5 (*60%*)
30	<lmxll>	21 (*76,19%*)	9 (*66,66%*)

* (% mapped on the integrated map)

(c)

Maps	**LG (no.)**	**Mapped Markers (no.)**	**SSRs (no.)**	**Mean LG size (cM)**	**Map Length (cM)**	**Average marker spacing (cM)**	**Maximum Gap**	**Observed genome coverage (%)**
'Olivière'	25 (34)	212 (197)	68 (34)	69.81 (61.3)	1745.3 (2210.2)	8,23 (11.2)	32,5 (48.5)	77,9
'Arbequina'	21 (31)	252 (191)	75 (30)	76.07 (63.4)	1597.6 (1966.2)	6,34 (10.3)	30,8 (40.4)	83,8
Integrated	26 (42)	450 (436)	103(26)	82.63 (91.0)	2148.4 (3823.2)	4,77 (8.7)	30,5 (81)	86,9

Correspondences with the first genetic map characteristics are indicated between brackets

Table 4. QTLs detected on 'Olivière' x 'Arbequina' integrated map.

BLUP	Linkage Group	LOD[a]	Var (%)[b]	Allelic effect[c]	A_f[c]	A_m[c]	D[c]	Cofactor[d]
Tree Scale								
Yield	**OA12**	4.35 (2,5)	15.1	Am	-0,7267	4,8602	-0,8794	*Zit053*
Yield_08	**OA2**	3.33 (3.4)	13.6	Af	-3,0397	1,1436	1,1755	*ACG/CAC-59
	OA6	2.39 (2.4)	9.6	Af ;D	2,6439	-0,3025	-1,4886	Zit482
Yield_09	**OA3**	3,80 (3,0)	16.8	Af; Am	3,4688	1,1943	0,3547	*Zit324*
Yield_10	OA4	5.14 (3,1)	13.4	Af; D; Am	2,4808	-2,5112	-1,8372	*ACT/CAT-78
	OA9	3.45 (2,8)	11.1	D	0,6723	-0,7503	-3,1309	*AGA/CTG-117
	OA3	3.37 (2,9)	10.8	Am ; D	-0,9899	2,9788	1,2178	*DCA3*
Yield_11	**OA25**	4.50 (2,8)	20.2	Af; D; Am	-5,284	4,008	-2,909	Zit388
GU Scale								
Inflo_tot	**OA25**	3,45 (2,6)	11.6	Am	0,0039	-0,0402	0,0156	**Zit342**
Inflotot_09	OA25	4.00 (2,8)	14.7	D	0,0370	-0,0324	0,1217	**Zit342**
	OA3	3.27 (3,0)	12.1	Am	0,0335	0,1062	0,0231	*Zit324*
Inflotot_10	**OA13**	4.11 (3,4)	16.6	Am	-0,0243	0,1241	0,0169	*Zit376*
Inflotot_11	**OA1**	**6.26** (3,3)	18.0	Am ; D	-0,0045	-0,0605	0,0570	***AGA/CAA_142**
	OA7	**5.06** (2,3)	14.3	Af; Am	0,0500	-0,0457	-0,0226	***ACT/CTT_197**
Inflo_direct	**OA8**	3,33 (3,0)	12,1	Am	0,0707	0,1156	0,0488	***ACT/CAC_142**
Inflodirect_09	**OA16**	4.01 (3,2)	15	Af	-0,1519	-0,0556	0,0482	*ACG/CTC-128
Inflodirect_10	OA3	3.70 (2,9)	13.9	Af; Am	-0,0782	-0,0827	0,0421	*ACA/CAA-376
	OA9	3.47 (2,7)	9.5	Af; D	-0,0694	0,0097	-0,0609	*AGA/CTG-117
	OA2	3.46 (3,2)	13.0	Af ; D ; Am	-0,0446	-0,0651	-0,0784	*ACA/CAT-152
Inflodirect_11	OA1	4.47 (3,3)	15.0	Am ; D	0,0151	-0,0471	0,0750	***AGA/CAA_142**
	OA9	3.64 (2,7)	12.0	Af	0,0800	-0,0139	-0,0223	*AGA/CTC-83
	OA16	3.03 (3,0)	9.8	Af	0,0652	-0,0074	0,0151	*ACG/CTT-326
Fruit_direct	**OA20**	3.12 (2,8)	13.1	Af; Am	0,1186	0,2819	-0,0169	***AGA/CTT_143**
Fruitset_AS	**OA2**	**4.90** (3.3)	15.3	Af; D	-0,2623	0,1104	-0,1618	*ACA/CTG-50
Inflo_M_09	**OA2**	3.48 (3,4)	13.2	Af; Am	0,2260	-0,1695	-0,0955	Zit105
Inflo_M_10	**OA3**	3.46 (2,9)	11.1	Am	-0,0084	-0,1079	0,0145	*Zit324*
Inflo_M_11	**OA20**	3.71 (3,0)	13.3	Am	0,0360	0,0698	0,0342	***ACA/CTG_330**
	OA7	3.47 (2,3)	12.4	Af ; D; Am	0,0366	-0,0387	-0,0519	**Zit447**
Inflo_S	OA8	**5.57** (2,9)	19.1	Af; D	-0,0082	0,0013	0,0225	Zit394
	OA10	4.19 (3,0)	14.1	Af	0,0202	0,0009	-0,0025	Zit402
	OA9	2.64 (2.6)	8.4	Af; D	-0,0088	0,0019	0,0140	*AGA/CTG-117
Inflo_S_09	OA20	**4.61** (2,9)	12.9	Af; D	-0,1697	-0,0416	-0,1447	**Zit417**
	OA11	3.08 (3.0)	8.3	D	0,1014	0,0961	0,1415	*Zit309*
	OA21	3.08 (3,1)	8.3	Am; D	-0,0122	-0,1788	-0,0891	**IAS_oli_11**
Inflo_S_11	**OA7**	3.35 (2,4)	10.1	Am	0,0335	-0,1024	-0,0333	**Zit447**

[a] Maximum LOD score value with the considered threshold in parentheses: Bold LOD score values are significant at genome wide threshold.
[b] Percentage of phenotypic variation explained by the QTL.
[c] Allelic effects were estimated as $A_{f=}[(\mu_{ac+}\mu_{ad})-(\mu_{bc+}\mu_{bd})]/4$ for female additivity; $A_{m=}[(\mu_{ac+}\mu_{bc})-(\mu_{ad+}\mu_{bd})]/4$ for male additivity and $D_{=}[(\mu_{ac+}\mu_{bd})-(\mu_{ad+}\mu_{bc})]/4$ for dominance where $\mu_{ac,}$ $\mu_{ad,}$ $\mu_{bc\ and}$ μ_{bd} are estimated phenotypic means associated to each of the 4 possible genotypic classes *ac*, *bc*, *ad* and *bd*, deriving for an *ab* x *cd* cross.
[d] markers used as cofactors in the MQM analysis: Bold cofactors are mapped on 'Arbequina' genetic map (male parent); Non Bold cofactors are mapped on 'Olivière' genetic map (female parent) and Italic cofactors are mapped on both parental maps.

Table 5. Global model estimations for reproductive traits with several QTLs detected.

BLUP	Linkage Group	Cofactor[a]	p-value[b]	Global R^{2c}
Tree scale				
Yield_08	OA2	*ACG/CAC-59	0.0002	0.164
	OA6	Zit482		
Yield_10	OA4	*ACT/CAT-78	0.002	0.188
	OA9	*AGA/CTG-117		
	OA3	*DCA3*		
GU Scale				
Inflotot_09	OA25	Zit342	0.0001	0.244
	OA3	*Zit324*		
Inflotot_11	OA1	*AGA/CAA_142	3.266e-05	0.190
	OA7	*ACT/CTT_197		
Inflodirect_10	OA3	*ACA/CAA-376	0.001	0.143
	OA9	*AGA/CTG-117		
	OA2	*ACA/CAT-152		
Inflodirect_11	OA1	*AGA/CAA_142	1.665e-05	0.225
	OA9	*AGA/CTC-83		
	OA16	*ACG/CTT-326		
Inflo_M_11	OA20	*ACA/CTG_330	2.728e-05	0.237
	OA7	Zit447		
Inflo_S	OA8	Zit394	8.959e-06	0.2563
	OA10	Zit402		
	OA9	*AGA/CTG-117		
Inflo_S_09	OA20	Zit417	2.875e-06	0.402
	OA11	*Zit309*		
	OA21	IAS_oli_11		

[a] Effects selected according to AIC and BIC minimization.
[b] Effect probability.
[c] Percentage of variation explained by the global model.

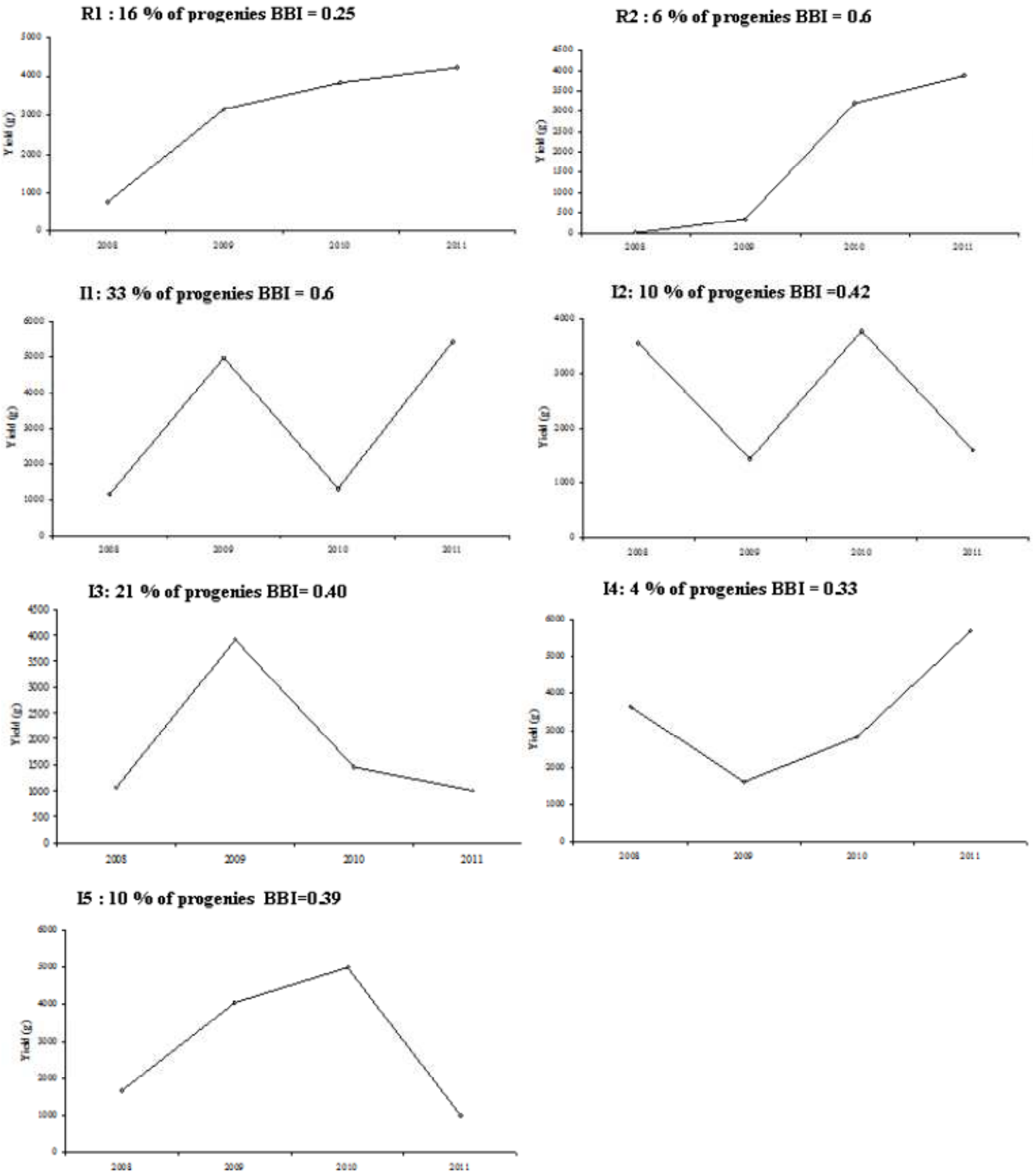

R1 : 16 % of progenies BBI = 0.25
R2 : 6 % of progenies BBI = 0.6
I1: 33 % of progenies BBI = 0.6
I2: 10 % of progenies BBI =0.42
I3: 21 % of progenies BBI= 0.40
I4: 4 % of progenies BBI = 0.33
I5 : 10 % of progenies BBI=0.39
Yield (g)
2008
2009
2010
2011

Figure 1

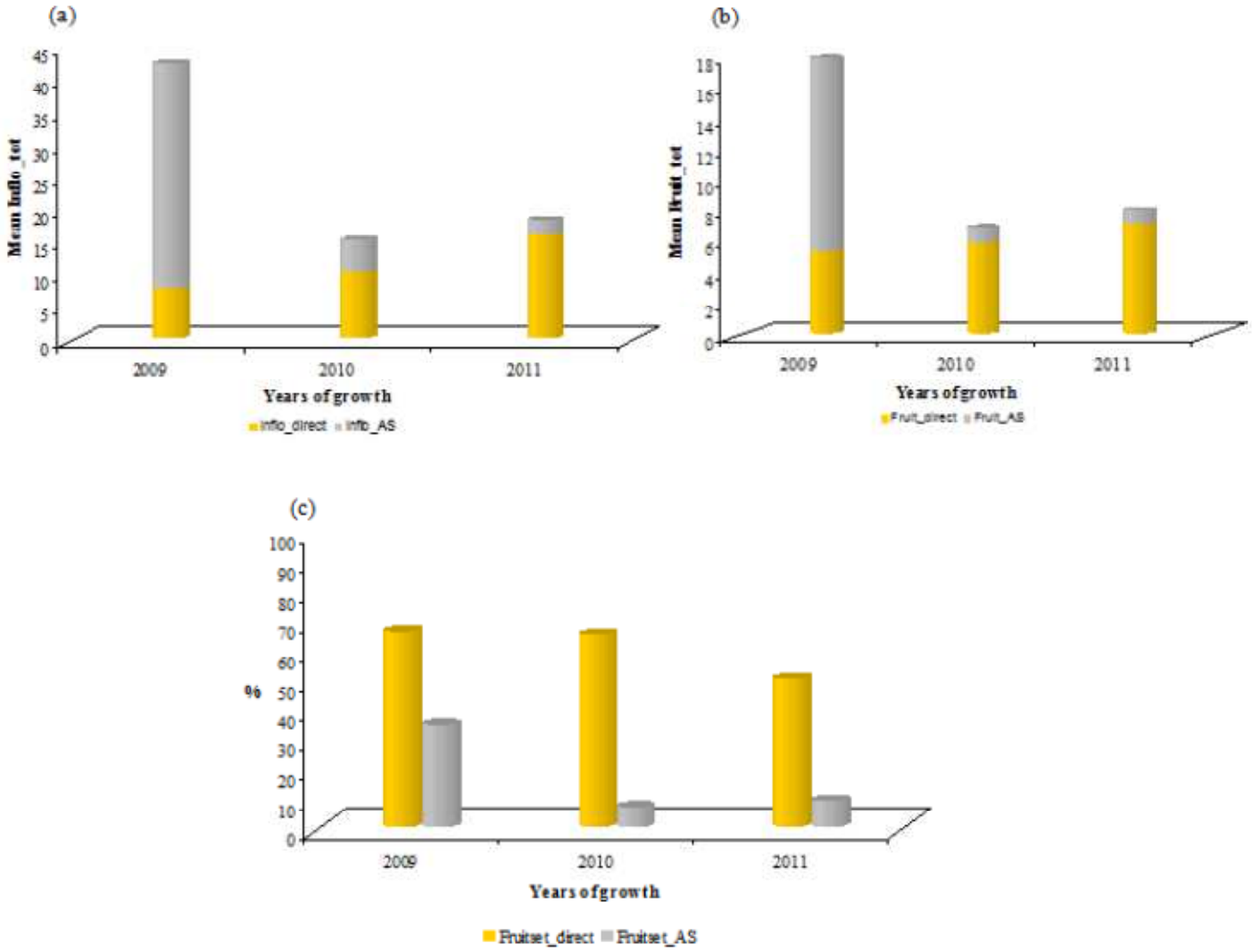

(a)
Mean Inflo_tot
45
40
35
30
25
20
15
10
5
0
2009
2010
2011
Years of growth
Inflo_direct
Inflo_AS
(b)
Mean Fruit_tot
18
16
14
12
10
8
6
4
2
0
2009
2010
2011
Years of growth
Fruit_direct
Fruit_AS
(c)
%
100
90
80
70
60
50
40
30
20
10
0
2009
2010
2011
Years of growth
Fruitset_direct
Fruitset_AS

Figure 2

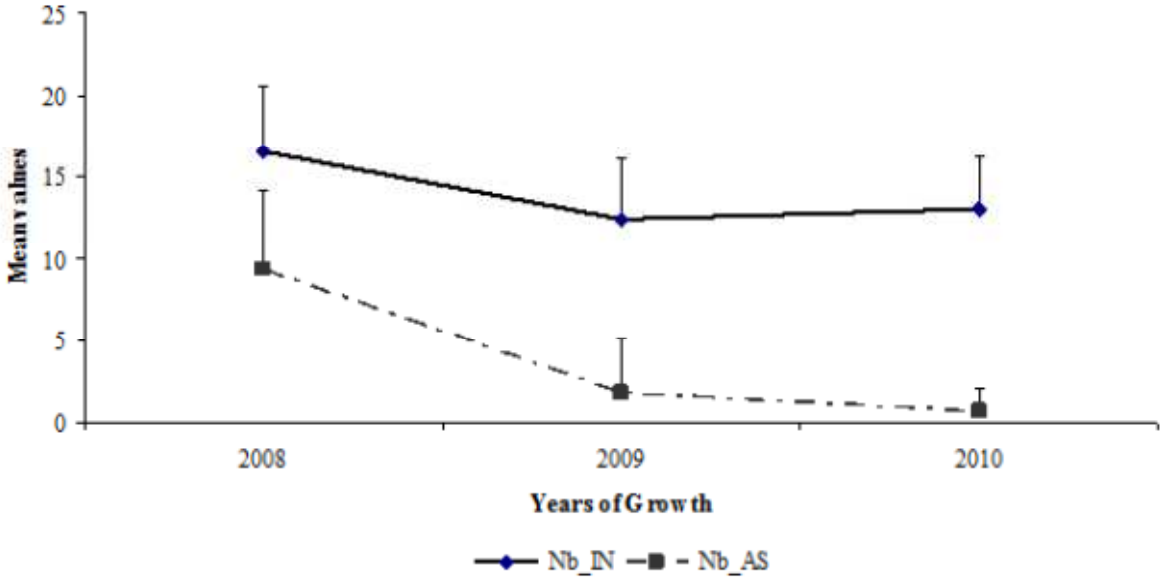

25
20
15
10
5
0
Mean values
2008
2009
2010
Years of Growth
Nb_IN
Nb_AS

Figure 3

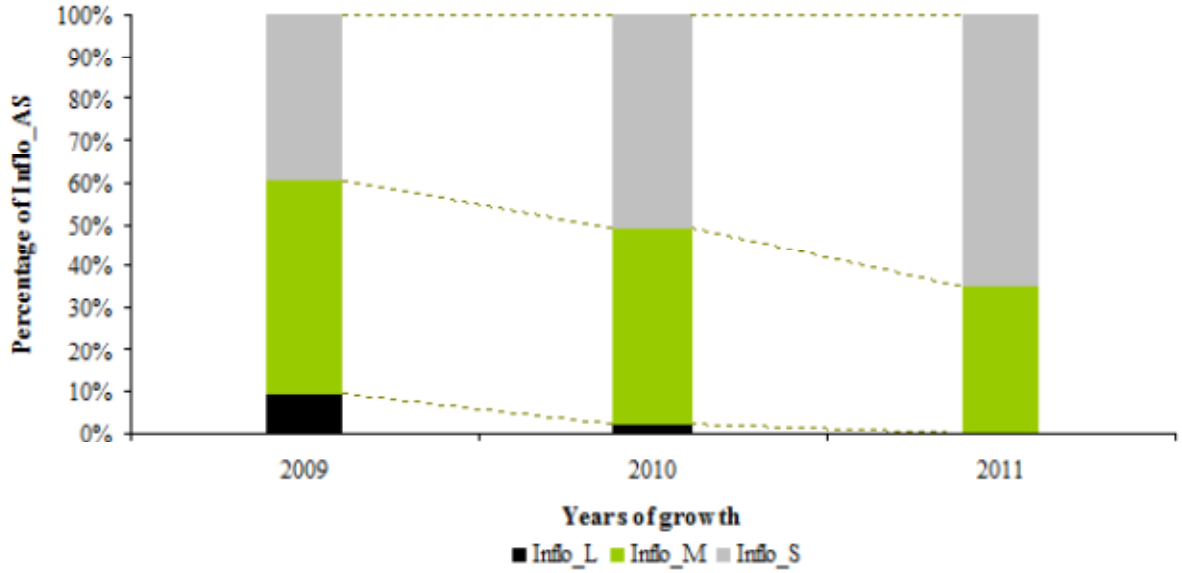

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Percentage of Inflo_AS
2009
2010
2011
Years of growth
Inflo_L
Inflo_M
Inflo_S

Figure 4

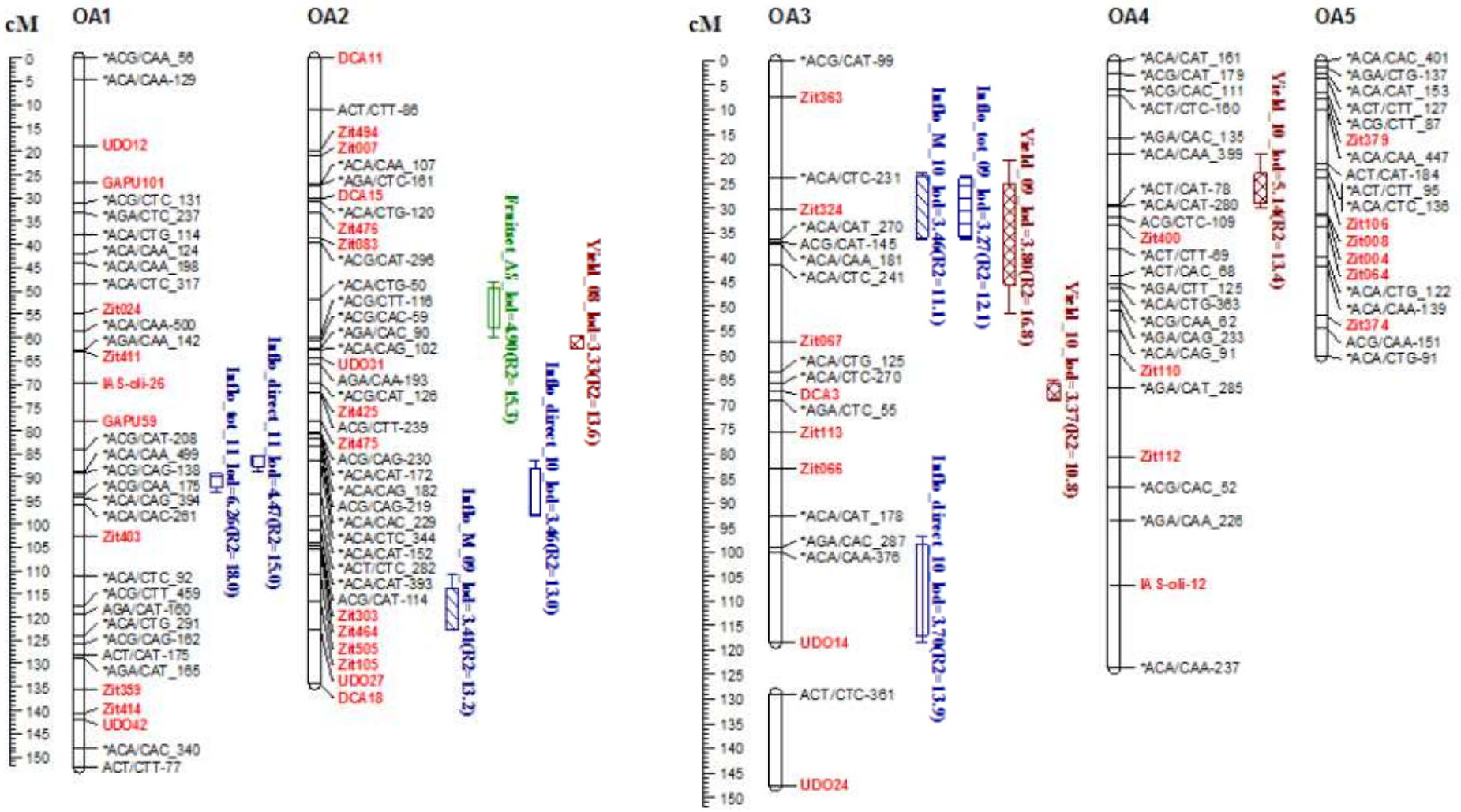

cM
OA1
OA2
cM
OA3
OA4
OA5
*ACG/CAA_56
*ACA/CAA-129
UDO12
GAPU101
*ACG/CTC_131
*AGA/CTC_237
*ACA/CTG_114
*ACA/CAA_124
*ACA/CAA_198
*ACA/CTC_317
Zit024
*ACA/CAA-500
*AGA/CAA_142
Zit411
IAS-oli-26
GAPU59
*ACG/CAT-208
*ACA/CAA_499
*ACG/CAG-138
*ACG/CAA_175
*ACA/CAG_394
*ACA/CAC-261
Zit403
*ACA/CTC_92
*ACG/CTT_459
AGA/CAT-160
*ACA/CTG_291
*ACG/CAG-162
ACT/CAT-175
*AGA/CAT_165
Zit359
Zit414
UDO42
*ACA/CAC_340
ACT/CTT-77
DCA11
ACT/CTT-86
Zit494
Zit007
*ACA/CAA_107
*AGA/CTC-161
DCA15
*ACA/CTG-120
Zit476
Zit083
*ACG/CAT-296
*ACA/CTG-50
*ACG/CTT-116
*ACG/CAC-59
*AGA/CAC_90
*ACA/CAG_102
UDO31
AGA/CAA-193
*ACG/CAT_126
Zit425
ACG/CTT-239
Zit475
ACG/CAG-230
*ACA/CAT-172
*ACA/CAG_182
ACG/CAG-219
*ACA/CAC_229
*ACA/CTC_344
*ACA/CAT-152
*ACT/CTC_282
*ACA/CAT-393
ACG/CAT-114
Zit303
Zit464
Zit505
Zit105
UDO27
DCA18
Frainet_A5_lod=4.90(R2= 15.3)
YieM_08_lod=3.33(R2=13.6)
InRo_direct_10_lod=3.46(R2=13.0)
InRo_M_09_lod=3.41(R2=13.2)
InRo_M_10_lod=3.46(R2=11.1)
InRo_tot_09_lod=3.27(R2=12.1)
YieM_09_lod=3.80(R2=16.8)
YieM_10_lod=3.37(R2=10.8)
InRo_direct_10_lod=3.70(R2=13.9)
YieM_10_lod=5.14(R2=13.4)
InRo_direct_11_lod=4.47(R2=15.0)
InRo_tot_11_lod=6.26(R2=18.0)
*ACG/CAT-99
Zit363
*ACA/CTC-231
Zit324
*ACA/CAT_270
ACG/CAT-145
*ACA/CAA_181
*ACA/CTC_241
Zit067
*ACA/CTG_125
*ACA/CTC-270
DCA3
*AGA/CTC_55
Zit113
Zit066
*ACA/CAT_178
*AGA/CAC_287
*ACA/CAA-376
UDO14
ACT/CTC-361
UDO24
*ACA/CAT_161
*ACG/CAT_179
*ACG/CAC_111
*ACT/CTC-160
*AGA/CAC_135
*ACA/CAA_399
*ACT/CAT-78
*ACA/CAT-280
ACG/CTC-109
Zit400
*ACT/CTT-69
*ACT/CAC_68
*AGA/CTT_125
*ACA/CTG-363
*ACG/CAA_62
*AGA/CAG_233
*ACA/CAG_91
Zit110
*AGA/CAT_285
Zit112
*ACG/CAC_52
*AGA/CAA_226
IAS-oli-12
*ACA/CAA-237
*ACA/CAC_401
*AGA/CTG-137
*ACA/CAT_153
*ACT/CTT_127
*ACG/CTT_87
Zit379
*ACA/CAA_447
ACT/CAT-184
*ACT/CTT_95
*ACA/CTC_136
Zit106
Zit008
Zit004
Zit064
*ACA/CTG_122
*ACA/CAA-139
Zit374
ACG/CAA-151
*ACA/CTG-91

Figure 5

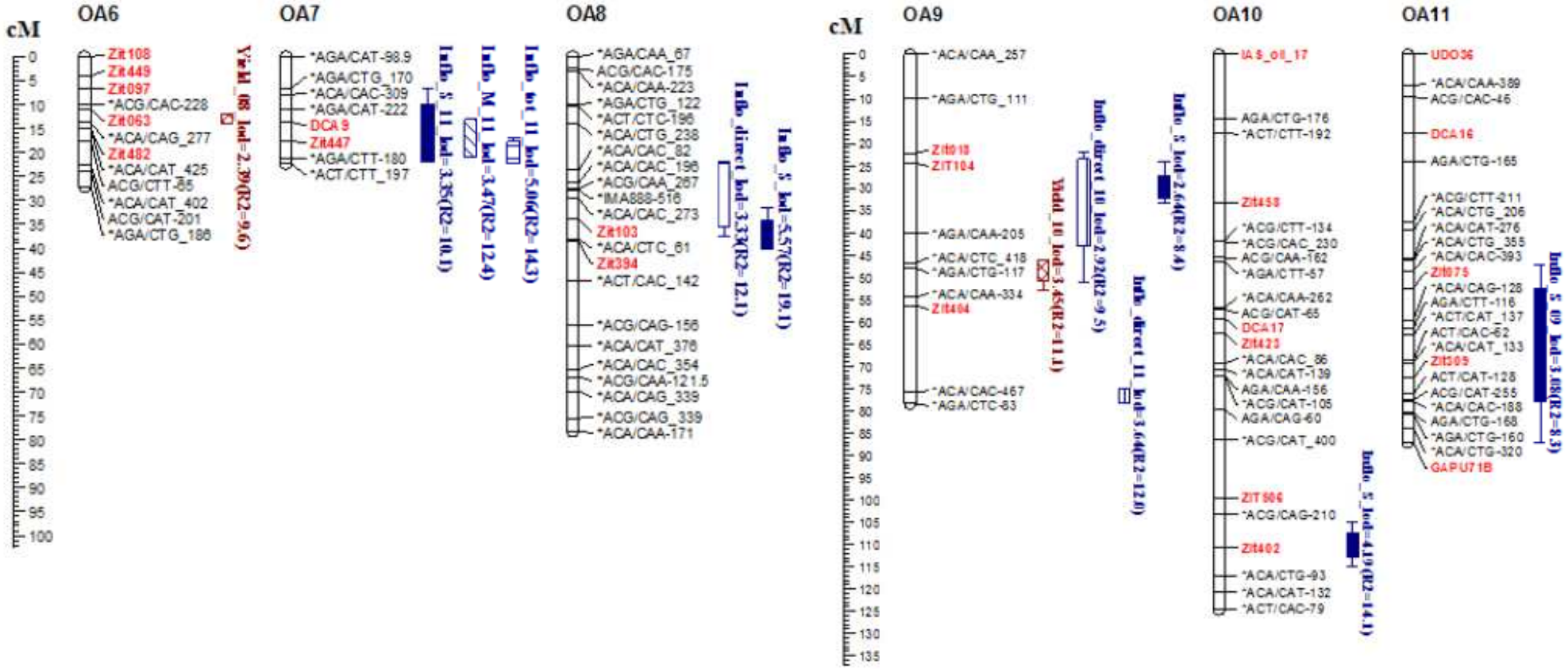
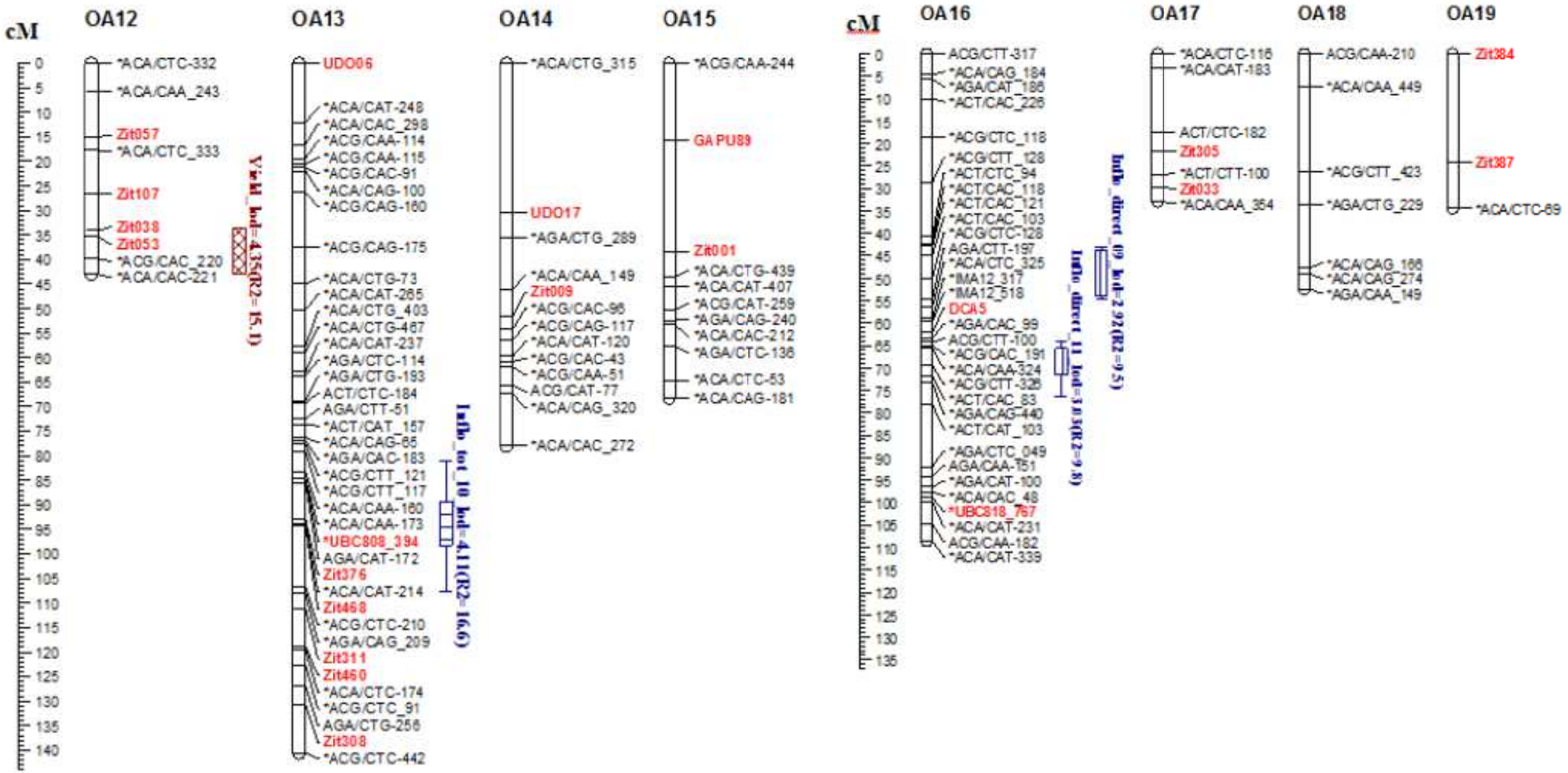


Figure 5 (continued)



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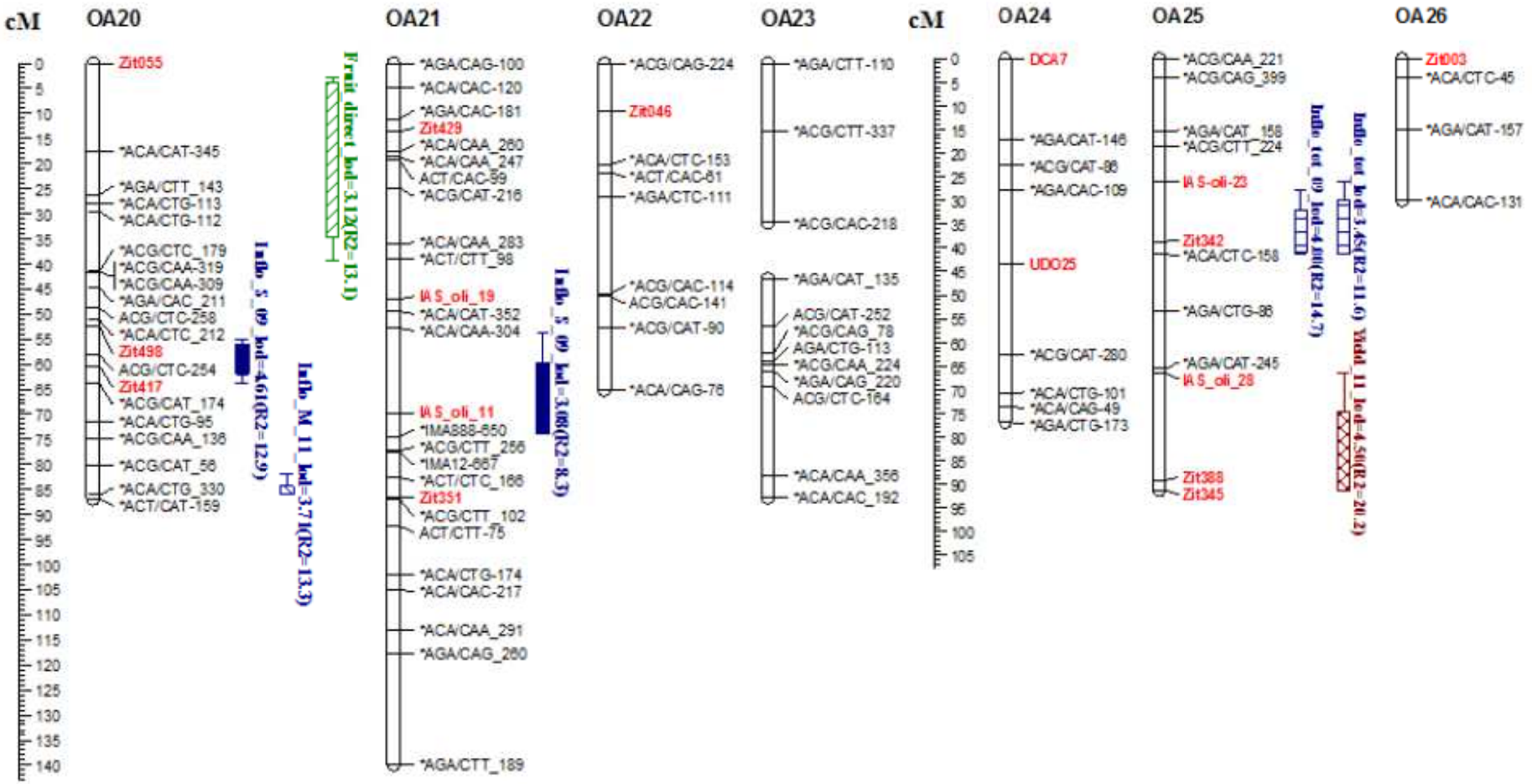


Figure 5 (continued)

FIGURES LEGEND

Figure 1. Seven bearing behaviours identified among genotypes within 'Olivière' x 'Arbequina' progeny based on the average Yield value per year and genotype. Progenies proportion within each class and average BBI value are indicated: BBI calculation takes into account the intensity of deviation in yield during successive years whatever its sign. Hence, similar BBI values were found for R2 and I2 class.

Figure 2. Illustration of the changes over time observed at flowering and fruiting periods on GUs in 'Olivière' x 'Arbequina' progeny: (a; b) Mean values of the number of inflorescences and fruits born along the leaf axils along GUs (Inflo(Fruit)_direct) and along GUs sylleptic laterals (Inflo (Fruit)_AS) as a function of years of growth (2009-2011); (c) Percentage of fruitset along the leaf axils along GUs and along GUs sylleptic laterals as a function of years of growth (2009-2011) .

Figure 3. Mean values and standard deviations of the number of internodes and the number of sylleptic laterals of bearing floral GUs as a function of years of growth in 'Olivière' x 'Arbequina' progeny (2008-2010).

Figure 4. Percentage of inflorescences born along GUs sylleptic laterals types (long. medium or short) depending on the year of growth in 'Olivière' x 'Arbequina' progeny. Inflorescences on sylleptic laterals (Inflo_AS) were mainly born on short and medium types (Inflo_S and Inflo_M, respectively) in each studied year (2009-2011).

Figure 5. Genomic positions of the QTLs detected on the linkage groups of the 'Olivière' x 'Arbequina' integrated map by multiple QTL mapping (MQM) for the best linear unbiased predictors (BLUPs) of reproductive traits measured at both tree and GUs scale. Map distances were derived using the Kosambi mapping function and SSRs markers were colored in red. QTLs are represented by boxes extended by lines representing the LOD-1 and LOD-2 confidence intervals. Boxes are coloured according to the process of the traits: Blue and Green for flowering and fruiting at GUs scale, respectively; Brown for production at whole tree scale. For each trait, a distinct fill style was used for boxes representing QTLs. For each QTL, corresponding BLUP (computed from genotype effect or from the interaction between genotype and year), LOD peak and R^2 were indicated as detailed in Table 4.

SUPPORTING INFORMATION DATA

QTL detection and mapping on parental maps

QTL detected on the integrated map as well as parental maps are indicated in bold in Table 4. Here are detailed the QTL not found on the integrated map but, detected on the parental maps (Table S.4).

Reproductive traits measured at tree scale

Yield

Besides the QTL detected on O12 (i.e. also found in the integrated map on OA12), a QTL was detected on O3 for the total fruit weight per tree (Yield, Table S.4), which explained 8.8 % of the variability. A global model was built for these QTL showing non significant interaction between cofactors and that explained 16.4% of the variability (Table S.4).

Two 2008 specific QTLs were detected on A13 and A21 (Yield_08, Table S.4) which explained 8.8 % and 8.5% of the variability, respectively. Together, these QTL explained 14.9% of the variability (Table S.4). Two 2009 specific QTLs were detected on O19 and A23 (Yield_09, Table S.4), which explained 13% and 12.4% of the variability, respectively.

Reproductive traits measured at GU scale

The total number of inflorescences

In addition to the QTL detected on A25 (i.e. found on OA25 in the integrated map), two QTLs were identified on A13.1 and O10 for the total number of inflorescences per GU (Inflo_tot, Table S.4), which explained 8.6% and 11.3% of the variability, respectively. The global model did not include any interaction between the QTL on A25 and A13.1 and explained 16.4% of the variability (Table S.4). An additional 2011 specific QTL was detected on O15 and explained 10.6% of the variability (Inflotot_11, Table S.4).

The number of inflorescences born at the leaf axils along GUs

A 2010 specific QTL was found on A8 for Inflo_direct (Inflodirect_10, Table S3), which explained 9.3% of the variability. This QTL co-localized with the QTL previously detected for the G effect BLUP of Inflo_direct (Table S.4). In addition to the two QTL previously found on O9.1 and O16 (i.e. OA9 and OA16 in the integrated map), a 2011 specific QTL was

found on O23, which explained 7.2% of the variability (Inflodirect_11, Table S.4). Together, these QTL explained 23% of the variability (Table S.4).

The number of inflorescences born along GUs medium and short laterals

Four QTLs were detected for the number of inflorescences per medium laterals on parental maps only (Inflo_M, Table S.4). Two of them were mapped on O2.1 and A2 and explained 10.6% and 8.8% of the variability, respectively. The two others were found on O15 and O16, which explained 5.8 % and 5.7% of the variability, respectively. These QTL co-localized with the QTL previously detected for the 2011 specific BLUP of Inflo_tot and Inflo_direct, respectively (Table S.4). A global model was built for the three QTL found on 'Olivière' map, which explained 27.3% of the variability (Table S.4). Further than the QTL found on O2.1 (i.e. OA2 in the integrated map), a 2009 specific QTL was detected on O15 and explained 7.7% of the variability. This QTL co-localized with the previously detected QTL for the G effect BLUP of Inflo_M (Table S.4). The global model did not include any interaction between the QTL and explained 16.8% of the variability (Table S.4). Considering the 2011 BLUPs, a QTL was detected on O6 for Inflo_M (Inflo_M_11, Table S.4), which explained 9.3% of the variability.

A QTL was detected on O3 for 2009 BLUPs of the number of inflorescences per short laterals (Inflo_S_09, Table S.4), which explained 6.7% of the variability. This QTL co-localized with the QTL previously detected for Inflotot_09 and Inflo_M_10 (Table S.4). Three 2010 specific QTLs were detected on O15, O11 and O13 (Inflo_S_10, Table S.4), which explained 8.6%, 7.9% and 6.8% of the variability, respectively. Together, these QTL explained 24.5% of the variability (Table S.4).The QTL mapped on O13 co-localized with the previously detected QTL for the 2010 BLUPs of Inflo_tot (Table S.4). A 2011 specific QTL was detected on O9.1, which explained 7.7% of the variability (Inflo_S_11, Table S.4). This QTL co-localized with the 2011 specific QTLs previously detected for Inflo_direct (Table S.4).

The total number of fruits

Four QTLs were detected on A20, A2, A23 and O11 for the total number of fruits per GU (Fruit_tot, Table S.4). These QTLs explained 8.9%, 8%, 6.9% and 6.3% of the variability, respectively. A global model was built for the three QTL found on 'Arbéquina' map, which did not include any interaction between the QTL and explained 19.4% of the variability (Table S.4).

The total fruit set

Three QTLs were detected on parental maps for the percentage of total fruit set (Total_Fruitset, Table S.4). The first QTLs was detected on both parental maps and mapped on O13 and A13.1, which explained 11.7% and 7.8% of the variability. These QTL co-localized with those previously detected for the 2010 BLUPs of Inflo_tot (Table S.4). The two others were detected on O11 and A23 and explained 10.4% and 7% of the variability. These QTL co-localized with those previously detected for Fruit_tot (Table S.4). A global model was built for the QTL of each parental map, which did not include any interaction between the QTL. Together, the QTL found on each parental map explained 14% of the variability (Table S.4).

The fruit set at the leaf axils along GUs

Four QTLs were detected for the percentage of direct fruit set (Fruitset_direct, Table S.4). The first three QTLs were mapped on O11, O13 and A13.1 and A23, which explained 9.8%, 9.4%, 5.8% and 5.7% of the variability, respectively. These QTL co-localized with those previously detected for the Total_Fruitset (Table S.4). The forth QTL was found on A20 and explained 7.5% of the variability. This QTL co-localized with the QTL previously detected for the Fruit_direct (Table S.4). A global model was built for the QTL found on 'Arbéquina' map, which did not include any interaction between the QTL and explained 18.9% of the variability (Table S.4).

The fruit set at GUs syllpetic laterals

In addition to the QTL detected on O2.1 (i.e. OA2 in the integrated map), two QTL were detected on O13 and A23 for the percentage of axillary fruit set (Fruitset_AS, Table S.4), which explained 10% and 7% of the variability, respectively. These QTL co-localized with those previously detected for Total_Fruitset and Fruitset_direct (Table S.4). Together, the QTL found on 'Olivière' map explained 15.6% of the variability (Table S.4).

Table S1. Mean phenotypic values and standard deviations (SD) for the parents 'Olivière and 'Arbequina' and the value range for the progeny.

Trait	Mean (SD)		Progeny Range	
	Olivière	**Arbequina**	**Minimum**	**Maximum**
Tree Scale				
Yield (Kg)	2,08 (1,34)	4,66 (2,05)	0	24,5
GU scale				
Flowering				
Inflo_tot	11,91 (4,75)	42,08 (36,33)	1	152
Inflo_direct	7,5 (6,00)	11,58 (7,80)	0	39
Inflo_AS	4,42 (6,24)	30,5 (41,30)	0	143
Inflo_L	0	0	0	113
Inflo_M	1,16 (3,73)	22,16 (32,58)	0	96
Inflo_S	3,25 (4,71)	8,33 (10,05)	0	61
Fruiting				
Fruit_tot	4 (4,97)	18,16 (23,22)	0	123
Fruit_direct	2,25 (3,74)	5,58 (4,75)	0	58
Fruit_AS	1,75 (4,09)	12,58 (22,94)	0	98
Total_Fruitset (%)	29,58 (32,89)	33,58 (19,41)	0	600
Fruitset_direct (%)	21,66 (31,38)	102,83 (153,54)	0	700
Fruitset_AS (%)	12,33 (25,39)	17 (23,61)	0	300

Table S2. Variance and correlation values between successive years of growth for the total yield per tree

variances	2008	2009	2010	2011
2008	793.00998	136.79404	120.75999	-11.67654
2009	136.79404	972.17294	90.67249	63.40609
2010	120.75999	90.67249	775.54128	50.87256
2011	-11.67654	63.40609	50.87256	1286.29039
correlations	**2008**	**2009**	**2010**	**2011**
2008	-	0.12524766	0.12074616	-0.01952145
2009	0.12524766	-	0.08644844	0.03221859
2010	0.12074616	0.08644844	-	0.03105333
2011	-0.01952145	0.03221859	0.03105333	-

Table S3a. Correlations between flowering and fruiting traits at Gus on the basis of mean phenotypic values per genotype and year scale

	Inflotot09	Inflodirect09	InfloAS09	InfloL09	InfloM09	InfloS09	Fruittot09	Totalfruitset09	fruitsetdirect09	fruitsetAS09	Inflotot10	Inflodirect10	InfloAS10	InfloL10	InfloM10	InfloS10	Fruittot10	Totalfruitset10	fruitsetdirect10	fruitsetAS10	Inflotot11	Inflodirect11	InfloAS11	InfloM11	InfloS11	Fruittot11	Totalfruitset11	fruitsetdirect11	fruitsetAS11
Inflotot09	-																												
Inflodirect09	*0,42*	-																											
InfloAS09	*0,96*	0,14	-																										
InfloL09	0,47	0,07	0,49	-																									
InfloM09	0,79	0,07	0,84	0,23	-																								
InfloS09	0,59	0,15	0,60	0,02	0,22	-																							
Fruittot09	0,26	0,08	0,26	0,18	0,29	0,00	-																						
Totalfruitset09	**-0,32**	-0,02	0,34	0,02	0,24	-0,37	0,48	-																					
fruitset direct09	-0,18	0,04	0,21	0,04	0,13	-0,24	0,62	**0,89**	-																				
fruitsetAS09	-0,10	-0,21	0,04	0,07	0,00	-0,13	0,77	0,47	0,48	-																			
Inflotot10	0,06	0,13	0,03	0,11	0,07	0,25	-0,35	-0,20	0,24	-0,33	-																		
Inflodirect10	0,06	0,41	0,07	0,01	0,15	0,08	-0,14	-0,04	0,02	-0,16	*0,54*	-																	
InfloAS10	0,04	-0,10	0,07	0,13	0,01	0,24	-0,33	-0,22	0,26	-0,29	*0,85*	0,02	-																
InfloL10	0,06	-0,02	0,07	0,05	0,06	0,10	-0,19	-0,12	0,14	-0,16	0,34	0,04	0,42	-															
InfloM10	0,06	-0,04	0,08	0,07	0,06	0,14	-0,27	-0,19	0,23	-0,23	0,66	0,08	0,83	0,40	-														
InfloS10	0,00	-0,13	0,04	0,14	0,04	0,25	-0,27	-0,17	0,21	-0,25	0,76	0,11	0,83	0,17	0,40	-													
Fruittot10	-0,05	0,22	0,12	0,01	0,11	-0,11	0,35	0,44	0,47	0,20	-0,01	0,24	-0,15	-0,11	-0,13	0,12	-												
Totalfruitset10	-0,12	0,06	0,15	0,03	0,04	-0,23	0,42	0,54	0,52	0,28	**-0,38**	0,19	-0,34	-0,16	-0,27	0,28	0,70	-											
fruitset direct10	-0,13	0,03	0,15	0,04	0,05	-0,22	0,42	0,53	0,51	0,28	-0,36	0,23	-0,29	-0,16	-0,22	0,25	0,71	**0,98**	-										
fruitsetAS10	-0,14	-0,08	0,13	0,00	0,09	-0,15	-0,06	0,01	0,08	-0,02	0,17	0,12	0,28	0,03	0,28	0,21	0,25	0,10	0,17	-									
Inflotot11	0,00	0,27	0,08	0,04	0,09	-0,01	-0,07	0,02	0,00	-0,19	0,43	0,42	0,25	0,07	0,19	0,23	0,14	-0,12	-0,09	0,07	-								
Inflodirect11	0,19	0,45	0,06	0,00	0,01	0,12	-0,10	-0,06	0,10	-0,18	0,40	0,42	0,22	0,15	0,29	0,07	0,04	-0,22	-0,21	-0,04	*0,74*	-							
InfloAS11	-0,19	-0,06	0,19	0,06	0,15	-0,14	0,00	0,10	0,10	-0,10	0,23	0,20	0,15	-0,06	-0,02	0,28	0,18	0,05	0,08	0,14	*0,71*	0,06	-						
InfloM11	-0,11	0,03	0,13	0,11	0,07	-0,10	0,01	0,13	0,08	-0,11	0,14	0,09	0,11	-0,02	0,01	0,17	0,23	0,22	0,25	0,14	0,60	0,06	0,83	-					
InfloS11	-0,21	-0,13	0,19	0,00	0,19	-0,13	-0,01	0,03	0,08	-0,06	0,24	0,23	0,14	-0,07	-0,05	0,29	0,07	-0,12	-0,10	0,11	0,61	0,04	0,86	0,43	-				
Fruittot11	-0,07	0,14	0,12	0,01	0,11	-0,09	0,42	0,43	0,45	0,30	-0,11	0,01	-0,14	-0,16	-0,17	0,04	0,63	0,53	0,57	0,18	0,27	0,07	0,33	0,39	0,18	-			
Totalfruitset11	-0,15	-0,05	0,14	0,05	0,11	-0,19	0,43	0,44	0,48	0,42	-0,33	0,23	-0,25	-0,12	-0,22	0,19	0,47	0,59	0,60	0,16	**-0,31**	-0,39	0,05	0,02	-0,11	0,73	-		
fruitset direct11	-0,16	-0,06	0,16	0,03	0,12	-0,20	0,44	0,47	0,50	0,40	-0,33	0,24	-0,24	-0,13	-0,22	0,18	0,47	0,63	0,64	0,18	-0,25	-0,39	0,04	0,12	-0,05	0,77	**0,98**	-	
fruitsetAS11	-0,11	-0,04	0,11	0,10	0,17	0,08	0,17	0,24	0,36	0,14	0,15	0,00	0,18	-0,07	0,05	0,28	0,24	0,17	0,17	0,14	0,07	-0,17	0,29	0,22	0,26	0,46	0,35	0,37	-

Table S3b. Correlations between Fruit weight per tree and flowering and fruiting traits at GUs on the basis of mean phenotypic values per genotype and year scale.

	Yield08	Yield09	Yield10	Yield11
Yield08	-			
Yield09	0,25	-		
Yield10	0,51	0,27	-	
Yield11	0,04	0,49	0,08	-
Inflotot09	-0,07	0,00	-0,20	-0,02
Inflodirect09	-0,01	0,02	-0,09	-0,05
InfloAS09	-0,07	0,00	-0,18	0,00
InfloL09	0,08	0,09	-0,04	0,17
InfloM09	**-0,17**	-0,10	-0,18	-0,14
InfloS09	0,02	0,06	-0,11	0,07
Fruittot09	0,09	**0,19**	0,04	0,13
Totalfruitset09	-0,01	0,04	0,01	0,02
fruitset direct09	0,05	0,11	0,09	0,02
fruitsetAS09	0,08	**0,21**	0,00	0,19
Inflotot10	0,11	**-0,15**	-0,06	-0,04
Inflodirect10	0,27	0,06	0,01	0,11
InfloAS10	-0,03	**-0,21**	-0,07	-0,11
InfloL10	-0,04	-0,10	-0,02	-0,11
InfloM10	0,00	-0,10	-0,02	-0,18
InfloS10	-0,05	**-0,24**	-0,10	0,00
Fruittot10	0,15	**0,34**	**0,15**	0,01
Totalfruitset10	-0,01	**0,26**	0,07	-0,04
fruitset direct10	-0,02	**0,26**	0,06	-0,03
fruitsetAS10	0,09	0,07	0,10	0,04
Inflotot11	-0,01	-0,09	**-0,17**	0,10
Inflodirect11	-0,01	-0,05	**-0,18**	**0,13**
InfloAS11	-0,01	-0,08	-0,06	0,01
InfloM11	-0,09	-0,07	-0,13	0,02
InfloS11	0,07	-0,07	0,02	-0,01
Fruittot11	0,07	0,13	0,10	**0,12**
Totalfruitset11	0,13	0,12	**0,23**	0,01
fruitset direct11	0,11	0,11	**0,22**	0,00
fruitsetAS11	0,07	0,03	0,14	0,04

Table S4. Parameters associated with the QTLs detected separately by multiple QTL mapping (MQM) on 'Olivière' and 'Arbequina' parental maps, for the best linear unbiased predictors (BLUPs) of reproductive traits measured at both tree and GUs scale over 4 and 3 years, respectively. For each trait, QTLs detected for the genotype effect BLUP were presented first followed by those detected for the Year-specific BLUPs.

BLUP	Linkage Group	LOD[a]	Var (%)[b]	Female Additivity[c]	Male Additivity[c]	Cofactor[d]	Global R^{2e} (p-value)
Tree Scale							
Yield	O12	3.60 (2,5)	13.7	4.5758		*Zit053*	0.164
	O3	1.95 (1,3)	8.1	-3.5208		*ACA/CAA-376	(0.0005)
	A12	2.46 (1,6)	11.4		-4.1351	*Zit053*	
Yield_08	O2.1	2.16 (1,8)	9.3	-2.8402		*ACG/CAC-59	0.164
	O6	1.84 (1,5)	7.9	-2.6193		Zit482	(0.0002)
	A13	2.04 (1,5)	8.8		-2.7545	*ACG/CTT_117	0.149
	A21	1.98 (2.0)	8.5		2.713	*ACA/CAA_283	(0.0002)
Yield_09	O19	2.85 (1,9)	13.0	-3.2997		*ACA/CTC-69	
	A23	2.70 (1,5)	12.4		-3.2399	*AGA/CAG_220	
Yield_10	O3	1.74 (1,7)	8.2	-2.5777		*DCA3*	
	A3	2.61 (2,1)	10.1		2.9454	*DCA3*	
Yield_11	O25	3.12 (1,3)	14.5	-5.1584		Zit388	
GU Scale							
Inflo_tot	A25	2.21 (1,8)	8,3		0.03732	Zit342	0.164
	A13.1	2.31 (1,4)	8,6		0.03743	*Zit376*	(0.0008)
	O10	2.62 (1,7)	11,3	0.0428		Zit402	
Inflotot_09	O3	2.13 (1,6)	9,3	-0.0987		*Zit324*	
	A3	2.13 (2,0)	9,3		-0.0988	*Zit324*	
Inflotot_10	O13	**3.80** (1,8)	15,9	0.1184		*Zit376*	
	A13.1	**4.17** (1,3)	16,8		0.1226	*Zit376*	
Inflotot_11	A1	2,93 (2,4)	11,6		0,0631	*AGA/CAA_142	0.190
	A7	2,52 (1,5)	9,9		-0,0585	*ACT/CTT_197	(3.266e-05)
	O15	2.46 (1,5)	10,6	0.0602		*ACG/CAT-259	
Inflo_direct	A8	2.15 (1,9)	9.3		0.1128	*ACT/CAC_142	
Inflodirect_09	O16	2.53 (1,5)	10.9	0.1385		*ACG/CTC-128	
Inflodirect_10	A8	2.44 (2)	10.5		0.0956	*ACT/CAC_142	
	O9.1	1.54 (1,3)	6.2	-0.0731		*AGA/CTG-117	
Inflodirect_11	O9.1	2.53 (1,4)	9.1	0.0676		*AGA/CTC-83	0.230
	O16	3.14 (1,4)	11.5	-0.0748		*ACG/CTT-326	(2.894e-06)
	O23	2.02 (1,6)	7.2	-0.0600		*ACG/CAC-218	
Inflo_M	O2.1	3.14 (1,8)	10.6	0.0360		Zit105	0.273
	O15	1.76 (1,5)	5.8	-0.0263		*ACA/CTC-53	(2.531e-07)
	O16	1.74 (1,5)	5.7	-0.0257		*ACG/CTT-326	
	A2	2.13 (2,0)	8.8		-0.0321	*ACA/CAC_229	

Table S4 (continued)

Trait	Linkage Group	LOD[a]	Var (%)[b]	Female Additivity	Male Additivity	Cofactor[c]	Global R^{2e} (p-value)
GU Scale							
Inflo_M_09	O2.1	2.10 (1,7)	8.4	0.2021		Zit105	0.168
	O15	1.94 (1,6)	7.7	-0.1886		*ACA/CTC-53	(5.309e-05)
Inflo_M_10	O3	2.07 (1,6)	9.0	0.0980		*Zit324*	
	A3	2.07 (1,9)	9.0		0.0980	*Zit324*	
Inflo_M_11	O6	2.13 (1,3)	9.3	-0.0710		Zit063	
	A20	1.99 (1,9)	7.3		0.0638	*ACA/CTG_330	
Inflo_S	O10	3.00 (1,7)	12.3	0.0179		Zit402	
Inflo_S_09	O3	1.66 (1,6)	6.7	-0.1613		*Zit324*	
	A21	3.04 (2,1)	12.9		-0.2287	IAS_oli_11	
Inflo_S_10	O15	2.44 (1,5)	8.6	-0.1335		*ACG/CAT-259	0.245
	O11	2.25 (1,8)	7.9	0.1303		*ACA/CAT-276	(4.466e-05)
	O13	1.96 (1,7)	6.8	0.1231		*Zit376*	
Inflo_S_11	O9.1	1.86 (1,4)	7.7	-0.0945		*ACA/CAC-467	
	A7	2.02 (1,5)	8.3		0.1010	Zit447	
Fruit_tot	O11	1.43 (1,8)	6.3	-0.1912		*ACA/CAG-128	
	A20	2.37 (2,1)	8.9		0.2322	*AGA/CTT_143	0.194
	A2	2.15 (1,9)	8.0		0.2153	Zit007	(0.0001)
	A23	1.86 (1,6)	6.9		0.1990	*ACG/CAG_78	
Fruit_direct	A20	2.28 (2,1)	9.9		0.2284	*AGA/CTT_143	
Total_Fruitset	O13	2.94 (1.7)	11.7	-0.8425		*Zit376*	0.14
	O11	2.64 (1.7)	10.4	-0.8000		*ACA/CAG-128	(0.001)
	A23	1.73 (1,6)	7.0		0.6409	*ACG/CAG_78	0.14
	A13.1	1.91 (1,4)	7.8		-0.6760	*Zit376*	(0.001)
Fruitset_direct	O11	2.41 (1,7)	9.8	-0.7191		*ACA/CAG-128	0.14
	O13	2.32 (1,8)	9.4	-0.7002		*Zit376*	(0.001)
	A13.1	1.52 (1,5)	5.8		-0.5408	*Zit376*	0.189
	A20	1.96 (2.0)	7.5		0.6325	*AGA/CTT_143	(0.0001)
	A23	1.51 (1,5)	5.7		0.5379	*ACG/CAG_78	
Fruitset_AS	O13	2.50 (1,8)	10.0	-0.1534		*ACG/CAG-160	0.156
	O2.1	1.95 (1,8)	7.7	-0.1369		*ACA/CTG-50	(9.074e-05)
	A23	1.58 (1,4)	7.0		0.1280	*ACG/CAA_224	

[a] Maximum LOD score value with the considered threshold in parentheses: Bold LOD score values are significant at genome wide threshold.
[b] Percentage of phenotypic variation explained by the QTL.
[c]markers used as cofactors in the MQM analysis: Italic cofactors are mapped on both parental maps.
[e] Percentage of variation explained by the global model.

Table S5. Correlations betweens reproductive growth traits on the bases of G and (GxY) BLUPs

	Total Fruitset	Fruitset direct	Fruitset AS	Fruit tot	Fruit direct	Inflo tot	Inflo tot09	Inflo tot10	Inflo tot11	Inflo direct	Inflo direct09	Inflo direct10	Inflo direct11	InfloM	InfloM 9	InfloM 10	InfloM 11	InfloS	InfloS 9	InfloS 10	InfloS 11	Yield	Yield 8	Yield 9	Yield 10	Yield 11
Total_Fruitset	-																									
Fruitset_direct	0,96	-																								
Fruitset_AS	0,51	0,45	-																							
Fruit_tot	0,92	0,91	0,55	-																						
Fruit_direct	0,87	0,93	0,33	0,91	-																					
Inflo_tot	-0,42	-0,32	-0,07	-0,12	-0,09	-																				
Inflo_tot_09	-0,13	-0,08	-0,10	0,02	0,01	**0,54**	-																			
Inflo_tot_10	-0,40	-0,36	0,00	-0,23	-0,22	0,55	-0,24	-																		
Inflo_tot_11	-0,01	0,03	0,02	0,08	0,16	0,26	-0,23	-0,03	-																	
Inflo_direct	-0,09	0,05	-0,26	0,12	0,35	0,66	0,28	0,32	0,38	-																
Inflo_direct_09	0,19	0,31	-0,06	0,26	0,46	0,25	0,41	-0,14	0,03	0,67	-															
Inflo_direct_10	-0,23	-0,18	-0,24	-0,13	-0,06	0,34	-0,13	0,61	-0,04	0,34	-0,27	-														
Inflo_direct_11	-0,21	-0,27	-0,01	-0,12	-0,20	0,24	-0,12	0,03	**0,66**	0,01	**-0,37**	**-0,22**	-													
Inflo_M	-0,32	-0,28	0,06	-0,19	-0,28	0,52	0,57	0,05	0,02	0,01	0,04	-0,17	0,16	-												
Inflo_M_09	-0,24	-0,19	-0,09	-0,12	-0,18	0,45	0,75	-0,13	-0,17	0,05	0,10	-0,08	0,01	0,86	-											
Inflo_M_10	-0,31	-0,35	0,17	-0,27	-0,36	0,20	**-0,19**	0,43	0,05	-0,11	-0,16	-0,20	0,37	0,29	-0,12	-										
Inflo_M_11	0,18	0,20	0,21	0,17	**0,20**	-0,02	-0,22	-0,10	**0,50**	0,07	0,07	0,03	-0,06	0,08	-0,17	-0,20	-									
Inflo_S	-0,36	-0,34	0,32	-0,18	-0,33	0,54	0,37	0,31	-0,01	-0,06	-0,10	-0,04	**0,15**	0,37	0,25	0,29	-0,03	-								
Inflo_S_09	-0,27	-0,25	0,00	-0,10	**-0,19**	0,49	0,67	0,01	-0,16	0,10	0,05	-0,01	0,09	0,28	0,35	0,05	**-0,25**	0,71	-							
Inflo_S_10	-0,26	-0,30	0,34	-0,20	-0,33	0,22	-0,13	0,52	-0,15	-0,22	-0,17	-0,15	0,12	0,19	-0,02	0,52	-0,10	0,62	0,06	-						
Inflo_S_11	-0,05	0,00	0,32	0,00	-0,04	0,16	-0,14	0,07	**0,47**	-0,03	-0,13	0,12	0,05	0,16	0,02	-0,07	**0,52**	0,35	-0,14	0,09	-					
Yield	0,64	0,65	0,33	0,69	0,69	0,03	0,01	-0,07	0,18	0,31	0,29	0,06	-0,12	-0,14	-0,06	-0,34	0,18	-0,30	-0,22	-0,30	0,02	-				
Yield_08	0,02	0,06	-0,02	0,05	0,15	0,32	0,10	0,24	0,14	0,40	0,15	0,44	-0,16	0,08	0,17	-0,26	0,09	-0,09	-0,27	-0,01	0,28	0,21	-			
Yield_09	0,11	0,07	0,05	0,14	0,09	0,13	**0,28**	-0,14	0,02	0,06	0,13	-0,21	0,11	0,15	0,20	-0,01	-0,12	0,02	0,23	-0,13	-0,20	0,36	-0,22	-		
Yield_10	0,05	0,06	-0,13	0,06	0,05	-0,03	-0,05	0,19	-0,32	0,06	0,06	**0,31**	-0,41	-0,17	-0,11	-0,14	-0,03	-0,11	-0,08	-0,12	0,02	0,17	0,15	-0,28	-	
Yield_11	0,39	0,38	0,35	0,37	0,34	**-0,28**	**-0,22**	-0,27	0,24	-0,14	0,00	-0,37	0,23	-0,14	-0,23	0,03	0,17	-0,11	-0,06	-0,06	-0,08	0,43	-0,45	-0,01	-0,43	-

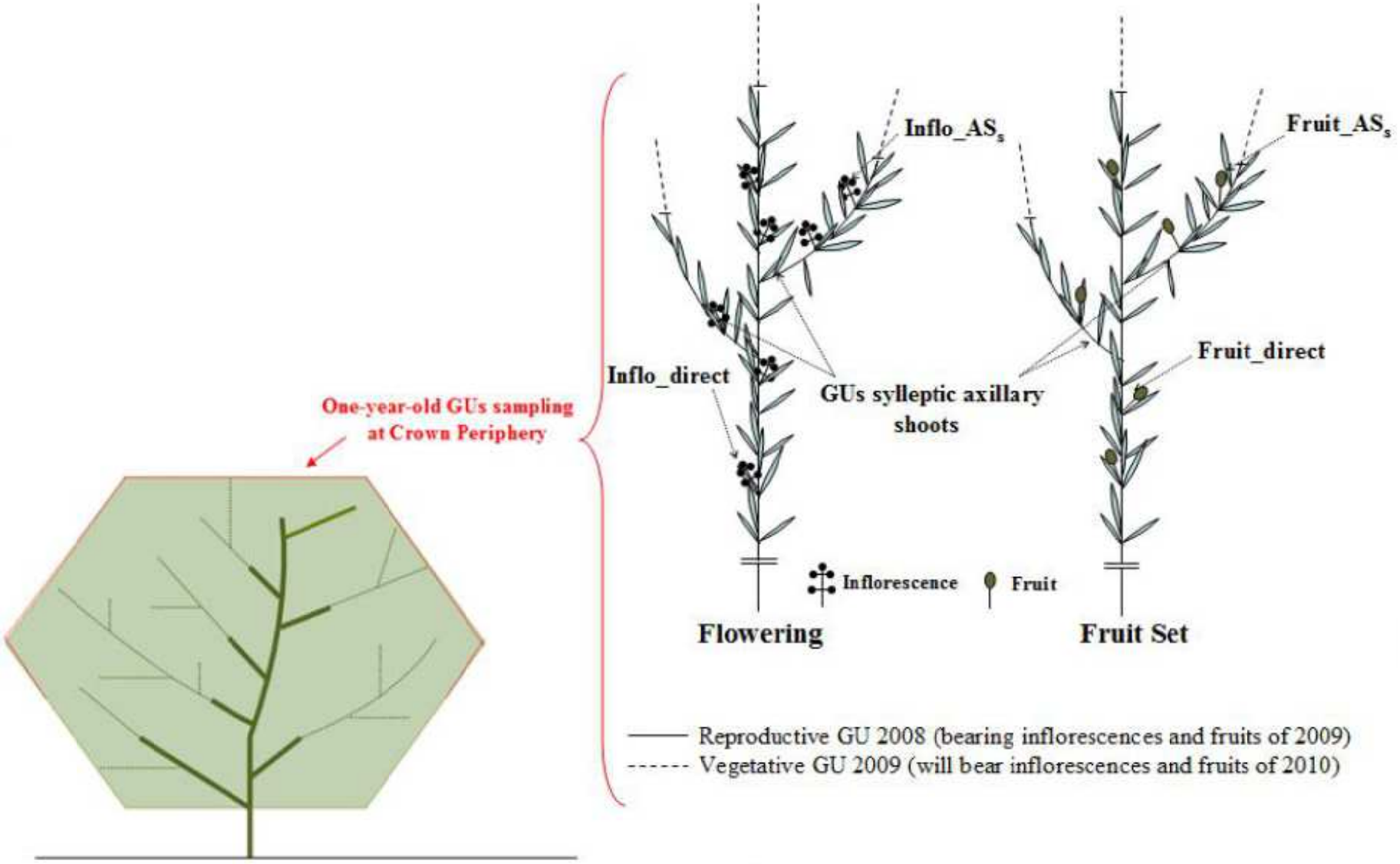


Figure S1. Schematic representation of a 5-year-old olive tree phenotyped in 2009: flowering and fruiting traits collected on 1-year-old GUs at flowering and fruit set periods; the number of inflorescences and fruits born along the floral GUs (Inflo(Fruit)_direct) or along their sylleptic laterals (Inflo(Fruit)_AS) were counted. Floral buds in year i (2009 in the present case) are induced during the summer of year i-1 (2008 in our case) and were thus born on GUs of year i-1. Their final differentiation occurs in year i [14].

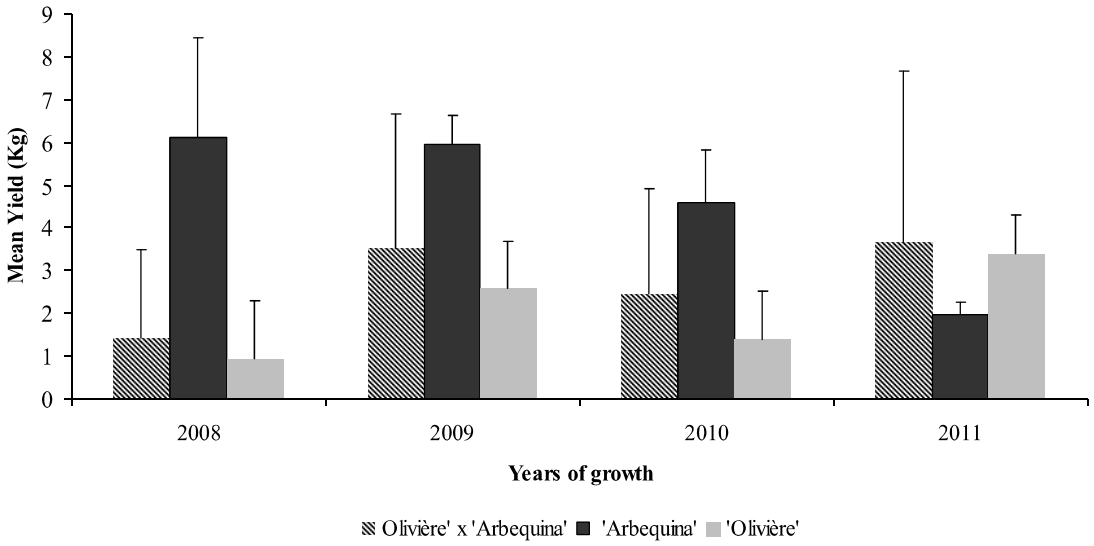


Figure S2. Mean Yield values and standard deviations (SD) for the parents ‘Olivière and ‘Arbequina’ and the progeny over four years (2008-2011).

(a)

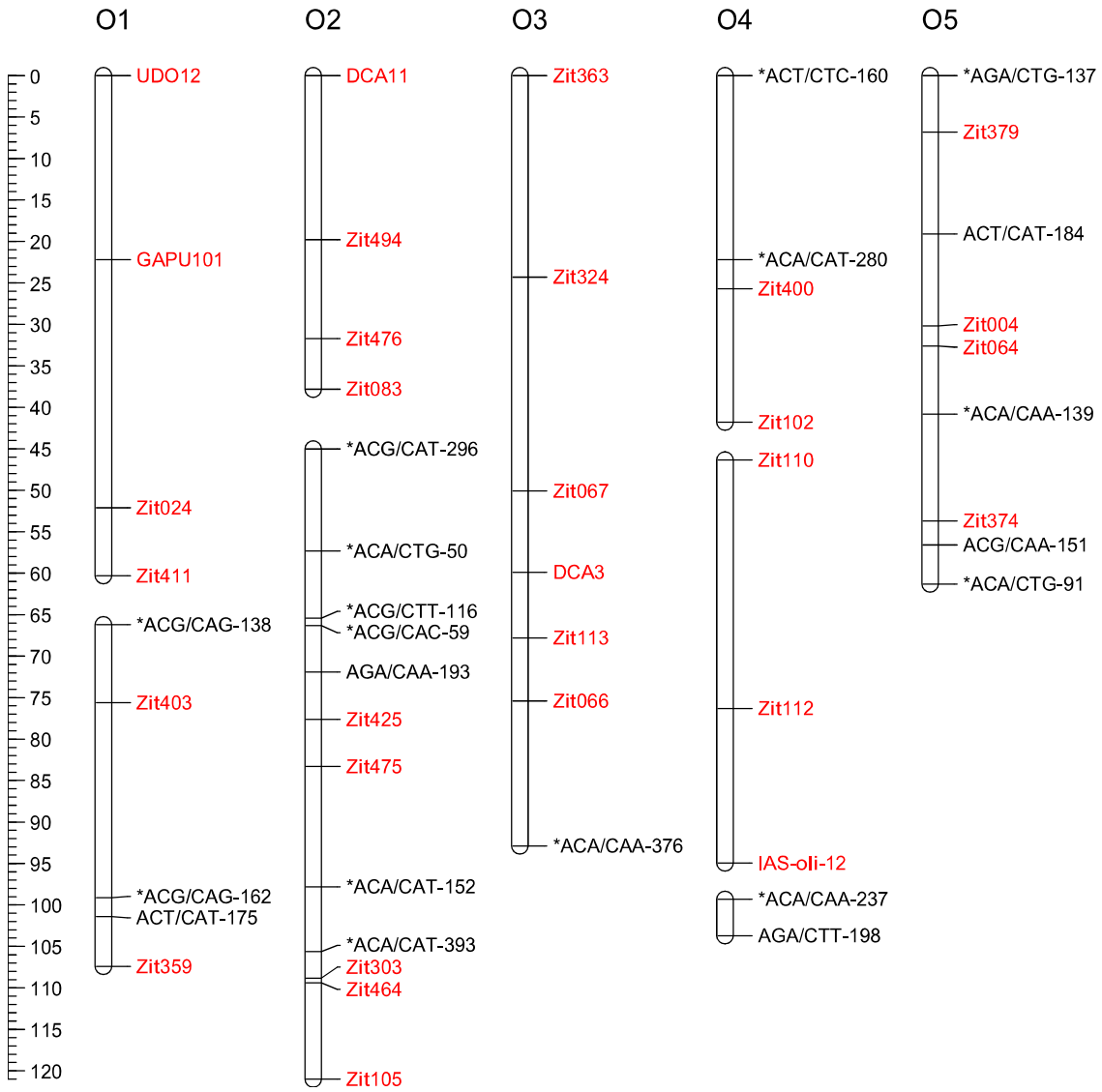


Figure S3. Representation of parental genetic linkage maps (a) 'Olivière' female parent map (b) 'Arbequina' male parent map. Map distances were derived using the Kosambi mapping function. AFLP markers presented without '*'are segregating in both parents. SSRs markers are colored in red

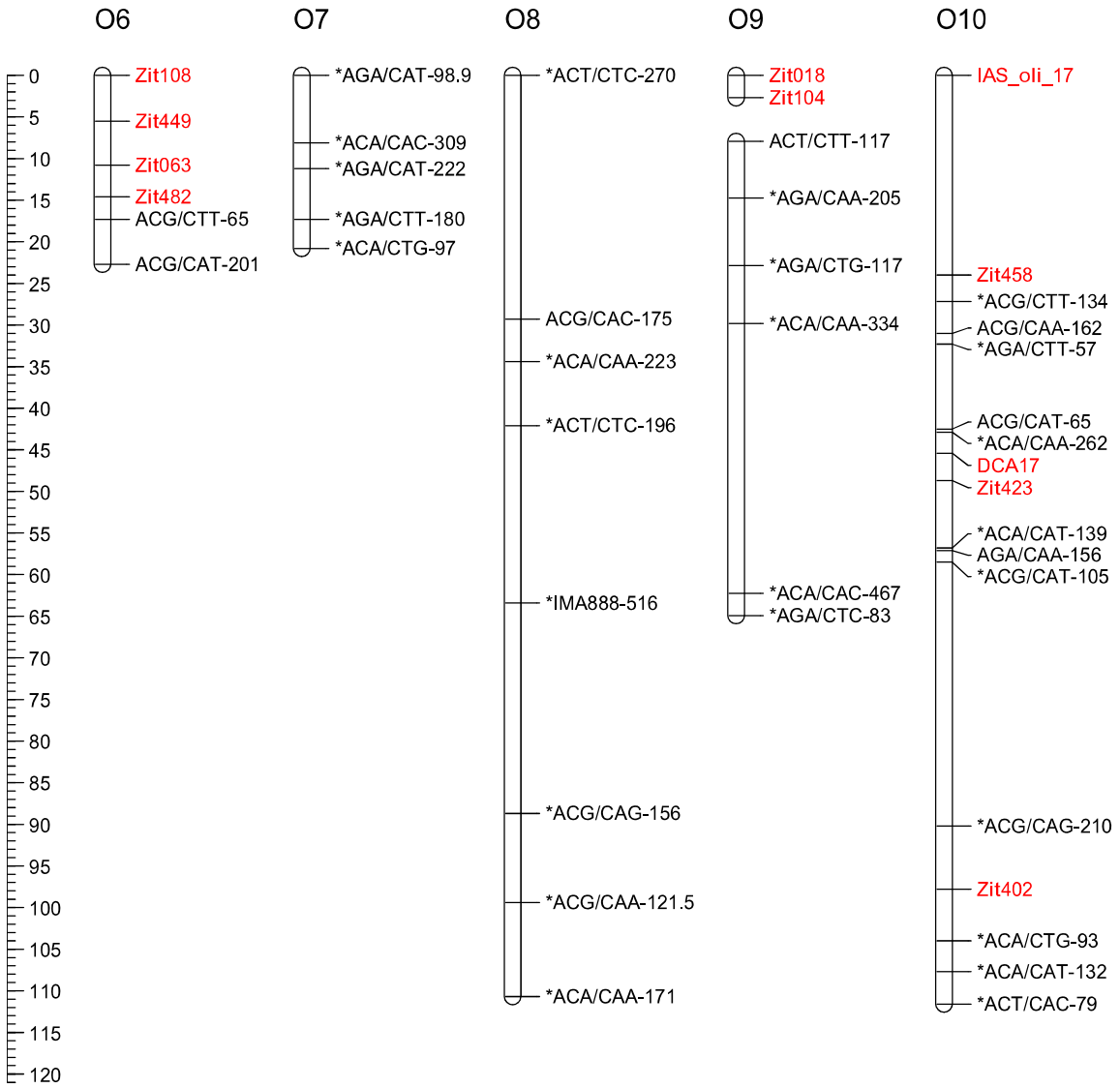


Figure S3a (continued)

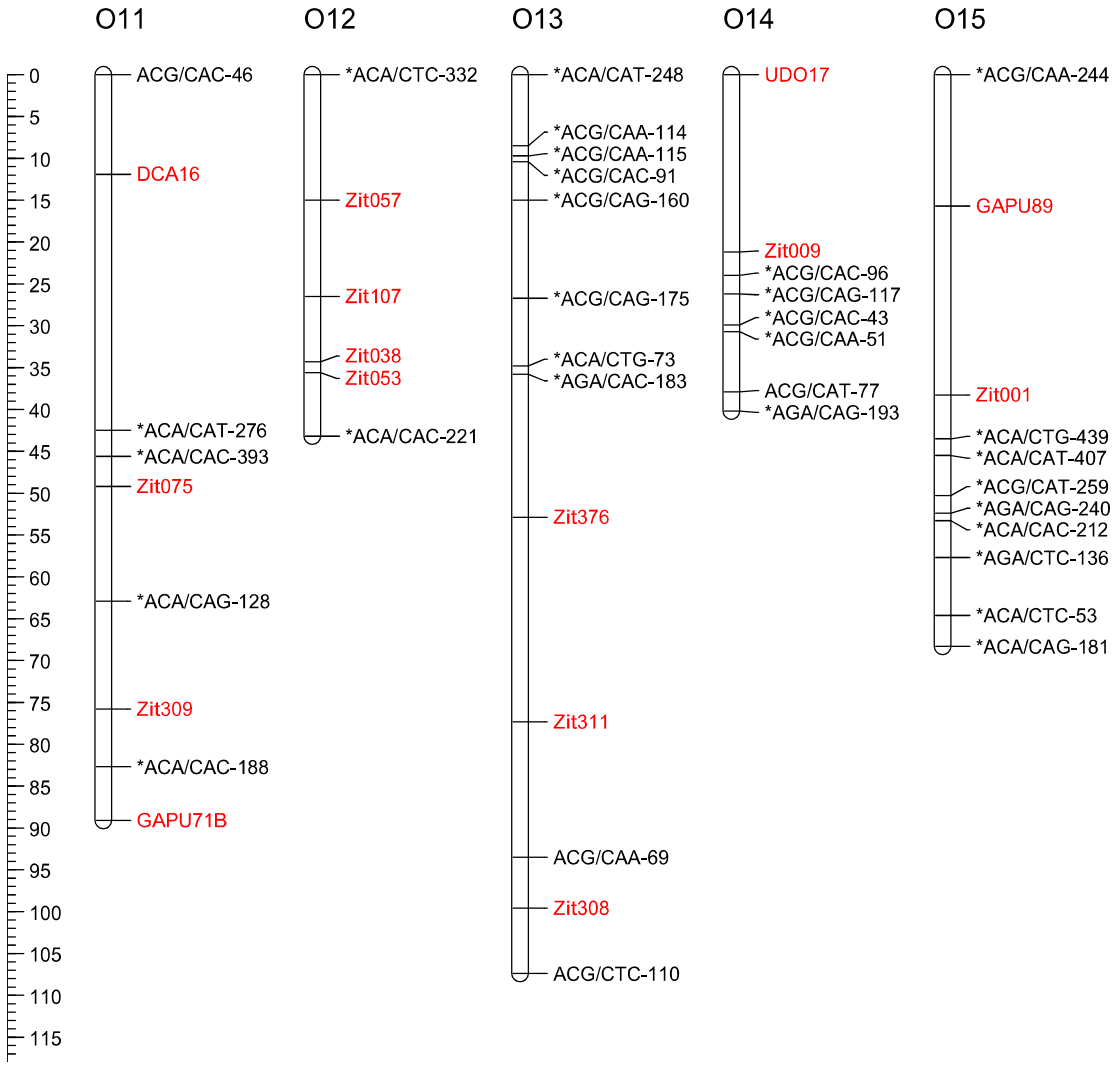


Figure S3a (continued)

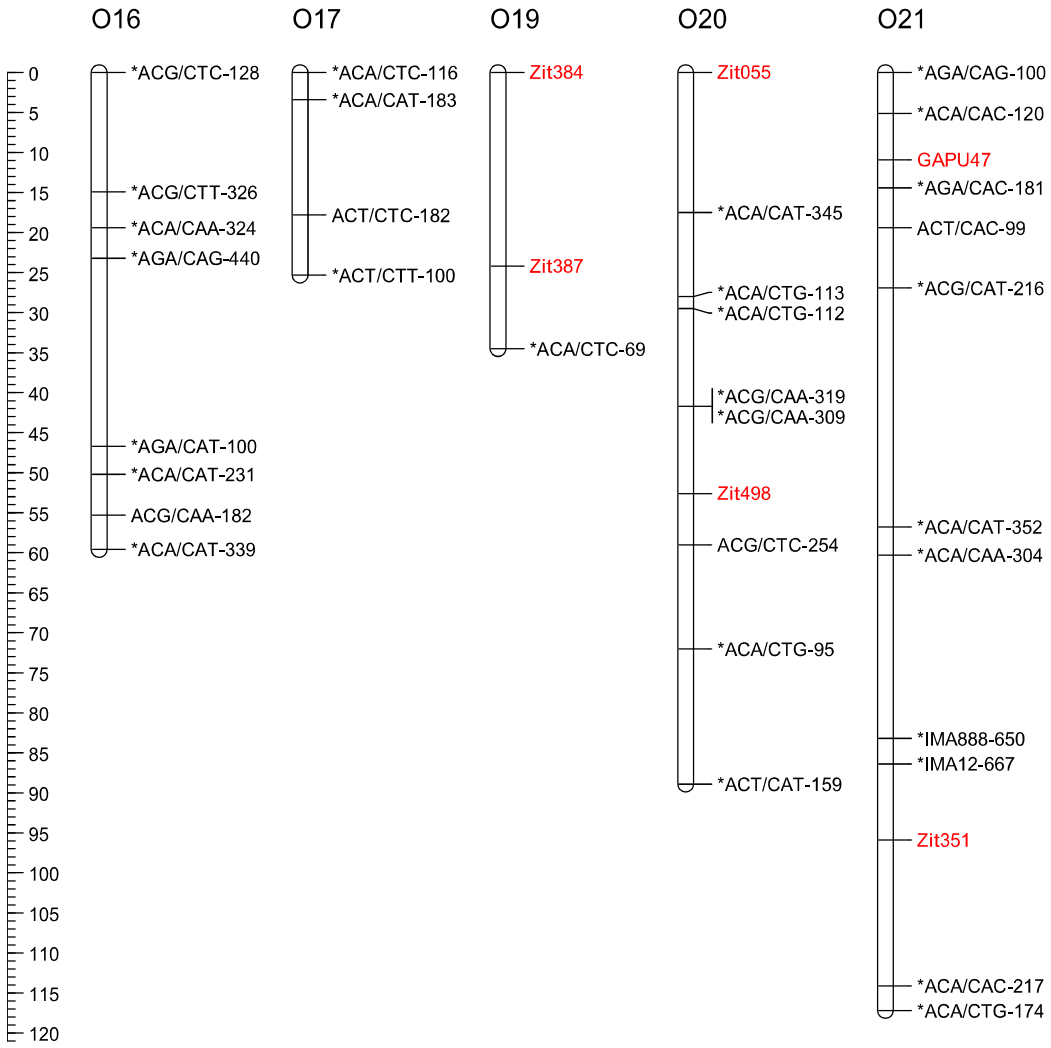


Figure S3a (continued)

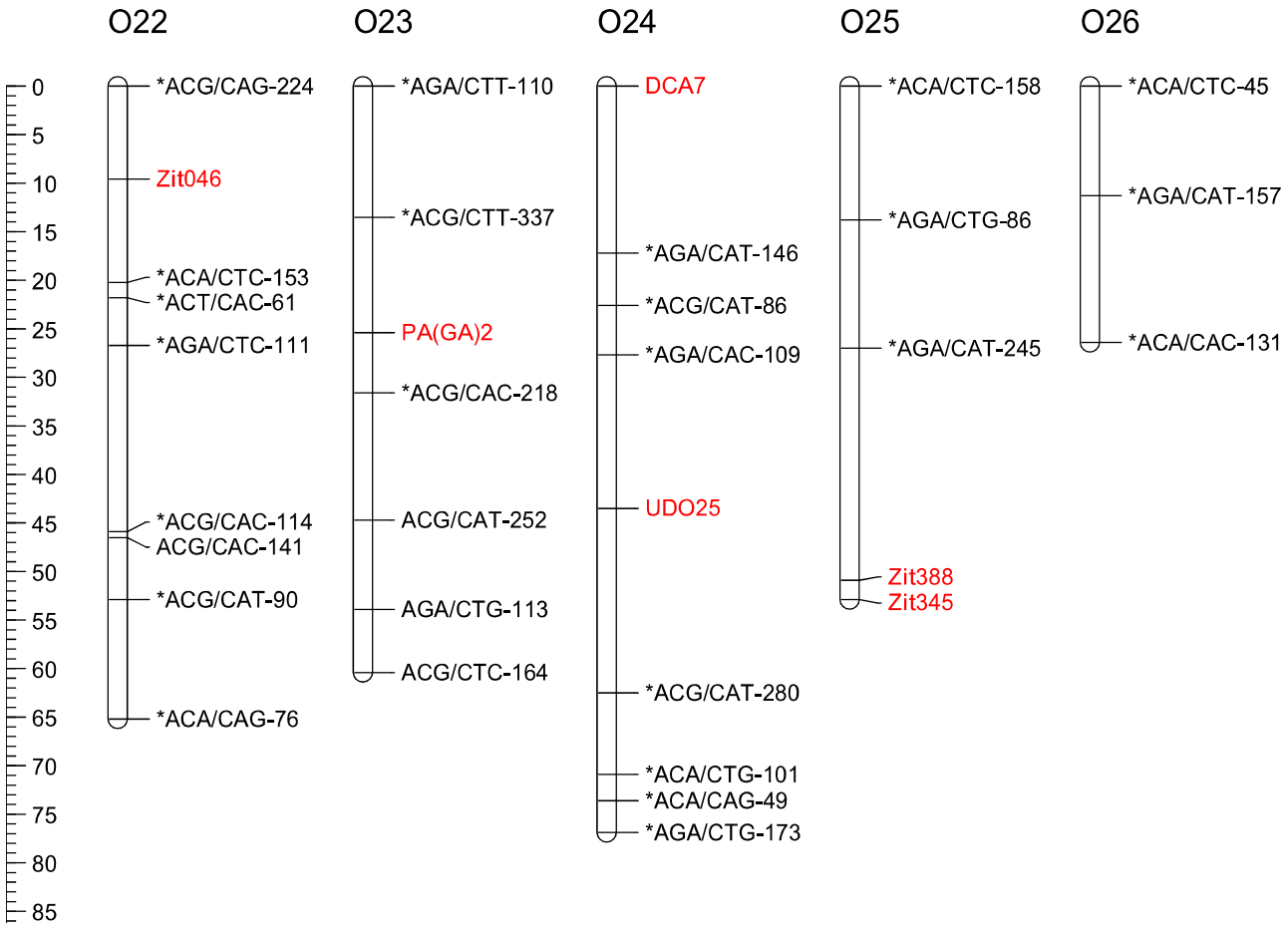
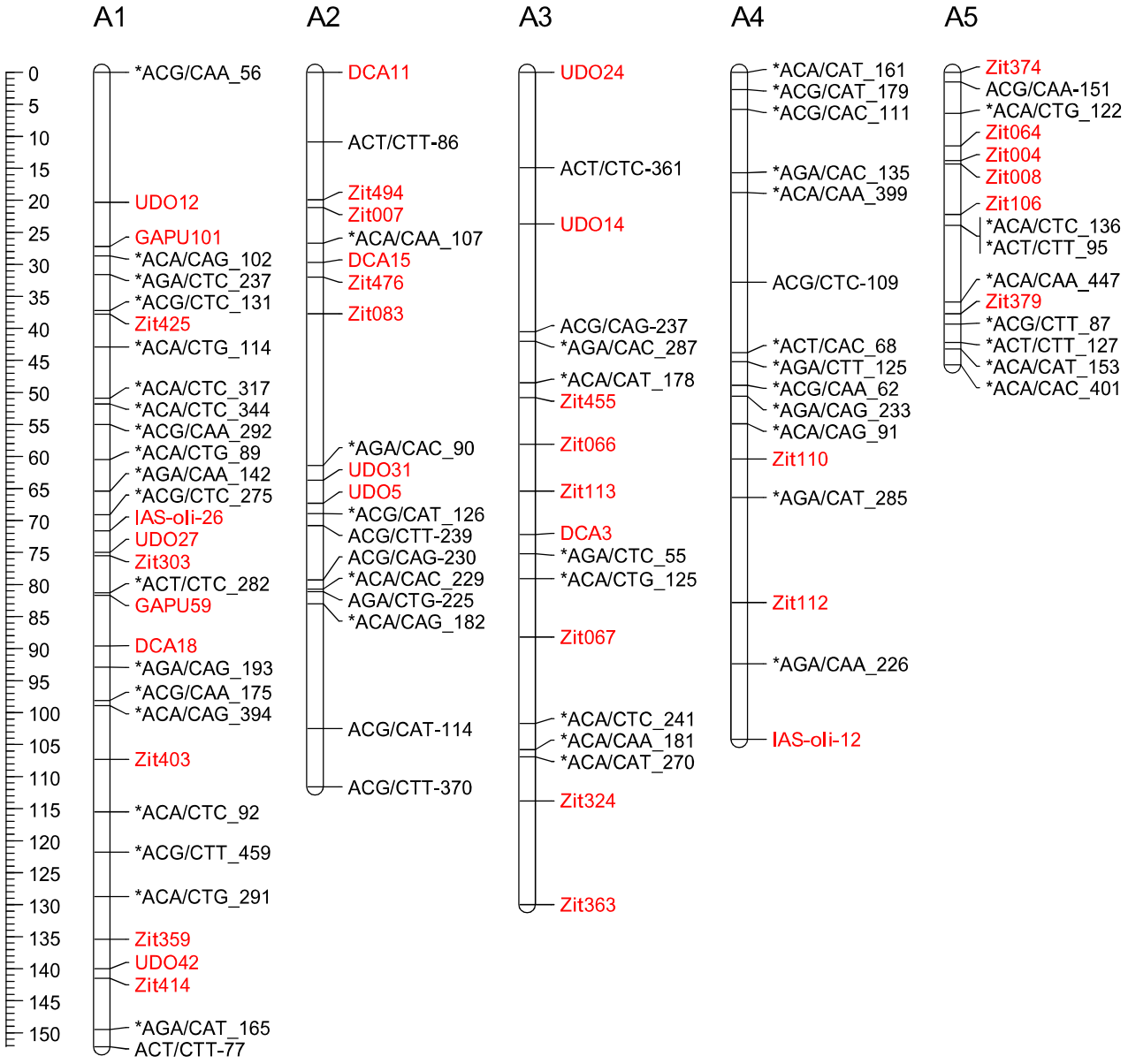


Figure S3a (continued)

(b)


A1
A2
A3
A4
A5
0
5
10
15
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25
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35
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45
50
55
60
65
70
75
80
85
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95
100
105
110
115
120
125
130
135
140
145
150
*ACG/CAA_56
UDO12
GAPU101
*ACA/CAG_102
*AGA/CTC_237
*ACG/CTC_131
Zit425
*ACA/CTG_114
*ACA/CTC_317
*ACA/CTC_344
*ACG/CAA_292
*ACA/CTG_89
*AGA/CAA_142
*ACG/CTC_275
IAS-oli-26
UDO27
Zit303
*ACT/CTC_282
GAPU59
DCA18
*AGA/CAG_193
*ACG/CAA_175
*ACA/CAG_394
Zit403
*ACA/CTC_92
*ACG/CTT_459
*ACA/CTG_291
Zit359
UDO42
Zit414
*AGA/CAT_165
ACT/CTT-77
DCA11
ACT/CTT-86
Zit494
Zit007
*ACA/CAA_107
DCA15
Zit476
Zit083
*AGA/CAC_90
UDO31
UDO5
*ACG/CAT_126
ACG/CTT-239
ACG/CAG-230
*ACA/CAC_229
AGA/CTG-225
*ACA/CAG_182
ACG/CAT-114
ACG/CTT-370
UDO24
ACT/CTC-361
UDO14
ACG/CAG-237
*AGA/CAC_287
*ACA/CAT_178
Zit455
Zit066
Zit113
DCA3
*AGA/CTC_55
*ACA/CTG_125
Zit067
*ACA/CTC_241
*ACA/CAA_181
*ACA/CAT_270
Zit324
Zit363
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*ACG/CAT_179
*ACG/CAC_111
*AGA/CAC_135
*ACA/CAA_399
ACG/CTC-109
*ACT/CAC_68
*AGA/CTT_125
*ACG/CAA_62
*AGA/CAG_233
*ACA/CAG_91
Zit110
*AGA/CAT_285
Zit112
*AGA/CAA_226
IAS-oli-12
Zit374
ACG/CAA-151
*ACA/CTG_122
Zit064
Zit004
Zit008
Zit106
*ACA/CTC_136
*ACT/CTT_95
*ACA/CAA_447
Zit379
*ACG/CTT_87
*ACT/CTT_127
*ACA/CAT_153
*ACA/CAC_401

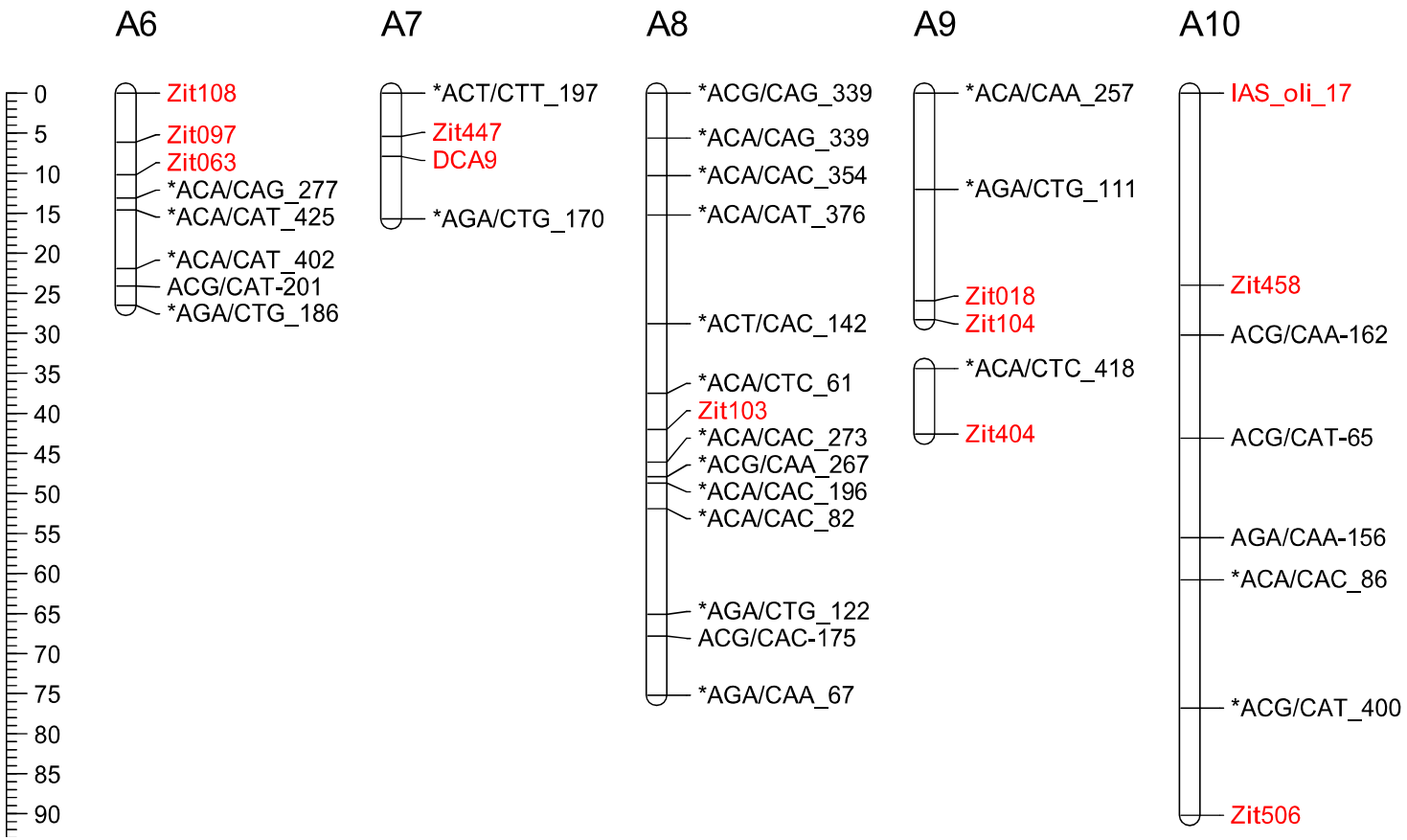

Figure S3b (continued)

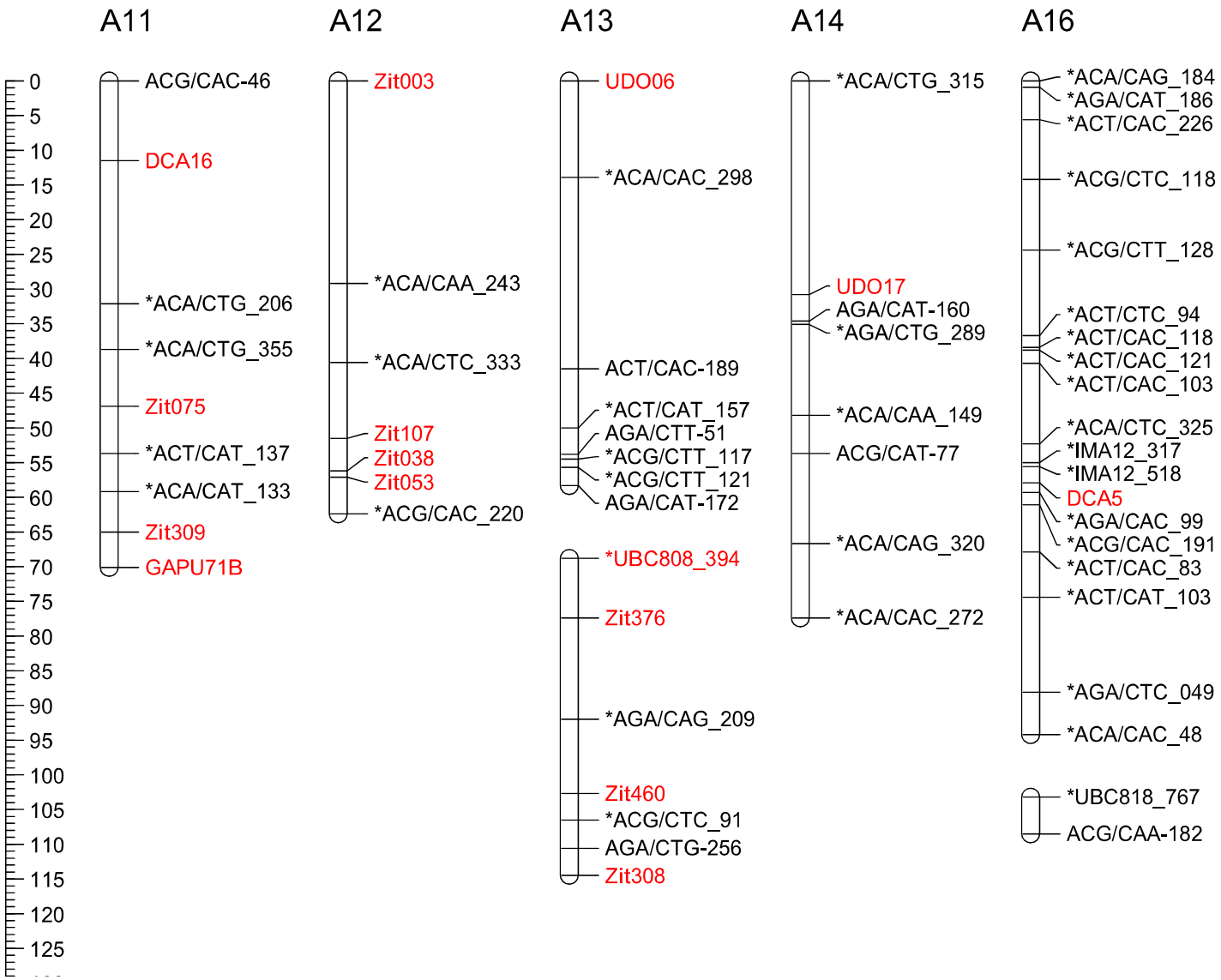

Figure S3b (continued)

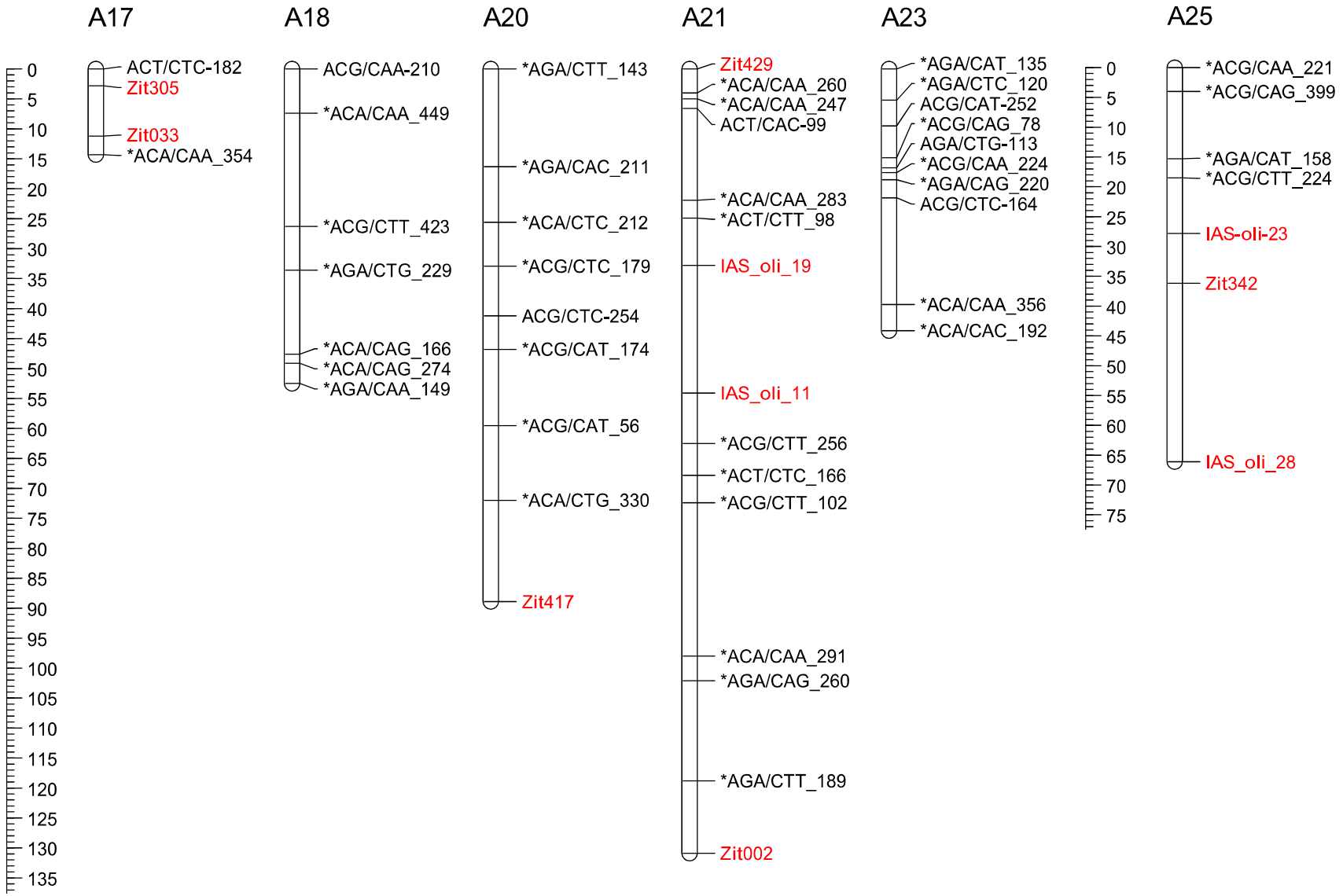

Figure S3b (continued)

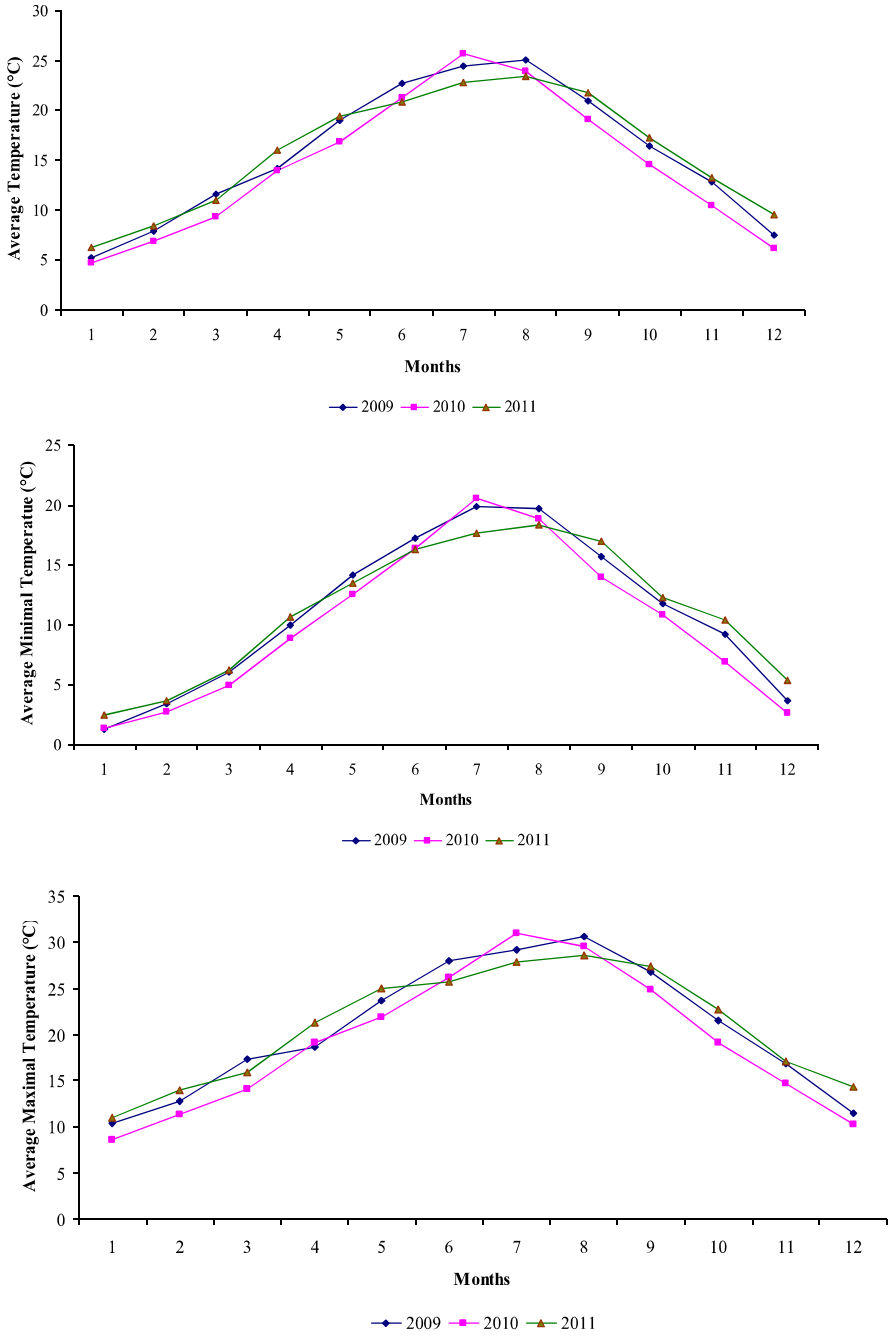

Figure S4. Temperature records during 2009-2011: (a) Monthly average temperature (b) Monthly maximal temperature (c) Monthly minimal temperature: Data were from meteorological stations at Melgueil INRA experimental station.

General Discussion and Perspectives

General Discussion and Perspectives

Methodological Framework Toward an efficient assess of the genetic effect on the olive tree:

Phenotyping Methodology

One of the initial objectives of my thesis was to establish a phenotyping strategy attaining the variability of the olive tree architecture and that can be easily applicable to a high number of progenies in quantitative genetics studies. To this end, the olive tree architecture was dissected in a number of elementary quantitative traits accounting for different developmental stages i.e. vegetative vs reproductive and different scales of observations within the tree i.e. trunk, sympode, growth unit (GU) and internode. The advantage of such a strategy is to acquire a substantial description of the entire topology on a large number of trees with relatively easy measurements. Moreover, this formalisation permits to define several architectural variables that can be calculated *a posteriori* for each of the studied years of growth, observation scales and environments. Up to now, the data base constituted during this thesis provides the most detailed description of the olive tree architecture from the juvenile period and until the first years of maturity. The repeated measurements over consecutive years were also appropriate to capture the architectural changes during the olive ontogeny and highlighting within tree dependencies associated to perenniality and trait correlations.

The chosen strategy combined the decomposition of complex traits into more elementary variables, as often performed for complex traits as for instance yield (e.g. Zhang et al. 2013), and the decomposition of plant architecture into entities corresponding to within-tree hierarchized organisation levels (Barthélémy, 1989). In the olive tree, local and global scales of observations, i.e. internode and whole tree respectively, were suitable to capture genetic effects contrary to intermediate scales such as growth units, at least during the exclusively vegetative phase of the trees. This result contrasts with those previously obtained in apple in which intermediate scales were appropriate for the genetic study of a number of morphological traits (Segura et al. 2007). It shows that, in the olive tree, high non-genetic variance mainly related to ontogenetic trends and climatic conditions was revealed when decomposing the trunk growth and branching over consecutive years into growth units or annual shoots which are themselves decomposed into internodes bearing or not lateral shoots

and positioned according to their branching order. Nevertheless, such decomposition could be considered for genetic studies when the variation over consecutive years and branching orders is low. As reported in apple by Segura et al. (2006), these finding suggest that during the first years of olive ontogeny, metamers appearance is more likely to be sensitive to environmental changes leading to phenotypic plasticity, whereas internode lengthening is mainly genetically determined. Also, the accumulation of metamers, irrespectively to their organisation in GUs and annual shoots, led to global variables such as trunk diameter and height or tree volume, which were discriminant between genotypes, consistently with previous studies on either fruit or forest trees (Liebhard et al. 2003; Lerceteau et al. 2000). These results suggest that adequate decomposition in scales and variables under genetic control depends on the species considered and their identification requires dedicated studies.

The study of the olive branching habit over the first five years of growth give an over view of the proportion and distribution in space and time of shoots length categories along trees main path of GUs. Axillary shoots of medium and short length located at tree crown periphery showed a significant genetic variance in both studied environments (Chapter 1 and 2), which makes these shoot length categories suitable for studying the genetic control of branching in olive (Ben Sadok et al. 2013). In the present study, branching was represented by syllepsis, whereas prolepsis was considered one year only (2008 GUs, Ben Sadok et al. 2013). In order to make a more comprehensive appraisal of the branching potential and the mixture of sylleptic and proleptic laterals in the olive tree, describing both syllepsis and prolepsis along trunks could be appropriate. Further characterisation of branching could also be related to the estimation of the production potential since branching is responsible for the number of shoots and their synchronism at the tree scale (see below).

The architectural description presented in this thesis gives a comprehensive multi-scale characterization of the olive vegetative development focusing on a main GU and axes pathway within the trees, from the establishment phase and until growth decrease. The morphogenetic gradients highlighted in the study could be complemented by the description of another fundamental morphogenetic process which is their capacity to duplicate and repeat their architectural units during their development (Barthélémy et al. 1991). This process is called reiteration and permits the tree to regenerate it self by developing reiterated complexes expresses again the juvenile growth pattern (Hallé et al. 1978). It can occur automatically during tree development after a threshold of differentiation or can be traumatic or adaptive (Barthélémy and Caraglio, 2007). In the case of olive tree, reiteration has been described as

responsible for the renewal of production units, through the development of new vigorous axes in proximal positions along bearer (Villemur et al. 1986; Lauri et al. 2001). In the present study, a description of reiterations has been performed on the 'Olivière'x'Arbequina' progeny, positioning along the trunk the first developed reiterated complex and describing its topology. The length and basal diameter of its growth units have been measured afterwards. Most of the progenies have developed a vigorous juvenile shoot at the basis of the trunk mainly located at GUs developed during the second year of growth. Considering that the data set obtained require additional analysis, this part could not be included in this manuscript because the data analysis was not fulfilled. However, the analysis of the number and relative development of reiterated complexes with respect to the main pathway I have described will constitute a step forward in understanding the dynamics of olive tree architectural development and of its production potential.

Considering the intense flowering and abscission characteristic of olive (Lavee et al. 1996; 1999) and the large population size, characterising the flowering and fruiting at GU scale on a sub-sample of one-year old GUs was suitable to quantify the flowering and fruiting potential at GUs over the first years of mature phase through simple measurements. It also permitted the appraisal of the fruit set in the progeny calculated on the basis of the number of inflorescences per GU. The data set collected allowed us to analyse the changes in inflorescences position along GUs (i.e. located at the leaf axils or at the GUs axillary shoots) during the olive ontogeny and the relationship between inflorescences position and fruit set. However, one-year old GUs did not reflect the bearing status of the whole tree in a giving year. Sampling branches could be more appropriate in this prospect. In fact, taking into account all or a sample of growing points along a branch could give access to more complex within-tree organisation of flowering and to synchronisms that could exist, especially at branch scale, as previously proposed for a range of fruit species (Couranjou, 1978; Monselise & Goldschmidt, 1982). Specific analyses of succession of shoot types (i.e. vegetative, flowering without fruiting and fruiting) have led to the development of alternation synchronism indices in apple (Lauri et al. 1997; Guitton, 2011). This approach could be tested in olive to address bearing regularity by the decomposition of alternation and non-alternation at the branch and whole tree. Knowing that branching architecture is one of the multiple factors determining productivity, it could be of great interest to investigate the effect of the balance between proleptic branching zones and sylleptic branching zones on the parent branches on the fruiting behaviour. In this thesis a first study of bearing regularity in an olive

segregating population has been initiated. Quantifying the total yield per tree and year had permitted to inquire on the bearing pattern of the progenies over the first four years of production and to classify the progenies accordingly. Whereas in apple tree alternative bearing mainly involved the within presence/absence of flowering (Guitton et al. 2012), in the olive tree considering yield and its variation depending on the years is thus suitable for characterising the fruiting behaviour in an olive tree progeny. This may lead to different biological and physiological assumptions underlying the alternation process that will need further investigations.

Eventually, the phenotyping performed in this thesis allowed us to initiate a first quantitative genetics study of complex traits in an olive segregating population. The variance of the architectural descriptors was partitioned into genetic and non-genetic components and their heritability was estimated. Furthermore, the repeated measurements allowed us to compare trait correlations and account of the within tree dependencies associated to perenniality. A segregation analysis and QTL mapping of heritable traits was finally initiated.

Considerations on the estimation of variance components

In this thesis, a comprehensive data analysis methodology adapted to complex and nested quantitative traits in a complete randomized design was proposed. As previously recognized in various perennials (Caraglio and Edelin, 1990; Heuret et al., 2000; Nicolini et al. 2000; Barthélémy and Caraglio, 2007; Segura et al. 2008), the olive tree architecture appeared to be governed by intrinsic organization rules of tree structure that evolves during the ontogeny. Considering that traits the same experimental units i.e. trees were involved in several non-randomized measurements over consecutive years, the development of a complex statistical modelling including a function of variance for traits showing heterogeneous variances and/or a covariance structure to account for serial correlation among observations on the same experimental unit was required. The repeated measurements were thus analysed within a mixed model framework (Jennrich and Schlutchter, 1986) using restricted maximum likelihood (REML) (Patterson and Thompson, 1971). The advantages of such method is the availability of a more parsimonious correlation (variance-covariance) model in comparison to the one implied by other methods e.g. multivariate ANOVA (MANOVA) procedures (Cole and Grizzle, 1966), the facility to accommodate several error terms and its robustness with regard to unbalancing in the dataset (Verbeke and Molenberghs, 2000). Also, each individual does not need to have the same number of observations, which is beneficial in our case as the

number of GUs developed per year and order depended on each tree. Mixed linear modelling of repeated data allowed us to dissect olive tree architectural plasticity into its genetic, ontogenetic and environmental components. However, some improvements could be considered in future studies. Giving that our randomized designs involve blocking, taking into account multiple block effect in the model could be favourable to treat the possible spatial dimension of correlations at the field level (Smith et al. 2001). Only few studies address this issue in the context of randomized block experiments in agriculture and biology (Berzsenyi et al. 2000; Ogliari and Andrade, 2001; Singh and Jones, 2002; Piepho et al. 2004) and a consensus as to the most suitable mixed modelling approach to repeated measures in such design is still missing.

The univariate analysis of variance presented in this thesis provides an extremely powerful and useful tool for statistical tests of factors and their interactions (Underwood, 1997). However, modelling the residual covariance between two traits could be interesting giving that most of the studied architectural traits were correlated. To this end, performing a multivariate REML analysis could be considered in future studies (Lee et al. 2012).

THE GENETIC DETERMINISM OF THE OLIVE TREE VEGETATIVE AND REPRODUCTIVE DEVELOPMENT

In the present thesis, the genetic determinism of the olive tree vegetative and reproductive development has been investigated in a F1 segregating population following a quantitative genetic approach. First, the analysis of the genetic parameters i.e. broad sense heritability or heritability of genotype mean was performed considering the architectural data recorded during juvenile phase of 'Olivière'x'Arbequina' progeny (i) collected in Melgueil experimental station over the first five years of growth and (ii) collected in a two-sites experimental design (i.e. Melgueil and Cordoba experimental stations) over the first three years of growth. Architectural traits appeared to be complex and substantially influenced by environmental factors (i.e. soil, temperature variations and water resources) and genotypic effect estimations were coherent between the two experiments. This confirms the power of the statistical analysis methodology and the quality of the data acquired in this thesis. However, assessing additive and dominant effects, narrow-sense heritabilities and genetic correlations could allow the computation of selection indices and the prediction of genetic gains (Falconer, 1981). In this prospect, traditional mating designs e.g. nested, factorial or diallel designs in olive (one-way diallel crosses, Zeinanloo et al. 2009) could be considered. The knowledge

that such experimental designs could provide can be complemented in the long term using wide-pedigree information as exhibited in apple (Durel et al. 1998).

This study presents the QTL detection performed on traits related to the reproductive development of the olive tree. Regarding traits related to the vegetative development, the assessment of QTL and QTL x environment effects is in preparation and results were not included in the manuscript as analysis needs to be completed. A two-stage approach has been chosen for QTL detection using permutation-based thresholds. This method is particularly interesting for data collected at different times or positions in the same individual as it dissociate modelling of autocorrelations and QTL mapping on the basis of Best linear unbiased predictors (BLUPs) estimation (Segura et al. 2009). In the present work, BLUPs of the genotypic effect (i.e. considered as independent of both ontogenetic and climatic effects) and its interaction with the year factor were obtained first from the mixed linear models of repeated data and then were used for interval mapping (IM) and multiple QTL mapping (MQM) analysis. This approach allowed the detection of several QTLs genotype specific and QTLs year specific (Chapter 3). Nevertheless, QTL analysis resolution could be improved through increases in sample size and marker number. This implies a great investment in time and money giving the long generation time of such perennial species. According to Verbyla and Cullis (2012), BLUPs of the genotype effect are often biased downwards, reducing accordingly the chance of QTL detection. Following the principle of regression-based QTL mapping (Haley and Knott, 1992), one-step modelling of phenotypes, in which genotype effect would be replaced by molecular marker information in the mixed models, could also be considered to optimize the statistical power to detect significant QTL and the magnitude of their effects. Giving that 'good' varieties combine optimal values of several traits, the determination of quantitative trait loci (QTL) for multiple traits or across multiple environments is of further interest for breeders. Multivariate methods increase the power for detection of QTL through correlation. Capitalizing on the between-traits genetic correlation could also allow the use of secondary traits to improve primary ones that are difficult to measure or have low heritability (Malosetti et al. 2008). Moreover, multivariate methods for QTL analysis would allow a deeper interpretation of QTL by trait or environment interactions, whether related to pleiotropy or a common QTL at a locus, an equal or unequal QTL size and closely linked QTL rather than being pleiotropic (Knott and Hayley, 2000; Verbyla and Cullis, 2012).

Past researchers have investigated multi-trait or multi-environment QTL mapping approaches (Knott and Haley, 2000; Piepho, 2000; Verbyla et al. 2003; Malosetti et al. 2004; Vargas et al. 2006; Emrich et al. 2007; Boer et al. 2007). However, these methods fail when the number of traits or environments increases and the problem is either reduced to the multi-trait (MT), or to the multi-environment (ME) dimension. In fact, approaches that fully exploit data from multi-trait multi-environment (MTME) trials are limited. Malosetti et al. (2008) addressed how to model QTL by environment interactions and the genetic basis of heterogeneity of variance and correlations between traits and environments by mixed models. These authors proposed to use the direct product of covariance matrices for traits and environments to construct genetic (co)variance matrices, giving a good fit to the data and reducing the computation time while considerably reducing the number of parameters. Nevertheless, most of the methods proposed both in the univariate and multivariate approach require multiple testing and genome scan implying the use LOD scores (Verbyla and Cullis, 2012).
Whole genome average interval mapping (WGAIM) in which all markers are used simultaneously was presented by Verbyla et al. (2007) for univariate QTL analysis and recently extended to multiple traits and/or environments QTL analysis (MVWGAIM) by Verbyla and Cullis (2012). This method uses all the intervals on a linkage map simultaneously and avoids the difficult issues regarding repeated genome scans that are part of composite interval mapping (CIM) and multiple interval mapping (MIM). Hence, the number of models that need to be fitted is greatly reduced. Moreover a simple stopping rule based on a likelihood ratio test is available and permutation-based methods are not required.

In the present thesis, numerous QTLs of small effects associated to flowering and fruiting behaviour were identified in this thesis and needs to be further confirmed on other segregating populations. Giving the upcoming of the next generation sequencing methods, using dense genetic markers, such as single-nucleotide polymorphisms (SNPs) will be soon possible in olive. This could allow the future densification of the available olive genetic maps and complement our linkage mapping study by association mapping approaches to enhance the mapping resolution and identify the underlying causes of quantitative trait variation. Association mapping also known as linkage disequilibrium mapping can be performed on populations of related individuals or on a selection of distinct unrelated genotypes representing the greatest olive diversity i.e. core collection. It requires extensive knowledge of SNPs within the genome of the organism of interest and is therefore difficult to perform in not well studied species or species that don't have well annotated genomes yet (Yu et al. 2008). In

order to initiate association mapping in olive, an in silico search of SNPs on the basis of olive RNA sequences has been initiated in the AFEF Team. This new project aims at developing first, a phenotyping network mainly based on the worldwide olive core collections grown in Marrakech (Morocco) and Montpellier (France) for the time being and will probably be enlarged to the olive core collections grown in Cordoba (Spain) and Turkey. Second, association mapping studies will be initiated on the various characters of interest. Moreover, the availability of several segregating populations having a common parent in olive could motivate launching association mapping in this species. Taking the example of 'Arbequina' male parent of the progeny studied in this thesis, several segregating populations are available in this prospect: 'Arbequine' x 'Picual'; 'Arbequine' x 'Zaity'; 'Zaity' x'Arbequine' (De la Rosa et al. 2004) and 'Olivière'x'Arbequina' (Ben Sadok et al. 2013). Finally, when the olive genome sequence will be available, it will be possible to initiate candidate genes research in the genomic regions of the detected QTLs.

PROGENIES PHENOTYPIC PLASTICITY AND BREEDING POTENTIAL

The identification of progenies showing promising characters with respect to the breeding targets e.g. low vigour, stable production could constitute valuable innovative genetic material for further genetic studies or breeding programs. Hence, heterosis has been investigated in the studied progeny revealing interesting superiorities in comparison to the highly heterozygous parents e.g. progenies exhibiting higher values for flowering and fruiting traits, or less vigorous than 'Arbequina' parent. Progenies showing regular bearing over the first four years of production are of great interest, although additional observations years need to be considered for a proper diagnosis of their potential. Moreover, progenies were able to tolerate the variation in the environmental through growth plasticity. Their reaction to different environments was mainly uniform i.e. non significant GxE effect (see Chapter 2). This suggests that the progeny under study have a broad adaptation, at least during the juvenile phase. The non significant GxE effect makes also possible the prediction of the genotypes performance. These findings give substantial material for future genetic studies and breeding programs in olive, whether the presence or not of genotype-by-environments is privileged. The evaluation of the general pattern of trait covariations between environments and within each environment allowed the identification of three groups of architectural traits, corresponding to the three observation scales considered: tree, GU and internode, from which progenies showing interesting trait combinations could be selected (Chapter 2). This approach

could be further thorough by partitioning the studied progeny into architectural groups containing trees of relatively similar form and branching as previously performed by Segura et al. (2006). Such classification could be useful for understanding the interaction between vegetative and reproductive habits in olive. Such classification could constitute an innovative source in the debate for defining architectural ideotypes in olive e.g. trees characterised by low global volume, primary growth, branching and internode length could be suitable for high density orchards. There are other possible ideotypes e.g. tall trees characterized by high branching potential suitable for extensive orchards. Likewise, trees showing high global volume and GU length and low branching could be appropriate for studying the reproductive habit of the adult olive tree giving that its flowering was mainly located at the leaf axils (see Chapter 2 and 3). Among all studied traits, internode lengthening variables (i.e. IN_Max and Mean_INL) were not affected by exogenous conditions and were mainly genetically determined. Their stability across environment could constitute an interesting supply for breeding programs control of canopy shape and density on orchard.

Finally, this PhD thesis constitutes a first study in quantitative genetics accounting for the long lasting tree development of olive tree and its developmental specificities. It also opens a number of perspectives either in terms of scientific investigation for others students and researchers or for the genetic improvement and breeding for olive tree that could be beneficial for countries all around the Mediterranean Basin.

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ANNEXES

Annexe 1
Conference Proceedings

BEN SADOK I., MOUTIER N., DOSBA F., REBAI A., GRATI-KAMOUN N., KHADARI B., COSTES E (2009) Studying the Genetic Determinism of Olive Tree Architecture in a F1 Progeny 'Olivière' x 'Arbequina'. OliveBioteq Proceedings: 416-421.

BEN SADOK I., MOUTIER N., DOSBA F., KHADARI B., COSTES E (2011) Studying the Genetic Determinism of Olive Tree Architecture in a F1 Progeny 'Olivière' x 'Arbequina'. Acta Hortic 924: 299-306.

S2-P18

Studying the Genetic Determinism of Olive Tree Architecture in a F1 Progeny Olivière x Arbequina

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Abstract

Tree architecture is of major importance for the agronomic performance of fruit tree orchards. It influences tree adaptability to cultivations systems, and determines yield and fruit quality. The study of the genetic determinism of architectural traits has been initiated in apple tree and has opened new perspectives for breeding programs. In the present study, we aim at investigating the existence of genetic determinism in the olive tree architecture. A F1 hybrid population was created at Montpellier (France) between two genotypes with contrasted architecture, Olivière and Arbequina. A phenotyping methodology of architectural traits was applied and led us to analyze traits related to growth, branching and fruiting behavior, that were measured during the 3 first years of tree growth after plantation. Analyses show the influence of tree ontogeny and morphogenetic gradients on the tree architectural development that must be taken into account during quantitative genetic analyses. The phenotypic variability observed within the population was decomposed into genotypic and residual (environmental) effects in order to identify the most heritable and discriminating phenotypic architectural characters depending on tree age and branching orders. In parallel, an initial genetic map based on the same hybrid population was constructed with 44 SSR loci and 494 AFLP (Khadari *et al*., in preparation). This will allow us to initiate QTLs mapping related to architectural traits in the next future, as we will continue further phenotyping campaigns for the most relevant traits.

Keywords: Olive tree architecture, Phenotyping Methodology, Quantitative Genetics, Growth, Branching, Flowering, Fruiting.

Résumé

L'architecture de l'arbre a une incidence majeure sur les performances agronomiques des arbres fruitiers en verger. Elle influence la capacité d'adaptation des arbres aux systèmes de culture et conditionne leur productivité et la qualité des fruits. L'objectif de la présente étude, est d'explorer le déterminisme génétique des caractères architecturaux chez l'olivier. Une population hybride F1 a été créée entre deux génotypes à architectures contrastées, Olivière et Arbequina. Une méthodologie de phénotypage a été appliquée, nous permettant d'analyser les caractères liés à la croissance, la ramification et la fructification, et qui ont été mesurés au cours des 3 premières années de croissance des arbres. Les analyses montrent l'influence de l'ontogenèse de l'arbre et des gradients morphogénétiques au cours du développement des arbres, qui doivent être pris en compte dans les analyses de génétique quantitative. La variabilité phénotypique a été décomposée en effets génotypiques et résiduel afin d'identifier les caractères les plus héritables.

1. Introduction

Genetic studies are needed to design strategies for including traits related to tree architecture such as regular bearing in breeding programs. In order to investigate the genetic determinism of architectural traits in the olive tree, a F1 progeny was created from a cross between two contrasted cultivars giving high architectural variability. During the 3 first years of growth, a phenotyping methodology was applied, inspired from previous studies apple tree, based on a detailed decomposition of tree architecture into topological an geometrical variables (Segura et al., 2006). This strategy highlighted the existence of morphogenetic gradients that must be taken into account in the estimation of genetic effects on architectural traits.
Finally, this estimation was carried out on both growth and branching traits and led us to identify the most heritable traits.

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2. Materials and methods

2.1. Plant material

The progeny under study derived from an 'Olivière' X 'Arbequina' cross and counts 162 genotypes. The parents have been chosen for their contrasted architecture (Moutier et al. 2004; 2008). 'Olivière' (female parent) is a vigorous French cultivar with a fast growth rate, a big fruit and a rapid entrance in production. The 'Arbequina' male parent is a Spanish cultivar with low vigor and small fruit. In 2005, the 162 hybrids have been randomly planted after cutting (6m x 2m; N/S orientation in plot) as 6 repetitions per hybrid at INRA- Montpellier experimental station. For the architectural study, trees were not pruned and trunks were cut back to 20cm. Standard irrigation and treatments were carried out.

2.2. Architectural description

A topological and geometrical description was performed on a total of 324 trees (2 repetitions/genotype) for the three first years of growth. All growth units (GUs), whatever the branching order[1], were observed in 2005 while a subsample corresponding to the main path of GUs in the tree and along the longest lateral branch was observed in 2006 and 2007. GUs were classified into three types according to their length: long (length ≥20 cm); medium (5 cm≤ length <20 cm) and short (length <5 cm). Bottom and top diameters, basal angle and cord were measured on the longest lateral branch (Figure 1). Tree topology was formalized according to the coding methodology defined by Godin et al. (1999). Thus, a multiscale tree graph (MTG) was used to calculate architectural traits at 3 different scales: branch, GU and internodes (Table 1). Data extraction from MTGs was performed using VPlants software (http://openalea.gforge.inria.fr).

2.3. Statistical Analysis

A *one-way ANOVA (with a* TYPE III sum of square for unbalanced design) was applied to estimate the genotypic effect on phenotypic variance for the architectural quantitative characters. Broad sense heritabilities (H^2) of growth and branching characters were calculated from the variance additive model decomposing phenotypic values into genotypic and environmental effect. Broad sense heritability corresponds to the ratio between the genotypic and phenotypic variances (Gallais, 1989) and can be approximate as a function of
Fisher statistic for the genotypic factor (Knapp et al., 1985):

$$H^2 = \sigma^2 G / \sigma^2 P = \sigma^2 G / \sigma^2 G + \sigma^2 \varepsilon; \; H = 1 - 1/F$$

A confidence interval based on the Fisher theoretical distribution is estimated for the heritability significance appraisal. Generally, a variable is considered heritable when the correspondent low limit for the confidence interval is greater than zero and when the heritability value is greater than 0.2 (Gallais, 1989). All statistical analyses were performed using R software[2]

3. Results

3.1. Architectural analysis

Every year after plantation, the olive trees developed axes at several branching orders (Figure 2). These axes did not show any morphological differentiation since they were all orthotropic with an opposite decussate phyllotaxy and a rhythmic growth, even though no or few morphological markers were visible (data not shown).

3.2. Primary Growth

The number of GUs developed per tree was the greatest in the second year after plantation in 2006 (Figure 2). Moreover, an increase in the average length, positively correlated to the internodes number, was found in the second year when compared to first year of growth (Figure 3). This primary growth launching was observed mainly at orders 0 and 1 and was followed by a decrease at order 2 in both first and second year of growth. A stable phase was reached from order 3 and in the third year of growth at all observed orders (2 to 5; Figure 3).

[1] Branching orders were incremented from axes developed after cutting, which were considered as order 0 (Figure 1).

3.3. Branching

Whatever the year and branching order, the three types of GUs (long, medium and short) were observed as laterals (Figure 3). However, lateral GU types changed depending on the age of their parent GU: the older the parent GU the higher the proportion of long lateral GUs (Figure 4). The mean number of lateral GUs per GU decreased with years and branching orders, in a similar way to that observed for GU length and number of nodes (data not shown).

3.4. Broad Sense Heritability

Due to significant effects of branching orders on all variables (data not shown), only particular combinations of orders were considered for estimating the genotypic effects: all measured orders were considered for the first year after plantation , order 2 and 3 for the second year of growth and orders 2 to 5 for the third year of growth. Topological variables showed intermediate to high heritability values (Table 2). The highest values were found in 2005 for the number of internodes per GU (Nb_IN) and the short GUs (Nb_S) in 2006, with H^2 value greater than 0.5 and a lower limit of the confidence interval of 0.38. The geometrical variables showed relatively low heritability values except for the mean

4. Discussion and Conclusion

The analysis of growth and branching during the three first years of ('Olivière' X 'Arbequina') progeny highlighted the existence of morphogenetic gradients related to tree ontogeny. A growth increase was observed in the second year of growth, showing an establishment phase after tree plantation that was followed by a more stable phase at high orders and in the third year of growth. These results are consistent with previous quantitative studies on fruit and forest trees (Costes et al., 1992, 2003; Barthélémy et al., 1997; Fournier et al., 1998; Seleznyova et al., 2002). High trait variability was also found depending on the branching order that can be interpreted as resulting from frequent sympodial branching in the olive tree. Both tree age and branching orders were thus accounted for in our quantitative genetic study, as previously proposed by Segura et al. (2008). This study will allow the next phenotyping campaigns to focus on the most heritable and less correlated variables. In addition, combined with a first genetic map built for the same progeny (Khadari *et al.,* submitted), this study will allow us to initiate QTLs mapping for architectural traits.

Table 1: List of quantitative variables related to tree topology and geometry with detailed formula for calculated variables.

Tree variables	Abbreviation	Formula	2005	2006	2007
Growth					
Number of Internodes / GU	Nb_IN		X	X	X
Branching					
Number of axillary shoots / GU	Nb_AS		X	X	X
Number of long ax. GUs Number	Nb_L		X	X	X
of medium ax. GUs Number of	Nb_B		X	X	X
short ax. GUs	Nb_S		X	X	X
Length of the longest Internode (mm)	IN_Max		X	X	X
Basal GU diameter (mm)	Bdiam		X	X	X
GU top diameter (mm)	Tdiam		X	X	X
Longest branch cord (mm)	cord		X	X	X
Longest branch basal angle(°)	B_angle *		X	X	X
Tree volume (m^3)	V	1/3* B_area*H		X	X
Trunk basal diameter (mm)	Tr_Bdiam		X	X	X

[2] R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

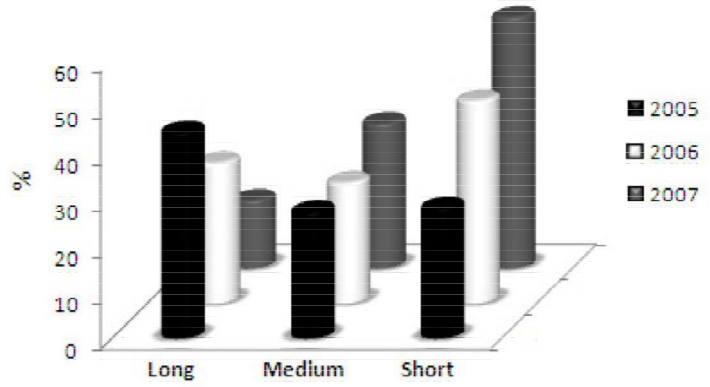


Fig. 3. Percentage of lateral growth unit (GU) types (long, medium or short) depending on parent growth units age, calculated on 3-year-old olive trees of an 'Olivière' × 'Arbequina' progeny.

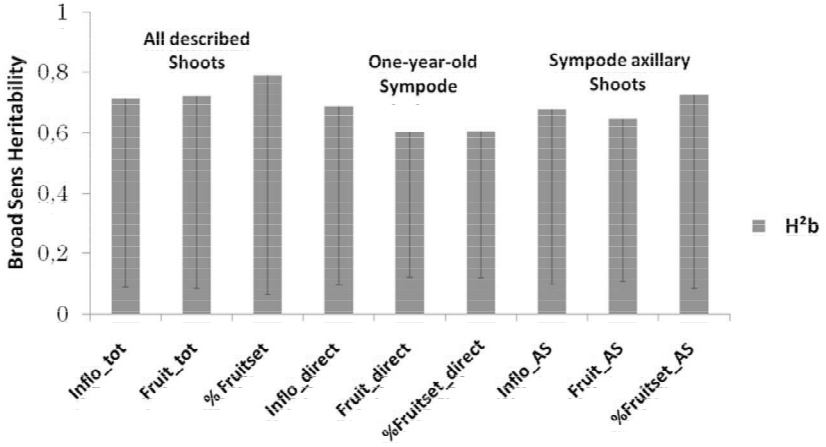


Fig. 4. Broad sense heritability values of flowering and fruiting variables measured on 1-year-old sympodes in 2008.

Annexe 2
Poster

BEN SADOK I., MOUTIER N., DOSBA F., KHADARI B., COSTES E (2010). Studying the Genetic Determinism of Olive Tree Architecture in a F1 Progeny 'Olivière' x 'Arbequina'. 28th International Horticultural Congress, August 22-27, Lisboa- Portugal.

Annexe 3

Valorisation Des Compétences Des Docteurs, NCT®

Inès Ben Sadok

Architecture, Génétique et Production
L'olivier de la juvénilité à la maturité



Ecole Doctorale SIBAGUE
Systèmes Intégrés en Biologie, Agronomie, Géosciences, Hydrosciences, Environnement
Spécialité : BIP- Biologie Intégrative des Plantes

Etablissements d'enseignement supérieur de rattachement :
Montpellier SupAgro (France) -Faculté des Sciences de Sfax (Tunisie)
Unité Mixte de Recherche : *Amélioration Génétique et Adaptation des Plantes méditerranéennes et tropicales (AGAP)*

Sujet académique de la thèse: *Etude du Déterminisme génétique de l'architecture de l'olivier en vue de l'amélioration de la production et de la qualité du fruit.*
Directrice de Thèse : *Evelyne Costes.*

DOCTOR TO BE…WHAT ELSE?

I. CADRE GENERAL DE LA THESE

THEME ET ENJEUX

RESUME DE MON THEME DE RECHERCHE :

Mes travaux de thèse consistent à comprendre et caractériser la part génétique impliquée dans l'architecture de l'olivier. Une description annuelle détaillée du développement végétatif et reproducteur d'une descendance issue du croisement entre la variété Française 'Olivière' et la variété Espagnole 'Arbéquina' a été effectuée dans deux environnements contrastés. Les principaux facteurs influençant la variabilité architecturale observée ont été identifiés. A l'aide d'outils statistiques, j'ai étudié l'héritabilité des caractères architecturaux et identifié les régions chromosomiques ou QTLs susceptibles de contenir le ou les gènes contrôlant le caractère d'intérêt associé. Ce travail aboutira au développement d'une base de connaissances génétiques exploitable dans des programmes d'amélioration pour les caractères d'intérêt agronomique tel que la régularité de production et économique tel que la réduction des coûts de conduite en vergers.

ENJEUX ECONOMIQUES, TECHNIQUES ET SOCIETAUX :

Chez les arbres fruitiers, l'architecture suscite un intérêt particulier car elle influence les caractères liés à la production. Le développement architectural et l'organisation interne des arbres influence l'efficacité de l'interception lumineuse (nécessaire à la production de biomasse et à l'élaboration de la qualité des fruits), le rendement et sa régularité.

Sur le plan technique, l'obtention d'arbres peu volumineux permet la facilitation de la récolte depuis le sol et l'augmentation de la densité de plantation des vergers.

La maîtrise du développement et de la forme des arbres fruitiers est généralement obtenue par les pratiques culturales manuelles: greffage, conduite et éclaircissage chimique ce qui engendre des coûts de main d'œuvre et un coût environnemental. Le développement de méthodes alternatives et non destructives devient de plus en plus une priorité pour les organismes de recherche en arboriculture fruitière. La description du développement naturel de l'arbre et l'identification du fondement génétique du développement végétatif et reproducteur constituent les bases de l'amélioration ciblée de la conduite de l'arbre.

L'étude du déterminisme génétique des caractères architecturaux a été initiée chez le pommier. Plusieurs caractères relatifs à la croissance et à la ramification de l'arbre avaient un contrôle génétique. Cependant, même si la variabilité architecturale a été quantifiée chez le pommier, le déterminisme génétique du port de l'arbre demeure inexploré chez l'olivier, en dépit de son importance

économique. L'olivier (*Olea europaea L. subsp. europaea*) est l'un des arbres fruitiers les plus anciens et le plus important autour du bassin méditerranéen, où se concentre 98% de la production mondiale d'olives (Standish., 1960; Doveri et al., 2008). La part du commerce international qui revient à l'huile d'olive est de 22 % par rapport aux autres huiles végétales fluides alimentaires, c'est-à-dire un niveau supérieur à celui de l'huile de tournesol ou de colza qui représentent pourtant respectivement 16 et 21 % de la production mondiale contre seulement 4,5 % pour l'huile d'olive (Touzani et al., 2004).

Ces connaissances pourraient permettre l'introduction des caractères architecturaux dans les programmes de sélection d'olivier pour la régularité du rendement et la réduction des coûts des conduites en vergers (Laurens et al., 2000; Costes et al., 2004).

ENJEUX SCIENTIFIQUES :

Ce travail de thèse a pour objectif premier, l'identification des déterminants génétiques impliqués dans l'architecture de l'olivier incluant son développement végétatif et reproducteur. Le second objectif est d'estimer l'effet de l'environnement et son interaction avec le génotype sur les caractères architecturaux. Mon analyse intègre l'évolution en fonction des années des différents caractères notamment ceux de floraison et fructification en relation avec la localisation des inflorescences sur les unités de croissances et les ramifications. A terme, l'objectif est d'initier l'étude de la régularité de production de la descendance étudiée.

Les résultats attendus permettront de compléter la connaissance de l'étude du port de l'arbre fruitier par les spécificités du modèle olivier et de se doter ainsi d'une base de connaissances génétiques en vue de mener des programmes d'amélioration pour les caractères d'intérêt agronomique tels que la production et de sa régularité.

Cette thèse est basée sur un dispositif génétique en ségrégation : 160 descendants + 2 parents étudiés à Melgueil (France) et Cordoue (Espagne) issus du croisement entre Olivière, variété très vigoureuse, et Arbéquina, variété de faible vigueur adaptée aux fortes densités de plantation. Une première carte génétique est disponible sur la même descendance.

CONTEXTE ET POSITIONNEMENT

Mon sujet de thèse s'insère dans la thématique globale de l'équipe : Architecture et Fonctionnement des Espèces Fruitières à savoir, l'étude du développement de l'arbre en vue

de l'amélioration de la production. Les deux espèces d'étude au sein de l'équipe sont le pommier (espèce principale d'étude) et l'olivier.

SITUATION AU REGARD DE LA CONCURRENCE:

Le manque de connaissances génétiques, les difficultés dans la manipulation des arbres et la complexité génétique (2n = 46), rend les programmes d'amélioration de l'olivier difficiles. Mon étude de génétique quantitative des caractères architecturaux chez l'olivier est une étude innovante qui se base sur la description pendant les 5 premières années de croissances d'un matériel génétique original de plus 324 arbres installés en verger. Ceci met en œuvre une méthodologie de phénotypage développée au sein de l'équipe et adaptée aux spécificités de l'olivier. Mon projet se déroule dans un cadre ouvert à l'international avec le transfert de méthodologies à Cordoue. Le phénotypage de la même descendance dupliquée me permet d'initier la première étude de l'effet de l'interaction du génotype avec l'environnement sur le développement végétatif de l'olivier. Mon projet de thèse constitue une étape importante dans l'identification des bases génétiques et moléculaires des caractères architecturaux chez l'olivier. Comparé à d'autres espèces pérennes tempérées, l'olivier a offert un modèle biologique intéressant du contrôle génétique d'une croissance annuelle rythmique sans la formation des bourgeons d'hiver.

COMPETENCES ET RESEAUX SCIENTIFIQUES MOBILISES POUR LE PROJET :

L'équipe AFEF compte toutes les compétences scientifiques et techniques nécessaires au bon déroulement du projet de thèse.

Le phénotypage sur terrain a été appuié par une équipe technique spécialiste dans le domaine de la description architecturale de l'olivier et le phénotypage à haut débit. Pour pallier aux difficultés liées au caractère pérenne de l'olivier, les campagnes de mesure des trois premières années de croissance de la descendance (2005-2007) ont été préalablement conduites par l'équipe AFEF avant le début du projet de thèse en 2009.

Le projet est dirigé par deux chercheurs, l'un spécialiste en analyse architecturale du pommier, génétique quantitative et modélisation, l'autre en génétique moléculaire et diversité du figuier et de l'olivier.

L'équipe AFEF évolue au sein de l'unité mixte de recherche : amélioration génétique et adaptation des plantes méditerranéennes et tropicales (AGAP) ce qui facilite les échanges avec d'autres équipes et ouvre les perspectives de réflexion.

Un comité constitué par quatre chercheurs (externes à l'équipe) et les deux encadrants s'est réuni au cours de la première et deuxième année de thèse afin d'examiner la stratégie d'étude, les méthodes adoptées et les résultats obtenus. La pertinence et la faisabilité du calendrier des travaux de thèse ont été validées. J'ai géré l'organisation des deux réunions annuelles (invitation des membres du comité, choix des dates en fonctions de leur disponibilité, réservation des billets de train, mise en place d'une visioconférence). J'ai aussi fourni aux membres du comité un rapport d'avancement qui contient une synthèse complète du travail accompli pendant l'année. J'ai enfin présenté tous mes résultats obtenus et détaillé mes interprétations pour nourrir la discussion avec le comité.

MA THESE ET MOI

POURQUOI UN DOCTORAT ?

J'ai choisi de préparer un doctorat pour être en charge de répondre à une question de recherche et contribuer à l'avancement de la connaissance sur un sujet qu'il me sera donné de nourrir et gérer sous forme d'un projet de 3 ans. Ce travail compte le développement de méthodes et stratégies d'étude transférables et reproductibles. La formation doctorale me permettra donc d'acquérir une autonomie et une maturité scientifique qui me préparera à m'engager dans mes futurs projets de carrière. Ce diplôme ouvre des perspectives professionnelles dans le monde de la recherche scientifique et de l'enseignement supérieur que je vise à moyen terme. J'affectionne le partage de la connaissance (Favoriser l'implication collective dans l'élaboration de projets de recherche et leur développement, valoriser les compétences et idées individuelles, lutter contre le renfermement et l'isolation scientifique) et l'innovation (développer des idées et outils qui répondent à des besoins concrets).

POURQUOI CE SUJET ?

J'ai choisi de travailler sur l'architecture de l'olivier car j'estime essentiel de connaître l'arbre et son développement naturel pour optimiser les travaux sur les caractères d'intérêts agronomiques tels que la production et sa régularité. Etant Tunisienne, travailler sur une espèce méditerranéenne emblématique telle que l'olivier est enrichissant et valorisant tant sur le plan national qu'international.

MON ROLE DANS LA DEFINITION ET LA PROGRAMMATION DU PROJET

J'ai contribué à la mise en place du projet de cotutelle qui regroupe l'équipe AFEF et l'institut de l'olivier de Sfax en Tunisie. Ma contribution compte la proposition et la défense du projet devant deux équipes de recherche en Tunisie et la mise en place des dossiers de candidatures pour l'octroi de la bourse de thèse. J'ai enfin décroché deux demi bourses de thèses : Programme Averroès pour l'échange Maghreb-Europe et Ambassade de France.

Les axes principaux du projet de thèse étaient définis avant mon arrivée dans l'équipe. Cependant, au cours de la première année j'ai pu améliorer le protocole de phénotypage initialement suivi en identifiant les caractères les plus héritables et en focalisant les futures campagnes de mesures sur une liste de variables plus pertinentes.

II. DEROULEMENT, GESTION ET COUT ESTIME DU PROJET

FACTEURS DE SUCCES ET DE RISQUES

FACTEURS DE SUCCES

- Compétences mobilisées par l'équipe pour le projet : connaissance de l'arbre, solide expertise dans l'analyse statistique des caractères quantitatifs, maitrise de la description architecturale des arbres.
- Le projet évolue dans un cadre collaboratif spécialisé dans l'étude de l'olivier : Institut de l'olivier (Tunisie), IFAPA (Cordoue).
- Motivation et sérieux de tous ceux qui s'investissent dans le projet.

FACTEURS DE RISQUES ET STRATEGIE DE MAITRISE

- **Risque naturel**: le projet se base sur une population en ségrégation d'olivier installée en verger : les conditions environnementales sont non maitrisées, risque de dégâts en cas d'intempéries.

⟶ Pour réduire ce risque, 6 répétitions de chaque génotype ont été planté de manière randomisée.

- **Risque intellectuel** : le projet met en œuvre des connaissances et techniques qui m'étaient inconnues.

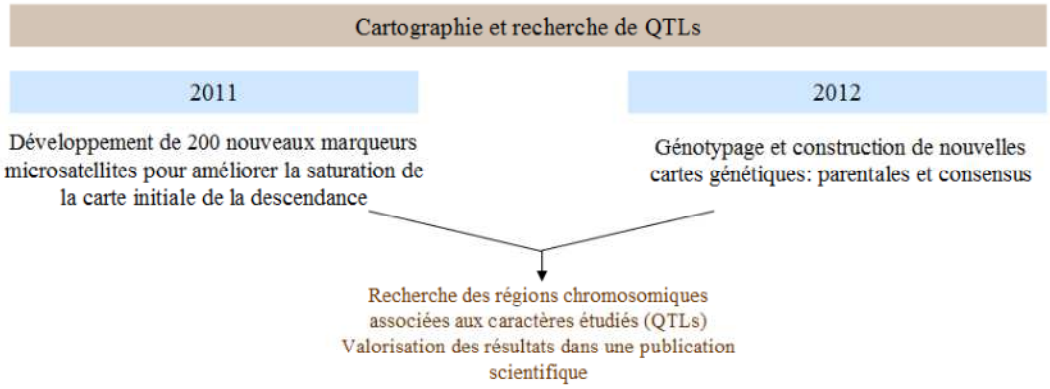
⟶ Pour acquérir ces connaissances, je me suis appuyée sur la bibliographie et les

méthodes utilisées sur les autres espèces. J'ai suivi des formations en génétique quantitative et j'ai aussi interagit avec les membres de l'équipe qui pouvait m'aider ainsi que des membres externes via des forums d'utilisateurs des logiciels que j'emploi.

- **Risque stratégique**: le projet est ambitieux et compte quatre parties impliquant des longues périodes d'acquisition et analyse de données.

⟶ Examen de l'avancement des travaux et des résultats obtenus par un comité de suivie de la thèse constitué d'experts externes à l'équipe

CONDUITE DU PROJET : PRINCIPALES ETAPES



ESTIMATION ET PRISE EN CHARGE DU COUT DU PROJET

Le coût de ma thèse est estimé à 149.5K€ dont plus de 90 % sont allouées aux ressources humaines. Le détail des dépenses figure dans le tableau de l'estimation du cout consolidé de la thèse en annexe.

Tableau 1 : Nature et coût des dépenses associées au projet de thèse

Nature de la dépense	Coût total (euros TTC)
Ressources humaines	138955
Matériel (amortissements)	3560
Déplacements	3509
Formations	1703
Infrastructure	700
Consommables	672
Documentation et communication	344
Total	**149443**

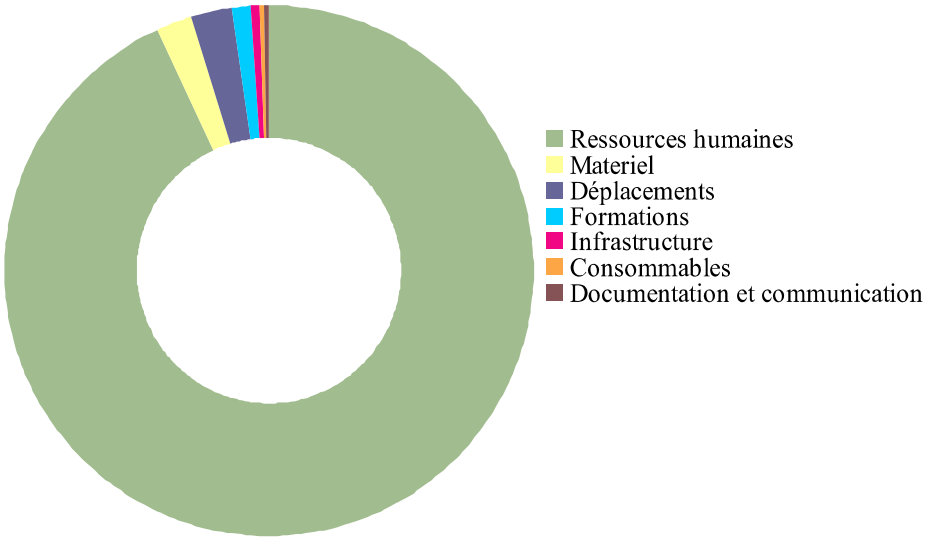


Figure 1 : Proportions des différentes catégories de dépense

III. SAVOIR, SAVOIR-FAIRE ET SAVOIR-ETRE

SAVOIR: SCIENTIFIQUE ET TECHNIQUE

Génétique quantitative :

Analyse de données quantitatives, construction de modèles statistiques adaptés aux caractères complexes, estimation des valeurs d'héritabilité des caractères et identification des régions chromosomiques ou QTLs associés aux caractères d'intérêt.

Phénotypage à haut débit de caractères complexe :

Gestion et réalisation de 3 campagnes de phénotypage de caractères relatifs au développement végétatif (une à Melgueil et deux à Cordoue) et de 6 campagnes de phénotypage de caractères relatifs au développement reproducteur (3 campagnes de floraison et 3 de fructification). Participation à une campagne de récole d'olives.

Bio- informatique :

- Extraction de variables à partir d'un graphe arborescent multi-échelle (MTG) par le logiciel Python 2.6.
- Manipulation du logiciel R v2.9.2 pour les analyses statistiques dans l'approche de génétique quantitative, estimation des Blups pour la détection de QTLs.
- Développement de marqueurs microsatellites SSRs : Manipulation des bases de données de séquences codantes, détection de motifs répétés et design d'amorces par Primer 3+ et SAT.
- Identification de QTLs et cartographie génétique par MapQTL4 et JoinMAP4.
- Détection de marqueurs AFLP et SSR polymorphes à l'aide de GeneMapper 3.

SAVOIR-FAIRE : ADMINISTRATIFS, ORGANISATIONNELS ET LINGUISTIQUES

- Gestion des procédures de renouvellement de carte de séjour étudiant.
- Gestion des procédures d'inscription dans les deux établissements Français et Tunisien.
- Gestion des dossiers de renouvellements des deux demi bourses : Rédaction de rapports d'avancement des travaux de thèse pour chacun des organismes et rassemblement rigoureux de chacune des pièces demandées.
- Contribution à la rédaction des rapports d'avancement pour des projets finançant le fonctionnement du projet.
- Organisation d'une mission à l'étranger : Prise de contact avec les collègues espagnols, choix des dates, rédaction du protocole à suivre et gestion des réservations pour le déplacement et l'hébergement.
- Montage de projet pour l'octroi de la bourse L'Oréal pour les femmes de sciences.
- Communication en arabe, français, anglais et italien.
- Contribution à l'organisation de journées jeunes chercheurs à Montpellier : Mise à disposition aux doctorants visiteurs de brochures et descriptifs des activités de recherches dans les principaux établissements Montpelliérains. Animation d'une session de communication orale pendant les journées. Présentation de mes travaux de recherche.

SAVOIR-ETRE

CREATIVITE ET INNOVATION

- Utilisation chez l'olivier de nouvelles méthodes d'analyse statistiques de caractères complexes. Proposition d'une méthode de phénotypage des caractères architecturaux adaptée à l'olivier.
- Montage et proposition au ministère de l'agriculture Tunisien d'un projet de réhabilitation d'un verger d'oliviers centenaires : Entretien avec l'adjoint du ministre de l'agriculture, réalisation d'une étude économique du projet sur une période de 5 ans pour une demande de soutien financier. Cette expérience m'a permis de réaliser les étapes clés pour le montage d'un projet agricole et de me sensibiliser aux contraintes associées.

SOCIABILITE

- Parfaite intégration dans l'équipe, disponibilité pour toute requête ou sollicitation (Je consacre le temps nécessaire pour répondre aux questions des collègues sur les analyses statistiques et leur fourni les documents qui m'ont permis d'acquérir ces compétences).
- Contribution à la convivialité et la cohésion de l'équipe par l'organisation de petits événements festifs.

ADAPTABILITE

- Prise en charge pendant mon parcours académique de projets très diversifiés impliquant l'acquisition rapide de nouvelles connaissances: Interaction interculturelle réussie avec les collègues et gestion des contraintes de délai et des différences de méthodes de travail.
- Interaction avec des botanistes amateurs et prospection sur terrain de feuilles de Tulipes sauvages.

APTITUDE A L'ENCADREMENT

- J'ai encadré un groupe de 14 étudiants (1ère Année- ingénieurs- Montpellier Supagro) pendant un module d'initiation à la Démarche Expérimentale chez les Arbres Fruitiers (TP+TD : 30,5h). A partir d'une problématique de recherche sur le Pommier, les étudiants devaient réfléchir à des stratégies expérimentales pour explorer la question. Mon travail était ensuite d'expliquer la stratégie retenue par l'équipe de recherche qui travaille sur la thématique et amener les étudiants à vivre l'expérience de recherche, de l'acquisition de la donnée sur terrain à l'exploitation des données et enfin à la rédaction d'un article. Cette expérience d'encadrement m'a permis de valoriser mes acquis dans le phénotypage et l'analyse génétique, de partager mes connaissances sur la rédaction scientifique et synthèse bibliographique et enfin de confirmer mon gout pour l'enseignement appliqué et interactif.
- J'ai pu initier des doctorants étrangers en courte formation dans l'équipe à l'analyse architecturale des arbres (organisation et gestion des données, logiciels et librairies nécessaires, explication et mise en route d'algorithmes d'extraction de variables).

APTITUDE A L'ANIMATION ET AU TRAVAIL EN EQUIPE

- J'ai instauré un brainstorming hebdomadaire d'une heure entre les doctorants et post-doctorants de l'équipe baptisé « What's up Doc ? ». Chaque séance est consacrée à un

doctorant qui discutera ses travaux sans support power point. L'animateur de la séance est différent à chaque fois et les 10 dernières minutes sont ouvertes aux éventuelles questions urgentes. Ces réunions informelles ont permis d'améliorer la communication entre doctorants et favorisé le partage des connaissances. Ceci à fortement renforcé la cohésion de l'équipe et crée un esprit d'équipe.

APTITUDES ARTISTIQUES

- J'ai fais 5 ans de conservatoire.
- Pratique de trois instruments de musique (Piano, Guitare et Chant).
- Pratique de la musique assistée par ordinateur et maitrise de plusieurs logiciels d'enregistrement et séquenceurs.
- Auteur compositeur, j'ai participé à divers spectacles de chant et de comédie musicale.
- Actuellement élève à L'école régionale de Jazz (JAM de Montpellier).

IV. RESULTATS ET IMPACT DE LA THESE

Les résultats de ma thèse constituent une avancée supplémentaire dans la compréhension du développement naturel des arbres fruitiers. L'olivier présente une complexité architecturale unique qui influence au fil de l'ontogénie de l'arbre, son comportement reproducteur. L'étude de la floraison et fructification en relation avec le développement végétatif chez l'olivier profite du caractère pionnier et fourni des résultats originaux et contrastés avec ceux obtenus chez d'autres espèces fruitières. Ceci permet d'élargir les perspectives d'étude et approfondir les connaissances acquises. Les résultats obtenus sur l'effet de l'environnement et son interaction avec le génotype sur la croissance et la ramification de l'olivier constituent la première base fondatrice qui fournira des pistes nouvelles de recherche pour la suite du projet. Dans le contexte de ma thèse, il a apparu nécessaire d'appliquer des méthodes d'analyse statistiques complexes adaptées à des données quantitatives répétées avec des variances hétérogènes. Ce qui apporte une avancée méthodologique transférable à d'autres études génétiques.

Ce projet a également été l'occasion de me former tant sur le plan scientifique et technique que sur le plan de gestion de projet et communication. Pendant ces trois années, j'ai pu évoluer dans la réflexion scientifique et acquérir de l'agilité dans la gestion des difficultés et risques liés au projet. J'ai pu définir mon projet professionnel en identifiant les actions qui me motivent le plus et ou je suis la plus efficace. Les différentes collaborations mise en place pendant ma thèse ainsi que mes communications dans les congrès internationaux m'a enfin permis de construire mon réseau professionnel.

V. IDENTIFICATION DE PISTES PROFESSIONNELLES

Une fois ma thèse soutenue, plusieurs pistes professionnelles sont envisageables selon mon profil et mes besoins.

1- CARRIERE DE RECHERCHE ET DEVELOPPEMENT DANS UN ETABLISSEMENT PUBLIC OU UNE ENTREPRISE PRIVEE :

✓ **Préparation pour les concours de CR ou IR à l'INRA :**

Afin de constituer un bon dossier pour prétendre au poste de chargé de recherches ou ingénieur de recherche en génétique quantitative, je compte élargir mon champ de compétences par des connaissances en sélection génomique ou en génétique d'association. Pour se faire, j'ai identifié deux établissements de recherche et développement qui proposent des projets innovants dans le cadre de la thématique recherchée adaptés à mon profil pour effectuer un post-doctorat :

- **Etablissement publique Français** : l'INRA d'Angers : Il s'agit là d'un projet professionnel adapté à mon profil et à ma vie personnelle.
- **Etablissement privé Néo-zélandais**: The New Zealand Institute for Plant and Food Research: Cette expérience me permettra de développer mon réseau international et de perfectionner mon niveau d'anglais.

La discussion avec les responsables de chacune de ces structures d'équipes d'amélioration s'est couronnée par deux propositions de post-doc dans un délai de 1à 2 ans.

✓ **Préparation pour les concours d'enseignant - chercheur:**

Je considère que le métier d'enseignant chercheur est un bon moyen d'allier deux missions complémentaires qui sont en phase avec mon ambition d'innover en recherche et ma volonté de partager mes connaissances. Je profite d'une aisance de communication et j'apprécie le contact des étudiants. Par contre, je compte cibler les postes en écoles agronomiques ou instituts techniques horticoles, car j'affectionne plus les enseignements appliqués.

✓ **Intégration d'une entreprise de recherche privée:**

Mon gout prononcé pour la création variétale, le management et la gestion de projet m'a amené à répondre à l'offre d'emploi proposée par une entreprise leader dans le domaine de l'amélioration génétique et la création variétale :

2- ACTIVITE COMPLEMENTAIRE D'AUTO ENTREPRENEUR :

✓ Dans le milieu agricole :

Ma formation d'ingénieur, mon désir d'indépendance et mon gout pour la production ont nourri mon ambition de monter un projet agricole et d'envisager une carrière d'auto entrepreneur en parallèle de ma carrière de chercheur. J'estime que ce projet m'apportera de l'équilibre et de la lucidité vis-à-vis des besoins des producteurs qui viendra enrichir mes projets de recherche.

✓ Dans le milieu audio-visuel:

Soucieuse de la vulgarisation de la science et n'approuvant pas la séparation du domaine de la science et de l'art, je pense à développer un concept d'émission radio mensuelle, qui alliera chroniques scientifiques, interviews de deux « artistes-scientifiques » et musique.

ANNEXE

ESTIMATION DU COUT CONSOLIDE DE LA THESE

Se référer aux règles de calcul avant de compléter le tableau

Montants en euros TTC

	Nature de la dépense	Détails *		Coûts totaux (euros TTC)			
				Nombre d'unités	Coût unitaire moyen	Quote-part utilisation	Total
1	**Ressources Humaines**						
1.1	Doctorant	1178		39	1178	100%	45975
1.2	Encadrant 1	4080	1748	36	5828	15%	31471
1.3	Prime Encadrement						
1.4	Encadrant 2	2536	1086	36	3622	5%	6519
1.5	Prime Encadrement						
1.6	Techniciens	2427	1039	15	3466		51990
1.7	Sous-traitance partenaire projet	750		4			3000
	Sous-total Ressources Humaines						**138955**
2	**Consommables**						
2.1	Fournitures expérimentales						600
2.2	Fournitures de bureau	200		36	200	0,50%	72
2.3	Autres achats						
	Sous-total Consommables						**672**
3	**Infrastructures**						
3.1	Entretien, gardiennage, secrétariat						700
3.2	Loyers des locaux						
3.3	Electricité, eau, chauffage, … (si non inclus dans les charges locatives)						
3.4	Autres						
	Sous-total Infrastructures						**700**
4	**Matériel (amortissements)**						
4.1	Matériel d'expérimentation (dont les ordinateurs et logiciels spécialisés)	5			2000	0,4	800
4.2	Ordinateur de bureau						
4.3	Logiciels de bureau						
4.4	Autre (cout parcelle : 1ha Oliviers)			84	4600	0,6	2760
	Sous-total Matériel						**3560**
5	**Déplacements**						
5.1	Missions en France	182	92				274
5.2	Missions à l'étranger	247	1106				1353
5.3	Congrès en France						
5.4	Congrès à l'étranger	857	1025				1882
	Sous-total Déplacements						**3509**
6	**Formation**						
6.1	Formations						608
6.2	Autres frais (Inscription à l'Université, Sécurité Sociale étudiante, etc.)						1095
	Sous-total Formation						**1703**
7	**Documentation et communication**						
7.1							
7.2	Affranchissements, Internet, téléphone et impressions			36	398	1%	144
7.3	Documentation (périodiques, livres, bases de données, bibliothèque, etc.)						200
7.4	Autres						
	Sous-total Documentation et communication						**344**
8	**Charges financières (intérêts des emprunts)**						
	Sous-total Charges financières						
9	**Charges exceptionnelles**						
	Sous-total Charges exceptionnelles						
10	**TOTAL**						**149443**

** de manière générale, toute dépense d'un montant supérieur à 2 000 euros doit être détaillée*