The olive growing in Spain and its genetic improvement
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Abstract
The olive area in Spain reached 2.35 million ha by 1965, decreased afterwards down to 2.1 million ha by 1985-86 and then increased steadily up to date. The recent expansion of olive plantations was promoted by the entrance of Spain in the EU in 1986 and by the continuous expansion of the olive oil and table olives market in the world. Now there is more than 2.4 million ha which represents the 28% of the Total World Area. This recent expansion has been associated with an intensification of the orchards. New orchards and training systems more adapted to mechanical harvesting have been established. A steady increase in drip irrigation occur in traditional and in new plantations doubling the production of olive oil and table olives in the last fifteen years. Relevant innovations in the processing systems of olive oil and table olives have also occurred. We are finally attending to a dramatic change in the cultivars planted in the new orchards which has been associated to the boom of the nursery industry and to the expansion of new intensive orchards. This lecture summarises the recent changes of the olive industry in Spain with particular reference to the studies on genetic resources and the olive breeding programs in progress.

From traditional to new olive growing

Traditional Olive Plantations. (Figure 1)
Several patterns characterize current traditional olive plantations in Spain and in other Mediterranean Countries. A big size of the tree, which is a common feature in many plantations, constitutes a difficulty for harvesting the crop. Many plantations are more than 50 years old, even sometimes they are centennial olives. This longevity represents a major constrain for the olive orchards to be adapted to the social and economical changes that are taking place nowadays. Therefore obsolescence becomes a common feature of many olive groves (Rallo, 1986).

Olive tree is very well adapted to the Mediterranean climate which is characterized by a mild and short winter and a long, dry and hot summer. Olive has developed adaptation mechanisms to summer water stress insuring the survival of the trees but reducing yield (Fereres, 1984). Up to recent years only 8-10 % of olive orchards were irrigated (Civantos, 2008). Olive has commonly been planted in low yield potential soils, many of them in sloping lands, without possibility of irrigation. These plantations yielded a low crop. Therefore, the increasing demand for olive oil and table olives has traditionally been met by planting olives in new more fragile and less yield potential soils. For instance, although more than one million ha of olive trees were planted in Spain between 1888 and 1972, the average yield per ha decreased (Rallo, 1986).

Harvesting olives has been a highly consuming hand labour task. More than 70% of total labour demand in traditional olive orchards is required for harvesting (García-Ortiz et al., 2008). In many
cases olive harvest has represented the main demand for labour in many rural olive areas. Consequently olive expansion has been historically associated to periods of demographic rising. The progressive concentration of plantations in many olive producing zones of the Mediterranean world has triggered a temporary demand of labour to attend olive harvesting. Temporary migration during the 2-4 months of olive harvest and cultural celebrations associated to the end of the harvest are common features in the olive world. Concomitantly, unemployment along the rest of the year, biennial trend of the olive tree crop and cyclic crisis of prices have represented causes for social instability in olive zones with monoculture.

Most of the traditional olive growing technology is local and empirically based. Cultivars used and pruning practices are clear examples. Olive cultivars in almost all Mediterranean countries have been locally selected among random seedlings and vegetative propagated by farmers since many centuries. As a rule, diffusion of most cultivars was confined around their supposed area of origin. For instance, 24 cultivars account for most of the crop in Spain. These cultivars have been diffused to only one continuous area (Barranco et al. 2005, Rallo and Barranco, 2006). Only two main cultivars, ‘Manzanilla de Sevilla’ and ‘Empeltre’, both propagated by grafting, have been largely cultivated out of their original area of diffusion. Thus, farmers have used the best performing cultivar among the selected in their growing areas. Also pruning practices are empiric, local and divers. Reducing tree size to facilitate the accession to the canopy at harvesting has been a common feature of many pruning practices. Divers local criteria led to many local pruning systems in different growing areas.

The new olive orchards (Figure 2)
Migration towards cities reduced rural population in olive growing areas of Southern Europe after War World II. First in Italy and afterwards in Spain, Portugal and Greece, the scarcity and increasing cost of man labour became a major economical problem in olive areas of these countries. Furthermore, low olive orchards yield and low prices of other vegetal oils from annual crops, such as soybean, sunflower and rape, triggered a deep crisis in the olive oil sector by 1970’s. In Spain, a public program to reconvert olive yards, aiming at increase yield and reduce costs by mechanical harvesting, was set up between 1972 and 1986. This year Spain and Portugal entered the EU. The European subsidies for the olive oil have then promoted high yield orchards. Since that time new olive plantations have transformed the Spanish Olive Orchards. The following paragraphs illustrated these major changes:

1. Mechanical harvesting and high density orchards. By the seventies, mechanical harvesting becomes a must for the new plantations. Trunk shaker associated to different frameworks to collect the dislocated fruits from the trees was the first approach to mechanical harvesting. Traditional olive orchards in Spain have been characterized by a density of 70-80 trees/ha, with 3 trunks per tree and a medium-low productivity (Navarro and Parra, 2008). Since the 1970’s new planting systems with 200-450 trees/ha and single trunk trees were established. These new orchards provide higher productivity and they also facilitate mechanical harvesting with tree trunk shakers.

In the first half of the 1990’s a new planting system: the high density hedgerow (>1.500 trees per ha) (De la Rosa et al, 2007; Tous et al., 2006), was promoted by the nurseries and the vine straddle harvester manufacturers. In this system ‘Arbequina’ is the standard cultivar. ‘Arbosana’ and ‘Koroneiki’ are also planted. Since 1993 the expansion of this system has been exponential and currently more than 60.000 ha have been planted in many countries according to the estimates of the nursery industry. This system requires high investment (>6000€/ha), yield the earliest crop (> 1.000 kg of oil /ha at the third year of planting and more than 1800 kg of oil per ha from the 5th to the 9th -10th years of planting) with 150-250 mm of applied water. One harvester may collect up to 2, 5 ha per day, thus,
reducing drastically harvest costs. When orchards get older than 9-10 years the height of the hedge-row requires canopy management to avoid shading of the incident solar radiation on the canopy and yield reduction. There has also been reported higher incidence of some pest and diseases, particularly *Colletotrichum* spp and *Pseudomonas savastanoi*. (Moral et al., 2006), than in other intensive systems. New ongoing harvester designs may allow orchard densities on the range of 450-1000 trees per ha that will allow a longer time without restriction for mechanical harvesting because of a big canopy size. Current breeding programs try to obtain new low vigour cultivars or dwarfing rootstocks to enhance mechanical harvesting and extend the age for maximum crop in this new plantation system.

2. *Irrigation*. Increasing planting density was the general strategy for high yield, early bearing and mechanical harvesting in the new orchards. However, water stress limited crop and oil yield. By the 1960's irrigation was the most efficient way to increase yield in California and Israel orchards. In Spain a shy increase in drip irrigation in the 1970's and early 1980's was followed by a steady increase. In 1997 more than 40% of the new orchards were drip irrigated and this percentage has risen continuously since that time (Consejería de Agricultura y Pesca, 2003). Currently, most of the new orchards are irrigated following a deficit irrigation strategy (Pastor, 2005). The new irrigated orchards represent about 450,000 ha. Irrigation is the main reason for the increase of the oil production per year in Spain from 600,000 t in 1980-1985 to 1,100,000 t in 2003-2008.

3. *Changes in processing*. Spain innovated the processing systems for oil and table olive production in the 1980's. New continuous systems based on centrifugation for oil processing increased both the rate and the capacity of the oil industry. This change carried out a notorious improvement of the olive oil quality (Alba, 2008). The table olive industry has also increased its capacity and diversified the types of table olive produced (Rejano and Garrido, 2008).

4. *Nursery Development, Varietal Change and Verticillium Wilt Spreading*. Propagation of big hardwood cuttings by the farmers has been the usual method for olive in Spain. A shy development of nurseries using semi hardwood and softwood cuttings under mist (Caballero y Del Río, 2008) began in the 1980's and triggered a continuous expansion of the nursery industry which production over passed thirty millions plants per year in 2007. The expansion of the nurseries was associated to the propagation of few traditional cultivars. Between 1975 and 1995 the varietal structure of the Spanish Orchard has been modified. This trend will continue in the near future. Thus propagation by the farmer has practically disappeared and only three oil cultivars (‘Arbequina’, ‘Picual’ and ‘Hojiblanca’) and one table olive variety (‘Manzanilla de Sevilla’) account for more than 95% of produced plants. For the first time, the risk of genetic erosion has dramatically appeared in olive growing.

The increasing incidence of Verticillium wilt in olive, a disease caused by the fungus *Verticillium dahliae* Kleb., has been associated to infected plants from nurseries among other factors (Trapero and Blanco, 2008). The certification of nursery plants is a main issue to limit the expansion of this disease. Certification programs are however far from being generalized in the country. Tests to identify the cultivars by SSR and morphological characteristics (Trujillo et al., submitted) and for early detection of asymptomatic and infected nursery plants by the main virus, by *Pseudomonas savastanoi* pv. *savastanoi* and by *Verticillium dahliae* with specific SSRs and RT-PCR are currently available (Bertolini et al., 2003; Mercado-Blanco et al., 2003). Certification should be compulsory for the Spanish nursery industry in the next years.

5. *Integrated Pest Management* (IPM). Major olive pests and diseases and their control have been
review by Alvarado et al. (2008) and by Trapero and Blanco (2008). The entomological fauna of traditional olive orchards is usually balanced. However, the intensification of the planting systems and the irrigation has modified the incidence of pest and diseases. For example, in new irrigated orchards Verticillium wilt has become the most prevalent disease. Anthracnose and shoot moth have also become important problems in the hedgerow orchards.

Pest and disease control is usually done with the standard traditional pesticides and fungicides because of their efficiency and low cost. However, the Integrated Pest Management (IPM) approach developed in the last 30 years. Initially, the Atria’s (local groups for common treatments) develop IPM according to the concept of economic threshold for pesticide application. Currently the concept of Integrated Crop Management (ICM), in which the whole orchard management is taken into consideration, is progressively extended to many local groups. Also organic farming is progressively extended in traditional olive orchards. The two later systems of production have been promoted by the regional governments. More than 20% of traditional orchards are cultivated following the controls required in both systems.

The genetic resources in Spain

The Spanish Germplasm and the World Olive Germplasm Bank (WOGB)

In 1970, within a cooperative Program with FAO, an International World Olive Germplasm Bank (WOGB) began to be established in Cordoba (Caballero et al., 2006). This initiative led to complete the systematic surveys and descriptions of the Spanish cultivars. The methodology of the survey and the morphological descriptions of Andalusia allowed a complete catalogue of the cultivars of the most important olive region of the world (Barranco and Rallo, 1984). Other similar works in Catalonia (Tous and Romero, 1993) and in Valencia (Iñiguez et al., 2001) has been followed by a general Élaiogrphie of the Spanish cultivars (Barranco et al.2005). As in many other olive-producing countries, plant material cultivated in Spain is characterized by the abundance of very old cultivars restricted to specific areas where they were originally cultivated. The diversity of cultivars - 262 cultivars with 501 varietal denominations have been identified in Spain (Barranco et al., 2005) - is due to their local origin and reduced pressure of selection by farmers throughout history. The limited spread of cultivars may have been due to difficulties involved in transporting the voluminous plant material required in traditional propagation procedures and the scant knowledge of the behaviour of cultivars in areas far from their place of origin.

The progressive use of molecular markers (isozymes, RAPDs, AFLPs and SSRs) supported the discriminating capacity of the morphological schedule used in the referred works and provide a more powerful tool that guarantee also the varietal identification of nursery produced plants (Rallo, P. et al., 2000, Belaj et al. 2004; De la Rosa et al., 2002; Trujillo et al., 2005).

The use of morphological and molecular markers allowed also the genetic discriminations of the accessions at the WOGB (Trujillo et al., submitted). 824trees, representing 499 accessions from 21 countries of origin were characterized by 33 SSRs markers and 11 morphological characteristics of endocarps. The accessions have shown 411 different SSR profiles and they correspond to 332 identified cultivars based on unique combinations of SSRs markers and endocarp morphologies, 201 were authenticated due to genotypic and phenotypic matches with authentic control samples, 28 cultivars did not match their respective authentic control samples, and 103 cultivars could not be authenticated because reference endocarp samples were not available. 130 SSR genotypes were considered molecular variants of 48 different cultivars and several pairs of cultivars present identical or very similar SSR profiles but different morphologies. A minimum set of 17 SSR markers was sufficient to
identify all of the cultivars. The history and the current state of the WOGB have been summarized (Caballero et al., 2006).

Two additional works have been undergone in the last years. A survey of ancient monumental olive and wild olive trees in Andalusia that were genotyped with 14 SSRs (Muñoz Díez, 2008) have evidenced that only 17% of cultivated genotypes were identified as known varieties, 32% was grafted mostly onto wild olives. Genetic analysis showed two major groups: cultivated and wild. The cultivated genotypes were associated between themselves according to their geographical origin, thus supporting the hypothesis of local selection and domestication of olive cultivars. In this work the author also genotyped wild trees from three different olive regions (Andalusia, Valencia and Cataluña) with the same SSRs. The observed pattern of genetic variation revealed differential clustering of wild and cultivated genotypes in the studied regions which points to autochthonous or allochthonous origin of the cultivated varieties accordingly. Higher variability was observed in wild than in cultivated olives and the tolerance to biotic and abiotic stress over the time strongly suggest the interest of wild olives for the current breeding programs.

The risk represented by the incidence of Verticillium in the field lead us to establish an isolated repository at the University of Córdoba with plants true to type and free from pathogens. We have propagated all the identified accessions of the WOGB. We are currently growing the rooted plants in containers with substrate free from pathogens, particularly from Verticillium, and testing them for trueness to type and for pathogen status of the genotypes before the plants are finally established in the repository. This material will be available for the scientific community and to the sector according to the international legal prescriptions.

Selection works
In Spain, evaluation of agronomic and oleotechnic characters of both the World Olive Germplasm Bank of the IFAPA in Córdoba and the Catalanian Bank of Germplasm of the IRTA in Reus are in progress since more than 20 years. In many cases a common protocol was adopted. These studies were carried out by the confluence of multidisciplinary teams belonging to R+D institutions located close to the Germplasm Banks. These results have recently been summarized (Tous et al., 2005a). Since 1975 several works on clonal selection within different olive cultivars were carried out (Tous et al., 2005 b) or are still in progress. The erratic results of these works, the uncertainty on the genetic basis of the observed agronomic variability and the scarce diffusion and incidence on yield of the known virus diseases question the differential value of these selections in respect to standard material true to type of the same cultivars. However, these selections have promoted the certification of true to type and free from pathogens nursery plant material.

Breeding programs in Spain

The Juvenile Phase
The length of the Juvenile Phase has been the main impediment for breeding fruit trees (Hackett, 1985). The length of the Juvenile Phase is even longer in olive than in other fruit trees (Humanes et al., 1967; Natividade, 1954; Ruggini, 1990). Bellini (1993) found only 20% of flowering seedlings from crosses among different olive cultivars 12-13 years after germination. This delay has been a major factor for the very few olive breeding programs undergone along the 20th Century. Also very few studies were carried out to shorten the Juvenile Phase in olive (Lavé et al., 1996).

Several breeding programs are currently in development in Spain. The first systematic breeding program was initiated in Córdoba in 1990-91 (Rallo, 1995). Later on other programs carried out by
the University of Seville (Rallo, P et al., 2006), by Agromillora (Cunill et al, 2006), an important Catalonian nursery, and by the IRTA (Tous, Pers. Commun.) are on progress.

The Córdoba Breeding Program (Figure 4)

This programme has been jointly developed by the University of Córdoba and the IFAPA, the Andalusia Research Institute for Agriculture and Fisheries. The programme included both methodological and agronomic purposes. Shortening the juvenile period, the search of criteria for early selection and for simplifying the breeding protocol have been methodological aims pursued since the beginning of the program. The initial agronomic objectives included early bearing, high oil content, high levels of oleic acid, adaptation to mechanical harvesting and resistance to Spilocaea oleagina Hughes (Cast) (León et al. 2005). Currently two new objectives have been added: resistance to Verticillium wilt and reduction in vigour and compact habit of growth (Rallo and Barranco, 2006).

1. Crosses. The first crosses were carried out among ‘Arbequina’, ‘Frantoio’ and ‘Picual’ between 1991 and 1993. These three cultivars belong to three different olive growing areas: Catalonia in North-East Spain, Toscana in Italy and Jaén in Andalusia. They are productive varieties with high oil content, differing in other traits such as earliness of bearing and oil composition. Later on, between 1994 and 2006, up to 83 new crosses, including selfing and open pollination, have been carried out among more than 20 different genitors among which ‘Koroneiki’, ‘Lechin de Sevilla’, ‘Manzanilla de Sevilla’, ‘Meski’ and ‘Zaity’ and ‘Arbosana’. In the last crosses we used also ‘Sikitita’ (Sin: ‘Chiquitita’ in USA), the first released cultivar of this programme (Rallo et al. 2008). Crosses were done bagging shoots of the female genitor several weeks before flowering and spraying pollen of the male genitors several times since the beginning of bloom in the female genitors. Since 2003 paternity test for verifying the male paternity were done.

2. Forcing protocol to shorten the Juvenile Phase. We followed a protocol of forcing growth similar to that proposed by Zimmerman for crab apple (1971). Santos-Antunes (1999) and Santos-Antunes et al. (2005) reported this methodology. In summary, naked seeds harvested by the beginning of November were stratified at 14°C in the dark for 45 days until emergence of the radicule and then grown at 20-22°C until expansion of the second pair of true leaves. Then the plantlets were placed in 9 L plastic bags with a mix of sand and peat moss (2:1) and placed in a greenhouse at 22°C in average under continuous light provided by metallic halogen lamps and drip fertirrigated. The seedlings stayed into the greenhouse until the plants reached more than 160 cm of height. During this time lateral shoots were pinching off when they were longer than 10cm. At this time the plants were placed into a lathouse until planted in the open field the following spring. The trees were planted at 1,5m distance between trees in a row and 4, 0 m between rows. The canopy was freely formed above 1,6m. This seedling forcing protocol has been able to produce flowering seedlings 28th months after germination with>93% of flowering trees 65 months after germination.

The different parents used influenced the length of the juvenile periods of the descendents (‘Frantoio’>‘Picual’>‘Arbequina’) in correspondence with the length of their unproductive periods in vegetative propagated plants. This protocol has recently being modified reducing the height of the canopy at 1, 00-1, 30 m that facilitate plant training without delaying flowering (Moreno- Alias, 2009). The new progenies proceeding from the crosses done after 1993 and in the program of breeding table olives in Sevilla (Rallo,P. et al., 2008) confirm the efficiency of the protocol described for shortening the Juvenile Phase.
3. Early Selection. Early selection is a very useful tool in breeding perennial fruit crops for reducing the number of genotypes during the Juvenile Phase and saving time, space and money in the breeding programs. Two recent works (De la Rosa et al., 2006; Rallo, P et al., 2008) indicate that the size of the progenies may be reduced in the greenhouse forcing stage according to the height of the seedlings as this trait is related to earliness of flowering. Selections of seedlings resistant to *Spilocaea oleagina* at the Juvenile Phase has also been done in our breeding program (Trapero, Pers. Comun.).

Also selection in the first years of bearing may save time and labour thus increasing breeding efficiency. Correlations among years for oil and oleic acid content indicate that data of two years for oil content and of only one year for oleic acid content may provide confident basis for early selection (León et al., 2004c, d).

4. Variability of the progenies. A common pattern of the evaluated progenies have been the large range of variability for any trait. The range of variability among genotypes within any cross bred progeny was always higher than the range of variability among cross bred progenies for the evaluated traits (León et al., 2004d, e). These results are in agreement the expected high degree of heterozigosity for the olive. Different studies with SSRs confirm this high heterozigosity of the cultivated olive (Rallo, P. et al., 2000; De la Rosa et al.,2002; Diaz et al., 2006 ) that is enlarged when wild olive populations are evaluated (Belaj et al., 2007, 2010; Muñoz Díez, 2008).

5. Response to selection. We have estimated heritability on the basis of repeatability among years for several evaluated traits (León et al., 2004 c, d). We observed differences among traits. Whereas some characters as for instance content in oleic acid showed high values other like crop load and relative humidity showed a low repeatability. On the other hand, the observed relation between the length of unproductive period of the genitors and the length of the Juvenile Phase of the corresponding progenies and more recent observations on vigour and habit of growth (Hammami, 2009) indicate the possibility for establishing selection criteria for different agronomic traits.

6. Simplifying the selection process. Field evaluation of progenies is a long and expensive process. The protocols used to shorten the Juvenile Phase and for early selection have represented useful tools for increasing the efficiency of our program. High correlations coefficients among different evaluated traits may also allow indirect selection reducing the characters to be recorded (León et al., 2004 b). The use of NIR spectrometry is very useful in the selection stage of breeding programs (Batten, 1998) as it provide quick, non destructive sampling and simultaneous analysis for many different characteristics. Since the evaluation of the first progenies we have used NIRS spectrometry for the simultaneous analysis of traits such relative humidity, oil content and fatty acid composition of our progenies (León et al., 2003 and 2004 a).

7. Preselections, advanced selections and cultivars release. The Joint Breeding Program of the University of Córdoba and the IFAPA include 10021 seedlings proceeding from 83 crosses and has yield up to date more than 400 preselections and 31advanced selections. Several intermediate field evaluation plots including the preselections with four to eight replications and several comparative field trials including different advanced selections are on progress. In 2006 a new cultivar ‘Sikitita’ (sin: ‘Chiq-uitita’ in the USA), selected for Super- High-Density Hedgerow was released (Rallo et al. 2008) and it was commercialized since the end of 2009. Currently 9 Spanish nurseries and one Italian nursery propagate ‘Sikitita’. Commercial orchards start bearing in 2012. A web site www.sikitita.es informs on this new cultivar.
In summary, these results indicate the high chance of obtaining valuable genotypes in a vegetative propagated plant like olive through a cross breeding program within a reasonable time.

Prospective and ongoing research
Olive growing is attending to a changing era in Spain. Since the crisis of the 1960’s, Spain olive growing has been progressively moving from traditional olive plantations to new intensive olive orchards. In this paper the changes undergone by the Spanish olive growing has been summarized with particular attention to the genetic resources and its improvement.

The efforts carried out in the collection, conservation, evaluation and sustainable use of the genetic resources since the beginning of the 1970’s allowed the progressive establishment of three Germplasm banks of cultivars. The first one initiated in Córdoba is known as World Olive Germplasm Bank (WOGB) include approximately 700 accessions from 21 countries (499 have already been identified by SSR and endocarps markers and correspond to 332 different cultivars) and two regional in Reus (Catalonia) with 98 genotypes and in Llíria (Valencia) with 78 genotypes. Morphological and molecular characterization of these banks is advanced. Agronomical evaluation is also progressing. A program has been proposed for the establishing a network of ex situ and in situ banks. The ex situ bank system will include a reserve lathouse with the genotypes identified and free from the main pathogens. The establishment of core collection in different ecologies is also considered. The in situ banks will pay attention to singular olives and to populations of wild olives. This framework will provide up dated information through a permanently actualized Data Base.

Since the beginning of the 1990’s a breeding program was initiated in Córdoba by the University and the current IFAPA. Later on several breeding programs have been also established in Seville and Catalonia. The breeding programs have allowed notable methodological progress, preselections and advanced selections and, up to date, a new cultivar for hedgerow orchards. A program on functional genomics has been initiated. All these activities herald the time of scientific breeding in olive. Currently resistance to Verticillium wilt and adaptation to new olive mechanized growing system are included as objectives in several breeding programs.

Finally the boom of the nursery industry has triggered a real and dramatic change in the varietal structure in Spain. Currently different regional certification programs of nursery plants are on development.

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Figure 1. Traditional low density (<100 trees/ha) rainfed orchards (A) are trained to maintain the canopy close to the ground (B and C) to enhance manual harvesting (D). Fig. 1D by courtesy of the family d’Abrunhosa.)
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Figure 2. Intensive irrigated (250-450 trees/ha) (A) and superintensive (>1,500 trees/ha) (B) have been designed for early and high yield and mechanical harvesting (C and D).
Figure 3. The exploration of genetic resources (A) and their identification by morphological and molecular markers (B) have allowed the catalogue (C) of the Spanish cultivars. Currently an isolated repository (D) and field collections (E and F) insure the permanent conservation and evaluation of the cultivars in the World Olive Germplasm Bank of Córdoba.
Figure 4. Some cross-breeding programs are currently in progress in Spain. The protocol (A) and the calendar (B) of the Joint UCO-IFAPA Program led to release a new cultivar: ‘Sikitita’ selected for superintensive plantations (C) by its early bearing, low vigor and habit of growth. In (D), ‘Sikitita’ is on the left in comparison to ‘Arbequina’ (center) and ‘Frantoio’ (right).