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Research review paper

Genetic improvement of olive (*Olea europaea* L.) by conventional and *in vitro* biotechnology methods

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ABSTRACT

In olive (*Olea europaea* L.) traditional methods of genetic improvement have up to now produced limited results. Intensification of olive growing requires appropriate new cultivars for fully mechanized groves, but among the large number of the traditional varieties very few are suitable. High-density and super high-density hedge row orchards require genotypes with reduced size, reduced apical dominance, a semi-erect growth habit, easy to propagate, resistant to abiotic and biotic stresses, with reliably high productivity and quality of both fruits and oil. Innovative strategies supported by molecular and biotechnological techniques are required to speed up novel hybridisation methods. Among traditional approaches the Gene Pool Method seems a reasonable option, but it requires availability of widely diverse germplasm from both cultivated and wild genotypes, supported by a detailed knowledge of their genetic relationships. The practice of "gene therapy" for the most important existing cultivars, combined with conventional methods, could accelerate achievement of the main goals, but efforts to overcome some technical and ideological obstacles are needed. The present review describes the benefits that olive and its products may obtain from genetic improvement using state of the art of conventional and unconventional methods, and includes progress made in the field of *in vitro* techniques. The uses of both traditional and modern technologies are discussed with recommendations.

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1. Introduction

Over 750 million olive trees are cultivated worldwide and 95% are located in Mediterranean Basin countries (between latitudes 30 and 45) characterized by hot dry summers and cool winters. The plants are sensitive to winter cold (−8 °C) but are able to tolerate drought and heat. Olive is cultivated both for oil extraction and table consumption with a world average production of 3–3.2 million tons per year. Europe produces 76.1% of all olive oil, followed by Asia with 12.2%, Africa with 10.7%, America 0.8%, and Oceania 0.1%. In Europe about 2.2 million tonnes of olive oil are produced by 1.9 million farmers which make up roughly one-third of all European Union (EU) farmers (Niaounakis and Halvadakis, 2006). Spain is the largest producer with an average 1 million tons per year, followed by Italy and Greece with 560 and 350 thousand tons respectively, and they together account for about 97% of EU olive oil production (FAOSTAT, 2013). Other countries that produce significant amounts of olive oil are Tunisia, Turkey, Syria, and Morocco. However, production drastically varies each year due to adverse climate conditions, particularly drought, and to pests and diseases, in addition to alternate bearing typical of the species.

In the traditional area of cultivation this species provides low anthropogenic pressures and agronomic inputs, being a biocenosis in equilibrium able to ensure the stability of the ecosystem with high homeostatic ability. This peculiarity has been held in high regard by the European Union which introduced the principle of “environmental conditionality”. The concept of conditionality tends to strongly bring out the link between agriculture and territory, as a strategic factor to create favourable conditions for mutual enhancement as the principal benefit of rural areas. In this context, the application of environmental issues is a priority in order to promote agricultural production methods aimed at reducing environmental impacts, encouraging conservation of natural habitats, biodiversity of the agricultural landscape and exerting an ecological and hydro-geological defence, while at the same time characterizing the landscape. In other words the olive plays food, environmental and rural functions.

In the past olives were extensively cultivated under traditional regimes, but over recent decades a limited number of groves have been progressively established with more plants per hectare under irrigation. In most areas of the Mediterranean Basin olive groves are still characterized by old plants of great size, with low yield and alternate bearing, located in steeply sloping areas, and shaped according to the vase training system. These are not suitable for full mechanization of harvesting and pruning, and so do not guarantee profits to the land owners. For these reasons the search for new ways to exploit and promote its main product, the oil, is fundamental to maintain the groves which often host monumental plants, and are part of historical memory and culture - representing art under different form.

2. New approaches of cultivation and specific requirements from cultivars and rootstocks

Notwithstanding the objective of maintaining some of the traditional groves, there are means to establish new groves with high density plantations, thus fulfilling the aim to increase oil production to both meet the increasing demand for a good extra-virgin olive oil and to guarantee more income for farmers. The use of available cultivars, that are productive although oil quality is not excellent, might be a temporary solution in order to assure the increasing demand for extra-virgin oil, perhaps used to blend with oil from traditional groves to improve the quality sought under the current imperfect oil classification based on acidity and extraction methods.

A complete review derived from experience with super high-density cultivation has been reported by Rallo (2014). Previously Fontanazza et al. (1998), and Moutier et al. (2010) reported various methods to improve training systems for the new orchards based on tree architecture, tree training and use of low vigour genotypes.

Among more than one thousand known varieties, of which there are about 600 in Italy, very few are suitable for high density and super high-density cultivation systems that require 250–400 and 900–1200 plants per hectare respectively; most of the traditional cultivars are suitable for 250 plants/ha, that are normally used in new plantations of several countries. At the present time these modern groves are mainly realized with a few genotypes chosen among the traditional cultivars, such as Arbequina, Arbosana and Koroneiki. Other new varieties are also promising, such as the Italian FS17 (Favolosa) and Don Carlo (Fontanazza et al., 1998), the Spanish Sikitita and Oliana (Bellini et al., 2008), and the Israeli Askal (Lavee et al., 2003). Varieties suitable for many of the required environments are not well known, because olive trees can change their general performance from one environment to another, including the production of secondary metabolites in the fruits which confer taste and health properties, or fatty acid composition, particularly oleic acid level. The reduction of this compound below a certain concentration compromises not only the quality but also marketability as extra-virgin olive oils.

Development through traditional cross-breeding of new cultivars suitable for mechanical pruning and harvesting is time consuming, whereas planting of vigorous traditional local cultivars grafted on dwarfing rootstocks appears a feasible strategy as an alternative to breeding of dwarf cultivars. In addition, this allows maintenance of the best local cultivars which are known for their oil quality, agronomic and healthy traits.

However, the availability of dwarfing rootstocks is very limited and their effectiveness cultivar-specific. A few accessions are currently under investigation, such as FS17 (Fontanazza et al., 1998) and LD (Nardini et al., 2006; Rugini et al., 1996), together with some selections among traditional cultivars, seedlings and *in vitro* plantlets (both diploids and tetraploids) derived from mutagenesis (Rugini et al., 2011a, 2011b). However, in order to speed up rootstocks selection it would be advisable to identify them among available cultivars for which the phenotypic stability of the adult phase is known, rather than among seedlings which will require time for reliable selection. Consequently a large number of genotypes, of both cultivars and rootstocks, are required for genetic selection to fit in different environments, requirements of modern farming techniques, and criteria of oil and table fruit consumers. Research on new cultivars of table olives is still limited to a few breeding programmes (Lavee, 2013; Medina et al., 2012; Rallo, 2014).

Variety renewal has been hampered by the extreme longevity of olive trees, the long period of juvenility of their offspring, and the diffidence of the public to accept genotypes obtained with advanced biotechnological approaches. Modern biotechnological techniques are suitable for olive improvement because they both allow direct correction of main defects, practising a sort of “gene therapy” on the existing known superior cultivars, and can also support traditional breeding using the great genetic variability present in the species, to guide crossing of genotypes chosen among the olive populations of different sites.

In general cultivars to be employed for modern high-density and super high-density cultivation should possess a number of characteristics. Their form should be of reduced size, medium-low vigour, reduced