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Characterization of olive progenies derived from a Tunisian breeding program by morphological traits and SSR markers

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ABSTRACT

An olive breeding program was conducted in Tunisia since 1994 using controlled crosses and open pollination on 'Meski' cultivar mainly used for table olives in northern Tunisia. From a total of 200 progenies, thirteen genotypes were subsequently selected and are potentially suitable for both oil processing and table olive uses. The study aimed to identify the aforementioned genotypes as well as the pollen donors used for cross breeding using morphological and molecular approaches. The morphological study showed high variation between genotypes and permitted their repartition into two groups according to the main discriminative parameters, which are the fruit and endocarp weights based on principal component analysis. The parentage of thirteen olive progenies and the molecular identification of 26 olive cultivars, including 8 olive varieties used as the parents in the cross breeding program, were analyzed using a set of six co-dominant polymorphic Simple Sequence Repeats (SSRs) selected among the most suitable markers for olive identification. SSR analysis revealed a high polymorphic information content value and a low probability of identity among genotypes, indicating the efficiency of SSR for detecting genetic diversity among genotypes and their potential use for olive breeding. Progenies were grouped into two groups, according to the crosses. Transmission of alleles from parents to offspring was observed, in which ten of the thirteen progenies sampled were matched consistently to their maternal parent 'Meski' and the paternity was confirmed. Three did not match, but their parents were identified and they appear derivatives from other breeding crosses. These genotypes remain to be evaluated for their agronomic performances.

1. Introduction

Olive trees (*Olea europaea* subsp. *europaea* var. *europaea*L.) are grown for oil and table olive production in the Mediterranean basin since ancient times, an enormous genetic diversity between varieties is resulting (Breton et al., 2006). The richness of the cultivated olive germplasm represents an unusual case among horticultural crops, as a consequence of tree longevity and little turnover with new breeding genotypes (Belaj et al., 2011). The need for more suitable cultivars prompted the development of olive breeding programs in the main olive-producing countries based in intra-specific cross-breeding between cultivars of known merit aiming at combining the good qualities of the progenitors in some of the genotypes of the progenies (De Medina et al., 2014; León et al., 2015). These programs aim at improving oil yield and quality, resistance to diseases, suitability to mechanical harvesting, early and low alternate bearing, high productivity and oil content, and shortening of the juvenile period (Fabbri et al., 2009). In the case of table olives, other features include shape, size, ripening time uniformity, or a high pulp:stone ratio (Lavee, 1994). Cross-breeding is considered the best strategy in olive breeding programs due to the high level of heterozygosity of varieties.

Olive trees are propagated by vegetative methods. Clonal propagation enables to multiply a single tree based on cuttings or suckers. Aims of clonal propagation is to obtain homogenous cultivars characterized by interesting features such as high yield, disease resistance and oil composition (Hannachi et al., 2011). However, Clonal propagation does not allow genotypes combining new traits. Therefore, this method could lead to reduction of variability. In this situation, genetic improvement based on breeding program would be more efficient to obtain new genotypes having interest characters.

Breeding programs by crossing and selection in the progenies are reported in Turkey (Arsel and Cirik, 1994), Spain (Rallo, 1995), Tunisia (Trigui, 1996), Iran (Zeinanloo, 2006), China (Shan-An et al., 1981), Ukraine (Sholokhova, 1984), Greece (Pritsa et al., 2003), Israel (Lavee et al., 1999, 2003), and Italy (Bellini et al., 2002; Fontanazza et al., 1999). However, until recently, very few cultivars have been emerged

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from formal olive breeding programs and were selected empirically within their region of cultivation (Marchese et al., 2016). To date, only a very small number of new cultivars and advanced selections, coming from different breeding programs, have been described (e.g. 'Moncita' in France (Besnard et al., 2000), 'Askal' in Israel (Lavee et al., 2003); 'Arno' in Italy (Bellini et al., 2000) and 'Chiquitita' in Spain (Rallo et al., 2008).

New cultivar selections largely depend on early identification and protection of olive varieties. Breeders have used morphological traits to identify hybrid progenies and selfing progenies. However, the use of morphological traits has limitations to identify varieties because they are largely affected by plant development and highly influenced by the environment. Alternatively, DNA molecular markers have added a new and powerful dimension to olive breeding and management. Among the molecular markers, microsatellites or SSR (Simple Sequence Repeats) are now widely accepted. Genetic studies using microsatellite markers have increased rapidly because they have codominant segregation and a high level of polymorphism in olive (Bracci et al., 2011). In fact, microsatellites were previously validated for olives to characterize the genetic diversity and cultivars identification (De la Rosa et al., 2002; Belaj et al., 2004; Díaz et al., 2006; Sarri et al., 2006; Baldoni et al., 2009; Khadari et al., 2010; Arbeiter et al., 2014; Fendri et al., 2014; Muzzalupo et al., 2014). In an other hand, Simple Sequence Repeats have become the markers of choice for paternity analysis in olive (Mookerjee et al., 2005; Arbeiter et al., 2014). They allow the early identification of true interspecific hybrid progenies for further evaluation. True hybrid progenies are detected by the presence of DNA sequences corresponding to one of the two alleles contributed by each of the two parents. The effectiveness of microsatellite markers in paternity testing of progeny obtained from an olive breeding program has been demonstrated (De la Rosa et al., 2004; Díaz et al., 2007).

In Tunisia, the olive tree is widely cultivated and it constitutes one of the principal economical and agricultural strategic sectors that are known for their richness of varieties (Abaza et al., 2005). A cross-breeding program has been carried out in 1994 within the context of the project "olive breeding" (supported by the IOOC) by crossing two local cultivars 'Meski' (table olive) and 'Chétoui' (oil olive) with both local and foreign pollen donors in the goal of selecting new dual purpose, as well as new oil or table olive varieties to meet the requirement of the international market. An exhaustive agronomic evaluation of 200 progenies of the above-mentioned crosses was undertaken and the best thirteen progenies were selected as those with better performance in terms of either yield or quality in either oil or fruits throughout several years of production.

The present work aimed to study the genetic diversity among the selections and their parents by using morphological traits and SSR markers, to check the paternity of the selections derived from olive crossing by using six SSR markers and to discriminate them from the 26 varieties presented in the olive collection studied.

2. Materials and methods

2.1. Plant material

The experimental material used in the present study comprised thirteen olive progenies, obtained through genetic controlled olive crossing using the combinations on 'Meski' as mother variety, their parents (8 varieties), and 18 olive varieties from the collection of 'Borj Amri' (North of Tunisia; 36° 42' 10" North, 9° 53' 16" East). The thirteen olive progenies were supposed to be the result of crossing 'Meski' with three local varieties: ('Besbessi', 'Chemlali' and 'Chétoui') and four foreign varieties ('Agezzi', Egypt; 'Ascolano tenera', Italy; 'Manzanillo', Spain; and 'Picholine', France). The progenies were obtained from the seven following crossings: 17 C ('Meski'x'Agezzi'); 16D ('Meski'x'Chétoui'); 10E ('Meski'x 'Chemlali'); 9 F and 14 F ('Meski'x'Besbessi'); 8 H ('Meski'x'Manzanillo'); 22 H, 16I, 22I, 23I and 12 J ('Meski'x'Picholine') and 21 K and IO2 ('Meski'x'Ascolano tenera') (Table 1).

Table 1
List of crosses.

Code	cross	Code	cross
17 C	'Meski' x 'Agezzi'	16I	'Meski' x 'Picholine'
16D	'Meski' x' Chétoui'	22I	'Meski' x 'Picholine'
10E	'Meski' x 'Chemlali'	23I	'Meski' x 'Picholine'
9 F	'Meski' x' Besbessi'	12 J	'Meski' x 'Picholine'
14 F	'Meski' x' Besbessi'	21 K	'Meski' x' Ascolano tenera'
8 H	'Meski' x' Manzanillo'	IO2	'Meski' x' Ascolano tenera'
22 H	'Meski' x 'Picholine'		

2.2. Morphological characterization

Morphological description was conducted on eight parent cultivars and thirteen olive progenies, according to the methodology for primary characterization of olive varieties proposed by the International Olive Oil Council (IOOC, 1997) on olive leaves, fruits and stones characters. The studied olive genotypes were evaluated for 29 morphological traits following the descriptors reported by the IOOC (1997a,b). Four characters related to the leaf (length, widht, shape and longitudinal curvature of the blade), Eleven related to the fruit (weight, length, width, shape, symmetry in position (A), position of maximum transversal diameter, apex, base, nipple presence, presence and size of lenticels) and thirteen related to endocarp (weight, length, width, shape, symmetry in position (A), symmetry in position (B), position of maximum transversal diameter, apex, base, surface, number of grooves, distribution of grooves and mucro presence (Table 3). The flesh to stone ratio (FSR) was also determined according to the pomological characterization procedure (IOOC, 1997b).

The investigation included 13 quantitative and 16 qualitative traits. All the measurements were evaluated for random samples of 30 leaves and fruits from each tree. Endocarps were removed and were subject of characterization.

All statistical analyses based on quantitative traits were performed using SPSS® 24.0 (IBM®). Average data, standard deviation, minimum and maximum values and coefficient of variation were calculated. Pearson correlation coefficients were calculated for each trait between all other traits in order to study the associations among thirteen quantitative traits used in the analysis of twenty-one olive genotypes. Principal Component Analysis (PCA) was carried out on quantitative traits in order to distinguish between groups of olive cultivars and progenies.

2.3. DNA extraction

Total genomic DNA was extracted according to the CTAB protocol previously established by Murray and Thompson (1980) with slight modifications described by De la Rosa et al. (2002), starting from 100 mg fresh leaves for all olive tree and progenies samples, previously ground in liquid nitrogen. Then, 500 µl CTAB/DTT mix was added in addition to 500 µl of chlorophormisoamyl alcohol (24:1). After centrifuging the suspension, DNA was precipitated by 2/3 vol of isopropanol from the aqueous phase at −20 °C. Finally, the dry pellet was re-suspended in 100 µl of TE solution with 5 µl RNAse and incubated at 37 °C for 1 h.

DNA concentration and purity were evaluated by NanoDrop 2000 spectrophotometer (Thermo Scientific). The extracted DNA was stored at −20 °C until use.