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Olive biodiversity in Colombia. A molecular study of local germplasm

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ABSTRACT

The olive tree has been present in Colombia for several centuries, however we still do not know the identity of old olive trees present in this territory.

This study aimed at characterizing and identifying the Colombian germplasm for the first time through molecular analysis. Thirty-nine olive accessions were subjected to genotyping with ten SSR (ssrOeUA-DCA, UDO099, GAPU, EMO series) markers. The set of chosen markers resulted as a whole highly polymorphic, and made possible the characterization of all studied accessions. Data were analysed by cluster analysis, and results revealed a good genetic variability within the olive germplasm. Nineteen genetic profiles were discriminated and among these different cases of intra-varietal clones. Only some accessions (about 28% of the studied germplasm) have shown genetic identity with known cultivars (Picual and Azapa). The remaining accessions were distinguished and assembled in 5 different genotypes of unknown origin. In addition, the majority of genotypes found does not correspond to the names given in the region, and several cases of homonymy and synonymy were revealed. The data obtained suggest that confusion on naming of the accessions as well as propagation errors might have occurred either in the countries of origin of the samples and/or in the Colombian region.

1. Introduction

The colonization of the Americas, mainly carried out in the first centuries by Spain, was characterized by a two way exchange of plant germplasm of economic species. Olive (*Olea europaea* L.) was one of those; its importance went beyond the food aspects of the crop, it was also important in catholic liturgy, and the missionaries who accompanied all expeditions made sure to establish olive cultivation in all territories where missions were founded (Taylor, 2000; Soleri et al., 2010). The olive has been vegetatively propagated, to maintain the agronomical and morphological features of selected genotypes, for millennia (Zohary and Hopf, 2000); however, in many instances seedlings have been utilized due to a series of reasons: unavailability of large quantities of planting material, voluntary attempts at obtaining new variability, occasional retrieval of feral olives from seed spread by animals or other causes. This was more common in new olive areas than in the traditional Mediterranean Basin area, where this kind of events was most likely common at the beginning of olive domestication (Soleri et al., 2010).

As far as the Americas are concerned, the first recorded introduction is that of Antonio Ribero in Mexico, 1560 (Taylor, 2000), although others contend that as early as in 1531 the olive had been introduced in Colombia, in the Boyacá Department, where centuries old olive trees still survive by the ancient Mission of Santo Ecce Homo (Garcìa Molano, 2010). The olive continued to receive attention and attempts of introductions, especially in the fields surrounding the towns of Villa de Leyva, Sachica and Sutamarchan of Alto Ricaurte province, although production was not reported until 1701 (D'Alessandro and Granados Medina, 2012); in 1875 the Spaniard Josè Maria Gutierrez planted five thousand olives in the same zones, obviously considered endowed with a suitable environment. By the end of the 1950s, several cultivars were introduced in the same towns from Portugal, Spain and Italy (Taguas, 2009; Garcìa Molano, 2010).

Like in all similar situations, this gave rise to a remarkable confusion of germplasm, with a number of synonyms and homonyms, making it difficult to distinguish original cultivars from cultivars somehow adapted to the local environment, and from new genotypes originated from seedlings that had proved to be particularly adapted to the local conditions (Barranco et al., 2008; Taguas, 2009).

Historical populations of fruit trees (as is the case for olive), display a genetic variability, usually little studied, which can be a precious source of biodiversity. A preliminary and unavoidable prerequisite for the exploitation of such biodiversity is a deep and unequivocal knowledge of the characteristics of local germplasm, starting with its morphological, agronomical and molecular characterization, and the identification of all synonyms

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and homonyms present. A prerequisite felt by all researchers which have attempted, also in recent years (Soleri et al., 2010; Koehmstedt et al., 2011; Trentacoste and Puertas, 2011; do Val et al., 2012) to study the olive germplasm, be it local (ancient) or imported at different times in various countries of the continent.

Numerous techniques are being used to study the genetic characteristics of olive germplasm (Ganino et al., 2006). Molecular markers have proved to be a powerful tool for assessing genetic variation, phylogenesis and genetic structure of many species; in particular, microsatellites or simple sequence repeats (SSR) markers, given to their abundance, high polymorphism, reproducibility, and codominant inheritance resulted to be the most suitable molecular markers for the characterization, identification and evaluation of genetic relationships among cultivars (Fabbri et al., 2009; Bracci et al., 2011; Hasnaoui et al., 2012; Beghè et al., 2013; Muzzalupo et al., 2014).

This research was carried out on the germplasm existing in the Villa de Leyva District, in the Province of Alto Ricaurte (Boyacá Department, Colombia). In this area olive growing has been practiced for over 200 years, and olive groves consist in about 18,000 trees, of which approximately 3000 are in new plantations planted in the course of the last decade, the rest consists in trees of over 30 years of age (Garcìa Molano, 2010; D'Alessandro and Granados Medina, 2012). These areas are characterized by a particular microclimate, typically equatorial with little temperature excursions, that didn't facilitate the development of a profitable olive culture (D'Alessandro and Granados Medina, 2012).

However, in spite of the climatic conditions, in the course of the last decade a great interest for this fruit crop and its products has developed in Colombia. The "olive oil" product is today appreciated mainly for its nutritional and health value, and we are in the presence of a constant increase of the demand for it, and of the relative import flow, mainly from the Mediterranean countries, firstly from Spain (75.95%), and from Italy (12.38%), but also from Chile (4.38%), Argentina (4.03%) and Peru (2.16%) (FAOSTAT, 2013). Hence the interest in developing a commercial olive culture in the country, able to meet the local demand.

Research has been carried on since the 1960s to ascertain which are the most promising accessions. A first study produced information concerning the productivity of trees of European and local origin grown in the Boyacá Department: the cultivars better adapted resulted to be "Picual", "Cordovil", "Passareira", two cultivars identified by local people with names of "Leyva de tronco amarillo" and "Leyva de tronco scuro" and others of which no recognized names exist (Taguas, 2009; Garcìa Molano, 2010). Other scientists studied the rooting ability of a number of olive accessions found in the same territory (Rache-Cardenal et al., 2008). To our knowledge, no molecular characterization research has been conducted so far for the identification of the existing trees, i.e., whether they belong to known cultivars or are of an obscure origin.

The purpose of this study was therefore to examine for the first time the genetic diversity of the germplasm existing in the Villa de Leyva District of Boyacá Department (areas of largest olive diffusion in Colombia) by utilizing simple sequence repeats (SSR) markers. These molecular markers were also instrumental to identify synonyms and homonyms.

2. Materials and methods

2.1. Area of study and plant material

The experiment was carried out in the Boyacá Department, precisely in the Villa de Leyva District of Province of Alto Ricaurte (04°39′10″E 07°03′17″N; 71°57′49″E 74°41′35″W) in the centre of Colombia, at an altitude of 2100 m a.s.l. (Fig. 1). This area is characterized by equatorial climate and in 2010, year of the study, the average of maximum temperatures was 26 °C, that of minimum temperatures was 7 °C, with a general average of 17 °C; the photoperiod is 11.5 to 12.5 h/day, relative humidity is around 73% for most of the time. We selected 39 olive trees: 24 accessions from olive groves planted 40 years ago, 15 additional younger trees from olive groves planted 3 or 4 years ago and propagated from ancient trees, just beginning to set fruit. The accessions were taken from 7 cultivated areas, each accession was also indicated by the alpha-numeric code (F1 to F7) as described in Table 1. All accessions were propagated from centuries old trees existing in the region, which had been imported from Spain, Italy and Portugal at different times, as gathered from local information. The olive trees were selected after a preliminary morphological and agronomical evaluation (data not shown), and thanks to the aid of information supplied by local growers; in particular, the plant material was collected from productive trees. Most accessions were identified by the grower with the name of a commercial cultivar (e.g., "Picual"), or with a local term such as "Acebuche", which in Spanish indicates the wild olive (*O. europaea* L. subsp. *europaea* var. *sylvestris)*, while locally means "rustic olive tree" and is used to indicate a variety with resistance characteristics typical of wild olives. Other accessions did not have a name and were of unknown origin, and were tagged with an alpha-numerical code (Table 1).

2.2. SSR analysis

Genome DNA of the 39 above described samples was extracted from young leaves collected from young, healthy and actively growing shoots. The samples, after immersion in liquid nitrogen, were stored at −80 °C until DNA isolation. Genomic DNA was extracted following the CTAB procedure (Doyle and Doyle, 1987) with minor modifications as described by de la Rosa et al. (2002).

In order to evaluate the validity of analyses (PCR amplification and sequencer analysis) a control was set through the insertion in each analysis of a varietal standard (cultivar Picual (CS) collected in CRA-Research Centre for the Olive Growing and Olive Oil Industry of Rende (Cosenza, Italy) and with well-known alleles (Ganino et al., 2008; Beghè et al., 2009, 2011; SSR Database of Parma University, unpublished data) to allow an internal control at each analysis.

The samples were subjected to characterization with SSR markers. For DNA amplification 10 couples of SSR primers were used which had shown a high discriminating capacity: ssrOeUA-DCA-(03, 05, 09, 16, 17, 18) (Sefc et al., 2000), UDO99-(043) (Cipriani et al., 2002), GAPU-(101, 103A) (Carriero et al., 2002) and EMO90 (de la Rosa et al., 2002). All SSR markers utilized were already well known in literature for being highly polymorphic and these microsatellites, with the exception of locus DCA17, had been listed by Baldoni et al. (2009) among the best SSR markers for olive genotyping.

The PCR amplification was performed in a 25 μl volume containing: 1× Reaction Buffer (Biotools, B&M Labs, S.A., Madrid, SP), 1.5 mM $MgCl_2$ (Biotools, B&M Labs, S.A., Madrid, Spain), 0.2 mM dNTPs (Amersham Biosciences, Piscataway, USA), 0.2 μM primer (MWG Biotech, Ebersberg), 20 ng genomic DNA and 0.6 U of *Taq* polymerase (Biotools, B&M Labs, S.A., Madrid, SP). For primer UDO-043 $MgCl_2$ the concentration was 2.5 mM, to obtain a better quality of amplification.

The PCR amplification was optimized in thermal cycler MJ PCT 100 Research (Watertown, Mass.), programming a first step at 95 °C for 5 min followed by 30 cycles of 45″ at 94 °C, 45″ at the specific annealing temperature for each couple of primers (Sefc et al., 2000; Cipriani et al., 2002; Carriero et al., 2002; de la Rosa et al., 2002), and 45″ at 72 °C, for denaturation, annealing and primer extension, respectively; at the end of the cycles were allowed 8 min of incubation at 72 °C.