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Combination of morphological and molecular markers for the characterization of ancient native olive accessions in Central-Eastern Tunisia

Association de marqueurs morphologiques et moléculaires pour la caractérisation d'anciens génotypes autochtones d'olivier dans le Centre-Est de la Tunisie

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ABSTRACT

Increasing olive germplasm erosion in the coastline of Tunisia has required an imperious conservation of the traditional genotypes before an ultimate disappearance. This region has been relatively neglected in the literature sources of olive identification. In this context, a prospection effort and a preliminary selection of olive accessions belonging to Central-Eastern Tunisia was carried out. Twenty-seven ancient olive accessions were studied by combining molecular and morphological data in order to fingerprint them, and to evaluate their relationships with classical cultivars. Compared to known classic Tunisian olive cultivars, the new prospected olive accessions were well distinguished, presenting a potential use as promising genotypes. The morphological and molecular data showed a high diversity between genotypes. 92 and 63 polymorphic bands were scored using 10 RAPD and 9 SSR markers, respectively. Significant correlation coefficients were obtained among fruit and stone sizes (r = 0.90) and among their shapes (r = 0.73). The genetic distances obtained with the two DNA marker systems were significantly correlated (r = 0.45) according to Mantel's test. No significant correlation was observed between distances based on molecular and morphological markers. UPGMA analysis based on molecular data showed no clear clustering trends according to morphological traits or fruit use. Despite the high genetic variation among accessions in each prospected area, geographical origin seemed to have significant impact on the observed variability. The relationship between morphological and molecular data has confirmed that each marker expressed different aspects of variability. Integration between all markers will be useful for distinguishing new accessions and genotyping local varieties.

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RÉSUMÉ

Mots clés: Olea europaea L Descripteurs morphologiques RAPD; SSR Test de Mantel Corrélation L'accélération de l'érosion génétique du germoplasme oléicole à la suite de l'urbanisation effrénée dans le Sahel tunisien nécessite une conservation pressante du patrimoine local avant sa disparition. De fait, la région du Sahel tunisien est considérée l'une des premières forêts oléicoles installées en Tunisie (Carthage) par les Phéniciens. Les prospections préliminaires ont permis la sélection de 27 génotypes centenaires d'olivier. Différents marqueurs morphologiques et moléculaires ont été combinés en vue de leur caractérisation et de leur identification. Ces génotypes ont présenté une diversité génétique intéressante et ils se différencient des cultivars classiques d'olivier en Tunisie. Dix marqueurs RAPD et neuf marqueurs SSR ont permis de ressortir respectivement 92 et 63 bandes polymorphes. Une corrélation significative (r = 0.45) a été notée entre les deux types de marqueurs moléculaires, tandis que les descripteurs morphologiques n'ont pas enregistré une corrélation significative avec les descripteurs moléculaires. Cependant, une forte corrélation est notée entre la forme (r = 0.73) et la taille (r = 0.90) du fruit et de l'endocarpe. L'analyse UPGMA basée sur les marqueurs moléculaires a permis de grouper les génotypes partiellement en fonction de leurs origines géographiques. La relation entre les données morphologiques et moléculaires a confirmé que chaque marqueur exprime un aspect différent de la variabilité observée. L'intégration de tous les marqueurs sera utile pour distinguer les nouvelles accessions et le génotypage des variétés locales.

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1. Introduction

The olive tree (Olea europaea L.) belongs to the family of Oleaceae and is one of the oldest known agricultural settlements in history. It is presently spreading from the Mediterranean region of origin to new production areas, due to the beneficial nutritional properties of olive oil and to its high economic value [1]. Despite the enlargement of the olive growing in new extensions all over the world, this enlargement is done mainly by few commercial cultivars. The increasing use of conventional cultivars added to the growing phenomenon of urban development in some areas is always at the expense of minor and ancient olive accessions that are generally located in restricted areas and are sometimes threatened by elimination. These minor and ancient olive accessions are generally characterized by a high genetic variability, which is important for the conservation of several adaptative and interesting traits that could support olive growing. In this context, various studies were interested in the identification and characterization of minor and ancient accessions all over the world [2-9].

In Tunisia, the cultivation of olive trees is millennial. The long history of olive cultivation and the differentiated pedoclimatic conditions from the North to the South of the country allowed a quite rich olive germplasm. The Tunisian cultivar sets can be split into those of local origins and those introduced from the Near-East and western regions, making Tunisia in central Mediterranean a key-place for olive and oleaster diversity [10]. However, olive cultivation in Tunisia depends mainly on two prevailing cultivars: Chetoui in the northern part of the country and Chemlali in the central and southern parts. The Tunisian olive germplasm is estimated to include about 56 cultivars [11]. Most of them are maintained in restricted areas. This number is probably underestimated because of the scarce information on minor local varieties widespread in the different olive growing areas.

Identification and characterization of Tunisian olive cultivars was developed by morphological [11–13] and molecular markers such as AFLP [14], RAPD [15], SSR [16–18] and SNP [19]. Those studies have focused mainly on central (Sidi Bouzid, Kairouan, Kasserine), southern and northern parts of Tunisia. However, Central-East Tunisia and especially the region of Sousse, 'capital of the Tunisian Sahel', has been neglected in the literature regarding olive characterization, although it is considered as a historical location of olive biodiversity [20]. Furthermore, the rapid and uncontrolled urbanization is spreading out on behalf of the important number of old olive groves that increased olive germplasm erosion in the coastline of Tunisia [21]. As a consequence, an imperious conservation of traditional genotypes before their disappearance is required.

In order to evaluate and exploit olive diversity in this region, a prospecting effort was carried out in many ancient and historical olive groves. In the present work, we performed the morphological, pomological and molecular characterization of some olive accessions selected for their fruit and oil characteristics. Additionally, a study of the relationship between the selected accessions and known classic olive cultivars was performed. A comparison of morphological and molecular markers was also performed to investigate the usefulness of molecular markers as a complement and/or an alternative for varietal distinctness based on morphological criteria.

2. Materials and methods

2.1. Plant material and DNA extraction

The study was carried out on twenty-seven autochthonous olive accessions (*Olea europeae* L.) collected from archeological sites and ancient olive groves localized in Central-Eastern Tunisia, which is actually subjected to a high risk of olive genetic erosion due to rapid and

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