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Pomology observations, morphometric analysis, ultrastructural study and allelic profiles of "*olivastra Seggianese*" endocarps from ancient olive trees (*Olea europaea* L.)

Observations pomologiques, morphométriques, ultrastructurales et génétique sur des endocarpes de vieux oliviers (Olea europaea L.) de « Olivastra Seggianese » cultivars

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ABSTRACT

Preliminary studies of historical sources and remote sensing were used to identify ancient olive trees near archaeological sites and heritage buildings in the Orcia Valley (Siena, Italy). Distinctive characters were assessed by traditional pomological observation. Trees with similar characters were selected on the basis of the features of endocarps, the only structure that survives aerobic deterioration and conserves useful botanical information for centuries. Non-invasive morphometric analysis of endocarp size and shape established morphological variations in individuals of different populations. Plastid organization in the endocarp and location of DNA in the endocarp tegument were detected by morphological and ultrastructural observations using light and electron microscopy. Cytoplasmic markers with high polymorphism were used to test similarity of endocarp and leaf DNA within individuals and to confirm low variability and minimal divergence between individuals. The ancient trees studied showed the same allelic profiles and therefore belonged to a distinct cultivar. The traditional pomological descriptions of the trees, leaves and fruits, morphometric analysis of size, and shape elliptic Fourier analysis of endocarp outline, ultrastructural observations and allelic profiles of endocarp tegument delineated the general species-specific qualities of the cultivar "*olivastra Seggianese*" of the Orcia Valley.

Mots clés :
Arbres antiques
Endocarpe

RÉSUMÉ

Des études préliminaires sur sources historiques et de télédétection ont été utilisées pour identifier les oliviers centenaires à proximité des sites archéologiques et des édifices historiques du patrimoine dans le Val d'Orcia (Sienne, Italie). Les caractères distinctifs des

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Vieux oliviers

oliviers ont été évalués au travers d'observations pomologiques traditionnelles. Arbres aux caractères similaires ont été sélectionnés sur la base des caractéristiques de l'endocarpe, la seule structure qui survit à la dégradation aérobie et conserve pendant des siècles des informations botaniques utiles. Avec l'étude morphométrique non-invasive de la taille et l'analyse de la forme de l'endocarpe, des changements morphologiques étaient établis chez les individus de populations différentes. L'organisation des plates dans le tégument de l'endocarpe et la localisation du DNA ont été détectées par des observations morphologiques et ultrastructurales avec microscopie optique et électrique. Marqueurs cytoplasmiques avec haut niveau de polymorphisme ont été utilisés pour tester des similitudes du DNA entre endocarpe et feuilles, et pour confirmer la faible variabilité et des divergences minimes entre les individus. Les vieux arbres étudiés présentaient les mêmes profils alléliques et donc appartenaient à un cultivar spécifique. Les descriptions pomologiques des arbres, des feuilles et des fruits, les analyses morphométriques avec l'étude de la taille et la forme de l'endocarpe d'olive, les observations ultrastructurales et les profils alléliques des endocarpes tégument, ont définit les qualités générales spécifiques à l'espèce de la variété « *olivastra Seggianese* » du Val d'Orcia.

1. Introduction

Ancient species of olive trees (*Olea europaea* L.), growing near archaeological sites and heritage buildings contribute to the fascination of the Orcia Valley. Centuries ago, these trees were selected and cultivated for their oil. Interesting studies have been conducted to investigate the nutritional properties of oil produced in the valley [1]. The survival of the ancient olive trees was due more to their symbolic than their nutritional value. Recognition of olive cultivars is difficult due to the wide variability of germplasm selected over the centuries by adaptation to microclimate, parasites, cultivation techniques and local production methods.

Studies of the Orcia Valley, combined with historical sources can provide insights into the relationships between archaeological sites and ancient cultivation of olive trees. In particular, the historical presence of cultivated olive trees in the area is said to go back to the seventh century AD, when Demaratus, father of Luchmon, fled from Corinth with riches, men and products that he sold in Etruria. Luchmon later became the fifth legendary king of Rome (616–578 BC) under the name of Lucius Tarquinius Priscus [2].

Remote sensing data can be useful for reconstructing archaeological landscapes. Archaeological surveys in the Orcia Valley did not discover amphoras but *dolia* of Etruscan and Imperial Roman age, indicating production of oil for local consumption [3,4].

Olive trees are usually characterized pomologically, which involves comparing the trees, leaves and fruits (especially the endocarp) with reference collections and obtaining information about the range of morphologically similar plants that could have originated from similar varieties [5]. Morphometrics (non-invasive analysis of endocarp size and shape) provide a model for investigating differences between archaeological and modern olive endocarps [6]. Quantitative analysis based on study of endocarp outline by elliptic Fourier shape analysis discriminates cultivar groups [7].

The morphology and ultrastructure of carpological specimens have recently been studied by light and electron microscopy, and DNA can also be detected in palaeobotanical material by cytochemical staining with 4', 6-diamidin-2-phenylindole [8,9,10]. Carpological samples can be used for molecular studies, and ancient olive pits can be used to find ancient DNA [11].

The olive is a prevalently allogamous species with high levels of heterozygosis and polymorphism in apparently similar individuals [12]. The genetic heritage and the numerous synonyms and anonyms have been studied in ancient varieties of olive by collecting material (fruits and leaves) and applying nuclear microsatellite markers [13]. Olive trees have also been the subjects of phylogeographic and phylogenetic studies [14], to develop and evaluate consensus chloroplast primer pairs with highly variable sequence regions in a diverse array of plant taxa [15]. Plastid DNA is generally transmitted via the maternal line and is found in tegument of pits, the only structure that resists aerobic deterioration in archaeological sites [10]. The geographical distribution attained by organelle molecular markers provides a better scenario of past migration history than nuclear markers due to their uniparental mode of inheritance [16].

The aim of the present study was to investigate ancient olive trees growing near archaeological sites and heritage buildings in the Orcia valley, Tuscany, using traditional pomology studies of the trees, fruits and leaves. Morphometric study of size and shape and quantitative analysis of endocarp outline by elliptic Fourier analysis provided a model for discriminating cultivars on the basis of endocarp features. Ultrastructural characterization of endocarp tegument morphology was useful to detect plastid organization in the endocarp and locate DNA fluorescence in plastids of tegument tissues. Molecular studies with cytoplasmic markers were conducted to test similarity and if possible detect variations between individuals. By multidisciplinary comparison of pomological descriptions, morphometric analysis, ultrastructural observations and molecular data, we endeavored to characterize a restricted group of trees.

2. Materials and methods

2.1. Sampling

The plants chosen for sampling (Fig. 1) were the oldest in the area (Mediterranean bioclimatic stage at 43° 10′ N,