

Cultivated olive and oleaster: two very closely connected partners of the same species (*Olea europaea*). Evidence from enzyme polymorphism

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Abstract

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Using enzyme polymorphism at 10 loci, genetic variation was analysed in both wild and cultivated olive growing around the Mediterranean Basin. Twenty five and 21 alleles were observed in the 333 wild olive trees analysed and in the cultivated material (418 trees) respectively. Twenty one of these alleles were common to the two forms. In addition, in most the situations of sympatry between wild and cultivated olive, the two forms possessed the same alleles which differed from one area to the other, suggesting the occurrence of close genetic relationships between these two partners of the same species.

Introduction

Olive cultivars (*Olea europaea* L. subsp. *europaea*) and wild olives (oleaster olives) (*Olea europaea* L. subsp. *sylvestris*) are both outcrossing long-lived trees. They show similar climatic requirements and have large common distribution areas in the Middle East and in the Mediterranean Basin (Zohary & Spiegel-Roy 1975 and references therein, Zohary 1994). In addition, cultivars and wild oleaster olives have the same chromosome number ($2n = 2x = 46$), grow frequently side by side, and are fully interfertile. Oleasters differ from cultivated olives only by their smaller fruits, their lower oil content and, often, by their shorter leaves. At the juvenile stage, oleaster olives also show spiny shoots. These close affinities between the two olive forms as well as archaeological evidence (Zohary & Spiegel-Roy 1975) support the assumption that the oleaster olive constitutes the progenitor stock from which the cultivated olive has been derived. Oleaster domestication probably began in prehistoric times (fourth and third millennia B.C.) in the eastern part of the Mediterranean Basin (Zohary & Spiegel-Roy 1975, Zohary & Hopf 1993) by empirical

selection of individual trees showing superior performance for fruit size and/or oil content. These individual trees were propagated vegetatively as clones, using cuttings which were planted directly or, more recently, grafted onto indigenous oleasters. From these propagated individuals, the different cultivars of olive trees have been developed and distributed by various human migrations, especially from East to West throughout the Mediterranean Basin. Such domestication has modified markedly the reproductive biology of the species since wild olives reproduce from seeds whereas cultivated varieties have been maintained vegetatively, as clones, for very long periods. Reproductive divergence between wild and cultivated olive may be at the origin of genetic differentiation between them, unless significant gene flow occurred between these two olive forms. These do in fact possess large areas of sympatric distribution and, for thousands of years, have probably had many opportunities to exchange genes by several ways which were reported in detail by Zohary and Spiegel-Roy (1975). Recently, enzyme polymorphism was analysed in wild and cultivated olives collected in several sites of the Mediterranean Basin, in order to assess the effect of olive domestication on the genetic organization of this species (Ouazzani 1994, Ouazzani & al. 1993, 1995, 1996). Using these genetic markers, one of the main objectives was to examine whether genetic variation in the cultivars is lower compared with that found in wild material, as has been observed in other wild-cultivated species complexes (e.g. Second 1982) or alternatively (and more probably) the two olive forms have similar patterns of enzyme variation due to gene flow between them. The first results obtained from comparison of wild and cultivated olives are summarized in the present paper.

Material and methods

Enzyme polymorphism was studied at 10 loci, namely LAP1, EST1, EST2, ADH1, MDH1, MDH2, PGI1-2 (duplicated loci showing the same alleles), PGM1 and PGM2. Two loci, MDH1 and PGM1, were monomorphic. Protein extraction (from leaves), electrophoretic and staining techniques used to detect allozymes as well as their inheritance have been described previously by Ouazzani & al. (1993).

Four hundred and eighteen cultivated trees were analysed individually for enzyme polymorphism. They came from Syria (1 tree), Turkey (4 trees), mainland Greece (4), Crete (5), Cyprus (16), Italy (4), Corsica (2), France (13), mainland Spain (6), Ibiza island (12), Portugal (7), Tunisia (14), Algeria (2) and Morocco (328). The cultivated trees corresponded most generally to distinct identified clonal cultivars, except in Crete, Cyprus, Portugal and Morocco where only local names were known. These often corresponded to a mixture of clones growing in the same locality and which showed high morphological variability among them. The multilocus genotypes obtained from most those individuals were already reported in previous papers (Ouazzani & al. 1995, 1996). 333 wild olive trees (i.e. individuals that originated from olive stones) were also analysed for the same loci. They were collected in Cyprus (39 trees), Crete (10 trees), Corsica (99 trees located in two distant sites), Ibiza (8), South of France (73 trees located in mainland Provence and Port-Cros island), Spain (21), Portugal (14) and Morocco (106 trees distributed in 6 sites). In addition, two wild individuals from Madeira, which were identified as *O. europaea* L. subsp. *cerasiformis*, were analysed. These two Madeiran trees and several others from the Atlas region in Morocco, from Cyprus, the South of Corsica,

Port-Cros and Andalusia were found growing in environmental conditions not disturbed by cultivation and should be considered as wild trees, whereas in the other locations there was no possibility to distinguish between wild and feral individuals (escapees from cultivation) because many of those oleasters were, at least, several centuries old and were used as stocks for grafting. In many situations the stock part and the graft part of the same tree were analysed separately.

Results and discussion

From the 25 distinct alleles observed at the 8 polymorphic loci in the whole olive samples, 21 were common to both the wild and cultivated olives. Oleaster olive possessed four additional alleles, one observed only in Cyprus, another one in Madeira, Andalusia and in the south of Corsica, and one located only in Andalusia. In most cases of sympatry between wild and cultivated olives, the two forms showed exactly the same alleles. Average heterozygosity per individual was significantly higher ($p < 0.01$) in wild than in cultivated olive. In Morocco where numerous cultivated individuals were studied from 10 sites along a transect from the North (Pre-Rif) to the South (Anti-Atlas), 68 multilocus-genotypes were observed. In Moroccan cultivated olive, genotype diversity and heterozygosity increased from the North where trees are usually grafted to the South where the cultivated trees grow directly from cuttings and are close to numerous wild populations (Ouazzani & al. 1996). Such high variability in the South of Morocco may be due to the numerous successive introductions of cultivated varieties from northern regions and/or, more probably, to gene flow between cultivated varieties and native oleaster olive present in that region (Ouazzani & al. 1996).

Six groups were constituted from the analysis of 47 cultivars located in the whole Mediterranean Basin, using multivariate analysis of the multilocus genotypes (Ouazzani & al. 1995). A single group gathered together cultivars from a specific geographic area, namely France. Also the 11 North African varieties tested formed a single group but this group also included other cultivars of different origins. The four other groups contained cultivars from various geographic origins, probably as the result of the occurrence of numerous human migrations which may have favoured the dispersion of cultivated olive throughout the Mediterranean Basin.

In contrast, multivariate analysis of genetic distances between the wild olive populations using allozyme frequencies showed marked geographic structure of the genetic variation (unpublished data). One group was made up of populations from the western part of the Mediterranean Basin, namely Madeira, Portugal, Spain, Morocco and Ibiza island, whereas a second group contained populations from the central part of the Basin (Nice region, Corsica and Crete). The wild populations from Cyprus showed a specific genetic diversity with peculiar alleles (e.g. EST10.96) and alleles also found in Turkey (e.g. PGI1-20.95).

From those preliminary studies, it can be concluded that wild and cultivated olives share approximately the same allele pool, more particularly if they grow sympatrically. This suggests the occurrence of gene flow between the two partners. On average, oleaster olive is much more heterozygous than cultivated olive, suggesting that intensive selection

involving inbreeding may have taken place under cultivation originally to obtain particular characteristics in the olive cultivars, sometimes at the expense of trees' vigour. In addition to collections of traditional cultivars (e.g. the International Olive Council's collection in Córdoba, Spain), the conservation of numerous genotypes selected among those observed in local cultivars and within the wild olive material should be carried out as soon as possible. These genotypes may constitute genetic resources for further breeding. New orchards should be started in several locations to provide a dynamic conservation of the genetic variation in olive.

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