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Genetic variation in the olive tree (*Olea europaea* L.) cultivated in Morocco

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Summary

Genetic variation was studied using enzyme polymorphism at 8 loci in ancient olive trees. These were being cultivated in 10 sites along a transect from North (Pre-Rif) to South (Anti-Atlas), involving the main ecological areas where the species is cultivated in Morocco. For the 328 trees studied, 16 alleles and 68 multilocus genotypes, of which one was present in more than half the individuals analysed, were detected. Eighty seven per cent of the total genetic diversity was attributable to within site variation and showed a high proportion of local genotypes. The highest values for both genotype number and heterozygosity were observed in the South of Morocco. Such high variation may be due to the partial domestication of olive material which may be derived from crosses between cultivars or between cultivars and 'feral' or wild olive (oleaster) trees growing frequently in the Southern region. Genetic diversity in Moroccan olive, constitutes an important genetic resource which must be conserved for further breeding.

Introduction

The olive tree (*Olea europaea* L.), is a species characteristic of the Mediterranean area. This species was thought to have been domesticated in the Bronze age in the Near-East Mediterranean region (Zohary & Spiegel-Roy, 1975) and it has been cultivated in Morocco for millennia. From archaeological and palynological studies, evidence was obtained that wild olive was present in Morocco in the ninth millennium BC (Camps-Fabrer 1974; Lenoir & Akeraz, 1984). Remains of olive-mills dating back from the Roman period revealed the economic importance of that fruit, more than 20 centuries ago, more particularly in the Roman town of Volubilis located in the North of Morocco. In addition to the use of local olive material, numerous introductions of olive accessions around the Mediterranean area as a result of commercial exchanges and human invasions are thought to have occurred.

At present, in Morocco, *Olea europaea* is cultivated over a range of environments and under very contrasting regional climates. It can be found under variable and irregular annual precipitation regimes ranging from 800 to 1000 mm in the North to less than 200 mm in the South. The species grows at elevations ranging from 200 m up to 1700 m in the Anti-Atlas region. Nowadays, in Morocco, the olive tree populations play a major socio-economic role as they consist of 37 millions of individual trees growing over 395,000 hectares, i.e. more than half the total fruit-tree area occurring in the country. Moroccan olive production has been estimated to be about 45,000 tons for oil and 80,000 tons for fruits in 1992–1993 (COI, 1993). Despite this economic importance, the number of distinct olive varieties is poorly known in Morocco and is commonly referred as a single variety called 'Picholine marocaine'. However, even at the beginning of the present century, it was found that several mountain tribes cultivated several other local races of olive which were identified according to their morphological variation (Maestratti, 1922; Tornézy 1922). According to these authors, the most important local varieties were: 'Meslala' near Meknès, 'Noukal' in the

Taza region, 'Bouchouika' near Sefrou, 'Hamrani' in the Chefchaouen region and 'Soussia' in the Souss region. More recently, three additionnal local varieties were recorded, 'Dahbia' from the Meknès region, 'Haouzia' and 'Menara' from the region of Marrakech (unpublished data). These occasional records suggest that varietal diversity in Moroccan olive material may be much higher than what was assessed previously. This idea is supported by the predominantly outcrossing reproductive system of the species (Griggs et al., 1975; Lavee & Datt, 1978), the occurrence of very diverse ecological conditions for olive growth in Morocco and, in several regions, by the existence of either substantial native wild (oleaster), or of secondary feral forms (Chevalier, 1948; Turrill, 1951; Green & Wickens, 1989; Zohary, 1994). These forms originate from seeds derived from crosses either between cultivated trees or between wild and cultivated trees and growing nearby the cultivated olive with which they can exchange genes as they belong to the same species (Zohary & Spiegel-Roy 1975). In such situations, local varieties may have been obtained by empirical selection of remarkable individual trees, and then their subsequent vegetative multiplication. This reproductive system and the occurrence of a very long life-span which may reach several millennia has been considered to be the main factors responsible for conservation of original genetic variation over time (Morettini, 1972; Browicz & Zielinski, 1990).

In the present work we have studied the geographic distribution of genetic variation in Moroccan cultivated olive by using allozyme polymorphisms as markers. Allozyme variation has a mono or oligogenic control and is mostly neutral and thus constitutes an appropriate means to study the geographic variation of genetic diversity (Wilson et al., 1977; Kimura, 1983; De Vienne, 1984). Our main objective is to estimate the total number of distinct multilocus genotypes which may correspond to ancient varieties cultivated in Morocco, and to assess their geographical distribution.

Materials and methods

Plant material

Sampling was restricted to the main areas where olive has been cultivated traditionally for a very long time and which were considered as areas of primary diversification (Maestratti, 1922; Tornézy, 1992; Elant, 1948) so that the material should reflect original genetic diversity as much as possible. Trees were sampled in 10 sites corresponding to large ancient orchards along a transect from the North (Pre-Rif) to the South (Anti-Atlas) involving the main ecological areas where olive is cultivated in Morocco (Figure 1). Plant material from trees growing in four, two and four sites was sampled in the northern, central and southern regions respectively. The precise geographical location and the main climatic characteristics of the collecting areas are indicated in Table 1. In most these areas, olive cultivation is limited to fruit harvest without pruning and manure spreading. In the areas of Chefchaouen and Ouazzane, grafting on the oleaster is commonly used whereas in the eight other areas, vegetative multiplication is made using shoots or cuttings which are planted directly. According to stump size and to local people assessments, all the trees sampled in the present study were at least several centuries old and the oldest trees sampled in Volubilis, a Roman town located within the Moulay-Idriss area, may originate in Roman times. However, in Chefchaouen and Ouazzane, several successive grafting treatments may have occurred in the past on the centenaries oleaster stumps so that age of the plant material collected in our study cannot be estimated.

In each region, two surveys were made during the flowering and harvest seasons respectively and the sampling strategy was based on field observation and on information from the farmers. Trees were selected according to their variation for morphology (vigour, fruit and leaf size or shape etc. . .) and/or phenological traits. Genetic uniformity in an olive orchard may result from vegetative propagation (grafting or cuttings) of a single cultivar (genotype). Conversely, genetic diversity within a specific orchard of olive trees may be due to (1) mixed plantings of several cultivars, each propagated vegetatively, (2) landraces, grown from seed, but only partly domesticated, and still retaining much of the genetic diversity inherent in their wild ancestors and (3) when seedlings have not been weeded out, progenies from crosses between two distinct cultivars or between wild or 'feral' oleaster and 'pure' domesticated trees. Those genotypes are expected to be heterozygous at many loci and, subsequently, they may be multiplied vegetatively and cover large areas.

To point out maximal genetic diversity occurring in a specific area by using a minimum or reasonable sample size, the following selective sampling design was used. In each collecting site, from 1 to 3 individuals (at least 500 m apart) of each variety (when these could be identified), 10 trees (at least 500 m apart) showing the most common phenotype and each tree showing

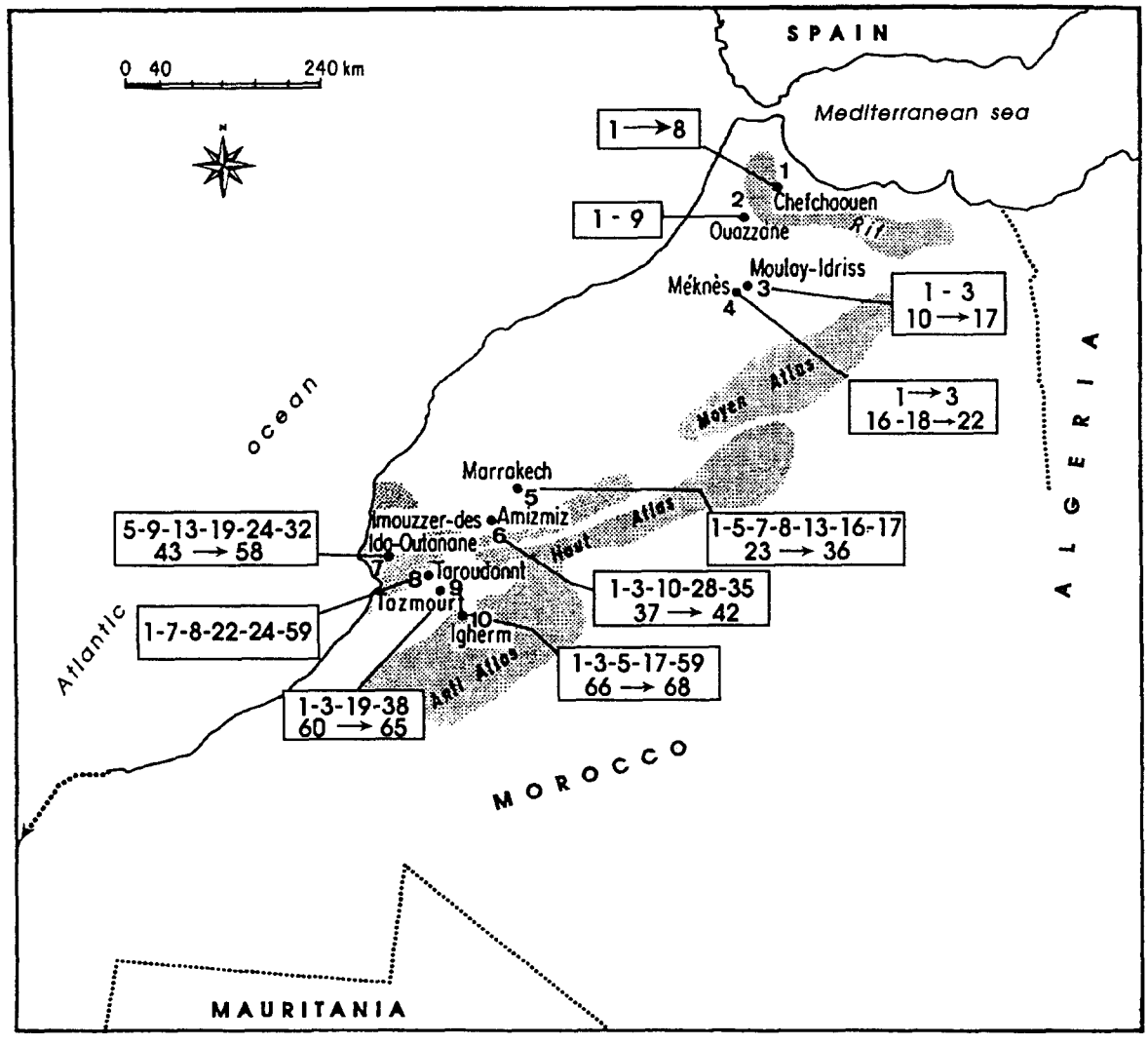

Figure 1. Geographical distribution of the 68 multilocus genotypes observed in the 10 olive sites studied in Morocco. The arrows indicate series of successive genotypes numbers.

a specific morphological trait and/or having particular agronomical interest were sampled and analysed separately. From 24 to 40 individual trees per site (328 in total) were sampled according to the observed range of morphological variation. Trees corresponding to the 'Hamrani' variety were collected in sites 1, 5, 7 and 10. 'Bouchouika' was collected in Moulay-Idriss (site 3), 'Dahbia' in sites 3, 4, and 5, 'Meslala' in sites 3 and 5 and, 'Picholine marocaine' was observed and collected in all the sites except No 7.

Allozyme analysis

Allozyme polymorphism was determined using starch gel electrophoresis of leaf protein extracts from 328 sampled trees. Protein extraction from fresh leaves, enzyme migration and staining, and genetic inheritance of the enzyme systems used in the present study have been described previously by Ouazzani et al. (1993). Plant material was scored for 8 polymorphic loci coding for leucine aminopeptidases, E.C.3.4.11.1. (LAP1 locus), esterases, E.C.3.1.1.2. (EST 1 and EST2), alcohol dehydrogenases, E.C.1.1.1.1. (ADH1), malate dehydrogenases, E.C.1.1.1.37. (MDH2), phosphoglucose isomerases, E.C.5.3.1.9. (PGI1-2, dupli-

Table 1. Locality, region (North (N), Centre (C) and South (S)), altitude, average annual rainfall (R), average minimum temperature of the coldest month (mT) and average maximum temperature of the warmest month (MT) of 10 collection sites of olive cultivated in Morocco

n°	Locality	Region	Altitude (m)	R (mm)	mT (°C)	MT (°C)
1	Chefchaouen	N	280	900	5.2	28.0
2	Ouazzane	N	300	740	6.1	29.0
3	Moulay-Idriss	N	600	760	4.0	33.0
4	Meknès	N	540	570	4.4	34.2
5	Marrakech-Aguedal	C	463	240	4.9	37.7
6	Amizmiz	C	950	300	3.1	34.7
7	Imouzzer	S	1310	532	2.0	32.0
8	Taroudant	S	255	230	5.1	36.3
9	Tazmourt	S	240	230	6.2	35.0
10	Igherm	S	1725	186	- 0.3	32.8

cated loci with the same alleles) and phosphoglucomutases, E.C.2.7.5.1. (PGM2).

Genetic data analysis

Because genotype distributions in cultivated populations of olive are predominantly dependant on the activity of man which has multiplied particular genotypes more than others, for each locus, genetic diversity and dissimilarity indexes were calculated from the observed genotypes and not from allele frequencies calculated over trees of each site.

Each individual tree was characterised by its multilocus genotype from which allelic richness, i.e. total number of alleles, and percentage of polymorphic loci were calculated. Multilocus genotypes observed over the 10 sites were compared to one another using correspondence analysis (CA) (Benzecri, 1973). Multilocus genotypes were encoded according to the method of Mathieu et al. (1990). More particularly, at each locus, the values 0, 1 and 2 were used for absence, presence of a specific allele in the heterozygous state and in the homozygous state, respectively. For PGI1-2 (duplicated loci with the same alleles), the 0, 1, 2, 3 and 4 values were used when a specific allele was absent or was present one, two, three or four times respectively at the 2 loci considered as a whole. To avoid too much distortion due to the occurrence of very rare multilocus genotypes, these were considered as additional elements in the analysis. The respective positions of the multiloci genotypes estimated by the distances between them were plotted in multidimensional space and then projected onto a plane. The same data also constituted the basis for a Hierarchical clustering analysis (HCA) (Ward, 1963). Clusters observed at the main levels of agglomeration (whole range from 0 to 100%) were mapped onto the diagram obtained from the C.A. so that the agreement between the results from the two data analyses could be compared. Data treatment was performed and analysed statistically using the Biomeco package 3.7 (Anon. 1989).

In each site, allelic diversity was estimated by the total number of distinct alleles and by the proportion of polymorphic loci. Genetic diversity was assessed by the total number of distinct multilocus genotypes per site. In addition, we used the relative genotype diversity index (GD) proposed by Pielou (1969). According to this author, $GD = 1 - \Sigma_i [n_i (n_i\text{-}1)/N (N\text{-}1)]$ where n_i is the number of individuals showing the i genotype and N the total number of individuals analysed in the site. This index ranges from 0 (all the trees possess the same genotype) to 1 when all the trees have distinct genotypes.

Variation in genetic diversity among sites was assessed by comparing their numbers of 'local multilocus genotypes' (observed only in a single region), of 'widely distributed' genotypes (occurring in several sites located in distinct geographical regions) and the proportion of individuals possessing a 'standard' genotype (observed in more than half the total number of individuals analysed). In addition, sites were compared for average heterozygosity as calculated by the proportion of heterozygous loci per tree. These data were averaged over all trees in the site studied.

Genotype dissimilarity between two sites was estimated from the genotypes observed at each locus, using

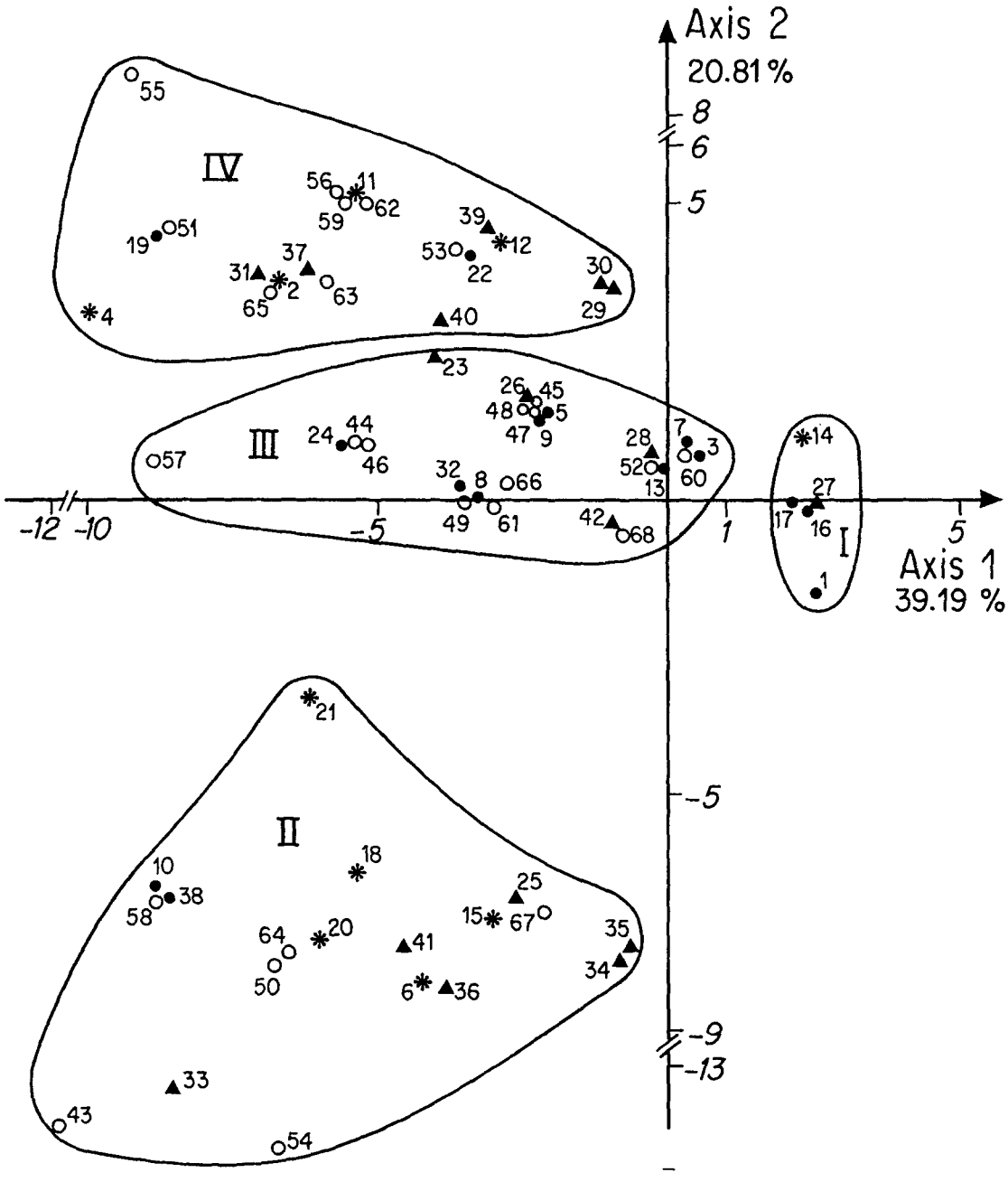

Figure 2. Position of the 68 multilocus genotypes observed in Moroccan cultivated olive according to polymorphism at loci LAP1, EST1, EST2, ADH1, MDH2, PGI1-2 and PGM2. Genotypes are clustered at level 24% of the hierarchical clustering. Widely distributed genotypes: (●), local genotypes specific to North (*), Centre (▲) and South (○) of Morocco are also indicated.

the Jaccard's distance (D_j) (Legendre & Legendre, 1979). $D_j = 1 - (a/(a + b + c))$ where a is the number of genotypes common to the two sites and b and c are the genotype numbers occurring only in either site. At each locus, presence and absence of a specific genotype were coded by 1 and 0 respectively. From the matrix of Jaccard's distance, the 10 studied sites were compared using a hierarchical cluster analysis (unweighted pair group means analysis in this case) (BIOMECO package (Anon., 1989)).

Table 2. Allele number, percentage of polymorphic loci and number of trees studied in 10 Moroccan collection sites of cultivated olive analysed for allozyme polymorphism at 8 loci

Site No	Allele number	Polymorphic loci (%)	N. trees
1. Chefchaouen	16	75.0	30
2. Ouazzane	12	37.5	30
3. Moulay-Idriss	15	75.0	34
4. Meknès	15	62.5	30
5. Marrakech	16	100.0	40
6. Amizmiz	15	75.0	30
7. Imouzzer	15	87.5	40
8. Taroudant	14	75.0	30
9. Tazmourt	16	62.5	40
10. Igherm	15	75.0	24
Region			
(1– 4) North	16	75.0	124
(5– 6) Centre	16	100.0	70
(7–10) South	16	100.0	134
Mean/pop.	14.9	72.5	33
Total pop.	16	100.0	328

Results

Allelic diversity in Moroccan olive orchards

At the 8 enzyme loci studied, 16 distinct alleles were observed overall in the olive material. These alleles were identified as: $LAP1^{0.92}$, $LAP1^{0.97}$, $LAP1^{1.00}$, $EST1^{1.00}$, $EST1^{1.04}$, $EST2^{0.94}$, $EST2^{1.00}$, $EST2^{1.03}$, $ADH1^{0.92}$, $ADH1^{1.00}$, $MDH2^{0.79}$, $MDH2^{1.00}$, $PGI1\text{-}2^{1.00}$, $PGI1\text{-}2^{1.60}$, $PGM2^{1.00}$ and $PGM2^{1.14}$ (for detail concerning alleles identification, see Ouazzani et al. 1993). No allele was observed specifically in a particular region. The total number of alleles per site ranged from 12 to 16 with the average being 14.9 (Table 2). In all the sites, the percentage of polymorphic loci was higher than 60% except in Ouazzane where it was 37.5%. The average value was 72.5% and the highest values were observed in Imouzzer and Marrakech areas. These high values are mainly due to polymorphism at the PGI1-2 loci occurring exclusively in these two sites. When olive material from the same region was clustered, all the loci were polymorphic in the Central and Southern regions whereas only 75% loci were polymorphic in the Northern region.

Multilocus genotype diversity

Sixty eight distinct multilocus genotypes were identified from the 328 trees analysed (Table 3). These genotypes had 2.2 alleles per locus on average and the mean proportion of heterozygous loci was 56.4% (range: 0–100%). A single 'standard' genotype (No 1) was observed in 52.4% of the trees which were usually called 'common olive' with no additional precision by the farmers and 'Moroccan Picholine' by the local technicians. Only five other genotypes (Nos 3, 5, 7, 13 and 19) were observed in more than 5 trees (from 8 up to 16 trees). Moreover, 72% of the multilocus genotypes were 'local' according to the definition indicated in the material and method section.

Results from the combined correspondence and hierarchical cluster analyses of the multilocus genotypes are shown in Figure 2. The two first axes of the CA accounted for 60% of the total variation. In the HCA, four very distinct genotype clusters were obtained at the 25% level of agglomeration (range from 0 to 100%). Apart from a single exception, the first set grouped the main widespread Moroccan traditional varieties such as 'Picholine marocaine' (genotype 1), 'Bouchouika' (genotype 14), 'Dahbia' (16), 'Meslala' (17). These genotypes were very close genetically to one another and were characterised by a low overall heterozygosity (average 0.20, range from 0 to 0.33) whereas each of the three other clusters regrouped a much higher number of genotypes with a much higher average heterozygosity (> 0.63, range from 0.33 to 1.00). The multilocus genotype 5 observed in cluster 3 corresponds to the traditional variety 'Hamrani'. The sets did not characterise specific geographic areas. However, it can be noticed that all the 'local' multilocus genotypes (except No 14) observed in the North of Morocco are located in cluster II and in cluster IV which groups genotypes (except No 55) showing the '13' allelic combination at the LAP1 locus.

Genotype variation within and among sites

The mean number of distinct genotypes per site was 10.7 (range from 2 to 22) (Table 4). The lowest values (2 and 6 genotypes) were observed in Ouazzane and Taroudant respectively whereas the highest values were found in trees from Marrakech and Imouzzer, with 21 and 22 multilocus genotypes respectively. Moreover, multilocus genotype numbers increased regularly from the North (22 genotypes) to the South of Morocco (39 genotypes) with the central region showing an inter-

Table 3. Identity and distribution (N) of multiloci genotypes observed at 8 enzyme loci in 328 trees from 10 collection sites of olive cultivated in Morocco

Geno-type	Locus							
	LAP1	EST1	EST2	ADH1	MDH2	PGI1-2	PGM2	N
1	33	11	12	22	12	1112	11	172
2	13	12	12	22	12	1112	12	3
3	33	11	12	12	12	1112	11	16
4	13	22	13	22	12	1112	12	1
5	33	11	12	12	12	1112	12	13
6	23	12	12	22	22	1112	11	1
7	33	11	12	12	22	1112	11	8
8	33	12	12	22	12	1112	12	4
9	33	11	22	12	12	1112	12	2
10	23	12	12	12	22	1112	12	2
11	13	11	11	12	12	1112	12	1
12	13	11	12	12	12	1112	11	1
13	33	11	12	22	12	1112	12	9
14	33	11	12	22	22	1112	11	3
15	23	11	11	22	12	1112	12	1
16	33	11	22	22	12	1112	11	3
17	33	11	22	22	22	1112	11	4
18	23	11	12	12	12	1112	12	1
19	13	12	12	12	12	1112	12	9
20	23	12	12	12	12	1112	11	1
21	12	11	12	12	12	1112	11	1
22	13	11	12	22	12	1112	12	3
23	33	11	11	22	12	1122	12	1
24	33	12	22	12	12	1112	12	5
25	23	11	13	12	12	1112	11	1
26	33	11	22	12	22	1122	12	1
27	33	11	12	22	12	1122	11	2
28	33	11	12	22	22	1112	12	2
29	13	11	12	22	22	1112	11	1
30	13	11	12	22	22	1122	11	1
31	13	12	22	22	22	1112	12	1
32	33	12	12	22	22	1112	12	1
33	22	11	13	12	12	1112	12	1
34	23	11	22	22	12	1112	11	1
35	23	11	12	22	12	1112	11	3
36	23	12	11	22	12	1112	11	1
37	13	12	12	12	12	1112	11	1
38	23	12	12	12	12	1112	12	3
39	13	11	12	12	22	1112	11	1
40	13	12	11	22	12	1112	11	1
41	12	12	12	22	22	1112	11	1
42	33	12	12	22	22	1112	11	1
43	22	12	12	12	12	1112	12	1
44	33	12	12	12	12	1122	12	1
45	33	11	12	12	22	1112	12	4
46	33	12	12	12	12	1112	12	3
47	33	11	12	12	22	1122	12	1
48	33	11	22	12	22	1112	12	1
49	33	12	22	22	12	1112	12	1
50	23	12	22	22	12	1112	12	1
51	13	12	11	12	12	1112	12	1
52	33	11	22	22	12	1112	12	1
53	13	11	22	22	12	1112	12	1
54	22	11	22	22	12	1112	12	1
55	11	11	22	12	22	1112	12	1
56	13	11	12	12	22	1112	12	2
57	33	22	12	12	22	1112	12	1
58	23	12	22	12	12	1112	12	1
59	13	11	12	12	12	1112	12	2
60	33	11	22	12	12	1112	11	1
61	33	12	22	12	12	1112	11	3
62	13	11	33	12	12	1112	12	1
63	13	12	13	22	12	1112	12	2
64	23	12	12	22	12	1112	12	2
65	13	12	22	22	12	1112	12	1
66	33	12	12	12	12	1112	11	2
67	23	11	12	12	12	1112	11	1
68	33	12	12	22	12	1112	11	1
Total	6	3	5	2	2	2	2	328

Allele mobility: LAP1: 1 = 0,92; 2 = 0,97; 3 = 1,00; EST1: 1 = 1,00; 2 = 1,04; EST2: 1 = 0,94; 2 = 1,00; 3 = 1,03; ADH1: 1 = 0,92; 2 = 1,00; MDH2: 1 = 0,79; 2 = 1,00; PGI1-2: 1 = 1,00; 2 = 1,60; PGM2: 1 = 1,00; 2 = 1,14.

mediate value (29 genotypes). Over the 10 sites, correlation between the number of distinct phenotypes collected in each site and that of corresponding multilocus allozyme genotypes was 0.64, and was significantly different from zero ($P < 0.05$). Genotype numbers were always lower than phenotype numbers but the difference between the two values was lower in sites located in the South of Morocco and in those where a large number of distinct local varieties could be recognized.

Genetic diversity (GD) over all the sites was 0.72 from which 87.5% was attributed to within-site genetic diversity. Mean genetic diversity per site was equal to 0.63 with values ranging from 0.07 (in Ouazzane) to 0.93 (in Imouzzer) (Table 4). More generally, GD values were observed to increase regularly from the North (GD = 0.50) to the South (GD = 0.86) of Morocco.

When considering 'local' (a), 'widely distributed' (b) and 'standard' (c) multilocus genotypes, more than half the genotypes observed in the several collection sites were widely distributed (b = 55.8% on average with a range from 27.3% in Imouzzer to 100% in Ouazzane) and more than half the trees per site showed a 'standard' genotype (c = 54.3% on average, range from 0% to 96.7%) (Table 4). The mean frequency of 'local' genotypes was 36.2% (range from 0% to 72.7%). Whereas some sites, (e.g. Ouazzane and Taroudant which are located far apart) are characterised by no 'local' genotype but by a high proportion of 'widely distributed' and 'standard' genotypes, conversely, other sites, and more particularly, that of Imouzzer, possess a very high proportion of 'local'

Table 4. Multilocus-genotype number, intra-site genotype diversity (GD), number and percentage (within parentheses) of local (a) and of widely distributed (b) genotypes, percentages of trees possessing the 'standard genotype' (c) and mean heterozygosity per individual in 10 sites of olive cultivated in Morocco

Site	Nb. multilocus genotypes	GD	Nb. genotype 'a'	Nb. genotype 'b'	% individuals with c genotype	Mean heterozygosity
1 Chefhaouen	8	0.59	2	5	63.3	40.6
2 Ouazzane	2	0.07	0	2	96.7	33.9
3 Moulay- Idriss	10	0.65	4	6	58.8	35.3
4 Meknès	9	0.56	3	5	66.7	46.7
5 Marrakech	21	0.82	10	9	42.3	39.6
6 Amizmiz	11	0.60	5	4	63.3	42.2
7 Imouzzer	22	0.93	16	6	00.0	60.8
8 Taroudant	6	0.55	0	5	66.7	41.7
9 Tazmourt	10	0.76	6	4	47.5	55.8
10 Igherm	8	0.76	3	4	37.5	45.8
Region						
North	22	0.50	10 (45.5)	12 (54.4)	71.0	39.0
Centre	29	0.74	17 (58.6)	12 (41.4)	51.4	41.0
South	39	0.86	26 (66.7)	13 (33.3)	35.8	52.4
Mean	10.7	0.63	4.9 (36.2)	5 (55.8)	54.3	44.0

genotypes and very few 'standard' and 'widely distributed' genotypes (Table 4). More generally, the proportion of 'local' genotypes increased and those of 'widely distributed' and 'standard' genotypes decreased regularly from the Northern to the Southern regions of Morocco. The central region of Morocco showed intermediate values for the three genotypes (a = 58.6%, b = 41.4% and c = 51.4%) (Table 4).

Average heterozygosity per individual (h) calculated over the 8 polymorphic loci ranged from 34% at Ouazzane to 61% at Imouzzer (Table 4). This parameter ranged from 39% in the Northern region to 52.4% in the South with an intermediate value (41%) obtained in the central region of Morocco. Average genotype dissimilarity between sites estimated by the Jaccard's distance was equal to 0.26 (range from 0.07 between Moulay-Idriss and Igherm, to 0.52 between Ouazzane and Imouzzer). Trees of this last site showed the highest distance values with those of the other sites (0.37 on average, range from 0.23 to 0.52).

Four sets of sites were obtained at the 64% agglomeration level (range from 0 to 100%) of the cluster analysis established from the distances matrix (Figure 3). Sets I and III clustered together sites characterised by high genotype diversity, high multiloci genotype numbers (more particularly set I) and high allele numbers (set III). Isolation of Ouazzane (set IV), in which trees showed a very low genotype diversity, may be due, at least partly, to the methodology used in the study as the Jacquard's index is based on the common occurrence and not on the common absence of a specific genotype in two groups of individuals. Moreover, the sets gathered together sites from distinct geographical regions of Morocco. For instances, sites located in the Northern and the Southern regions were grouped in set III.

Discussion

Allelic diversity in olive cultivated in Morocco

The allelic variation observed in cultivated olive trees sampled in the present study can be considered as probably representative of that occurring in olive traditionally cultivated in Morocco. Total allele number (16) is slightly lower than that (21) observed at the same polymorphic loci in 47 olive varieties distributed in the whole Mediterranean Basin (Ouazzani et al., 1995). As compared to those varieties, the five alleles absent in the traditional Moroccan olive material are rare alleles showing a local geographical distribution, namely $EST1^{1.07}$ located in Italy, $ADH1^{0.96}$ and $ADH1^{1.04}$ observed in Turkey and $PGI1\text{-}2^{0.95}$ and $PGI1\text{-}2^{1.05}$ found in a few varieties from France, Spain, Turkey and Greece. Conversely, $EST2^{1.03}$ was observed only

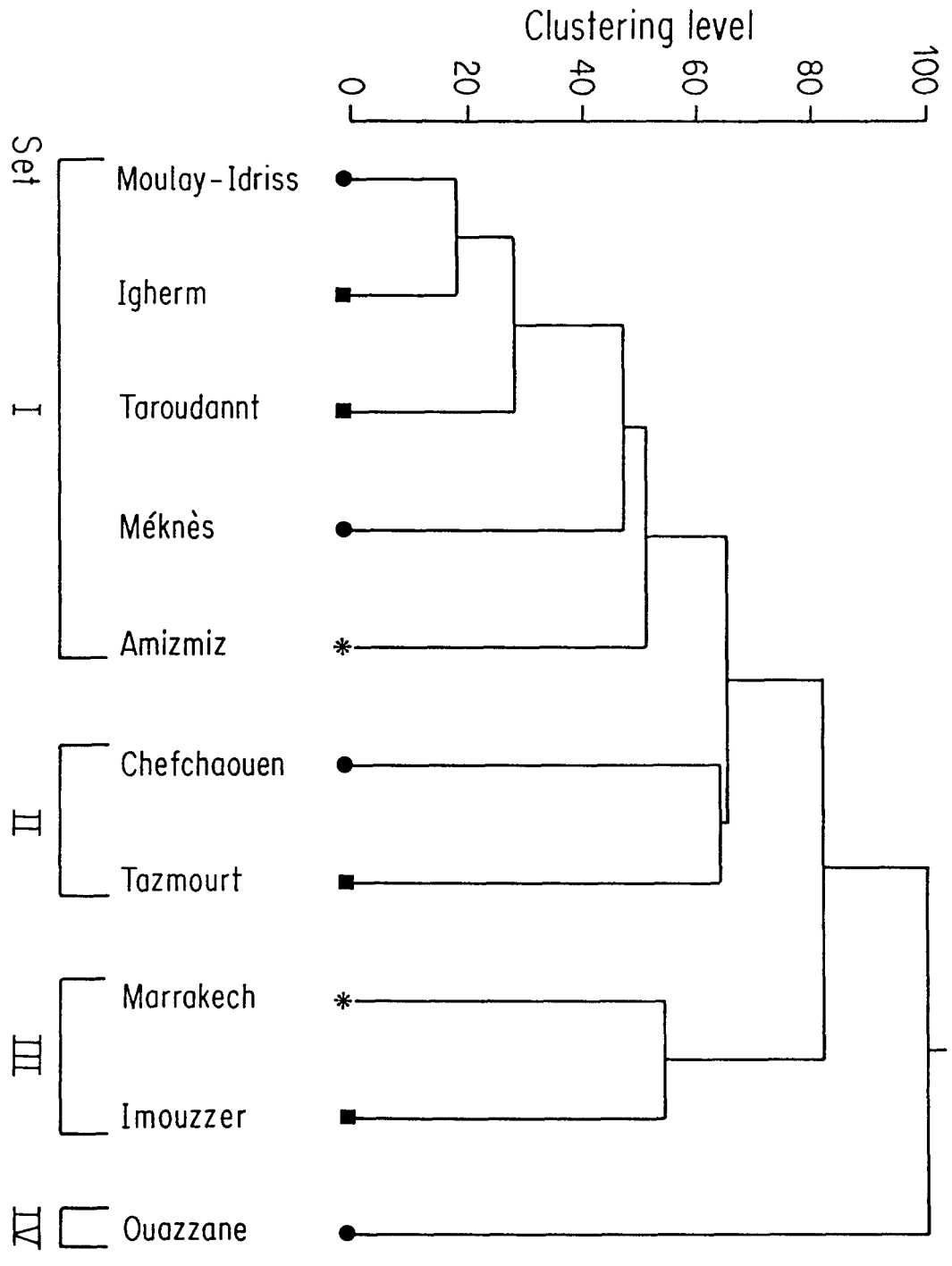

Figure 3. Phenogram showing the relationships between the 10 collection sites of olive trees studied in Morocco. Hierarchical clustering is based on Jaccard's distance calculated from the 8 studied enzyme loci. The symbols indicate populations from North (●), Centre (*) and South (■) of Morocco. Clustering level × 100.

in Morocco (i.e. was never found in wild or cultivated olive material analysed from numerous localities distributed over the whole Mediterranean basin (Ouazzani et al., 1995; and unpublished data) and its occurrence was restricted to the individuals from Chefchaouen, Marrakech and Tazmourt located in the Northern, central and Southern regions, respectively. This allele was also observed very frequently in wild olive trees

(oleaster) growing in the forests of the same areas, and in several other areas in Morocco (Ouazzani, 1993; Ouazzani et al., 1993; and unpublished data) suggesting that a part of the cultivated olive may originate from local domestication of trees derived from wild olive ancestors native to Morocco (Zohary & Spiegel-Roy, 1975; Green & Wickens, 1989; Zohary, 1994).

Multilocus-genotype diversity in Moroccan cultivated olive

Of the 68 multilocus genotypes identified in Morocco, one (No 1) was present in all the sites except Imouzzer, and was observed in more than half the trees analysed in the present study. This genotype was also found in the variety called 'Moroccan Picholine' originating from Morocco (Ouazzani et al., 1995). Trees growing in this country and possessing the genotype No 1 were usually called 'common olive' and reflected a large range of variation for morphological and physiological characters. A larger number of polymorphic enzyme markers may be necessary to improve discrimination among these morphologically variable trees.

In the CA analysis, the multilocus genotype No 1 was located in set I together with a few other genotypes among which, Nos 14, 16 and 17 were also found in the main traditional and widely cultivated varieties growing in Morocco, namely 'Bouchouika', 'Dahbia' and 'Meslala', respectively (Ouazzani et al., 1995). The five genotypes of set I show a high genotype homogeneity (GD = 0.12) which may reflect their common origin. The three other sets obtained from the CA analysis consist of a large number of genotypes out of which nearly 80% are 'local' genotypes which are specific to one of the three regions.

Only eight genotypes (Table 3) observed in 11.3% of the individuals analysed were also found in varieties growing in other countries of the Mediterranean Basin. These varieties were 'Belgentier' and the French 'Picholine' originating from France (genotypes No 9 and 49 respectively), 'Picual' and 'Arbiquine' from Spain (genotypes 13 and 24 respectively), 'Barouni' and 'Meski' from Tunisia (genotype 17 for the both), 'Sigoise' from Algeria (genotype 27), 'Koroneiki' from Greece (genotype 29) and 'Ayvalik' from Turkey (genotype 17) (Ouazzani et al., 1993; 1995). Five of these genotypes (Nos 13, 17, 24, 27 and 29) were found in the Aguedal olive grove (site No 5 located in Marrakech). Since the 12th century, this grove had been established by the several successive Moroccan Sultans as an olive collection constituted from cuttings of various geographic origins (Tornézy, 1922; Elant, 1946). Moreover, 6 Algerian and Tunisian varieties were identified in 1913 in the same region (Tornézy, 1922). Therefore, at least some of the eight genotypes which were also found outside Morocco may correspond to olive material introduced into Morocco.

Within and among populations genotype variation

The results obtained in the present study indicate that 12.5% of the genotypic diversity (GD) observed in the olive trees cultivated in Morocco results from genotypic differentiation among sites. The substantial variation for multilocus genotype diversity per site among the 10 samples studied may reflect ecological and historical differences and also variation in vegetative multiplication techniques. In the regions where ecological conditions (more particularly rainfall and edaphic conditions) favour the occurrence of wild olive trees which possess high genetic diversity (Ouazzani, 1993; Ouazzani et al., 1993; Ouazzani et al., 1994) both very high and very low genotype diversity was observed in cultivated olive material. The former situation was found in the mountain regions of the South of Morocco (e.g. at Imouzzer, Tazmourt and Igherm) where the grafting method was not used. In these areas, olive cultivation is not intensive and is limited to fruit picking (no pruning). In that region, olive groves are often neglected so that seeds from crosses between distinct cultivars or between wild/or 'feral' trees and cultivars have a high probability to produce adult trees. Local selection of noticeable trees derived from crosses involving wild material and which were subsequently multiplied vegetatively may have occurred and may be partly responsible for the high genotype diversity observed. In previous studies dealing with the same enzyme loci as those analysed in the present study, average heterozygosity was systematically found to be significantly higher in wild than in cultivated olive material. (Ouazzani, 1993; Ouazzani et al., 1993). This difference was considered to be probably due to the series of combinations of both sexual (mostly allogamous) reproduction in the wild trees and empirical selection in the cultivars leading to the fixation of genes in a homozygous condition (Ouazzani et al., 1993). The high average heterozygosity observed in cultivated olive from the South of Morocco, more particularly in Imouzzer and Tazmourt, may thus constitute further evidence for empirical (very weak) selection of wild or of 'feral' trees in this region and their use as cultivated varieties. Conversely, in the Ouazzane region (North)

where olive production is more intensive and where grafting on wild olive has predominated for many centuries, the low genotype diversity could be related to this method of multiplication which allows easy and fast propagation of a single high quality variety (i.e. genotypes showing a low average heterozygosity) over large areas. In the other regions, long shoots (3 or 4 per each new tree) were used for vegetative multiplication. To cover large areas, these shoots were cut from numerous trees which corresponded probably to several distinct genotypes. Moreover, as reported above, the high genetic diversity observed in the Marrakech orchard is probably due to the introduction of allochtonous olive material to that area.

Genotype variation and genetic resources in olive trees cultivated in Morocco

The present study was restricted to the analysis of a few polymorphic enzyme loci in ancient olive populations cultivated in several geographically and ecologically different regions of Morocco. Sixty of the 68 distinct multilocus genotypes observed in that country, which were mostly 'local' genotypes, were not present in the 47 varieties studied previously (Ouazzani et al., 1995) from several other countries located around the Mediterranean Basin. Such large original genotype variation reveals a substantial complexity (many local cultivars even within one population) in the varietal structure of the Moroccan olive, as is also the case in other Mediterranean countries (Ruby, 1918; Rugini & Lavee, 1992). More particularly, from the results of the present study, evidence was obtained to refute the common idea that only a single variety called 'Picholine marocaine' occurs in Morocco. Several other more localised varieties which can be distinguished both morphologically and by their multilocus genotypes at the eight enzyme loci, are also present in that country.

The substantial genetic variation observed in the main traditional Moroccan olive populations constitutes a wide range of potential genetic resources available for breeding programs. For instance, agronomic evaluation and the conservation of the several genotypes observed in the present study should be carried out as soon as possible. Gardens should be established in several locations to provide dynamic conservation of the genotypes. Moreover, the number of polymorphic enzyme loci studied should be increased to improve varietal discrimination and a further survey of olive populations should be organised in Morocco, in other areas of traditional cultivation which have not been yet investigated.

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