Molecular Characterization of Common Olive Varieties in Israel and the West Bank Using Randomly Amplified Polymorphic DNA (RAPD) Markers

Z. Wiesman¹

Institutes for Applied Research, The Institute of Agriculture and Applied Biology, Ben-Gurion University of the Negev, P.O. Box 653, Beer-Sheva 84105, Israel

N. Avidan and S. Lavee

Institute of Horticulture, ARO, The Volcani Center, Bet Dagan, Israel

B. Quebedeaux

Department of Natural Resources Sciences and Landscape Architecture, University of Maryland, College Park, MD 20742

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ABSTRACT. RAPD analysis was performed on the main traditional and introduced varieties of olive grown in Israel and the West Bank. Primers were identified and used in combination to discriminate between different varieties. Significant biodiversity was demonstrated among 'Nabali' olive trees growing along the central mountain ridge of the West Bank, suggesting that the grouping known as 'Nabali' is actually a mixture of genetically distinct variants. On the other hand, RAPD profiles of selected variants of 'Souri', cultivated mainly in the northern mountains of Israel, revealed a high degree of similarity, indicating that these variants represent environmental phenotypes of the same genome. Molecular differences were demonstrated between the 'Nabali' group variants and 'Souri'. Other more recently developed or introduced varieties showed individually distinct RAPD profiles. Possible future developments based on these data are mentioned.

Olive cultivation originated in antiquity on the eastern shores of the Mediterranean basin (Zohary and Spiegel-Roy, 1975), and the olive is still a basic constituent of the diet in most of the region. The current perception that olive oil is a healthy source of fats has led to its adoption in many countries situated outside the confines of the Mediterranean basin, and today production of olive oil is valued at ≈ 10 billion dollars. The statistics indicate that the volume of production is still rising (International Olive Oil Council, 1992).

Many of the olive trees growing in Israel and the West Bank are hundreds of years old. Their oil yield is high and they are naturally adapted to arid conditions (Zohary and Hof, 1988), but they are highly variable in fruit yield. The main traditional varieties are 'Souri' and 'Nabali'. 'Souri' trees are mainly distributed in the northern part of Israel, while 'Nabali' trees predominate along the central mountain ridge of the West Bank. The fruit of 'Souri' trees is of small to medium size (2 to 3 g), with a very high oil content (30% to 32%). 'Souri' is well adapted to the dryland cultivation system prevailing in many regions of Israel, but it is slow growing and very susceptible to *Cycloconium elaginum*, L. the pathogen that causes leaf spot disease (Miller, 1949; Papo and Peleg, 1952; Shabi et al., 1994). In humid valleys this disease causes excessive defoliation during the winter months, resulting in severe reduction in fruit production. 'Nabali' trees are faster growing, but they too are susceptible to *C. elaginum*. Their fruit are somewhat bigger (≈ 3 to 4 g), the stone is smaller but

they contain less oil than 'Souri' fruit (Gur, 1948).

'Barnea' is a new variety selected in recent years from seedling populations thought to have originated from the free pollinated 'Chimlali' (Lavee et al., 1986). The trees of 'Barnea' are vigorous and highly productive, and their fruit serve both as a source of oil and as a black table olive. The oil content of the 'Barnea' olive at maturation is $\approx 19\%$ to 22% even when irrigated to produce high fruit yields, and oil yield per hectare is therefore high. 'Manzanillo', the Spanish table olive variety introduced to the region in the early 1930s from the US (Gur, 1948), is the major pickling olive in Israel. Other introduced varieties, e.g., 'Santa Caterina', 'Kalamata', and 'Picholine', as well as the locally selected 'Muhasan' and 'Maalot', can be found in some olive orchards.

There is much confusion and uncertainty concerning the identity of the olive trees in the region. In view of the considerable variability in performance and phenotypic development observed by Gur (1948) and Lavee (1990) among 'Souri' and also among 'Nabali' trees, it has been suggested that they belong to groups or mixtures of different variants. On the other hand, trees locally assigned to different varieties could prove to be genetically identical. Fortunately, PCR-based marker systems which might shed light on the problem have recently become available, the most widely used system being RAPD (Random Amplified Polymorphic DNA) (Newbury and Ford-Lloyd, 1993; Welsh and McClelland, 1990; Williams et al., 1990). In this method, short oligonucleotides of arbitrary sequences are used singly to amplify regions of the test plant genome. The amplification products are then separated by gel electrophoresis, differences between genotypes being reflected as differences in the number and size of the fragments amplified. RAPD technology has been used to detect polymorphisms in wheat (D'Ovidio et al., 1990), maize (Shattuck-Eidens et al., 1990), barley (Weining and Langridge, 1991), cocoa (Wilde et al., 1992), grape (Gogorcena et al., 1993) and other crops. Recently, Bogani et al. (1994), Fabbri et al. (1995), Cresti

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¹To whom reprint requests should be addressed.

Fig. 1. RAPD patterns obtained for 'Nabali' variants growing in three different villages in the West Bank using primers OPZ-10, OPZ-12, OPZ-9 and OPZ-11. Lanes 1 to 8: S-5, S-7, S-8, F-4, F-5, F-6, SA-6, and SA-10. Lane M: DNA size markers (Marker IV, BM, Germ.).

et al. (1996), and Wiesman et al. (1997) reported about the advantages of using RAPDs for identification of olive varieties. The present study represents an attempt to characterize important olive varieties grown in Israel and the West Bank at the molecular level using the RAPD system, with particular emphasis on the two local olives 'Souri' and 'Nabali'

Materials and Methods

PLANT MATERIAL. The olive plants used in this study were propagated vegetatively. Germplasm evaluated included: 'Nabali' variants originating from three West Bank villages, Shwaiki (S), Farcha (F), and Salfit (SA); four commercial 'Souri' variants of well-defined origin ('Amka', 'Buchbinder', 'Latrun', and 'Lud'); and six accessions, from the olive collection in Israel, namely 'Manzanillo-IT' (local variant of the Spanish 'Manzanillo'), 'Souri' (nonselected), 'Barnea', '80-B-69' (hybrid of 'Barnea'), 'Muhasan', and 'Maalot'.

DNA ISOLATION. Olive leaf tissue was collected in the greenhouse and/or the orchard and placed in a container with liquid nitrogen. For each variety, genomic DNA was extracted from at least three different individuals (unless otherwise specified) using the protocol described by Doyle and Doyle (1987). The concentration of DNA was determined by subjecting the samples to 0.7% agarose gel electrophoresis in TAE buffer (Sambrook et al., 1989) and comparing band intensities to lambda DNA standards, and by fluorometry using a Hoefer DNA fluorometer.

POLYMERASE CHAIN REACTION. A set of 20 decanucleotides of arbitrary sequence (Kit Z) obtained from Operon Technologies (Alameda, Calif.) were employed for PCR amplification. The 25- μ L reaction mix contained approximately 5 ng of template DNA,

0.2 mM of a single decanucleotide, 200 mM each of deoxyribonucleotide triphosphate (Boehringer, Mannheim, Germany), and 0.75 units of Taq polymerase in the incubation buffer provided by the manufacture of the enzyme (Boehringer, Mannheim, Germany). The mixture was overlaid with two drops of mineral oil. Amplification was achieved in a PTC-100 thermal cycler (M.J. Research) programmed as follows: step 1, 2 min at 94 °C; step 2, 30 s at 94 °C; step 3, 1 min at 35 °C; step 4, 2 min at 72 °C; step 5, repeat from step 2, 45 cycles; and step 6, 10 min at 72 °C. Amplification products were subjected to electrophoresis in 1.4% agarose gels and detected by ethidium bromide staining, viewed under ultraviolet light and photographed using a Sony CCD video camera. Molecular weights were estimated using a DNA molecular weight marker VI kit (Boehringer, Mannheim, Germany). The reactions were repeated at least three times. Each reproducible amplification fragment that could clearly be observed was assigned a code-name reflecting the primer used and its approximate size in base pairs and was scored for its presence or absence in the genotype analyzed.

STATISTICAL ANALYSIS. Data generated from RAPD gels were analyzed using the Nei similarity index (Nei and Li, 1979), which excludes common negative data, according to the following equation: Similarity = $2Nab/(Na + Nb)$, where Nab is the number of scored amplification fragments with the same molecular weight common to genotypes a and b, Na the number of scored amplification fragments in genotype a, and Nb the number of scored amplification fragments in genotype b. Confidence intervals were calculated using binomial variance analysis. Cluster analysis of olive varieties common in Israel was performed using a JMP program (SAS Institute, Cary, N.C.), and a dendrogram was drawn.

Results and Discussion

RAPD MARKER CHARACTERIZATION OF 'NABALI' VARIANTS ORIGINATING FROM DIFFERENT VILLAGES IN THE WEST BANK. RAPD marker profiles were generated from eight 'Nabali' variants collected from three different villages in the West Bank (Fig. 1). Among the 20 primers used, 14 revealed polymorphisms in the accessions examined. Eighty bands out of a total of 198 (40.4%) were found to be polymorphic.

The amplification products obtained with the 14 primers were analyzed pairwise to compare genetic relationships between the eight 'Nabali' variants (Table 1). Based on similarity matrix and confidence interval analysis, it was difficult to find significant differences within groups. Among the individuals collected from the village of Shwaiki, the pair S-7/S-8 scored the highest similarity values. Values for the pair F-4/F-5 are higher (0.906 ± 0.04) than for the other pairs of individuals collected from Farcha, with the pair F-4/F-6 occupying the bottom of the range (0.799 ± 0.06).

Table 1. Similarity matrix for eight 'Nabali' variants collected in the West Bank villages Shwaiki (S), Farcha (F), and Salfit (SA). Values are given $\pm$ confidence interval (0.95).

	S-5	S-7	S-8	F-4	F-5	F-6	SA-6	SA-10
S-5	1.000							
S-7	0.809 ± 0.03	1.000						
S-8	0.807 ± 0.02	0.852 ± 0.03	1.000					
F-4	0.819 ± 0.05	0.824 ± 0.05	0.870 ± 0.06	1.000				
F-5	0.757 ± 0.06	0.763 ± 0.05	0.828 ± 0.02	0.906 ± 0.04	1.000			
F-6	0.712 ± 0.04	0.729 ± 0.03	0.786 ± 0.04	0.799 ± 0.09	0.846 ± 0.05	1.000		
SA-6	0.753 ± 0.04	0.785 ± 0.04	0.841 ± 0.05	0.876 ± 0.06	0.891 ± 0.07	0.875 ± 0.03	1.000	
SA-10	0.746 ± 0.06	0.740 ± 0.03	0.782 ± 0.04	0.863 ± 0.05	0.874 ± 0.04	0.859 ± 0.05	0.954 ± 0.04	1.000

Fig. 2. RAPD patterns obtained for commercial 'Souri' variants using primers OPZ-10, OPZ-12, OPZ-18 and OPZ-20. Lanes 1 and 2: 'Souri Buchbinder'; lanes 3 and 4: 'Souri Latrun'; lanes 5 and 6: 'Souri Amka'; lanes 7 and 8: 'Souri Lud' (two different trees of the same variant are represented in each of lane of every variant). Lane M: DNA size markers (Marker IV, BM, Germ.).

The two individuals from Salfit exhibit a very high level of similarity (0.954 ± 0.04), suggesting that they were propagated from the same mother source. The interaction between the Shwaiki and Farcha groups suggests that a roughly uniform genetic distance separates the individual Shwaiki accessions from F-5 and F-6. S-8 lies at the top of the similarity range in the interaction with F-5 (0.828 ± 0.02), while S-5 scores near the bottom in the interaction with F-6 (0.712 ± 0.04). Interestingly, F-4 lies in the upper range of similarity, thereby showing a higher degree of similarity to the individuals from Shwaiki than the other two from Farcha. Once again, the interaction between the Shwaiki and Salfit groups points to a generally uniform genetic distance between individuals from the two villages, with S-8 lying at the top of the similarity range. The interaction between the Farcha and Salfit groups uncovered a relatively narrow range of similarity. From the similarity matrix shown in Table 1, it is possible to make out a group that includes the individuals from Shwaiki and, unexpectedly the individual F-4 (from Farcha), which may suggest on a relatively close relationship. It is also possible to distinguish another group composed of the three individuals from Farcha interacted with the two from Salfit, and of course the uniform group of two individuals from Salfit.

The relatively large number of polymorphic markers found for the eight 'Nabali' variants suggests that the horticultural diversity which they display is at least partly attributable to genetic causes. Similar biodiversities have been detected among olive varieties common in the Middle East using diverse indicator methods (Fabbri et al., 1995; Pontikis et al., 1980; Quazzani et al., 1993; Trujillo et al., 1995).

In many orchards in the West Bank, the practice has been to graft scions onto seedlings taken from the local tree population. Shoots from these seed-propagated rootstocks may conceivably have been used as scions over the years. Because 'Nabali' trees are mostly self fertile (Lavee, unpublished), phenotypic variation is often relatively small, and new genetic variants tend to remain within the broad limits of phenotypic expression of the variety. Consequently, the pattern of genetic diversity obtained for 'Nabali' in this study (Fig. 1, Table 1) could eventually tell us something about how and where local plant material was propagated and distributed among growers of the West Bank.

RAPD MARKER CHARACTERIZATION OF VARIOUS 'SOURI' VARIANTS. Analysis of the 'Souri' accessions revealed a very high degree of similarity, or even genetic identity. As shown in Fig. 2,

the RAPD profiles obtained for the 'Souri' variants 'Buchbinder', 'Amka', 'Lud' and 'Latrun' were identical for four different primers (OPZ-10, OPZ-12, OPZ-18, OPZ-20). A very similar picture emerged when all 20 primers were examined. From the similarity matrix constructed for the four variants (Table 2), it can be seen that similarity values were close to 1.000 for all pair combinations. Based on our RAPD data, then it would appear that the method of vegetative propagation practiced in Israel for many years (Wiesman and Lavee, 1995) has maintained the genetic trueness-to-genotype of the 'Souri' variety. Hence the diversity of horticultural characteristics observed in the variants tested here cannot be attributed to genetic differences, and phenotypic variations are probably due to differential gene expression in response to varying environmental conditions and growing practices. However, the possibility cannot be ruled out that examination of additional primers will uncover polymorphisms in the future; a study currently under way on additional variants of 'Souri' could reveal molecular differences between them.

Figures 1 and 2 illustrate the very considerable differences found between the RAPD profiles of 'Souri' and those of 'Nabali'. Primer OPZ-10 indicated a relatively low level of polymorphism among 'Nabali' variants, while primer OPZ-12 showed considerable polymorphism in the same accessions (Fig. 1). In 'Souri', on the other hand, primer OPZ-12 revealed uniformity (Fig. 2). Pairwise analysis between 'Souri' and 'Nabali' variants (not shown) produced similarity values ranging between 0.635 and 0.738, again pointing to significant genetic diversity between the two groups. These results are in agreement with data reported by

Table 2. Similarity matrix for four 'Souri' variants common in different nurseries in Israel. Values are given $\pm$ confidence intervals (0.95) for each pair.

	Buchbinder	Latrun	Amka	Lud
Buchbinder	1.000			
Latrun	0.948 ± 0.04	1.000		
Amka	0.986 ± 0.02	0.961 ± 0.04	1.000	
Lud	0.964 ± 0.02	0.950 ± 0.05	0.977 ± 0.02	1.000

Fig. 3. RAPD patterns obtained using primers OPZ-9, OPZ-11 and OPZ-13 for six olive varieties commonly grown in Israel. Lane 1: 'Barnea'; lane 2: 'Souri'; lane 3: 'Muhasan'; lane 4: 'Maalot'; lane 5: '80-B-69'; and lane 6: 'Manzanillo-IT'. Lane M: DNA size markers (Marker IV, BM, Germ.).

Table 3. Similarity matrix for six olive varieties commonly grown in Israel. Values are given $\pm$ confidence intervals (0.95) for each pair.

	Barnea	Souri	Muhasan	Maalot	80-B-69	Manzanillo-IT
Barnea	1.000					
Souri	0.862 $\pm$ 0.03	1.000				
Muhasan	0.839 $\pm$ 0.04	0.859 $\pm$ 0.05	1.000			
Maalot	0.836 $\pm$ 0.09	0.803 $\pm$ 0.05	0.847 $\pm$ 0.04	1.000		
80-B-69	0.930 $\pm$ 0.04	0.847 $\pm$ 0.07	0.846 $\pm$ 0.09	0.838 $\pm$ 0.05	1.000	
Manzanillo-IT	0.772 $\pm$ 0.02	0.818 $\pm$ 0.05	0.848 $\pm$ 0.08	0.809 $\pm$ 0.04	0.779 $\pm$ 0.02	1.000

Fabbri et al. (1995). Our findings regarding the RAPD profiles attest to significant genetic variability within part of the local olive germplasm in Israel and the West Bank, an important finding with relevance for the development of new varieties with improved horticultural traits.

SPECIFIC FINGERPRINTING OF SOME OLIVE VARIETIES COMMONLY GROWN IN ISRAEL. Using the 20 different arbitrary primers detailed above, RAPD PCR was performed on six olive varieties commonly grown in Israel. Each of the varieties was found to be distinguished by RAPD polymorphisms. Nineteen primers yielded polymorphic banding patterns, and out of a total of 239 amplified fragments, 125 (52.3%) proved to be polymorphic.

RAPD profiles obtained for the six olive varieties using three different primers are shown in Fig. 3. Preliminary relationships among the six olive accessions were determined based on pairwise analysis of the amplification products obtained with the 20 primers. '80-B-69' is located in the upper range (0.930 $\pm$ 0.04) of similarity values generated by pairwise analysis of 'Barnea' and the other varieties (Table 3). Since '80-B-69' is a hybrid of 'Barnea', this result is not surprising. 'Souri', 'Muhasan' and 'Maalot' lie in the middle range of similarity values, while 'Manzanillo-IT' is located in the lower range (0.772 $\pm$ 0.02). Similarities obtained for 'Souri', 'Muhasan' and 'Maalot' paired with the other varieties were restricted to a narrow range. In general, among the varieties tested, those presumed to originate in Israel ('Barnea', 'Souri', 'Muhasan', 'Maalot', and '80-B-69') showed a relatively high level of mutual similarity, although common parentage (from 'Chimlali') is probable only for 'Barnea', '80-B-69' and 'Maalot' (Lavee, unpublished). Our data are in agreement with reports indicating that the native olive trees distributed in the hills and mountains of Israel and the West Bank share many basic horticultural characteristics—presumably related to adaptation to the dry environment—while showing significant genetic diversity (Gur, 1948; Lavee, 1990).

Interestingly, a relatively high level of similarity was found between 'Maalot', a recently selected variant resistant to *C. elaginum*, and the somewhat resistant olive varieties 'Barnea' and '80-B-69' (similarity values of 0.836 $\pm$ 0.09 and 0.838 $\pm$ 0.05, respectively, Table 3). However, it should be kept in mind that all three varieties probably descend from 'Chimlali', which is resistant to leaf spot disease. On the other hand, similarity of the same order was also found between 'Maalot' and 'Muhasan' (0.847 $\pm$ 0.04), which is highly susceptible to the disease. A research currently under way for specific markers for tolerance to leaf spot disease may shed some light on this topic.

Cluster analysis of the six olive varieties (Fig. 4) revealed two well-defined clusters, one consisting of 'Barnea' and '80-B-69' and the other of 'Souri' and 'Muhasan'. The two latter varieties have long been known to have originated locally (Gur, 1948). 'Maalot' was found to be closely related to 'Barnea' and '80-B-69'. These findings are readily interpreted in the light of the common 'Chimlali' parent (Lavee, unpublished data). Fabbri et al.

(1995) reported that in their study molecular differences in olive varieties were not correlated with geographic origin. In the present case the fact that 'Manzanillo-IT' was not included in either cluster (Fig. 4) and seems to be genetically distinct from most of the other varieties tested (Table 3) suggests that it may be possible in some cases to relate certain genetic differences to geographic origin. To resolve this issue, further studies involving additional varieties and geographical areas will have to be carried out.

In total, 20 RAPD markers were analyzed for specificity to the six varieties examined. All the markers had been observed in the RAPD profiles of at least three different individuals of each variety. Two RAPD markers (OPZ13 530 and OPZ20 480 bp) were identified as specific to 'Barnea'. Four RAPD markers were characterized for 'Souri' (OPZ6 1100, OPZ7 700, OPZ11 1100, OPZ13 830 bp) and for 'Maalot' (OPZ5 360, OPZ17 720, OPZ19 280, OPZ20 900 bp). Three markers were specific to 'Manzanillo-IT' (OPZ3 600, OPZ11 235, OPZ17 225 bp), and two to '80-B-69', the hybrid of 'Barnea'. Five markers specific to both 'Barnea' and '80-B-69' were also characterized. No specific markers were discovered for 'Muhasan', but for that variety bands were specifically absent in three markers (OPZ10 650, OPZ15 390, OPZ18 920 bp). These markers could be exploited to select horticulturally improved olive genotypes with high adaptation to dry cultivation and/or unique oil quality, etc., among olives now growing in Israel and the West Bank. In addition, the local plant material identified and characterized in this study could be used in the future to develop a breeding program.

In summary, RAPD profiles revealed significant biodiversity among 'Nabali' olive trees, indicating that they belong to several genetically different variants and should be regarded as constituting a group. 'Souri', on the other hand, seems to be a genetically well-defined variety. Significant genetic differences were found between the 'Souri' and the 'Nabali' variants. Typical molecular fingerprinting analysis using a combination of RAPD primers was successfully used to discriminate between all the olive varieties commonly grown in Israel and tested in this study.

Fig. 4. Dendrogram illustrating genetic distances between six olive varieties commonly grown in Israel. The dendrogram was obtained on the basis of 20 RAPD markers using the JMP program for cluster analysis. The values included in the dendrogram indicate the distances between clusters.

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