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Intra-Genetic Variation within Olive Cultivar 'Nabali' in Palestine by Microsatellite and Random Amplified Polymorphic DNA

Aziz SALAMEH ***Soha GEZAEIL*** ***Alaa LAHLOOH*** ***Dina ARAFAT***

Department of Plant production and Protection, National Agricultural Research Center, PALESTINE

** Corresponding author (Sorumlu yazar): azbarghouthi@hotmail.com*

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ABSTRACT: *Over seventy olive trees (Nabali) from different regions in Palestine were used in this study. Intra-genetic variation within different olive Nabali variants were approved by SSR and RAPD markers. Four SSRs bands were monomorphic revealing a true-to-genotype of Nabali cultivar. Ten RAPD markers produced 60 reproducible bands with an average of 6 bands / marker. Only 24 were polymorphic. The percentage of polymorphic bands was 38% which is relatively high. Similarity matrix for studied populations ranged from moderate (0.610) for Jalkamous and Karawa Bani Zaid (2) to highly genetic similarity or even identity (1.000) in some cases as Aqraba and Aseerah (N). The interaction between different variants trends to be high. The effect of geographic location was absent in this study and has no significant contribution. Dendogram based on Jaccards coffiecnt revealed three main clusters, the biggest group consisted of the majority of variants including Bieta, Karawa Bani Zaid (1), Salfeet, Salfeet (h), Aqraba, Aseereh (N), Jalkamous, and Alaroub. Second group consisted of two variants Alaar and Nahaleen. The third group is containing only Karawa Bani Zaid (2). The relative high polymorphic bands of RAPD markers (40%) and moderate genetic similarity among different Nabali variants suggested attribution of genetic background. Selection for new traits within Nabali is suggested.*

Keywords*: Olive, Intra-Genetic Variation, SSR, RAPD.*

INTRODUCTION

Olive (*Olea europaea* L.) is as an important oil-producing crop in the Mediterranean region whose domestication occurred during the Chololithic period (5700–5500 years BC) in the Near-East (Zohary and Hopf, 1994). In Palestine, olive represents the most important fruit trees and growing for hundreds of years. Nabali is the most predominant cultivar and met around 75% of the total planted olive cultivars in Palestine. Nabali is adapted well with the environmental conditions with high productivity of olive oil (22-28%). The chemical parameters of its oil are met the International Olive Council (I.O.C.) standards for extra virgin olive oil except Δ-7-stigmastenolw which shows values higher than 0.5% (Qutub *et al*., 2010). Nabali is susceptible to olive leaf spot disease (*Spilocaea oleaginea*) and showed alternating bearing.

A high genetic diversity level and the presence of homonyms and synonyms cases observed in olive germplasm (Gomes *et al*., 2012), therefore, an efficient and rapid discriminatory methods are urgent. Molecular markers have been used successfully in olive characterization (Angiolillo *et al*., 1999; Khadari *et al*., 2003; Abdel hamid *et al*., 2012). Genetic variability among olive cultivars within a country was higher than olive cultivars from different countries (Belaj *et al*., 2003; Owen *et al*., 2005). Variability of 27 clones of the Portuguese olive cultivar was investigated by Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeat (SSR), and Inter Simple Sequence Repeat (ISSR) markers (Gomes *et al*., 2008), and the intra-variation within an olive cultivar has been approved (Muzzalupo *et al*., 2010; Ipek *et al*., 2012).

Though RAPD provides an inexpensive and reliable method for routine screening of large number of cultivars for olive germplasm collection (Belaj *et al.* 2004) and detecting genetic similarities in olive (Belaj *et al.* 2003). It's reproducibility still under question. Therefore, providing RAPD profile with other molecular technique could be a good option to support the obtained results. Recently, SSRs have become one of the most useful molecular markers in plant breeding, cultivar fingerprinting, and genome mapping genetic (Rallo *et al*., 2003).

In Palestine, genetic variation for Nabali has been studied and approved previously on eight olive trees from two sites only (Wiesman *et al*., 1998). No other studies were done to determine the intra-genetic variation within Nabali cultivar by using a large number of trees on large scale of area. Identification of genetic variation within Nabali could be a useful tool for breeding program in future in Palestine.

The objectives of this work are;

To identify by RAPD and SSR markers DNA fingerprints of Nabali cultivar, figure out the genetic relationship between them, and define the clones within the studied cultivar.

MATERIALS AND METHODS

Plant material consisted of fresh leaves of 72 trees tagged as Nabali cultivar. Trees were selected from thirty two villages located in eight governorates in Palestine (Table 1). The villages and trees were selected according to the extension agent's recommendations and knowledge. The samples were stored in paper bags in the field and at cold temperature in the lab until DNA extraction.

Genomic DNA was isolated from 100 mg leaf by ground into fine powder using pistil and mortar in the presence of liquid nitrogen. The leaf powder of each individual sample was then subjected to DNA extraction using DNeasy Plant Mini Kit (Qiagen). DNA quality was determined by visualization on 0.7% ethidium bromide agarose gel. Concentration of DNA for all samples was measured by S-30 spectrophotometer (BOECO, Germany) and uniformed to 20 ng/µl. Resulting DNA solutions were stored at -20 °C.

Amplification and genotyping four RAPD primers (opx-09, opj-05, opi-12, and opx-03) were tested (Table 2). All the 72 samples were amplified and analyzed with four RAPD primers. PCRs were performed in a total reaction mixture of 25 µL volumes containing 10 mM Tris-HCl, pH 8.2,50 mM KCl, 1.5 mM MgCl2, 200 mM primer, 0.2 unit of Taq DNA Polymerase (sigma) and 20 ng DNA. Amplifications were performed in PTC 100 thermocycler (MJ Research), initial denaturation at 94 °C for 1min, then 45 cycles at 92 °C for 1 min, 36 °C for 1 min, and 72 °C for 2 min and finally one cycle for 8 min at 72 C for elongation. Amplified products were fractionated by electrophoresis in 2% ethidium bromide Agarose gels (1× TAE buffer) and photographed.

Table 1. The Governorates and the villages where the leaves of Nabali olive trees were collected.

No	Governorate	Villages
1	Jenin	Biet Qad, dier Abu Daaf, Jalkamous, Maithloun, Sier, Alyamoun, Bourqeen, Yaabed, Arabeh, KuferRaa, and Markaa
2	Qalqelia	Jayous, BaqatAlhatab, and koufer Qadoum
3	Nablus	Qabalan, Aqraba, Biet Foureek, and Aseerah(N)
4	Tulkarem	Dier Ghosoun, Alaar, and Ras Rouman
5	Salfeet	Alwaraam, Salfeet[1], and Dierstya
6	Ramallah	Karawa Bani Zaid[2], Dier Gassaneh, and Biet Laqya
7	Bietlahem	Biet Jala, Nahaleen, and Tkouaa
8	Hebron	Alaroub and Dora,

[1]: In salfeet village two different olive variants were found. Thereafter in the text, salfeet and salfeet (h) used to distinguish between both.

[2]: In Karawa Bani Zaid village two different olive variants were found. Thereafter in the text, Karawa Bani Zaid (1), and Karawa Bani Zaid (2), used to distinguish between both.

Later on, in order to minimize the size of population (72 samples), only samples which showed at least a different RAPD profile bands or more were selected for regenotyping again with ten RAPD and six SSR primers in order to assess the genetic diversity (Table 2). Out of seventy two, eleven samples of Nabali were selected for second cycle of genotyping. SSR primers were described and used by Cipriani *et al*. (2002). SSR primers were amplified by PTC 100 thermocycler (MJ Research), initial denaturation at 94 °C for 2 min, then 35 cycles at 92 °C for 45s., 57 °C for 45s., and 72 °C for 45s., and finally one cycle for 8 min at 72 °C for elongation. Amplified SSR bands were fractionated by electrophoresis in 4% ethidium bromide garose gels (1×TAE buffer).

DATA analysis

Each SSR and RAPD fragments was treated as a unit character and was scored presence or absence of the band (1 or 0). The 1/0 matrix was prepared for all fragments scored and the data were used to generate Jaccard's similarity coefficients (1908) for RAPD bands depending on the following formula:

$$S_{ij} = a / (a+b+c)$$

Where Sij: standard Jaccard between two individuals i and j;

a = bands shared by both individuals;

b = bands present in i but not in j; and

c = bands present in j but not in I

Jaccard's coefficients were subjected to unweighted pair-group method using arithmetical averages (UPGMA) to generate a dendrogram using linkage procedure. The RAPD data were analyzed using fingerprint analysis missing DATA (FAMD version1.25) (Schlüter and Harris, 2006).

RESULTS

The aim of this work is determining the genetic diversity within Nabali cultivar in Palestine. Seventy two variants of Nabali from different areas were scored with four RAPD markers. All produced RAPD profiles were selected and analysed in next step with SSR and RAPD markers. Eleven Nabali variants were selected as a result of first RAPD running.

Table 2. RAPD and Olive SSR primers characterized in the study.

Type of primers	primer	Primer sequences (5′ to 3′)	Size of sequenced alleles (bp)
RAPD	OPA-19	CAA ACG TCG G	
	OPK-16	GAG CGT CGS A	
	OPX 09	GGT CTG GTT G	
	OPJ-06	CTC AGT CGC A	
	OPZ-11	GGG AAT TCG G	
	OPF-06	CCA GGA GGA C	
	OPJ 05	CTC CAT GGG G	
	OPZ-07	TCG TTC CGC A	
	OPI 12	AGA GGG CAC A	
	OPX 03	TGG CGC AGT G	
SSR	UDO99-006	TCA GTT TGT TGC CTT TAG TGG A TTG TAA TAT GCC ATG TAA CTC GAT	172
	UDO99-008	AAA AAC ACA ACC CGT GCA AT AAA TTC CTC CAA GCC GAT CT	159
	UDO99-024	GGA TTT ATT AAA AGC AAA ACA TAC AAA CAA TAA CAA ATG AGC ATG ATA AGA CA	188
	UDO99-031	TAT CCT CTA TGT GGC GAT TTG GTT AAA AGC ATT GAT ACA	151
	UDO99-043	TCG GCT TTA CAA CCC ATT TC TGC CAA TTA TGG GGC TAA CT	174
	UDO99-039	AAT TAC CAT GGG CAG AGG AG CCC CAA AAG CTC CAT TAT TGT	170

Only four SSR markers produced readable bands (UDO99-006, UDO99-024, UDO99-039, and UDO99-043). All SSR markers were monomorphic with the eleven Nabali variants. This outcome confirmed that all studied and selected olive trees were Nabali and any presented genetic variation due to intra-variation within Nabali rather than mis–selection with other cultivar. Ten RAPD primers produced 60 reproducible bands with an average of 6 bands / marker. Out 60 bands only 24 were polymorphic. The percentage of polymorphic bands was 40% which is relatively high.

Based on Jaccards coefficient, similarity matrix for studied populations was done. Similarity ranged from moderate (0.610) for Jalkamous and Karawa Bani Zaid (2) to highly genetic similarity or even identity in some cases such as Aqraba and Aseerah (N) (1.000) (Table 3). Interaction between different variants showed high trends. For example the interaction between Nabali variants from Jalkamous and Alaroub was high (0.925) the same trend was found between variants Salfeet and Salfeet (h) (0.953), Aqraba and Aseerah (N) (0.952). On other side some exceptions were found, the interaction for Nabali variants from Karawa Bani Zaid, Bieta and Alaroub was (0.675).

The effect of geographic location was absent in this study and has no significant contribution. For instance for two variants from Aqraba and Aseerah (N) the similarity was 1.000. As well variant from Nahaleen (in the south of Palestine) with variants from Akarab, Aseerah (N), and Alaar (North of Palestine) were 0.914, 0.914, and 0.912, respectively. On other side, Karawa Bani Zaid (1) and Karawa Bani Zaid (2) are two variants from the same village showed low genetic similarity (0.667). The high similarity in this study suggested a genetic similarity or even identity between different variants from different locations. This could be due to fact that these variants come from the same gene pool.

Dendogram based on Jaccards coffiecnt revealed three main groups (Figure 1), the biggest group consisted of the majority of variants including eight Nabali varints; Bieta, Karawa Bani Zaid (1), Salfeet, Salfeet (h), Aqraba, Aseerah (N), Jalkamous, and Alaroub. Second group consisted of two variants Alaar and Nahaleen. While the third group is contains only Karawa Bani Zaid (2).

DISCUSSION AND CONCLUSIONS

Two types of molecular markers were used in this study, SSR and RAPD markers. SSR markers were monomrphic and produced the same profile for all Nabali variants. In opposite, microsatellites were effective tool to discriminate on intra-varietal genotypes level (Muzzalupo *et al.*, 2010; Ipek *et al.*, 2012). The negative result of SSR may be due to the low number of used markers and the small size of the population. The same copies of profile of microsatellite for all samples confirmed that, all selected trees belong to the same olive cultivar Nabali. As well, any existed genetic variation within the olive population were due to the

Table3. Similarity matrix of Jaccard's coefficient of 11 olive trees (variants) in different sites in Palestine.

	1	2	3	4	5	6	7	8	9	10	11
1	1.000										
2	0.868	1.000									
3	0.610	0.667	1.000								
4	0.770	0.829	0.788	1.000							
5	0.925	0.892	0.675	0.842	1.000						
6	0.814	0.893	0.675	0.842	0.864	1.000					
7	0.878	0.917	0.692	0.865	0.864	0.826	1.000				
8	0.878	0.865	0.692	0.865	0.910	0.867	0.953	1.000			
9	0.829	0.914	0.730	0.914	0.860	0.864	0.952	0.952	1.000		
10	0.829	0.914	0.730	0.914	0.860	0.864	0.952	0.952	1.000	1.000	
11	0.780	0.757	0.722	0.912	0.773	0.778	0.860	0.860	0.857	0.857	1.000

Abberiviation: 1: Jalkamous, 2: Karawa bani zied (1), 3: Karaw abani zied (2), 4: Nahaleen, 5: Alaroub, 6: Bieta, 7:Salfeet, 8: Salfeet (h), 9: Aqraba, 10: Aseerah(N), and 11: Alaar.

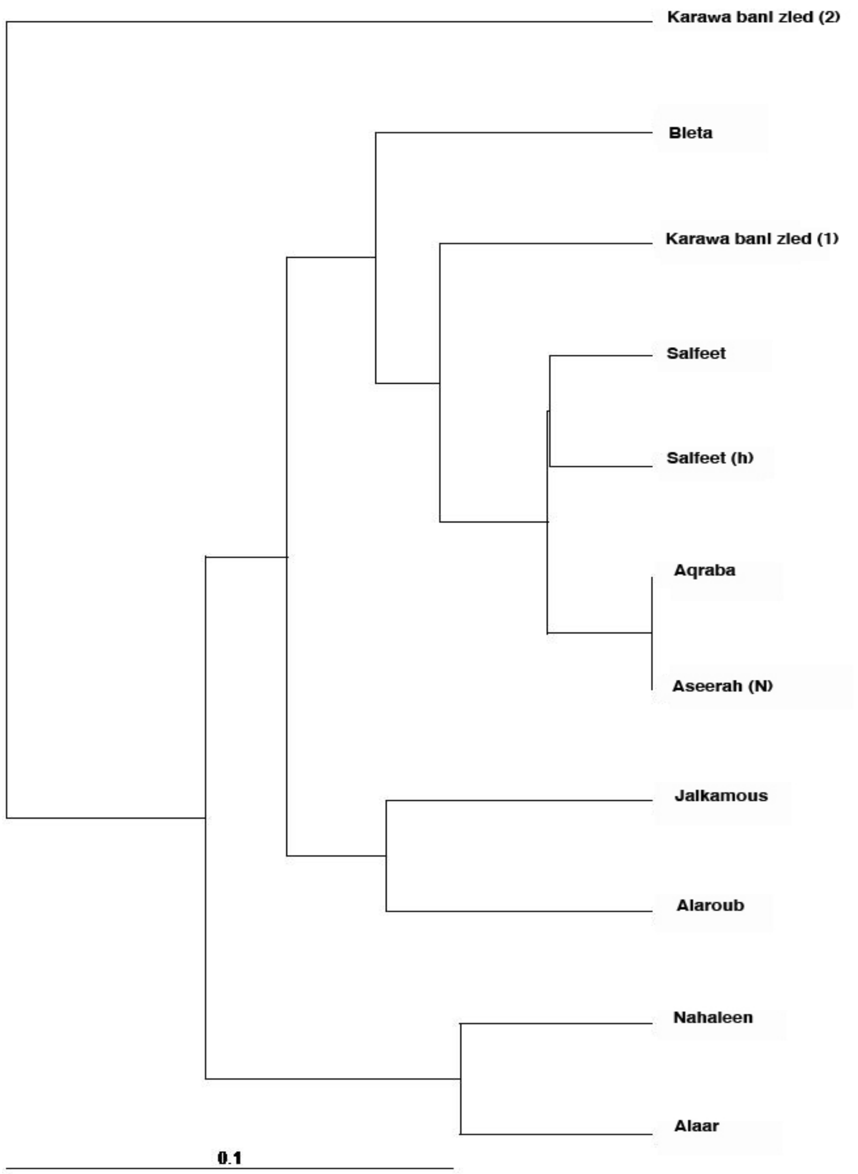

Figure 1. Dendogram based on Jaccards coefficient illustrating genetic similarity among eleven Nabali variants in Palestine based on 10 RAPD markers.

intra-genetic rather than inter-genetic variation level. In this study, RAPD were able to distinguish the genetic variation among different Nabali variants. Over seventy samples were screened with RAPD in first time. RAPD marker provides a cheap and fast tool for screening large population, which offers a good choice for researchers in developing countries (Belaj *et al*., 2003; Belaj *et al*., 2004). Combining two types of Markers SSR and RAPD in this study was good strategy to overcome the low reproducibility of RAPD.

Out of fifty eight, twenty two bands were polymorphic, the relative high polymorphic bands (40%) and moderate genetic similarity among different Nabali variants suggested attribution of genetic background. In Palestine, Nabali known as difficult rooting olive cultivar and grafting is the only and common vegetative propagation method. As well, sexual propagation is not used. Therefore, the variation due to somatic mutation within Nabli cultivar is possible since somatic mutations considered as an important source of intra-plant genetic variation (Salomonson, 1996). Genetic contribution within Nabali variants or other olive cultivars was indicated before. (Wiesman *et al*., 1998; Muzzalupo *et al*., 2010; and Ipek *et al*., 2012). The low-moderate genetic variation obtained in this study could be due to two main reasons. First, the predominant propagation method for olive in Palestine is grafting. Second, Nabali is self-pollinated cultivar. Therefore, the maintenance of cultivar is expected and could lead to minimize the genetic diversity within Nabali olive trees in Palestine.

In this study, two Nabali olive trees from Akarab and Aseerah (N) were completely genetically identity (1.00). This may due to the fact that Aseerah is known as a high quality olive oil producer village in Palestine and containing very old olive orchards which back to the Roman period. Therefore, trees from Aseerah (N) are expected to be used as a good source for scions which are used in grafting in many olive orchards. Oppositely, the similarity for two Nabali trees from Karawa Bani Zaid was moderate (0.667). In other cases, the genetic similarity was high for two Nabali variants from too far distance areas, Nahaleen (south of Palestine) and Aqraba (North of Palestine) (0.914). Therefore, the geographic contribution in genetic variation within Nabali variants is absent and eliminated.

The intra- genetic variation within Nabali variants is approved in this study. Few groups or lines of Nabali were found. The main group contains the majority of studied lines (eight variants). These group representatives the predominant Nabali variant in Palestine. Other lines of Nabali were found in this study. The available of these lines or genotypes is less.

Because Nabali variant is adapted well to the local conditions and producing oil with high quality, Nabali still the preferred olive variant for farmers in Palestine. Nabali is known as susceptible to peacock disease and showed an alternative bearing phenomena. The fact that there intra-genetic variation is existed within Nabali variants, justifies the selection for new lines with promising traits. In this study, several new lines (variants) of Nabali were identified. These variants will be subject for more research in order to evaluate its performance against important agronomic traits such as, alternative bearing phenomena and resistant to peacock disease, consequently, could be integrated in future in national breeding program.

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