Using Two Retrotransposon Based Marker Systems (IRAP and REMAP) for Molecular Characterization of Olive (Olea europaea L.) Cultivars

Ergun KAYA¹,², Emel YILMAZ-GOKDOGAN¹

¹Mugla Sıtkı Koçman University, Faculty of Sciences, Molecular Biology and Genetics Department, Kotehlı, 48000, Mugla, Turkey; ergunkaya@mus.edu.tr (corresponding author); emelyilmaz@mus.edu.tr
²Gebze Technical University, Molecular Biology and Genetics Dept, 41400, Gebze, Kocaeli, Turkey

Abstract

Olive (Olea europaea L.) is one of the most characteristic agricultural trees of the Mediterranean region and has a large number of cultivar diversity. Olive cultivar characterization is very important especially for the fruit productivity and olive oil quality. In the present study, 46 clones belonging to Turkey (eight cultivars, each having five clones) and Italy (two cultivars, each having three clones) were assessed for cultivar characterization via inter-retrotransposon amplified polymorphism (IRAP) and retrotransposon-microsatellite amplified polymorphism (REMAP) marker systems using 10 LTR and 10 ISSR primers. In total, 368 band profiles were obtained, 358 of which are polymorphic (97.28% polymorphism). The cultivars were segregated into three main groups, each group having several branches, where all the clones of each cultivar were belonging to the same main group. The only exception to that was the distribution of the clones of cultivar Yaglık, ‘Yaglık 4’ and ‘Yaglık 5’, into different main groups. IRAP and REMAP analysis showed a high level of genetic variability among the olive cultivars in this study and this marker systems would be useful tool for clonal selection programs.

Keywords: LTRs, olive cultivar diversity, molecular marker

Abbreviation list: CTAB (Cetyl trimethyl ammonium bromide); IRAP (Inter-retrotransposon amplified polymorphism); ISSR (Inter simple sequence repeat); LTR (Long terminal direct repeat); REMAP (Retrasposon-microsatellite amplified polymorphism).

Introduction

Olive (Olea europaea) has more than 2600 cultivars, and has been cultivated since the ancient times in the Mediterranean region, where it is the most significant oil-producing crop, the region accounts for not less than 97% of the world production and 91% of world consumption of olive oil (Luchetti, 1993; Rugini et al, 2001; Giordani et al, 2004). They cover about 50% of repetitive DNA of the whole genome in higher plants (Kumar and Bennetzen, 1999). Their unstable genomic locations, flexible copy numbers, length, described and conserved sequences provide them with an advantage to be a more specific genetic marker for plant biodiversity and genome analysis (Queen et al, 2004; Agarwal et al, 2008; Vukich et al, 2009; D’Onofrio et al, 2011; Corrado et al, 2013), SNPs (Reale et al, 2006), DArTs (Atienza et al, 2013).

Retrotransposons are mobile genetic elements through the eukaryotic genomes, especially in plants, they are considered to act an important role in genome evolution (Flavell et al, 1992; Vicent et al, 2001; Giorgetti et al, 2004). They cover about 50% of repetitive DNA of the whole genome in higher plants (Kumar and Bennetzen, 1999). Their unstable genomic locations, flexible copy numbers, length, described and conserved sequences provide them with an advantage to be a more specific genetic marker for plant biodiversity and genome analysis (Queen et al, 2004; Agarwal et al, 2008; Vukich et al, 2009; D’Onofrio et al, 2010; Kalender et al, 2011). Retrotransposons can be classified into three types according to their structural organization and amino acid similarities. Copia-like (Kumar et al, 1996) and gypsy-like retrotransposons (Suomi et al, 1998) belong to long terminal direct repeats (LTRs), they encode proteins similar to the retroviruses, and they are present over...
PCR DNA amplification was performed using a
Institute), Italy.

Plant Material

46 olive clones belonging to 10 olive cultivars were analysed. 8
cultivars were obtained from different cities of Turkey (Balıkesir cv.
‘Edincik’, Bursa-Gemlik cv. ‘Gemlik’; Hatay cv. ‘Edremit’; Mardin
cv. ‘Halhalı’, Muğla cv. ‘Domat’; Samsun cv. ‘Alacam’; cv. ‘Tekir’
and cv. ‘Yaglık’, obtained from Olive Research Institute, Izmir) while
the other 2 (cv. ‘Canino’ and cv. ‘Francois’) were obtained from CNR
(National Research Council) / IVALSA (Trees and Timber
Institute), Italy.

DNA Extraction

The total genomic DNA was extracted by using CTAB
method (Doyle and Doyle, 1987) after grinding the young leaf tissue
to a fine powder. DNA sample concentration was determined using
a nanodrop spectrophotometer (BioSpec-nano; Shimadzu-
Biotech). DNA samples were diluted to 50 ng/µl prior to IRAP and
REMAP PCR amplifications.

IRAP (Inter-Retroelement Amplified Polymorphisms) PCR

IRAP-PCR DNA amplification was performed using 10 IRAP
primers (LTR 1-13; Smykal et al., 2011; Table 1). Amplifications
were performed according to Kalendar et al. (2011) in a 25 µl
reaction volume, containing PCR Buffer (1x final concentration,
inviotrogen), 2.5 mM MgCl₂, 0.4 mM of each dNTP, 0.4 mM IRAP
primer, 50 ng genomic DNA, and 2 unit Taq DNA polymerase.
Amplification conditions (thermocycler Model-9700, Perkin-
Elmer, Boston, MA, USA) were as follows: initial denaturation at 95
°C for 3 min, 35 cycles at 95 °C for 15 sec, 55 °C for 30 sec, a ramp to
72 °C reaching in 3 min, followed by a 10 min lag at this
temperature, and an indefinite holdi
temperature, and an indefinite holdi

REMAP (Retrotransposon-Microsatellite Amplified
Polymorphism) PCR

REMAP-PCR DNA amplification was performed using a
combination of 10 LTR primers (0.2 mM for each reaction) and 10
ISSR primers, each primer at the concentration of 0.2 mM for each
reaction (Martins-Lopes et al., 2009; Smykal et al., 2011; Table 2).
Amplification conditions and separating were the same as for IRAP
PCRs (see above). DNA fragments of IRAP and REMAP PCRs
were scored by their presence (1) or absence (0), and the ones at low
intensities were scored only if they were reproducible in both the
PCR runs. Cluster analysis was performed to construct dendograms, with the unweighted pair-group method by
arithmetic averages (UPGMA) from the similarity data matrices
using Jaccard’s coefficient (D-UPGMA, 2002).

Results and Discussion

Molecular fingerprinting of forty six clones belonging to ten
cultivars was carried out using IRAP and REMAP analysis, and very
high polymorphism (97.28%, in average) was detected by both the
methods. The total of 368 reproducible bands, ranging from 125 to
3600 bp, were scored. 126 bands were obtained by IRAP and 242
were by REMAP techniques, with a similar polymorphism rates of
96.82% (122 polymorphic bands) and 97.52% (236 polymorphic
bands), respectively. The highest polymorphism rate was obtained
by REMAP PCR 2 amplification, and produced 23 polymorphic bands
(Fig 1).

The dendrogram of 46 clones belong to 10 olive cultivars
amplified by the IRAP markers is shown in Fig. 2. The genetic
similarities ranged from 0.013 (between Y1 cv. ‘Yaglık’ and T4-T5
cv. ‘Tekir’; between H1 and H2 cv. ‘Edremite’) to 0.75 (between B1
cv. ‘Edincik’ and S1 cv. ‘Alacam’), while T3 and T4 cv. ‘Tekir’ were
shown to be similar. The cultivars were grouped into eight clusters;
Cluster I, B1-5, (Balıkesir cv. ‘Edincik’); Cluster II, G1-5 (Bursa-
Gemlik cv. ‘Gemlik’), H1-5 (Hatay cv. ‘Edremite’); Cluster III, Ma
Kaya E et al. / Not Bot Horti Agrobo, 2016, 44(1):167-174

Cluster II and III divided into two sub-clusters (Fig. 2). On the other hand, according to the dendrogram of the REMAP markers, the cultivars were grouped into five clusters (Fig. 3; Cluster I, B1-5, (Balıkesir cv. ‘Edincik’); Cluster II, G1-5 (Bursa-Gemlik cv. ‘Gemlik’), H1-5 (Hatay cv. ‘Edremit’); Cluster III, Ma 1-5 (Mardin cv. ‘Halhali’), Mu 1-5 (Mugla cv. ‘Domat’); Cluster IV, S1-5 (Samsun cv. ‘Alacam’), T1-3 (cv. ‘Tekir’); Cluster V, Y1-3 (cv. ‘Yaglık’); Cluster VI, Y4-5 (cv. ‘Yaglık’), Ca1 (cv. ‘Canino’), Fr1-3 (cv. ‘Frantoio’); Cluster VIII, S1-3 (Samsun cv. ‘Alacam’). However, each cluster divided into some sub-clusters, for example, Cluster II and III divided into two sub-clusters (Fig. 2). On the other hand, according to the dendrogram of the REMAP markers, the cultivars were grouped into five clusters (Fig. 3; Cluster I, B1-5, (Balıkesir cv. ‘Edincik’); Cluster II, G1-5 (Bursa-Gemlik cv. ‘Gemlik’), H1-5 (Hatay cv. ‘Edremit’); Cluster III, Ma 1-5 (Mardin cv. ‘Halhali’), Mu 1-5 (Mugla cv. ‘Domat’); Cluster IV, S1-5 (Samsun cv. ‘Alacam’), T1-3 (cv. ‘Tekir’); Cluster V, Y1-3 (cv. ‘Yaglık’); Cluster VI, Y4-5 (cv. ‘Yaglık’), Ca1 (cv. ‘Canino’), Fr1-3 (cv. ‘Frantoio’); Cluster VIII, S1-3 (Samsun cv. ‘Alacam’). However, each cluster divided into some sub-clusters, for example, Cluster II and III divided into three sub-clusters (Fig. 3).
Genetic similarities/varieties were obtained with combined (IRAP and REMAP) UPGMA algorithm using Jaccard's coefficient (Fig. 4). The genetic similarities ranged from 0.068 (Ma 1 – Ma 2, Mardin cv. 'Halhali') to 0.705 (B1, Balıkesir cv. 'Edincik' – S1, Samsun cv. 'Alacam'). The cultivars were grouped into four major clusters: Cluster I, B1-5, (Balıkesir cv. 'Edincik'); Cluster II, G1-5 (Bursa-Gemlik cv. 'Gemlik'), H1-5 (Hatay cv. 'Edremit'), Ma 1-5 (Mardin cv. 'Halhali'), Mu 1-5 (Mugla cv. 'Domat'); Cluster III, S1-5 (Samsun cv. 'Alacam'), T1-5 (cv. 'Tekird'), Y1-3 (cv. 'Yaglık') and Cluster IV, Y4-5 (cv. 'Yaglık'), Ca1-3 (cv. 'Canino'), Fr1-3 (cv. 'Frantoio'). However, each cluster divided into some sub-clusters, for example Cluster I and IV divided into two sub-clusters, Cluster II divided into four sub-clusters, Cluster III divided into three sub-clusters (Fig. 4).

Some close relationships between cultivars were constant in all IRAP and REMAP analyses performed; for instance, cv. 'Gemlik' and cv. 'Edremit' (similarity ranges from 0.393 to 0.581); cv. 'Halhali' and 'Domat' (similarity ranges from 0.319 to 0.587) and Italian
Retrotransposon-based marker techniques have been extensively used to determine genetic relationships between numerous plant species and cultivars (Queen et al., 2004; Branco et al., 2007; Agarwal et al., 2008; Vukich et al., 2009; D’Onofrio et al., 2010). However, there have been a few reports on olive cultivar identification via retrotransposon-based marker techniques and one of them used SCAR marker (Hernández et al., 2001). Accordingly, the present report will be the first comprehensive study on molecular characterization of olive cultivars using both IRAP and REMAP molecular markers. All primers gave obvious amplification patterns. The co-dominant nature of these markers detected to higher levels of expected heterozygosity.

Although the two marker systems produced different cluster numbers in all cultivars according to the dendrogram analyses, high compatibility was obtained from both and their polymorphism rate was very similar (96.82% for IRAP and 97.52% for REMAP). The high level of polymorphism was detected with B1-5, Balıkesir cv. 'Edincik' by both the two marker systems; indeed this cultivar was very distant from the others and was grouped into different cluster (it cultivars 'Canino' and 'Frantoio' (similarity ranges from 0.245 to 0.379). On the contrary, B1-5, Balıkesir cv. 'Edincik' (Cluster I) indicated independent branches from the other cultivars (Fig 1) and this cultivar had many polymorphic bands in the most of PCR gel analysis (Fig 5A). On the other hand, the combined dendrograms indicated that clones Y1, 2 and 3 (Cluster III) and Y4 and 5 (Cluster IV) of cv. 'Yağlık' were in different groups. This was not surprising as there were many polymorphic bands in PCR gel analysis (Fig 5B).

Table 1. LTR and ISSR primers (Martins-Lopes et al., 2009; Smykal et al., 2011)

<table>
<thead>
<tr>
<th>LTR Primers</th>
<th>Sequence 5’-3’</th>
<th>Gene Bank accession number</th>
<th>ISSR Primers</th>
<th>Sequence 5’-3’</th>
<th>Gene Bank accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTR 1</td>
<td>ACCCCTTGAGCTAACCTTTGAGGTAAG</td>
<td>GU735096</td>
<td>ISSR 1</td>
<td>(AG),F</td>
<td>UBC 807</td>
</tr>
<tr>
<td>LTR 2</td>
<td>CTTAGCTGAAAGTGTGAGAGG</td>
<td>GU929374</td>
<td>ISSR 2</td>
<td>(GA),G</td>
<td>UBC 809</td>
</tr>
<tr>
<td>LTR 3</td>
<td>TGTTATTGACCTTAGTCGGGAAACCA</td>
<td>DQ767972</td>
<td>ISSR 3</td>
<td>(GA),T</td>
<td>UBC 810</td>
</tr>
<tr>
<td>LTR 4</td>
<td>AGCCCTGAAGAATTTTTGAGGAGG</td>
<td>GU980548</td>
<td>ISSR 4</td>
<td>(GA),C</td>
<td>UBC 811</td>
</tr>
<tr>
<td>LTR 5</td>
<td>CTGGCATTTTCTAGTGCAGT</td>
<td>GU980588</td>
<td>ISSR 5</td>
<td>(GA),A</td>
<td>UBC 817</td>
</tr>
<tr>
<td>LTR 6</td>
<td>GGCAGCCTGAGCCAGCTGTCC</td>
<td>GU929378</td>
<td>ISSR 6</td>
<td>(TC),C</td>
<td>UBC 823</td>
</tr>
<tr>
<td>LTR 7</td>
<td>CACCTCAAAATTTGAGCCAGCAGGACGATC</td>
<td>GU735096</td>
<td>ISSR 7</td>
<td>(AC),C</td>
<td>UBC 826</td>
</tr>
<tr>
<td>LTR 8</td>
<td>TCGAGATACACCCTTGCAGT</td>
<td>GU929377</td>
<td>ISSR 8</td>
<td>(AG),CTT</td>
<td>UBC 846</td>
</tr>
<tr>
<td>LTR 9</td>
<td>ATTCCTGTCCTGCTGCGCCCTTACA</td>
<td>GU980590</td>
<td>ISSR 9</td>
<td>(CA),AGT</td>
<td>UBC 855</td>
</tr>
<tr>
<td>LTR 10</td>
<td>TGAGTTGGAGCTGACAGGACGATC</td>
<td>GU980587</td>
<td>ISSR 10</td>
<td>(GT),CTA</td>
<td>UBC 856</td>
</tr>
</tbody>
</table>

Table 2. REMAP primer combinations

<table>
<thead>
<tr>
<th>Primer Combination</th>
<th>Primer Combination</th>
<th>Primer Combination</th>
<th>Primer Combination</th>
</tr>
</thead>
<tbody>
<tr>
<td>REMAP1</td>
<td>LTR 1 and ISSR 1</td>
<td>REMAP11</td>
<td>LTR 2 and ISSR 2</td>
</tr>
<tr>
<td>REMAP2</td>
<td>LTR 1 and ISSR 2</td>
<td>REMAP12</td>
<td>LTR 3 and ISSR 3</td>
</tr>
<tr>
<td>REMAP3</td>
<td>LTR 1 and ISSR 3</td>
<td>REMAP13</td>
<td>LTR 4 and ISSR 4</td>
</tr>
<tr>
<td>REMAP4</td>
<td>LTR 1 and ISSR 4</td>
<td>REMAP14</td>
<td>LTR 5 and ISSR 5</td>
</tr>
<tr>
<td>REMAP5</td>
<td>LTR 1 and ISSR 5</td>
<td>REMAP15</td>
<td>LTR 6 and ISSR 6</td>
</tr>
<tr>
<td>REMAP6</td>
<td>LTR 1 and ISSR 6</td>
<td>REMAP16</td>
<td>LTR 7 and ISSR 7</td>
</tr>
<tr>
<td>REMAP7</td>
<td>LTR 1 and ISSR 7</td>
<td>REMAP17</td>
<td>LTR 8 and ISSR 8</td>
</tr>
<tr>
<td>REMAP8</td>
<td>LTR 1 and ISSR 8</td>
<td>REMAP18</td>
<td>LTR 9 and ISSR 9</td>
</tr>
<tr>
<td>REMAP9</td>
<td>LTR 1 and ISSR 9</td>
<td>REMAP19</td>
<td>LTR 10 and ISSR 10</td>
</tr>
<tr>
<td>REMAP10</td>
<td>LTR 1 and ISSR 10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Retrotransposon-based marker techniques have been extensively used to determine genetic relationships between numerous plant species and cultivars (Queen et al., 2004; Branco et al., 2007; Agarwal et al., 2008; Vulich et al., 2009; D’Onofrio et al., 2010). However, there have been a few reports on olive cultivar identification via retrotransposon-based marker techniques and one of them used SCAR marker (Hernández et al., 2001). Accordingly, the present report will be the first comprehensive study on molecular characterization of olive cultivars using both IRAP and REMAP molecular markers. All primers gave obvious amplification patterns. The co-dominant nature of these markers detected to higher levels of expected heterozygosity.

Although the two marker systems produced different cluster numbers in all cultivars according to the dendrogram analyses, high compatibility was obtained from both and their polymorphism rate was very similar (96.82% for IRAP and 97.52% for REMAP). The high level of polymorphism was detected with B1-5, Balıkesir cv. 'Edincik' by both the two marker systems; indeed this cultivar was very distant from the others and was grouped into different cluster (it...
was seen in "Cluster I" for three dendrograms). This cultivar is very different from the others also for the morphological characteristics; it has relatively bigger fruits, low oil and high water content (Isik et al., 2011).

The dendrogram analyses almost fully matched with same clones, however there was some evidence for clustering of clones derived from different branches. Clone (cv. 'Yaglık') Y1-3 and Y4-5 were in different groups and their similarity ranges were between 0.419 and 0.480. These differences could be the result of cross-pollination with local populations (Contenuto et al., 2002), somatic mutations (Belaj et al., 2004), and sometimes could also be due to the presence of a high level of homonymy in the collection. This is a significant problem and is a great risk for olive producers, as the renewal of certified orchards should be based on certified plants (Gemas et al., 2004; Martínez-Lopes et al., 2007; Hannachi et al., 2008). However, classical olive certification system is based on morphological and agronomic procedures, which are affected by the environmental conditions, and mislabeling accessions can negatively affect certification of olive products (Hannachi et al., 2008).

Molecular marker systems are of great importance to overcome such problem, and is necessary to determine the polymorphism level of olive cultivars and homonymy and synonym problems in olive germplasm. High values of observed heterozygosity were recorded for all the IRAP and REMAP markers investigated.

Determination of genetic relationships among cultivars eases efficient sampling, operating and using of germplasm resources. In the present study, IRAP and REMAP analysis displayed a high level of genetic variability among olive cultivars, indicating a potential resource for the use of this germplasm in clonal selection programs.

Acknowledgments

The study was supported by Mugla Sıtkı Koçman University, Scientific Research Projects Coordination Unit (Mugla, Turkey, MSKU-BAP 15-005) and Gebze Technical University, Molecular Biology and Genetics Department, Plant Biotechnology Laboratory (Kocaeli, Turkey).

Conflict of interest

The authors declare that they have no conflict of interest.

References


Flavel AJ, Dunbar E, Anderson R, Pearce SR, Hartley R, Kumar A (1992). Tyl-1-copia group retrotransposons are ubiquitous and heterogeneous in...
higher plants. Nucleic Acids Research 20:3639-3644.


and Insertionally Polymorphic in Plants. Genome Resources 11(12):2041-2049.


