ISSR Analysis for Determination of Genetic Diversity and Relationship in Eight Turkish Olive (Olea europaea L.) Cultivars

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Abstract

Olive cultivar identification is very important especially for fruit productivity and olive oil quality. Up to date, numerous techniques have been used for characterization of olive cultivars and detection of genetic variability. In the present study, 40 clones specific to Turkey (8 cultivars, each having 5 clones) were assessed for cultivar determination via inter simple sequence repeat (ISSR) marker systems using 10 ISSR primers. In total, 217 band profiles were obtained, 206 of which were polymorphic (94.9% polymorphism). The cultivars were segregated into two main clusters, each cluster being subdivided into two groups, while all the clones of a cultivar belong to the same main cluster. The only exception was the distribution of the clones of cultivar ‘Tekir’: ‘Tekir 1’, ‘Tekir 2’ and ‘Tekir 3’ on cluster III, while ‘Tekir 4’ and ‘Tekir 5’ were on cluster IV, therefore grouped into different main clusters. In the present study, ISSR analysis displayed a high level of genetic variability among Turkish olive cultivars, indicating a potential resource for the use of this germplasm in clonal selection programs.

Keywords: cluster, ISSR, molecular marker, polymorphism, variability

Introduction

Olive (Olea europea L.), with more than 2,600 cultivars, is one of the oldest cultivated plants characteristic of the Mediterranean area, where it is the most important oil-producing crop (Bartolini et al. 2005; Rugini and Lavec, 1992; Zohary and Hopf, 1994). It is determined in two forms, former is wild (O. europaea subsp. europaea var. sylvestris) and the latter is cultivated (O. europaea subsp. europaea var. europaea). The cultivated olive (O. europaea L. var. europaea) is propagated by cutting or grafting whereas wild olive (O. europaea L. var. sylvestris) is reproduced from seeds (Green, 2002).

Olive trees have a high level of heterozygosity and genetic polymorphism is common among cultivars, so that they are predominantly allogamous (Angiolillo et al., 1999; Diaz et al., 2006; Rallo et al., 2000). This variability, coupled with the confusion in olive cultivar nomenclature, make necessary the evaluation and characterization of olive genetic resources that have been recognised as very important, since both olive productivity and oil quality are traits inherent to a variety (Fiorino and Rallo, 1999).

Inter simple sequence repeat (ISSR) markers have been effectively used to identify genetic variation among some olive cultivars (Gomes et al., 2008; Martins-Lopes et al., 2007; Martins-Lopes et al., 2009; Terzopoulos et al., 2005). ISSRs are repeated sequence motifs that are ubiquitously distributed throughout the plant genome. They can be easily amplified by PCR reactions using DNA nucleotide primers, the unique sequences flanking the repeated motifs. Polymorphic DNA fragments can be produced due to differences in the number of the repeat units. A number of ISSR markers have already been developed in olive and their primer sequences have been published (Hess et al., 2000; Gemas et al., 2004).

Determination of genetic relationships among cultivars eases efficient sampling, operating and using of germplasm resources. This study aims to determination of genetic diversity and relationship in foury Turkish olive (Olea europaea L.) clones belonging to eight cultivars via inter simple sequence repeat (ISSR) marker systems using ten ISSR primers.

Materials and methods

Plant material

Forty olive clones belonging to eight different olive cultivars (‘Edincik’, ‘Gemlik’, ‘Edremit’, ‘Halhali’, ‘Domat’, ‘Alacam’, ‘Tekir’ and ‘Yaglik’) were analysed. All samples were obtained from different parts of Turkey: Black Sea, Aegan (obtained from Aquaculture Central Research Institute), Izmir, Mediterranean and Southeast Anatolia.

DNA extraction

The total genomic DNA was extracted by using CTAB method (Doyle and Doyle, 1987) by 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/ml prior to ISSR PCR amplifications.
Table 1. ISSR primers and GenBank accession numbers

<table>
<thead>
<tr>
<th>ISSR Primers</th>
<th>Sequence 5’-3’</th>
<th>GeneBank accession</th>
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</thead>
<tbody>
<tr>
<td>ISSR 1</td>
<td>(AG)7T</td>
<td>UBC 807</td>
</tr>
<tr>
<td>ISSR 2</td>
<td>(AG)G</td>
<td>UBC 809</td>
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<tr>
<td>ISSR 3</td>
<td>(GA)T</td>
<td>UBC 810</td>
</tr>
<tr>
<td>ISSR 4</td>
<td>(GA)C</td>
<td>UBC 811</td>
</tr>
<tr>
<td>ISSR 5</td>
<td>(CA)A</td>
<td>UBC 817</td>
</tr>
<tr>
<td>ISSR 6</td>
<td>(TC)C</td>
<td>UBC 823</td>
</tr>
<tr>
<td>ISSR 7</td>
<td>(AC)C</td>
<td>UBC 826</td>
</tr>
<tr>
<td>ISSR 8</td>
<td>(AG)CTT</td>
<td>UBC 846</td>
</tr>
<tr>
<td>ISSR 9</td>
<td>(CA)AGT</td>
<td>UBC 855</td>
</tr>
<tr>
<td>ISSR 10</td>
<td>(GT)CTA</td>
<td>UBC 856</td>
</tr>
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</table>

ISSR (Inter Simple Sequence Repeat) analysis

ISSR-PCR DNA amplifications (Martins-Lopes et al., 2009) were performed using ten ISSR primers (Table 1) and PCR reactions were carried in a 25 ml reaction volume, containing PCR Buffer (1x final concentration, invitrogen), 2.5 mM MgCl2, 0.4 mM of each dNTP, 0.4 mM ISSR 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 holding at 4 °C, respectively. Amplicons were separated on 1.5% agarose gel at 80 V. They were then stained with 0.5 ml/ml ethidium bromide solution, visualized by illumination under UV light, and documented using a gel documentation and image analysis system (BIORAD, Molecular Imager, ChemiDocTM XRS+ with Image LabTM Software).

Data analysis

DNA fragments of ISSR PCR reactions were scored by their presence (1) or absence (0) and the ones at low intensities were scored only if they were reproducible in the PCR runs. Cluster analysis was performed to construct dendrograms, with the unweighted pair-group method by arithmetic averages (UPGMA) from the similarity data matrices using Jaccard's coefficient (Rohlf, 1998).

Results and discussions

Molecular fingerprints of 40 clones belonging to 8 Turkish cultivars were carried out using ISSR analysis and very high polymorphism (94.9% polymorphism in average) was detected by the method. The total of 217 reproducible bands, ranging from 100 to 4,250 bp, were scored with the ISSR technique. The highest polymorphism rate was obtained by ISSR 3 primer which produced 27 polymorphic bands (Table 2).

Genetic similarities/varieties were obtained with UPGMA algorithm using Jaccard’s coefficient. The genetic similarities ranged from 0.32 (between Mugla cv. ‘Domat’ clone 2 and Bahlkesir cv. ‘Edinecik’ clone 5) to 0.96 (Samsun cv. ‘Alacam’ clone 4 and 5) (Fig. 2. A and B).

The cultivars were grouped into four clusters: Cluster I, with Bahlkesir cv. ‘Edinecik’ 1-5 and Bursa-Gemlik cv. ‘Gemlik’ 1-5; Cluster II, Hatay cv. ‘Edremit’ 1-5 and Mardin cv. “Halhali’ 1-5; Culuster III, Mugla cv. ‘Domat’ 1-5, Samsun cv. ‘Alacam’ 1-5 and cv. ‘Tekir’ 1-3; Cluster IV, cv. ‘Tekir’ 4-5 and cv. ‘Yaglik’ 1, 2 (Fig. 1).

Some close relationships between cultivars were constant in all ISSR analyses performed; for instance, cv. ‘Edinecik’ and cv. ‘Gemlik’ (similarity ranges from 0.544 to 0.803); cv. ‘Edremit’ and ‘Halhali’ (similarity ranges from 0.475 to 0.658); cv. ‘Domat’ and cv. ‘Alacam’ (similarity ranges from 0.523 to 0.806) and cv. ‘Tekir’ with ‘Yaglik’ (similarity ranges from 0.480 to 0.762). On the other hand, the dendrogram indicated that cv. ‘Tekir’ clones 1, 2 and 3 (Cluster III) and ‘Tekir’ clones 4 and 5 (Cluster IV) were in different groups. This was not surprising as there were many polymorphic bands in PCR gel analysis (Fig. 2C).

ISSR marker system has been extensively used to determine genetic relationships between numerous plant species and cultivars (Galvan et al., 2003; Martins et al., 2004; Qian et al., 2001; Uysal et al., 2010; Wiesnerova and Wiesner, 2004). However, there have been many reports on olive cultivar identification via ISSR marker techniques (Essadki et al., 2006; Gemas et al., 2004; Gomes et al., 2009; Hess et al., 2000). Accordingly, the present report is to be a comprehensive study on molecular identification of olive cultivars using ISSR molecular marker.

Although all ISSR PCR reactions produced different band profiles in all cultivars according to the agarose gel analyses, high compatibility was obtained from them and their polymorphism rate was very similar (94.9%). The highest level of polymorphism was detected with cv. ‘Yaglik’ clone 3, 4 and 5 by all band profiles; indeed, this cultivar was very distant from the others and was grouped into different cluster (it was found in Cluster IV of the dendrogram). This cultivar was very different from the others also by morphological characteristics; it has different fruit size from the others, high oil and low 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. ‘Yaglik’ Y1-3 and Y4-5 were in different branches, while their similarity ranges were between 0.58 and 0.71.

These differences could be the result of cross-pollination with local populations (Contento 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; Hannachi et al., 2008; Martins-Lopes et al., 2007).

Classical olive certification system is based on morphological and agronomic procedures which are affected by environmental conditions, and mislabelling accessions can negatively affect certification of olive products (Hannachi et al., 2008). Molecular marker systems are of great importance to overcome such a problem, therefore is necessary to determine the polymorphism level of olive cultivars and to identify the homonymy and synonymy problems in olive germplasm.

Conclusions

Determination of genetic relationships among olive cultivars cases efficient sampling, operating and using of germplasm resources. In the present study, ISSR analysis displayed a high level of genetic variability among Turkish olive cultivars, indicating a potential resource for the use of this germplasm in clonal selection programs.
Table 2. Analysis of ISSR PCR band profiles

<table>
<thead>
<tr>
<th>Primer</th>
<th>Total band profiles</th>
<th>Polymorphic band numbers</th>
<th>Monomorphic band numbers</th>
<th>Maximum length (bp)</th>
<th>Minimum length (bp)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>18</td>
<td>18</td>
<td>-</td>
<td>3,600</td>
<td>220</td>
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<tr>
<td>ISSR 2</td>
<td>25</td>
<td>23</td>
<td>2</td>
<td>4,250</td>
<td>250</td>
</tr>
<tr>
<td>ISSR 3</td>
<td>28</td>
<td>27</td>
<td>1</td>
<td>4,100</td>
<td>100</td>
</tr>
<tr>
<td>ISSR 4</td>
<td>9</td>
<td>9</td>
<td>-</td>
<td>3,650</td>
<td>170</td>
</tr>
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<td>24</td>
<td>2</td>
<td>2,800</td>
<td>125</td>
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<tr>
<td>ISSR 6</td>
<td>24</td>
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<td>1</td>
<td>2,900</td>
<td>125</td>
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<tr>
<td>ISSR 7</td>
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<td>18</td>
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<td>3,150</td>
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<td>23</td>
<td>21</td>
<td>2</td>
<td>3,800</td>
<td>125</td>
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<tr>
<td>TOTAL</td>
<td>217</td>
<td>206</td>
<td>11</td>
<td>4,250</td>
<td>100</td>
</tr>
</tbody>
</table>

Fig. 1. UPGMA dendrogram based on Jaccard’s coefficient illustrating the genetic similarities and distance among olive cultivars obtained by ISSR data, generated by the UPGMA cluster analysis (NTSYS)

Fig. 2. Agarose gel image of PCR products using ISSR 1 (A), ISSR 3 (B) and ISSR 9 (C) primers, amplification products obtained in 1.5% agarose gel
Acknowledgements

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References


