Discrimination and genetic polymorphism in several cultivar of grapevine by RAPD markers

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Abstract

Some RAPD markers were used for molecular characterization of several grapevine cultivars from Romania. The following cultivars: Feteasca Alba, Feteasca Regala, Muscat Ottonel and Riesling Italian were provided by Research Station for Viticulture and Vinification, Blaj, Romania. DNA was isolated through the CTAB method. The amplification was accomplished by using 18 primers and the specific working programme. Some of the RAPD markers were useful for cultivar discrimination being non polymorphic or low polymorphic. Most of the RAPD markers studied showed different level of genetic polymorphism. The lowest genetic polymorphism was detected in the population Feteasca Regala cv. showed with OPA-13, OPB-12 and OPB-17 primers. The highest polymorphism was detected in the population of Muscat Ottonel, most of the primers being polymorphic.

Keywords: molecular markers, RAPD, grapevine, polymorphism.

Introduction

Accurate grapevine identification is necessary because of the global problem which has arisen as a result of the long history of cultivation, distribution of vegetative cuttings from new cultivars that were wrongly identified and renamed. The spread across cultural boundaries has also increased the problem due to different countries or regions adopting different names for the same cultivar. In Romania, ampelography was formalized at the beginning of the twentieth century to describe and identify cultivars based on phenotypic traits (CONSTANTINESCU [1, 2]). However, the difficulties associated with this phenotype-based ampelographic system are due, in large part, to the subjectivity of the methodology and the global distribution of grape growing regions. There are upward of 24,000 names for a Vitis vinifera population in the world thought to contain around 5000 genuine cultivars (TRUEL & al. [3]).

DNA marker technology has the potential to reduce or remove these limitations by providing accurate genetic identification and estimation of genetic polymorphism useful for selection. DNA polymorphisms appear to be particularly useful tools for distinguishing cultivars because 1) the results directly reflect the genotype; 2) the results are independent of the environment; 3) a large number of potential polymorphic sequences or markers are available; 4) DNA can usually be extracted from nearly every tissue. DNA polymorphisms generated by the RAPD technique (WILLIAMS & al. [4], WELSH & MCCLELLAND, [5]) are of great interest because this technique requires minimal amounts of template DNA, it is simple, and it is capable of detecting a high level of genetic variation. There have been numerous reports on the use of RAPD markers for the detection of genetic variation among grapevine cultivars (CASTRO & al. [6], AĞ AOĞLU & al. [7], BISZTRAY & al. [8], ZOGHLAM & al. [9]). RAPD and microsatellite polymorphisms have also been shown to be useful in grapevine cultivar identification (GOGORCENA & al. [10], THOMAS & SCOTT [11]).
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In some cases different polymorphic RAPD markers were associated with the resistance trait to the root-knot nematode Meloidogyne arenaria (GAMBINO & al, [18]).

Up to our knowledge there is a previous study regarding the molecular analysis of grapevine cultivars indigenous in the Carpathian basin using SSR markers (KISS & al, [19]), but this study reports data about Hungarian cultivars. There are few studies about molecular characterization of grapevine cultivars from Romania (BUTIUC-KEUL & al, [20], CRĂCIUNĂŞ & al, [21], GHEORGHE & al, [22]) but unfortunately, there is no available a Romanian data base with molecular fingerprints of grapevine cultivars from Romania.

In this study, RAPD markers were used to detect polymorphisms in the population of several cultivars of grapevine from Romania and also to identify valuable markers useful for cultivar discrimination and polymorphism in order to complete the data available for grapevine cultivars from Romania.

Material and methods

Plant material

Plant material was provided by the Research Station for Viticulture and Vinification, Blaj, Romania. Several valuable cultivars as Feteasca Alba, Feteasca Regala, Muscat Ottonel, and Riesling Italian, usually grown in Romania, have been studied.

DNA isolation and amplification

DNA was isolated through the CTAB method described by DOYLE & DOYLE [23]. The amplification was accomplished by using different oligonucleotide primers (synthesized by Eurogentech) and the specific working programme. RAPD marker were obtained by PCR amplification, performed in 25 μl of mixture containing 2 mM MgCl2, 1 μM of each primer, 200 μM of each dNTP, 1.0 U of Taq (Fermentas) in reaction buffer (10mMTrisHCl pH 8.8, 50 mM KCl, 1.5 mM MgCl2) and 25 ng of genomic DNA. Amplification programme: 1. T=95°C, 5 min; 2. T=94°C, 45 s; 3. primer alignment at 37°C, 45 s; 4. elongation T=72°C, 90 s; repeat steps 2-4, 35X; 5. final elongation at 72°C, 8 min. Hold at 4°C. 18 primers have been tested but in this paper we will show only the best results obtained with 6 primers as it can be seen in Table 1. Amplicons have been separated on 1.5 % agarose gel, stained with 0.5 μg/ml ethidium bromide.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Length</th>
<th>Tm°C</th>
<th>Tm°C of annealing</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPA-13</td>
<td>5'-cagcaccccac-3'</td>
<td>10</td>
<td>32.4</td>
<td>36°C</td>
</tr>
<tr>
<td>OPB-04</td>
<td>5'-ggactggaggt-3'</td>
<td>10</td>
<td>27.2</td>
<td>36°C</td>
</tr>
<tr>
<td>OPB-07</td>
<td>5'-ggtgaagcag-3'</td>
<td>10</td>
<td>32.9</td>
<td>36°C</td>
</tr>
<tr>
<td>OPB-12</td>
<td>5'acctgacgca-3'</td>
<td>10</td>
<td>31.1</td>
<td>36°C</td>
</tr>
<tr>
<td>OPB-17</td>
<td>5'-aggggacagc-3'</td>
<td>10</td>
<td>28.2</td>
<td>36°C</td>
</tr>
<tr>
<td>OPF-04</td>
<td>5'-ggtgatcagg-3'</td>
<td>10</td>
<td>25.5</td>
<td>36°C</td>
</tr>
</tbody>
</table>

Results and discussion

The RAPD markers obtained in the case of Feteasca Alba cv. are showed in Fig 1 and Table 2. By amplification with OPB-12 primer, nine polymorphic bands were obtained, having 800-200 pb and by amplification with OPB-07 primer, six polymorphic band of about 800-300 pb were obtained (Fig. 1a). These two amplicons are very polymorphic, each individual showing different pattern of amplification. DNA amplification with OPF-04 primer...
shows six bands of about 860-340 pb. As it could be seen in Fig 2b, the pattern is similar in all individuals, only the last fragment is present in only one individual, so this marker is non polymorphic. By DNA amplification with OPB-04 primer, four fragments of about 400 pb were obtained (Fig 1b). The fragments 2-3 are present in all individuals, but the fragments 1 and 4 are present only in 2 individuals, this marker being low polymorphic. By amplification with OPB-17 primer, five polymorphic fragments were obtained. The RAPD fragments are almost identical in all individuals (Fig. 1b). With OPA-13 primer the amplification was not obtained. Most of the primers generated polymorphic patterns which means that genetic polymorphism in the population of this cultivar is generally high.

Analysis of RAPD markers obtained by amplification in case of Feteasca Regala cv. showed that the fragment generated with OPA-13 primer is non polymorphic, being present in all individuals (Fig. 2c). The markers generated with primers OPB-12 and OPB-17 are low polymorphic; two fragment of about 300-400 pb were obtained by amplification with OPB-12 primer (Fig. 2b) and three fragments with OPB-17 of about 600 and 300 pb (Fig 2a). With OPB-04, OPB-07 and OPF-04 primers the amplification was not obtained (Table 2).

![Fig. 1. DNA amplification of Feteasca Alba cultivar with different primers (a: 1-molecular marker, fragments: 3000, 2000, 1500, 1200, 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100 pb, 2-6-OPB-12, 7-11-OPB-07, b: 1-molecular marker: plasmid PQE 60 digested with Dra I endonuclease, fragments: 1522, 859, 622, 339 pb, 2-6-OPF-04, 7-12-OPB-04, 13-molecular marker Fermentas, fragments 3000, 2000, 1500, 1200, 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100 pb, 14-18-OPB-17, 19-20-negativ controls).](image1)

![Fig. 2. DNA amplification of Feteasca Regala cultivar with different primers (a: 1-molecular marker, plasmid PQE 60 digested with Dra I endonuclease, fragments: 1522, 859, 622, 339 pb; 2-6-OPB-17, b: 1-5-OPB-12, 6-molecular marker, fragments: 1522, 859, 622, 339 pb; c: 1-3-OPA-13, 4-molecular marker Fermentas, fragments: 150, 100, 90, 80, 70, 60, 50, 40, 30, 20, 10 pb).](image2)
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RAPD amplification in the case of Muscat Ottonel cv. with OPA-13 primer shows five fragments of about 400-200 pb. The pattern is identical in four individuals, only one of them showing only two bands (Fig. 3a). This primer generated low polymorphic markers. By amplification with OPB-12 primer, eight different fragments of about 800-300 pb were obtained, and by amplification with OPB-07 other eight fragments were obtained of about 900-300 pb. These two primers generated polymorphic markers, they detected high polymorphism in the population of this cultivar (Fig. 3b). High genetic polymorphism was also detected by amplification with OPF-04 primer, seven different fragments of about 360-100 pb being obtained (Fig. 3c). All individuals show distinct pattern of RAPD fragments. By OPB-04 and OPB-17 primers the amplification was not obtained.

![Fig. 3. DNA amplification of Muscat Ottonel cultivar with different primers (a: 1-molecular marker, plasmid PQE 60 digested with Dra I endonuclease, fragments: 1522, 859, 622, 339 pb; 2-6-OPA-13, b: 1-molecular marker Fermentas, fragments: 3000, 2000, 1500, 1200, 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100 pb, 2-6-OPB-12, 8-11-OPB-07, 12-molecular marker; c: 1-molecular marker, plasmid PQE 60 digested with Dra I endonuclease, fragments: 1522, 859, 622, 339 pb; 2-6-OPF-04).](image)

DNA amplification of Riesling Italian cv. with OPB-04 primer showed seven polymorphic bands of about 800-200 pb (Fig. 4a). These fragments are present in almost all individuals, so these markers are low polymorphic. In Fig. 4b the pattern of amplification with OPB-17, OPB-12 and OPB-07 primers is shown. By amplification with OPB-17 primer...
nine fragments were obtained, this primer showing a high level of polymorphism in the population of this grapevine cultivar. The other two primers show low genetic polymorphism, thus with OPB-07 primer seven fragments were obtained, and with OPB-12, other six fragments were obtained. With OPA-13 and OPF-04 primers, the amplification was not obtained (Table 2).

Our results are summarized in Table 2. These results complete the previous data regarding molecular characterization of grapevine cultivars usually grown in Romania as Muscat Ottonel and Riesling Italian and also provide data about Romanian native cultivars as Feteasca Alba and Feteasca Regala, that were never characterized previously by the RAPD method. RAPD markers are useful and valuable tools for cultivar discrimination, and detection of genetic polymorphism for marker assisted selection of new cultivars and clones.

Table 2. RAPD markers obtained by amplification with different primers.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Cultivar</th>
<th>No. of bands</th>
<th>Obs.</th>
<th>No. of bands</th>
<th>Obs.</th>
<th>No. of bands</th>
<th>Obs.</th>
<th>No. of bands</th>
<th>Obs.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Feteasca Alba</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OPA-13</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>no polymorphism</td>
<td>5</td>
<td>polymorphism</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>OPB-04</td>
<td>4</td>
<td>low polymorphism</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7</td>
<td>low polymorphism</td>
<td></td>
</tr>
<tr>
<td>OPB-07</td>
<td>6</td>
<td>polymorphism</td>
<td>-</td>
<td>-</td>
<td>8</td>
<td>polymorphism</td>
<td>5</td>
<td>low polymorphism</td>
<td></td>
</tr>
<tr>
<td>OPB-12</td>
<td>9</td>
<td>polymorphism</td>
<td>2</td>
<td>low polymorphism</td>
<td>8</td>
<td>polymorphism</td>
<td>6</td>
<td>low polymorphism</td>
<td></td>
</tr>
<tr>
<td>OPB-17</td>
<td>5</td>
<td>low polymorphism</td>
<td>3</td>
<td>low polymorphism</td>
<td>-</td>
<td>-</td>
<td>9</td>
<td>polymorphism</td>
<td></td>
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<tr>
<td>OPF-04</td>
<td>6</td>
<td>no polymorphism</td>
<td>-</td>
<td>-</td>
<td>7</td>
<td>polymorphism</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Conclusions

Some of the RAPD markers are useful for cultivar discrimination being non polymorphic or low polymorphic.

Most of the studied RAPD markers showed different level of genetic polymorphism. The lowest genetic polymorphism was detected in the population Feteasca Regala cv., showed with OPA-13, OPB-12 and OPB-17 primers.

The highest genetic polymorphism was detected in the population of Muscat Ottonel, most of the markers being polymorphic.

Acknowledgements

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References