The RAPD techniques used to assess the genetic diversity in *Draba dorneri*, a critically endangered plant species

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ABSTRACT

A successful management and preservation of the natural populations is depending on accurate assessment of genetic diversity. Knowledge of genetic diversity within a population is important for the conservation of the species. Our aim was to assess the genetic diversity in *Draba dorneri* Heuff. population (*Brassicaceae* family)—an endemic plant species of conservative interest using Random Amplified Polymorphic DNA (RAPD) technique. The plant species is strictly protected at national level as well as at international level through “Convention on the Conservation of European Wildlife and Natural Habitats”, Bern, 1979 European Council. In this study, a total of 52 primers were scored initially. A total of 77 reproducible bands with an average of 6.41 bands per primer were obtained from the 12 primers selected. A cluster analysis (UPGAMA) was used to generate a dendrogram based on Dice coefficient. We found 67% similarity between the samples from the two analyzed slopes. Comparing with other rare plants species, our data revealed a higher level of genetic diversity in *D. dorneri* population in Retezat Mountains.

Keywords: *Draba dorneri*; RAPD Techniques; Genetic Diversity; Endemic Plant Species; Retezat Mountains

1. INTRODUCTION

*Draba* L., one of the largest genus of vascular plants in the Arctic, hosted about 350 species primarily in the N hemisphere, especially arctic, subarctic, alpine and subalpine regions [1]. Eleven species of genus *Draba* occur in Romania, nine of them being continental endemics. *D. dorneri* is an endemic plant restricted to Southern Carpathians (Romania). The distribution area is restricted to alpine and subalpine area of Retezat, Bucegi and Făgăraș Mountains [2]. It is a perennial caespitose plant, populations consists in small number of individuals found in the crevices of rock outcrops. The recognition characters are given by glabrous stem and leaf surface and ramified hairs on the leaf margins. The geological substrate is represented by both siliceous, granite or conglomerate rocks. Plant community includes species of Saxifraga, Thymus, Huperzia, Silene, Symphyandra, Phyteuma, Cetraria a.a.

According to 1997 IUCN Red List of Threatened Plants, the world status of *D. dorneri* Heuff is extinct and following the Red Book of Vascular Plant from Romania is considered a critically endangered plant. It is also mentioned on Anexe IIb, IVb of Habitats Directive, and the mountain ranges where *Draba* occurs are part of Natura 2000 network. There, the requirements for long-term conservation of *D. dorneri* are of prime importance. Currently, taking into account our monitoring in area of distribution during to last two years, the population is very poor being formed of few individuals distributed on a very small surface.

During the time, several studies concerning different aspects of *Draba* sp. were performed. In this way diverse aspects like systematics [3-8], cytology [4,7,9-11], breeding systems [12], molecular phylogeny [13-15], phylogeography [5], taxonomy [2,17-29], evolution of arctic *Draba* species using the microsatellites and QTL-mapping population [29,30] was covered.

Understanding the genetic variation within populations is essential to establish the proper conservation strategies for plant species, the preservation of genetic diversity of the populations being a fundamental goal of conservation biology [31]. The maintenance of genetic diversity has a considerable significance for the long-term survival of endangered plant species [32]. The ability of a species to adapt to environmental changes depends greatly on the genetic diversity in the species [33,34].

The analysis of the populations through RAPD showed the highly variable of the identified markers and the potential of them in population studies. Comparing with allozyme analysis, the RAPD method provides a much higher number of markers and it is now well established as a sensitive method for detecting genetic diversity [35]. The RAPD technique, a quick and relatively
inexpensive method, is extensively used to analyze the genetic variability as bacteria, fungi and plants [36]. Despite of lower reproducibility, the RAPD method is generally more desirable because of cost-effectiveness studies involving a smaller number of samples. The RAPD technology was used to investigate the genetic diversity within and between populations and has been applied to many of rare or threatened plant species [37-46].

The present paper deals with investigations related to genetic variability in D. dorneri population, an endemic species of conservative interest from Retezat Mountains, in Romania. According to the authors knowledge this is first description into field of genetic variability of D. dorneri from this area.

2. MATERIALS AND METHODS

Plant material: D. dorneri plant material was collected from 7 individuals of the natural population in Retezat Mountains. There, it occurs in a small area on the ridge that ascends from Glade Valea Reasca to Retezat peak at an altitude of approximately 1850 m. Being a critically endangered plant species, the number of individuals from the natural population was reduced. The tissue was stored in silica gel at 25°C until the DNA was extracted.

DNA extraction: Extraction of total genomic DNA was obtained by grinding small quantities of dried plant material (0.08 - 0.1 g) with quartz sand to obtain a powder that was re-suspended in 0.1 M TE buffer pH 8. For DNA isolation was used Genomic DNA purification kit, Fermentas, following the manufacturer protocol. The concentration of obtained DNA was between 16.7 - 973.45 ng/µl and 15.6 - 591.4 ng/µl after RNA-se treatment.

RAPD analysis: In order to evaluate the genetic diversity in D. dorneri population the RAPD techniques was used. The primers used for PCR amplification were purchased from Bio Basic Inc. The PCR reaction was performed using Fermentas kit (Dream TaqPCR master Mix and Dream Taq Green PCR Master Mix) in a BioRad C 1000 thermocycler. Each reaction was performed in a 12, 5 µl volume containing: 10 - 20 ng/µl DNA, 2.5 mM MgCl2, and 0.2 mM each dNTP, 1.5 µM primer, 0.312U Taq DNA polymerase or Dream Taq polymerase, PCR buffer and distilled water.

The amplification program consist in an initial denaturation step at 94°C for 3 minutes, followed by 45 cycles of denaturation at 94°C for 30 seconds, annealing at 37°C for 30 seconds and elongation for 2 minutes at 72°C and a final extension step at 72°C.

The amplification products were separated by electrophoresis in 2% agarose gels in TBE buffer pH 8, added with 50 µg/ml ethidium bromide. The DNA fragments were visualized under UV light using a device DocuGel from BioRad. Molecular size of the amplification products were estimated by using a 100 bp Ladder from BioScience with fragments between 100 - 2000 pb.

Data analysis: The DNA profiles were analyzed using Bio-Rad Quantity One software package for imaging and analyzing 1-D electrophoresis gels. The method for computing similarity in Quantity One is the Dice coefficient [47]. The formula is

$$S = 2n_{xy}/(n_x + n_y),$$

where $n_x$ and $n_y$ are the total number of fragments analyzed in individuals $x$ and $y$, respectively, and $n_{xy}$ is the number of fragments shared by the two individuals. For the visualization of the similarity of samples, a phylogenetic tree was constructed based on unweighted pair group method using arithmetic averages (UPGAMA).

3. RESULTS

From a total of 52 primers scored initially, 25 RAPD primers were selected for analysis based on their amplification products. A total of 77 reproducible bands with an average of 6.41 bands per primer were obtained from the 12 primers selected (Table 1). The size of amplified fragments varied between 200 and 1500 bp. The percentage of polymorphic loci in the analyzed samples was 78.94%. The highest number of polymorphic bands (10) was obtained by primers S69 and S235 and lowest (1) by primer S90 and S197 (Table 1, Figure 1).

Nowadays, many rare plant species are currently threatened by extinction due to the destruction, the fragmentation of habitats and the isolation of predominantly small plant populations [48], the ecological parameters having influences on the genetic differentiation of populations [49].

In this study, 12 primers were analyzed for assessing the genetic diversity in D. dorneri samples collected from the eastern and western slope of the Retezat Mountains. The presence of the species on the eastern slope summarizes a small number of individuals where it grows in association with less sun-loving plants. On the western slope, where solar radiation is high are installed clonal individuals more developed, that include even over 100 floral stems.

A number of 77 amplified products were produced, ranging in size from 200 to 1500 bp. From 12 primers tested, only one primer (S6) produced amplification products that were monomorphic across all the plants. Most of the primers generated high percent of polymorphic loci, which means that genetic polymorphism in the population, is generally high.

The percentage of polymorphic bands (78.94%) obtained in D. dorneri samples was higher than in other endangered plants like Paeonia suffruticosa 22.5%, P.
Table 1. Total number of amplified fragments and number of polymorphic fragments generated by 12 random primers in *D. dorneri* samples.

<table>
<thead>
<tr>
<th>Name of primer</th>
<th>Primer sequence (5' - 3')</th>
<th>Total number of amplified fragments</th>
<th>Number of polymorphic fragments</th>
<th>Polymorphic loci (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S6</td>
<td>5'TGCTCTGCCC3'</td>
<td>1</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>S33</td>
<td>5'CAGCACCCAC3'</td>
<td>6</td>
<td>6</td>
<td>100%</td>
</tr>
<tr>
<td>S69</td>
<td>5'CTCACCGTCC3'</td>
<td>12</td>
<td>10</td>
<td>83.33%</td>
</tr>
<tr>
<td>S90</td>
<td>5'AGGGCCGTCT3'</td>
<td>2</td>
<td>1</td>
<td>50%</td>
</tr>
<tr>
<td>S105</td>
<td>5'AGTCTCCCC3'</td>
<td>12</td>
<td>5</td>
<td>41.66%</td>
</tr>
<tr>
<td>S113</td>
<td>5'GACGCCACAC3'</td>
<td>5</td>
<td>5</td>
<td>100%</td>
</tr>
<tr>
<td>S197</td>
<td>5'TGGGGACCAC3'</td>
<td>6</td>
<td>1</td>
<td>16.66%</td>
</tr>
<tr>
<td>S201</td>
<td>5'GGGCACTCA3'</td>
<td>4</td>
<td>4</td>
<td>100%</td>
</tr>
<tr>
<td>S235</td>
<td>5'CAATGCGGT3'</td>
<td>10</td>
<td>10</td>
<td>100%</td>
</tr>
<tr>
<td>S285</td>
<td>5'GGCACTGAGG3'</td>
<td>8</td>
<td>8</td>
<td>100%</td>
</tr>
<tr>
<td>S245</td>
<td>5'TGGGCGGCT3'</td>
<td>6</td>
<td>5</td>
<td>83.33%</td>
</tr>
<tr>
<td>S312</td>
<td>5'TCGCCAGCCA3'</td>
<td>5</td>
<td>5</td>
<td>100%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>76</td>
<td>60</td>
<td>78.94%</td>
</tr>
</tbody>
</table>

Figure 1. The RAPD profiles of *Draba dorneri* Heuff. generated by S105 primer. Samples 1 - 7 *D. dorneri* individuals. MG-100 bpDNA ladder.

Figure 2. Dendrogram of the seven individuals from natural population of *D. dorneri* from Retezat Mountains, constructed using UPGAMA based on Dice coefficient. 1, 2, 3—samples from Western slope; 4, 5, 6—samples from Eastern Slope, 7—sample from the second site. The scale indicates the genetic distance between individuals.

The similarity between the samples from Eastern Slope (samples 4, 5, 6) is 76% and 74% for the samples from Western slope (samples 1, 2, 3). The sample 7, collected from a few meters from the sample 2 present a high similarity (80%) with this. The similarity between the samples from the slopes is 67%. This percent of polymorphism coincide with the small distance between the 2 slopes. We may conclude that the samples are part from the same population. Despite of the high percentage of polymorphic bands identified by RAPD, *D. dorneri* have no spreading power.

*rockii* 27.6% [37], *Lactoris fernandeziana* 24.5% [50], *Allium aseae* 40% - 63% [47], *Cathaya argyrophylla* 32% [51], *Dacydium pierrei* 33.3% [48]. Comparing with these percentages our data showed that the genetic variability of this species is not low. The maintenance of existing levels of genetic diversity has a considerable significance for the long-term survival of endangered plants.

In order to represent the relationships among samples, a cluster analysis (UPGAMA) was used to generate a dendrogram based on Dice coefficient (Figure 2).
Our results showed that the similarity represented by the polymorphism of RAPD bands between the individuals from the two slopes of the Retezat Mountains is 67%. The cluster analysis (UPGAMA) used to distinguish between individuals showed that samples can not be clustered in different populations.

Despite the small size of the population, which generally exhibit lower levels of genetic diversity, our results based on the RAPD techniques showed that the genetic diversity of this critically endangered plant species is not low.

A good strategy to protect this critically endangered plant species is to protect more of their habitat.

4. FUTURE PERSPECTIVES

We intend to increase the number of samples from the same and different locations. Also, we intend to check our results concerning the genetic diversity founded using RAPD through other molecular tools.

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REFERENCES


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