The Use of RAPD Markers in the Study of Polymorphism of Mountain Populations of Dandelion Officinalis

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Abstract. Molecular markers are used in plant science in a variety of ways. The method of molecular genome marking based on RAPD-PCR makes it possible to determine the genetic status of populations and establish intrapopulation relationships. In this work, we studied the genetic polymorphism of the dandelion Taraxacum officionale using RAPD markers. Five markers of random amplified polymorphic DNA (RAPD) were used to determine the genetic diversity and genetic relationship between two populations of T. officionale growing on the slopes of the Andean Range. A total of 26 fragments were amplified, and 25 of them were polymorphic, with an average of 6 amplified bands per primer. The percentage of total polymorphism was 96.1%. The size of the amplified fragments varied from 150 to 1200 bp. It has been shown that molecular genetic analysis makes it possible to identify RAPD markers of the dandelion genome, which can be used for population identification of genotypes.

1 Introduction

Determination of genetic variability and relatedness between and within plant species is important information for the conservation and development of valuable plant resources [4, 7]. PCR) [15]. The RAPD method is simple and efficient. It requires a very small amount of genomic DNA without the use of radioactive labels, and the analytical process is fast and simple [14]. In addition, RAPDs can cover a large part of the genome and have the advantage that no prior knowledge of the genome under study is required [6]. The RAPD technique has been widely used in plant genetic research. In particular, it has found application for identifying varieties of a number of horticultural and vegetable crops, determining genetic purity and sex, building genetic maps, and for analyzing somatic hybrids [13, 3]. RAPD and other molecular markers are of great importance for the selection of desired traits, including in long-lived species that take a long time to mature and display phenotypic traits. RAPD markers have been used to identify disease resistance genes in plants [8, 11]. RAPD analysis is an important tool for characterizing biodiversity.

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Many scientific studies have shown the usefulness of RAPD markers in the study of intraspecific plant polymorphisms based on differences in the DNA sequence [12, 10].

The aim of this work is to study the genetic polymorphism of dandelion Taraxacum officinale Wigg using RAPD markers. with wide ecological plasticity., which is found everywhere in the North Caucasus.

2 Research Methodology

Object of study. Natural populations of the Taraxacum officinale plant, which is a source of various biologically active components with proven pharmacological properties [1, 2, 5]. Plants were collected in the highlands of the Czech Republic at an altitude of 875 to 2200 m above sea level. A total of three were studied.

Isolation of DNA. For DNA extraction, 3 plants were used from each population of T. officinale plants growing at an altitude of 875 to 950 m, 1900 and 2175–2200 m above sea level. Total cellular DNA was isolated using the DNeasy Plant Pro Kit (50) (Qiagen) according to the manufacturer's instructions. Advanced DNA extraction technology results in high yields of inhibitor-free DNA that is ready for immediate use in a variety of assays, including RAPD analysis.

Conducting RAPD analysis. DNA samples were analyzed using five RAPD primers, the design of which was taken from open sources (Table 1) [9]. For amplification, GenPak™ PCR Core kits (Moscow) were used for 96 PCR DNA amplifications in a volume of 20 µl. 10 µl of PCR buffer, 0.1 µl of each primer, 7.9 µl of deionized water, and 2.0 µl of DNA samples of different dandelion genotypes were added to the master mixes. The thermal profile consisted of 1 cycle of denaturation for 7 minutes at 94°C, 35 cycles of 94°C for 30 seconds, 35°C for 30 seconds and 72°C for 1 min followed by a 7 min extension at 72°C. The imprint of RAPD fragments was obtained by horizontal electrophoresis in 1.7% agarose gel stained with ethidium bromide. The fragment size was estimated using the DNA marker Step50 plus (Biolabmix, Novosibirsk). After electrophoresis, the gel was photographed in ultraviolet light using a TCP-20.LM transilluminator (Vilber Lourmat, France).

Table 1. Primer Design and Annealing Temperature for RAPD Analysis

Primer name	Subsequence 5'–3'	Annealing temperature
OPC-04 OPC-05 OPD-07 OPC-08 AFK-03	CCGCATCTAC GATGACCGCC TTGGCACGGG TGGACCGGTG GCGTCCATTC	35°C

Analysis of results. In the analysis of electrophoresis results, the co-migrating bands were counted as the same locus and thus were considered as the same band for scoring. The presence of the amplified product was designated as "1" and the absence as "0". Similarity between samples was assessed using the Jaccard coefficient and calculated as: KJ = A /(C+D) − A), where A is the number of positive matches (i.e., the presence of bands in both samples), D is the number of bands in one sample , C is the number of bands in another sample.

3 Results and Discussions

RAPD studies of dandelion officinalis (T. officionale) genotypes were performed with only 5 random oligonucleotide primers. Only 4 of them produced measurable fingerprinting and polymorphism. A total of 26 fragments were amplified, and 25 of them were polymorphic, with an average of 6 amplified bands per primer. The percentage of total polymorphism was 96.1%. The size of the amplified fragments varied from 150 to 1200 bp. Of the selected primers, OPD-07 gave the maximum number of polymorphic (average 9) bands (Fig. 1). The smallest number of polymorphic bands (7 bands) was obtained using primer OPC-04. The studied samples were located at different heights, so it could be assumed that there could be genetic differences between the samples of dandelion officinalis. However, analysis of the visualized amplicons showed that there is a high genetic similarity between plants of different populations. Amplicons generated with primer AFK-03 showed more similarity within populations and showed more matches between populations. Dandelions growing at an altitude of 875 m above sea level. were phylogenetically closer to the dandelion population of the foothills of Lake Kazenoy-Am (height 2175 masl) (Fig. 1 (B)). Polymorphic loci were identified, which indicated the possible genetic heterogeneity of the Caucasian population of the studied species. At the same time, no genetic similarity was found between plants that are geographically close (height 875 m a.s.l. and height 950 m a.s.l.). (Table 2).

Table 2. Jaccard index for nine genotyped T. officionale accessions with primers AFK-03; OPC-04, OPD-07

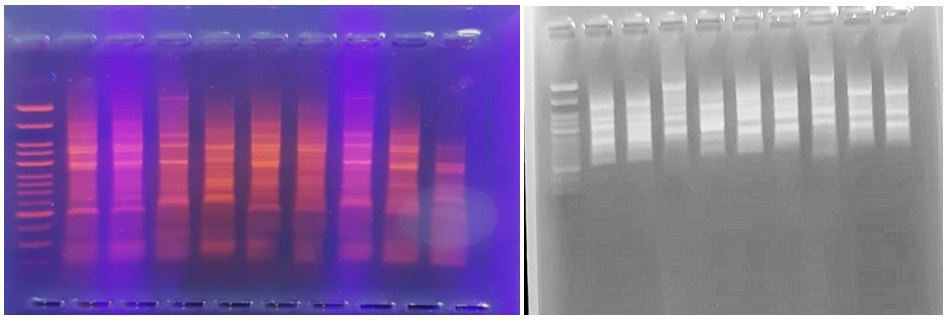
	Primer AFK-03								
	1	2	3	4	5	6	7	8	9
1	1								
2	1	1							
3	0,125	0,125	1						
4	0	0	0	1					
5	0,6	0,6	0	0	1				
6	0,6	0,6	0	0	1	1			
7	0,125	0,125	0	0.14	0,125	0,125	1		
8	0,4	0,4	0	0	0,75	0,75	0,14	1	
9	0,4	0,4	0	0	0,75	0,75	0,14	1	1
	Primer AFK OPC-04								
	1	2	3	4	5	6	7	8	9
1	1								
2	0,86	1							
3	0	0	1						
4	0,71	0,57	0	1					
5	0,57	0,43	0	0,5	1				
6	0	0	0,75	0	0	1			
7	0	0	0.75	0	0	0,5	1		
8	0.57	0,66	0	0,5	0,33	0	0	1	
9	0.11	0,125	0.4	0,125	0,17	0,2	0,5	0,17	1
	Primer AFK OPD-07								
	1	2	3	4	5	6	7	8	9
1	1								
2	1	1							
3	0,29	0,29	1						
4	0,22	0,22	0,29	1					
5	0,54	0,54	0,23	0,25	1				
6	0,67	0,67	0,23	0,25	1	1			
7	0,13	0,13	0,3	0,42	0,25	0,25	1		
8	0,54	0,54	0,33	0,18	0,78	0,78	0,25	1	

9	0,07	0,07	0,33	0,36	0,18	0,18	0,78	0,08	1

The similarity index, taking into account all the bands obtained using decameral primers, showed the following results. The lowest value of the similarity coefficient (25.0%) was observed between samples No. 1 and 9, 4 and 8, samples No. 1, 2 with 5, 6 showed the greatest similarity among themselves (1.0; 1.0; 0.75; 0 .65 respectively); 5 with 6, 8 (0.86; 0.67, respectively) and 6 with 8 (0.785), and the maximum value (88.1%) is between genotypes 5 and 6 (Table 3).

Table 3. Jaccard coefficient for samples of dandelion officinalis, taking into account all primers

Sample number								
№1	№2	№3	№4	№5	№6	№7	№8	№9
1								
1	1							
0,40	0,40	1						
0,45	0,45	0,30	1					
0,75	0,75	0,37	0,5	1				
0,65	0,65	0,38	0,43	0,86	1			
0,27	0,27	0,44	0,32	0,30	0,35	1		
0,59	0,59	0,47	0,25	0,67	0,785	0,32	1	
0,25	0,25	0,35	0,42	0,28	0,44	0,44	0,375	1



A **B**

Fig. 1. (A) RAPD analysis of Dandelion officinalis with primer OPD07 (lanes 1-4 - altitude 870-950 above sea level; lanes 5-9 - altitude 1900-2200 above sea level); (B) RAPD analysis of dandelion officinalis with primer AFK03 (lanes 1-4 - altitude 870-950 m a.s.l.; lanes 5-9 - altitude 1900-2200 m a.s.l.)

4 Conclusions

RAPD markers have great potential in determining genetic variability and have been used effectively to obtain reliable and reproducible results for the assessment of variation. In an attempt to explore the potential of RAPD markers to assess the variability of lettuce genotypes, we used a set of 4 random decamer RAPD primers for 9 dandelion genotypes growing in the mountains of the Chechen Republic. The percentage of polymorphism was 96.1%. In total, out of 26 developed bands, 25 were polymorphic. In the course of the present studies, four unique bands were obtained in RAPD.

Thus, the information obtained using RAPD markers in this study demonstrates the presence of polymorphism among samples of wild dandelion officinalis. However, further analysis is required, using other types of markers, to assess the similarity and diversity of

plants from different populations, which will be of great importance for conservation programs and the selection of valuable plant resources.

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