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Detection of polymorphic RAPD markers for *Pistacia atlantica* Desf.

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SUMMARY – The genus *Pistacia* has eleven species and among them, *Pistacia atlantica* Desf. is one of the important rootstock species. Randomly Amplified Polymorphic DNA (RAPD) technique is one of the powerful and useful molecular methodologies saving time and money in molecular characterization of plant genetic resources and germplasm. In this study, 76 RAPD primers were screened to find the most polymorphic RAPD primers to characterize *P. atlantica* germplasm and apply them to future breeding programmes. All the primers produced amplification patterns. From the 76 primers tested, 1156 RAPD bands were produced and 800 of them (69.2%) were reproducible. Among them, 247 reproducible polymorphic bands were scored for an average of 3.25 scorable polymorphic bands per primer. One primer did not give polymorphic bands and the rest of them amplified between 1 and 8 polymorphic markers whose estimated sizes ranged from 150 to 2500 bp. Results of this study clearly indicated that RAPD markers are powerful tools for the detection of genetic diversity and germplasm characterization in *P. atlantica*.

Key words: *P. atlantica*, RAPD, fingerprinting, *Pistacia*, polymorphism.

RESUME – *"Détection de marqueurs RAPD polymorphes pour* Pistacia atlantica *Desf.". Le genre* Pistacia *présente onze espèces et parmi elles,* Pistacia atlantica *Desf. est l'une des espèces les plus importantes de porte-greffe. La technique RAPD (randomly amplified polymorphic DNA) est une des méthodologies moléculaires les plus puissantes et utiles, car elle économise du temps et de l'argent pour la caractérisation moléculaire des ressources phytogénétiques et du germoplasme végétal. Dans cette étude, 76 amorces RAPD ont été examinées afin de trouver les amorces RAPD les plus polymorphes afin de caractériser le germoplasme de* P. atlantica *et de les appliquer à de futurs programmes d'amélioration. Toutes les amorces ont produit des formes d'amplification. Pour les 76 amorces testées, 1156 bandes RAPD ont été produites, 800 d'entre elles (69,2%) étant reproductibles. Parmi elles, 247 bandes polymorphes reproductibles ont été évaluées avec en moyenne 3,25 bandes polymorphes évaluables par amorce. Une amorce n'avait donné aucune bande polymorphe et le reste avait amplifié entre 1 et 8 marqueurs polymorphes dont les tailles estimées allaient de 150 à 2500 bp. Les résultats de cette étude indiquent clairement que les marqueurs RAPD sont des outils puissants pour la détection de la diversité génétique et la caractérisation du germoplasme chez* P. atlantica*.*

Mots-clés : P. atlantica*, RAPD, fingerprinting,* Pistacia*, polymorphisme.*

Introduction

The genus *Pistacia* L. is a member of the Anacardiaceae family and consists of at least eleven species (Zohary, 1952). *Pistacia* species are very valuable crops for the Mediterranean and North African countries. Since they are drought resistant plants and they can be grown in the regions where the annual precipitation is low. *Pistacia vera* L., the pistachio, has edible nuts and considerable commercial importance. The other species grow in the wild and their seeds are used mainly as rootstock seed source and rarely for fresh consumption, oil extraction and soap production (Kaska *et al*., 1996). Mixed populations of *P. vera* and these wild species have been growing in many locations for hundreds of years.

P. atlantica Desf. is one of the common wild species in the genus and economically important rootstock seed source in main pistachio producer countries. It grows naturally in Aegean, Mediterranean, Marmara, Black Sea and Central Anatolia regions of Turkey (Kaska and Bilgen, 1988), and in Algeria, Morocco, Syria, Israel, Greece and Egypt in the world (Browicz, 1988). It is used as rootstock either by top-working of old trees to *P. vera* or by seedling production from its seeds for budding to *P. vera*. The top-working of *P. atlantica* trees to *P. vera* has been done for many years in Turkey. The seeds of *P. atlantica* are used as rootstock sources especially in California (Ferguson,

1995) Syria (Hadj-Hassan and Kardouch, 1995), Morocco (Abderahmane, 1990) and currently it is getting an importance in Turkey.

Advances in DNA sequencing, data analysis and PCR (polymerase chain reaction) have resulted in powerful techniques, which can be used for the characterization and evaluation of germplasm and genetic resources. PCR technology has promoted the development of a range of molecular assay systems, which detect polymorphism at the DNA level. The Randomly Amplified DNA Polymorphism (RAPD) technique is one of them and is technically simple, it requires small quantities of DNA and no previous sequence information on the target genome. (Williams *et al*., 1990).

There are only a few molecular studies that address genetic and taxonomic relationships of *Pistacia* species (Parfitt and Badenes, 1997; Kafkas and Perl-Treves, 2001; 2002) and *P. vera* varieties (Hormaza *et al.,* 1994a; Dollo *et al*., 1995), and on the screening of sex markers (Hormaza *et al*., 1994b; Kafkas *et al*., 2001). With this study, 76 RAPD primers were screened, and scorable and consistent RAPD markers are detected for economically important rootstock species*, P. atlantica*.

Materials and methods

DNA extraction and RAPD reactions

Leaf samples were collected from ten genotypes of *P. atlantica* from the wild in Adana, Manisa and Aydın provinces of Turkey, frozen in liquid nitrogen and stored at -70°C until use. Genomic DNA was extracted from leaf tissue by the CTAB method of Doyle and Doyle (1987) with minor modifications (Kafkas *et al.,* 2001). RAPD analysis was performed according to Williams *et al*. (1990) with minor modifications (Kafkas and Perl-Treves, 2002). Totally 76 (23 from University of British Colombia and 53 from Operon Technologies) RAPD primers were screened.

Gel electrophoresis and data analysis

After the completion of polymerase chain reaction (PCR), RAPD products were size separated with 1.8% agarose gel electrophoresis in 1 x TBE buffer. Electrophoresis was run at a constant voltage, 80 V. The gels were stained with 0.5 μg/ml ethidium bromide solution for 15-20 min and destained with water for 20 min. The size of RAPD markers was estimated by comparing to the standard marker included in the gel. The gel was photographed under UV light. Each amplified RAPD marker was identified by the primer used to produce it and the appropriate size in base pairs (bp).

Scoring of RAPD markers was based on reproducible and consistent RAPD bands from all replicated assays. Each DNA sample was amplified at least twice, and markers that were present in all amplifications were scored. Markers that were present in one amplification but absent in another were considered as ambiguous markers and were not included in the analysis.

Results and discussion

In total, 76 randomly chosen RAPD primers were screened to select the most reliable and reproducible polymorphic primers for characterizing ten *P. atlantica* genotypes. The screened RAPD primers and their characteristics in the nuclear genome of *P. atlantica* are given in Table 1.

All the primers produced amplification patterns. From the 76 primers tested, 1156 different RAPD bands were amplified and 800 of them (69.2%) were reproducible. The number of reproducible bands produced by each primer ranged from 5 to 17 for an average of 10.5 bands per primer. Among the reproducible markers, 247 polymorphic bands were scored for an average of 4 polymorphic bands per primer, which is similar to the study in kiwi fruit by Cipriani *et al.* (1996) and in pecan by Conner and Wood (2001). The number of polymorphic bands per primer in this study is higher than lemon (Deng *et al.,* 1995), walnut (Machado *et al*., 1996; Nicese *et al*., 1998), mandarin (Coletta-Filho *et al.,* 1998), and olive (Sanz-Cortés *et al*., 2001; Belaj *et al.,* 2001). The low level of polymorphism in the other tree crop species can be explained by the reaction conditions and by the type of the genomic DNA used.

Table 1. The amplification characteristics of 76 RAPD primers by fingerprinting of ten *P. atlantica* genotypes

No	Primers	No of total bands	Marker sizes		Reproducible markers	Marker sizes		Polymorphic bands
			Min.	Max.		Min.	Max.	
1	OPE01	10	410	2040	7	480	1510	2
2	OPE04	14	160	1660	8	510	1660	2
3	OPE06	13	290	1280	10	290	1280	2
4	OPE18	17	250	2710	13	440	2060	6
5	OPE19	11	110	1860	7	210	1590	2
6	OPE20	16	100	1370	10	260	1220	0
7	OPL02	19	110	1900	14	310	1290	5
8	OPL04	13	260	1370	8	340	1230	2
9	OPL07	12	430	2750	8	660	1860	3
10	OPL14	13	230	1650	7	420	1250	2
11	OPR08	13	280	1890	8	280	1890	4
12	OPR12	15	310	1920	10	310	1920	4
13	OPR13	13	270	2340	10	510	2070	4
14	OPAC13	16	770	2750	13	770	2750	4
15	OPAC14	16	480	3050	11	740	2770	4
16	OPAC15	22	420	2090	15	540	1740	5
17	OPAF12	19	290	1980	14	290	1980	5
18	OPAF14	17	710	3250	9	710	1680	2
19	OPAF16	11	490	1660	7	490	1420	1
20	OPAF18	18	240	2090	14	360	2090	5
21	OPAF19	13	340	1350	9	460	1350	3
22	OPAG03	16	110	1890	9	700	1590	4
23	OPAG04	8	480	2070	5	830	1360	1
24	OPAG07	9	900	2150	8	900	2150	2
25	OPAG13	12	180	1610	9	180	1560	2
26	OPAG20	12	580	2780	11	580	2780	1
27	OPAH03	27	220	2760	17	430	1910	5
28	OPAH06	20	360	2540	11	480	2540	4
29	OPAH11	14	350	1870	9	660	1870	2
30	OPAH15	19	180	2270	11	320	2020	4
31	OPAH18	22	170	2620	14	430	1960	4
32	OPAI05	14	290	2620	9	710	2220	2
33	OPAI06	15	280	1990	11	640	1990	4
34	OPAI12	22	520	2970	13	520	2780	4
35	OPAI14	13	310	2750	8	740	1960	1
36	OPAI17	12	510	1970	8	510	1680	4
37	OPAI18	16	210	2800	12	210	2800	3
38	OPAJ01	24	110	2750	11	210	1860	1
39	OPAJ03	10	520	2190	7	520	2130	2
40	OPAJ09	16	260	2090	12	260	2090	1
41	OPAJ10	12	270	2660	9	370	2660	4
42	OPAJ12	13	360	1980	9	490	1780	3
43	OPAJ20	17	440	1880	12	440	1880	5
44	OPAK04	8	700	1990	6	700	1810	1
45	OPAK06	23	260	2870	15	260	2160	6
46	OPAK08	13	240	2320	8	360	2030	2

Table 1 (cont.). The amplification characteristics of 76 RAPD primers by fingerprinting of ten *P. atlantica* genotypes

No	Primers	No of total bands	Marker sizes		Reproducible markers	Marker sizes		Polymorphic bands
			Min.	Max.		Min.	Max.	
47	OPAK09	19	420	2760	15	420	1970	3
48	OPAK11	13	230	2160	11	230	1870	2
49	OPAK15	12	250	1950	10	250	1250	7
50	OPAK16	12	380	1740	8	710	1740	2
51	OPAK18	17	310	2830	13	450	1630	8
52	OPAK19	19	230	2230	13	570	2230	4
53	OPAK20	17	140	1810	10	460	1340	4
54	BC138	14	570	2850	8	570	1950	3
55	BC146	10	410	2040	7	410	2040	4
56	BC147	14	160	1960	10	160	1960	4
57	BC156	16	310	2280	9	450	1400	2
58	BC181	15	230	2030	12	320	1510	5
59	BC189	10	310	1730	8	310	1590	3
60	BC301	13	360	1360	9	490	1160	2
61	BC302	18	160	2030	14	160	2030	4
62	BC304	17	210	1980	13	210	1980	4
63	BC308	14	150	1750	11	150	1750	6
64	BC320	12	210	1480	10	210	1480	2
65	BC322	15	270	2250	10	350	1160	3
66	BC331	19	220	2080	13	420	2080	3
67	BC338	17	130	1490	11	260	1140	4
68	BC345	20	160	2320	14	160	1530	3
69	BC346	18	150	2810	13	150	1420	3
70	BC348	18	190	1760	14	280	1980	4
71	BC353	17	100	1680	13	220	1680	4
72	BC354	17	270	2010	13	270	2010	3
73	BC360	9	270	2790	7	270	1980	1
74	BC376	13	150	1310	10	150	1260	4
75	BC381	15	260	1750	11	410	1750	4
76	BC383	18	90	1840	14	240	1840	3
TOTAL		1156			800			247

One primer did not give any polymorphic band and the rest of them amplified between 1 and 8 polymorphic markers whose size ranged from 150 to 2500 bp. The sizes of the markers in this study agreed with the reported size range of RAPD markers (Deng *et al.,* 1995; Machado *et al*., 1996). These RAPD results confirmed a high level of polymorphisms in dioecious *P. atlantica* that is an important rootstock species for *P. vera*.

The efficiency of a molecular technique is usually measured in terms of progress per unit of time and associated cost, which is also of concern to most breeding programs. Numerous factors affect the costs and benefits of using molecular markers, one of which is type of the marker. RAPD methodology does not depend on the use of radioisotope and therefore it is more readily transferable to developing countries such as Turkey. It is relatively simple to generate a large number of polymorphisms to determine genetic relationships. The use of automation to run routine reactions may enhance the reliability of the technique.

Although a problem in the use of RAPD technique has been DNA pattern reproducibility, which is greatly influenced by the PCR reaction mixture and amplification conditions (Skroch and Nienhuis,

1995), our experience demonstrates that assay can be satisfactorily reproducible with sufficient repetitions.

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