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Genomic Diversity among Yew (*Taxus baccata*) Genotypes of Iran Revealed by Random Amplified Polymorphism DNA Markers

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ABSTRACT

English yew (*Taxus baccata* L.) is a European gymnosperm with a wide range and a discontinuous distribution. We used RAPD markers to analyze the genetic variation among 35 yew genotypes assembled from natural yew forests in northern region of Iran/Gorgan. Seventy 10 mer primers were used as single primers for amplification. A total of 130 bands were obtained for the 19 primers assayed of which 84 were polymorphic. The primers OPB08 and MG11 amplified maximum and minimum number of polymorphic bands, respectively. Cluster analysis of the genotypes was performed based on data from polymorphic RAPD bands, using Jaccard's similarity coefficient and UPGMA clustering method. The highest and lowest similarities detected between genotypes were 0.83 and 0.32, respectively. This study indicates that high levels of genetic polymorphism and genetic differentiation revealed by RAPD analysis in *T. baccata* and this marker was a suitable tool for genetic diversity studies in the yew population of Iran.

Key Words: Yew (*Taxus baccata);* RAPD; Genetic diversity; Polymorphism

INTRODUCTION

Yew (*Taxus baccata)* is a long-lived native tree species to western, central and southern Europe, northwest Africa, northern Iran and southwest Asia known as yew in English. However the later discovery of other very similar related species has led to qualification of yew as European Yew, Common Yew or English Yew (Rushforth, 1999). There is an increased tendency to consider the different species of yew, a fossil tree abundant in Triassic some 200 million years ago. Yew is also called the 'tree of death' because the tree is poisonous, valued for a variety of medicinal purposes mainly in the treatment of chest complaints and symbolic of eternal life due to its evergreen nature exceptional longevity and resistance wood to decay (Hartzell, 1991). Modern research has shown that the yew plants contain a substance 'taxol' in their shoots. Taxol has shown exciting potential as an anti-cancer drug, particularly in the treatment of ovarian cancers (Bown, 1995). Developing a reliable and discriminatory method for identifying cultivars of *T. baccata* has become increasingly important for plant breeders and those in the nursery industry who need sensitive tools to differentiate among and identify cultivars for plant patent protection. In the past, cultivars were identified primarily based on horticultural, morphological and physiological descriptions. In most cases, the descriptions and measurements varied considerably due to environmental fluctuation and human judgment. Methods developed over the past 20 years can detect differences in DNA sequences between individuals. Different types of marker systems have been used for genetic analysis and genotyping, including morphological, cytological, biochemical and DNA markers. The value of markers depends on their heritability and the level of polymorphism they can reveal. DNA markers are independent from environmental interactions, un-limited in number and show high level of polymorphism. Therefore, they are considered invaluable tools for determining genetic relationships/diversity. The random amplified polymorphic DNA (RAPD) technique can reveal polymorphism between very closely related genotypes. Since 1990, RAPD markers have been successfully used to identify cultivars and/or clones of barley (Tinker *et al*., 1993), wheat (He *et al.,* 1992), broccoli, cauliflower (Hu & Quiros, 1991; Demeke *et al*., 1992), apple (Koller *et al*., 1993; Autio *et al.*, 1998; Oraguzie *et al*., 2001), pear (Monte-Corvo *et al.,* 2000), almond (Bartolozzi *et al*., 1998), apricot (Takeda *et al.,* 1998), olive (Claros *et al*., 2001; Besnard *et al.*, 2001; Belaj *et al*., 2001), pistachio (Hormaza *et al.,* 1998), walnut (Nicese *et al*., 1998) and bluegrass (Rajasekar *et al*., 2006).

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Few studies on genetic variation in *T. baccata* are available to date. These studies, based on the analyses of isozymes, concern either only a single stand (Lewandowski *et al*., 1995; Rajewski *et al*., 2000) or few populations and ornamental trees to determine genetic variation (Hertel & Kohlstock, 1996). More recent studies in *Taxus canadensis* and *Taxus brevifolia* have used isozymes, RAPDs, restriction fragment length polymorphisms (RFLPs), or amplified fragment length polymorphisms (AFLPs) to elucidate clonal variation within populations, population dynamics, or metapopulation structure (El-Kassaby & Yanchuk, 1994; Wheeler *et al.,* 1995; Scher, 1996; Senneville *et al.,* 2001; Corradini *et al.,* 2002). RAPD marker was also used for regional differentiation in Swiss populations of English yew (Hilfiker *et al*., 2004) and to assess the genomic diversity of individual plants within a *T. cuspidata* population (Li *et al*., 2006).

Yew (*T. baccata*) can be found in northern forests in Iran, which includes Gorgan (Ghozghol, Afra akhteh, Siahroodbar), Ardabil (Arasbaran) and Gilan (Ghale roodkhan). Accurate identification of yew genotypes is very useful during all the steps of breeding from initial parent selection to the final utilization of cultivars in production schemes. Diversity analysis is an essential process for clear and sound identification of the genetic relatedness of the available genetic resources. It is also required for effective choice of parents for subsequent crossing and selection of the progenies. RAPD technology is feasible for the identification of the phylogenetic relationship among plant genotypes. Therefore, to obtain the pattern of genetic variation and population differentiation in supporting conventional plant breeding programs, we studied genetic diversity among of Iranian yew genotypes using Random Amplified Polymorphic DNA (RAPD) molecular marker for the first time. The objective of the present research was to study the genetic diversity of yew genotypes in Iran/Gorgan by RAPD marker.

MATERIALS AND METHODS

Plant materials and DNA extraction. Leaf material from 35 old yew trees was collected from natural yew forests in northern region of Iran/Gorgan including 21 samples from Siah roodbar, three samples from Afra takhteh and 11 samples from Ghozlog (Table I). Genomic DNA was extracted from yew leaves according to a modified procedure from the CTAB method (Murray & Thompson, 1980). The purity and quantity of genomic DNA was determined spectrophotometrically and confirmed using 0.8% agarose gel electrophoresis against known concentrations of lambda DNA/EcoR1+MindIII Marker, 3.

RAPD analysis. Seventy 10 mer single primers were used for amplification. The PCR reaction was performed in an Eppendorf (Mastercycler gradient) Thermal Cycler, in a 25 µL volume containing 2.5 µL of 1 X reaction buffer (100mM Tris-HCl, 15 mm $MgCl_2$, 500 mm KCl, pH 8.3, 0.5 mm $MgCl_2$), 200 µm each of dNTPs, 0.4 µm of 10 mer primer, 0.75 units of *Taq* DNA polymerase (Roche Co., Germany) and 50 ng of template DNA. Amplifications were performed as follows: initial step of denaturation at 94ºC for 2 min., followed by 40 cycles of denaturation at 92ºC for 1 min., primer annealing at 35ºC for 1 min. and extension at 72ºC for 2 min., followed by an extended elongation step at 72ºC for 5 min. The PCR fragments were separated on a 1.5% agarose gel in TBE buffer and stained with ethidium bromide and agarose gels were photographed with UV light. All amplifications were repeated at least twice and only reproducible and scorable bands were considered for analysis.

Data analysis. Polymorphic bands were considered as binary characters and scored as '1' for presence and '0' for absence of each band in individual lanes. These scores were entered as a binary matrix for analysis by the software NTSYS-pc version 2.02 (Raholf, 1998). The data were analyzed with the SIMQUAL option, on the basis of Jaccard's coefficients to estimate the genetic similarities. The similarity matrix was run on SAHN clustering, using the Un-weighted Pair Group Method with Arithmetic average (UPGMA) clustering algorithm to generate a dendrogram.

RESULTS AND DISCUSSION

Genetic variation of some major yew species, such as *Taxus baccata* L. (Hilfiker *et al.,* 2004) and *T. cuspidata* (Li *et al*., 2006) has been studied using RAPD markers. In this research 19 of the 70 primers screened were selected for DNA amplification reactions, because they yielded highly repeatable polymorphic bands in the subset samples (Table II). A total of 130 bands were obtained for the 19 primers studied of which 84 were polymorphic. The primers OPB08 and MG11 amplified maximum and minimum number of polymorphic bands, respectively. The number of fragments per primer ranged from three (CGS-16 & OPA18) to 13 (CGS-36) with an average of seven (Table II). The size of the fragments obtained varied from 400 to 3000 bp (Fig. 1). A dendrogram for these 35 cultivars was prepared using the similarity coefficient of RAPD markers (Fig. 2). Using Jaccard's coefficient (Table III) the highest genetic similarity (0.83) was obtained for 3A and 3C genotypes and the lowest genetic similarity (0.32) was observed between 2B and 7E Genotypes. The genetically homogenous 3A and 3C genotypes were grown in the same location (Siah roodbar forest) with same geographical conditions and had similar morphological traits such as leaf length and width, trunk height and thickness and fruit and seed size. These observations were justified between 8D and 8C genotypes, which had a similarity of 82% and were collected from Ghozlogh forest. The most different genotypes 2B and 7E belonged to different region with some different phenotypic characteristics (2B leaves form was long & 2E leaves shape was short) according to the dendrogram (Fig. 2).

Table I. collected samples from Iran/Golestan

	Samples	*Origin of samples*	*Height (m)*	*Geographical character*	
				N	E
1	1	Siah roodbar forest	1140	3650287	550529
2	2A,2B,2C,2D,2E,2F,2G,2H	Siah roodbar forest	1255	365047	550611
3	3A,3B,3C,3D	Siah roodbar forest	1330	365049	550703
4	4A,4B,4C ,4E,4F	Siah roodbar forest	1402	365101	550730
5	5A,5B	Siah roodbar forest	1087	365027	550515
6	6	Siah roodbar forest	914	365013	550442
7	7B, 7D,7E,	Afra takhteh forest	1502	364651	545725
8	8B,8C,8D,8E,8F,8G,8H	Ghozlogh forest	1415	364159	543429
9	9A,9B,9C	Ghozlogh forest	1923	364119	543402
10	10	Ghozlogh forest	1043	364159	543517

Table II. RAPD primers and the number of total and polymorphic bands produced in taxus cultivars

Primer	*Sequence (5'-3')*	*Total bands*	*Polymorphic bands*	*Polymorphic percentage*	*Monomorphic bands*
CGS-02	GGTGACGCAG	10	6	60	4
CGS-03	TCCGCTCTGG	7	7	100	0
CGS-04	GAAGCCAGCC	9	6	66.6	3
CGS05	CAGTGCCGGT	9	7	77.7	2
CGS-06	ACCGGCTTGT	8	7	87.5	1
CGS-07	CAGACTGGTC	6	5	83	1
CGS-09	GGAGCCTCAG	6	3	50	3
CGS-10	GACTAGGTGG	4	2	50	2
CGS-16	CCGATATCCC	3	3	100	0
CGS-36	AGCGCCATTG	13	2	15.4	11
CGS-44	TGGACCGGTG	5	2	40	3
CGS-46	CCAGTACTCC	5	3	60	2
OPA11	CAATCGCCGT	5	5	100	0
OPA16	AGCCAGCAAC	8	5	62.5	3
OPA18	AGGTGACCGT	3	3	100	0
OPAE10	CTGAAGCGCA	6	4	66.6	2
OPB08	GTCCACACGG	9	9	100	0
MG01	AGCGCCGACG	6	4	66.6	2
MG11	AGGAGCTGCC	8	1	12.5	7
Total		**130**	**84**		**46**
Mean		**6.84**	**4.42**	**64.6**	**2.42**

Table III. Similarity coefficient among of 35 yew genotypes based on 19 random RAPD primers

	1	2A	2B	2C	2D	2E	2F	2G	2H	3A	3B	3C	3D	4A	4B	4C	4E	4F	5A	5B	6	7B	7D	7E	8B	8C	8D	8E	8F	8G	8H	9A	9B	9C	10
1	1																																		
2A	0.62	1																																	
2B	0.52	0.6	1																																
2C	0.79	0.65	0.53	1																															
2D	0.66	0.61	0.52	0.71	1																														
2E	0.5	0.48	0.44	0.53	0.51	1																													
2F	0.61	0.78	0.45	0.71	0.67	0.47	1																												
2G	0.45	0.44	0.42	0.5	0.52	0.38	0.5	1																											
2H	0.56	0.57	0.38	0.6	0.64	0.45	0.62	0.64	1																										
3A	0.72	0.67	0.63	0.76	0.75	0.54	0.66	0.56	0.68	1																									
3B	0.59	0.62	0.53	0.61	0.6	0.4	0.57	0.39	0.47	0.71	1																								
3C	0.63	0.63	0.48	0.63	0.74	0.52	0.61	0.52	0.64	0.83	0.63	1																							
3D	0.52	0.61	0.38	0.62	0.59	0.35	0.55	0.46	0.55	0.62	0.66	0.65	1																						
4A	0.64	0.57	0.43	0.61	0.6	0.44	0.63	0.49	0.55	0.64	0.54	0.61	0.57	1																					
4B	0.57	0.58	0.53	0.6	0.58	0.38	0.59	0.47	0.48	0.58	0.53	0.57	0.56	0.63	1																				
4C	0.66	0.52	0.48	0.69	0.66	0.44	0.74	0.55	0.65	0.69	0.63	0.69	0.67	0.7	0.55	1																			
4E	0.7	0.62	0.43	0.71	0.74	0.51	0.71	0.55	0.67	0.75	0.54	0.67	0.6	0.71	0.65	0.65	1																		
4F	0.63	0.66	0.52	0.7	0.71	0.46	0.71	0.58	0.63	0.77	0.62	0.67	0.7	0.76	0.61	0.72	0.73	1																	
5A	0.63	0.56	0.43	0.6	0.58	0.51	0.52	0.45	0.58	0.64	0.52	0.52	0.53	0.6	0.56	0.51	0.63	0.58	1																
5B	0.6	0.63	0.53	0.67	0.66	0.46	0.65	0.49	0.6	0.7	0.57	0.65	0.61	0.64	0.6	0.65	0.7	0.65	0.56	1															
6	0.72	0.67	0.58	0.74	0.7	0.48	0.66	0.51	0.66	0.77	0.61	0.72	0.62	0.68	0.61	0.75	0.71	0.74	0.63	0.71	1														
7B	0.66	0.64	0.45	0.68	0.64	0.49	0.63	0.53	0.59	0.76	0.62	0.69	0.63	0.7	0.63	0.7	0.65	0.68	0.68	0.62	0.7	1													
7D	0.64	0.61	0.43	0.6	0.67	0.5	0.57	0.51	0.59	0.71	0.55	0.64	0.51	0.68	0.58	0.66	0.71	0.69	0.59	0.58	0.65	0.67	1												
7E	0.54	0.55	0.32	0.56	0.57	0.41	0.6	0.42	0.52	0.59	0.52	0.55	0.57	0.47	0.46	0.49	0.52	0.55	0.6	0.48	0.55	0.51	0.43	1											
8B	0.63	0.59	0.51	0.7	0.7	0.52	0.66	0.6	0.63	0.67	0.6	0.66	0.59	0.63	0.6	0.67	0.67	0.68	0.61	0.69	0.7	0.6	0.57	0.59	1										
8C	0.63	0.63	0.6	0.7	0.69	0.46	0.6	0.62	0.58	0.78	0.65	0.68	0.57	0.68	0.57	0.59	0.66	0.65	0.65	0.66	0.66	0.6	0.71	0.7	0.64	1									
8D	0.65	0.57	0.53	0.67	0.7	0.42	0.62	0.53	0.59	0.72	0.59	0.56	0.58	0.56	0.58	0.6	0.63	0.63	0.7	0.69	0.59	0.63	0.64	0.67	0.63	0.82	1								
8E	0.6	0.55	0.5	0.65	0.63	0.5	0.53	0.44	0.53	0.71	0.53	0.58	0.56	0.62	0.53	0.56	0.65	0.63	0.61	0.58	0.65	0.62	0.57	0.52	0.69	0.65	0.66	1							
8F	0.73	0.58	0.54	0.73	0.73	0.57	0.62	0.44	0.54	0.75	0.64	0.66	0.57	0.66	0.65	0.63	0.73	0.7	0.63	0.68	0.79	0.67	0.66	0.58	0.68	0.74	0.69	0.76	1						
8G	0.57	0.47	0.48	0.57	0.6	0.38	0.52	0.53	0.54	0.63	0.53	0.56	0.5	0.56	0.52	0.55	0.62	0.58	0.53	0.54	0.58	0.62	0.59	0.45	0.56	0.73	0.77	0.58	0.64	1					
8H	0.63	0.53	0.59	0.63	0.64	0.45	0.54	0.5	0.48	0.67	0.51	0.54	0.54	0.61	0.54	0.6	0.6	0.66	0.56	0.51	0.63	0.54	0.59	0.52	0.56	0.7	0.7	0.66	0.72	0.65	1				
9A	0.54	0.53	0.54	0.51	0.59	0.43	0.49	0.6	0.53	0.64	0.52	0.61	0.54	0.56	0.58	0.48	0.64	0.61	0.57	0.57	0.54	0.58	0.58	0.44	0.59	0.65	0.63	0.55	0.57	0.68	0.57	1			
9B	0.64	0.55	0.43	0.66	0.67	0.47	0.64	0.51	0.59	0.68	0.58	0.64	0.59	0.56	0.54	0.69	0.67	0.63	0.53	0.59	0.68	0.65	0.6	0.49	0.58	0.63	0.62	0.58	0.66	0.55	0.58	0.55	1		
9C	0.54	0.52	0.49	0.66	0.64	0.49	0.55	0.51	0.54	0.64	0.47	0.56	0.53	0.58	0.56	0.57	0.62	0.68	0.48	0.56	0.65	0.57	0.6	0.46	0.61	0.65	0.64	0.56	0.65	0.6	0.6	0.58	0.62	1	
10	0.66	0.64	0.63	0.63	0.64	0.47	0.63	0.44	0.54	0.75	0.63	0.65	0.56	0.66	0.62	0.58	0.63	0.64	0.61	0.59	0.69	0.65	0.59	0.56	0.62	0.67	0.62	0.71	0.69	0.54	0.59	0.58	0.58	0.58	1

Fig. 1. RAPD profiles of 23 *Taxus baccata* cultivars with primer CGS-06.The products were separated in a 1.2% agarose gel stained with ethidium bromide

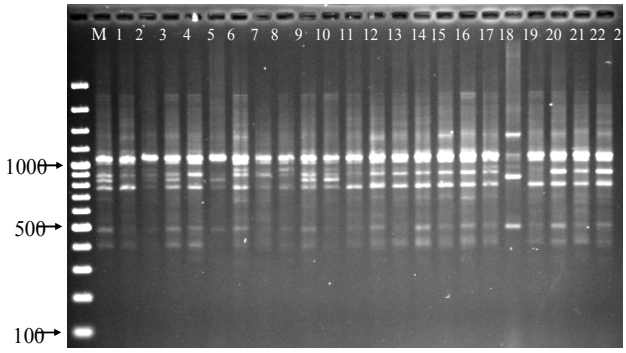
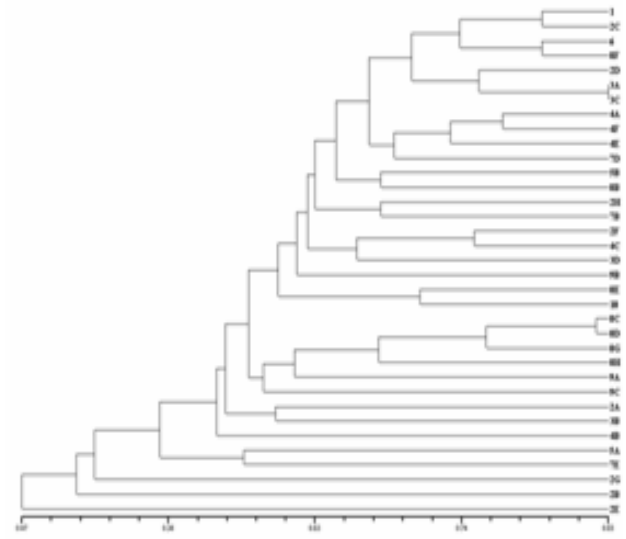


Fig. 2. UPGMA dendrogram of 35 yew genotypes based on 19 random RAPD primers



The genotypes 2E, 2B and 2G had a high distance with other genotypes. These three genotypes despite of having similar forms and longer leaves comparing with other genotypes did not show genetic similarity with each other. These morphological resemblances could be attributed to the same climatic conditions (all three genotypes were in Siah roodbar forest). This phenomenon was observed among of 7B, 7E and 7D or 5A and 5B genotypes, which had similar growing location and same phenotypic characteristics but did not show genetic similarity. It was interesting that the genotypes 9A, 9B and 9C with shrub form and different branching habit were morphologically distinguished from all other genotypes. They were in the highest region of Ghozlogh forest but did not show much genetic similarity. These results indicated that there are some differences between grouping by RAPD markers and morphological traits. Similar phenomena have been reported in banana (Uma *et al*., 2004), strawberry (Garcia *et al*., 2002), ryegrass (Roland-ruiz *et al*., 2001), grape (Martinez *et al*., 2003) and pomegranate (Sarkhosh *et al*., 2006). It can be related to some reasons; the different geographical conditions effect on morphological traits, which do not influence genetic markers (Kumar, 1999; Gupta & Rustgi, 2004). Previous researches showed that application of molecular markers can not detect mutations and genetic changes, which affect phenotypic traits such as tree size, leaf form and branching habit (Garcia *et al*., 2002; Sarkhosh *et al*., 2006). Moreover the molecular markers such as RAPD can distinguish fragments of any region of the genome, while only 1-2% of the genome is expressed. It should be noted that post-transcriptional modifications and non-nuclear inheritance of some characteristics can deprive the fitting of morphological markers with molecular markers (Gupta & Rustgi, 2004). However, during the last few years, emphasis shifted towards the development of molecular markers from the transcribed region of the genome. The availability of a large number of cDNA clones in a variety of plant systems and accumulation of a large number of expressed sequence tags (EST) have made this possible (Gupta & Rustgi, 2004).

This study was an attempt to establish the genetic diversity background in yew populations of Iran/Gorgan with RAPD markers. High levels of polymorphism and the existence of population differentiation of *T. baccata* found in the present work showed that RAPD markers are a suitable tool for genetic diversity studies in the yew and can be useful for accumulation and management of genetic-breeding resources of this species; Although, markers from the expressed region of genomes may be increasingly used in plant phylogenetic studies in future.

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