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Genetic relationships between selected Turkish mulberry genotypes (*Morus* spp) based on RAPD markers

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ABSTRACT. Mulberry (*Morus* spp, Moraceae) is an important horticultural crop in Turkey, which is one of the main world producers of mulberry fruit. We evaluated the genetic relationships among 26 mulberry genotypes selected for agronomic characteristics, using RAPD markers. A total of 367 DNA markers were generated with 34 random primers. The highest genetic similarity (0.80) was observed between Oltu58 (*M. nigra*) and Olur90 (*M. nigra*) genotypes. The genotypes Oltu3 (*M. alba*) and Oltu18 (*M. rubra*) were the most distant (0.36). We found that the RAPD technique is a useful tool to discriminate mulberry genotypes at both the intra- and interspecific level. This type of information will aid in accurate identification of useful genotypes for breeding programs.

Key words: *Morus alba*; *Morus nigra*; *Morus rubra*; RAPD; Genetic relationship

INTRODUCTION

Mulberries are highly adaptable species in different soil and climatic conditions. Therefore, they can be found in a wide area of tropical, subtropical, and temperate zones in Asia, Europe, North America, South America, and Africa (Kafkas et al., 2008). The trees have been used for both fruit production and also for sericulture especially in east, central, and south Asia. Although around 30 mulberry species have been found in the world, a few of them, for example, *Morus alba*, *M. nigra*, *M. rubra*, *M. indica*, and *M. laevigata*, are used for direct fruit consumption (Awasthi et al., 2004). Most of these species, except *M. alba*, has a distinct flavor with juicy and acidic characteristics making them attractive for use in the processing industry for products such as fruit juice, ice cream, jelly, and jam. However, *M. alba*, with the highest sugar content, is used for making traditional foods such as ''mulberry pekmez'', ''mulberry pestil'', and ''mulberry kome'' in Turkey (Ercisli, 2004; Ercisli and Orhan, 2007).

Turkey has very old mulberry cultivation, and mulberry plants can be found in most regions of Turkey, with a high morphological diversity, and it is believed that mulberry cultivation in Turkey was started 400-500 years ago (Ercisli and Orhan, 2007). Among the mulberry species found in Turkey, *M. alba* is the most dominant (95% of total mulberry production in Turkey), because its fruits are very high in soluble solid contents, and thus they are the most suitable for processing to make traditional local mulberry products in Turkey. More recently, *M. nigra* has grown in importance because it is believed to be a more healthy fruit among mulberry species due to its high bioactive content (Ercisli and Orhan, 2008).

In Turkey, particularly Eastern Anatolia, mulberries have been the main source of processed fruits for human consumption because of the cold temperate climate characteristics of the region. The people in this region consume mulberry products during the long winter season. On the other hand, the mulberry genotypes are very diverse, where they are obtained from seeds from the past in the region. This process may lead to a great number of landraces adapted to different conditions and to different uses throughout the region. In this region, little information is available about genetic resources in mulberries.

Previously, mulberry genotypes have been described based on morphological characters, but it is well known that such characters are strongly influenced by environment factors. More recently, molecular markers have gained more importance, and they are commonly used to characterize mulberry genotypes throughout mulberry-growing countries (Vijayan and Chatterjee, 2003; Vijayan, 2004; Vijayan et al., 2004a,b,c; Zhao et al., 2006, 2007; Kafkas et al., 2008; Kar et al., 2008).

The aim of this study was to use random amplified polymorphic DNA (RAPD) fingerprinting to detect and characterize polymorphisms among mulberry genotypes sampled from diverse environments of the Erzurum region in Turkey, and in addition, to investigate the genetic relationships among the genotypes sampled.

MATERIAL AND METHODS

Leaf samples from 26 selected mulberry genotypes (22 *M. alba*, 3 *M. nigra* and 1 *M. rubra*) found in the towns in Oltu and Olur in Erzurum provinces located in the Eastern

Anatolia, Turkey, were collected and immediately stored at -80°C for DNA extraction.

Genomic DNA was extracted from powdered plant materials using a modified method described by Lin et al. (2001). Approximately 10-15 mg tissue samples from each plant species were snap frozen in liquid nitrogen in 2-mL Eppendorf tubes. A volume of 1000 µL DNA extraction buffer [100 mM Tris-HCl, pH 8.0; 50 mM EDTA, pH 8.0; 500 mM NaCl; 2% SDS (w/v); 2% 2-mercaptoethanol (v/v); 1% PVP (w/v)] was added and the contents mixed well. The mixture was incubated at 65°C in a water bath for 40 min with intermittent shaking at 5-min intervals. The mixture was centrifuged at 12,000 *g* for 15 min at 4°C, the supernatant was transferred to a new 1.5-mL tube, mixed with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1), and centrifuged. The supernatant was collected and mixed with 1/10 volume 10% CTAB-0.7 M NaCl in a new tube. After centrifugation, the supernatant was collected and an equal volume of chloroform:isoamyl alcohol (24:1) was added with gentle mixed. The DNA was precipitated by the addition of 0.6 volume of freezer-cold isopropanol and left at -20°C for 10 min. The DNA was pelleted by centrifugation (12,000 *g*, 10 min), and the isopropanol was poured off; the DNA was allowed to air-dry before being dissolved in 100 µL TE buffer.

Samples were screened for RAPD variation using standard 10-base primers supplied by Operon. Thirty microliters of reaction cocktail was prepared as follows: 3.0 µL 10X buffer, 1.2 µL dNTPs (10 mM), 1.2 µL magnesium chloride (25 mM), 2.0 µL primer (5 µM), 0.4 µL Taq polymerase (5 U), 19.2 µL water, 3.0 µL sample DNA (100 ng/µL). A total of 34 RAPD primers were tested in this study (Table 1).

The thermocycler (Eppendorf Company) was programmed for 2 min at 95°C; 2 cycles of 30 s at 95°C, 1 min at 37°C, 2 min at 72°C; 2 cycles of 30 s at 95°C, 1 min at 35°C, 2 min at 72°C; 41 cycles of 30 s at 94°C, 1 min at 35°C, 2 min at 72°C; followed by a final 5-min extension at 72°C and then cooling down to 4°C.

The polymerase chain reaction (PCR) products (27 µL) were mixed with 6X gel loading buffer (3 µL), loaded onto an agarose (1.5%, w/v) gel in 0.5X TBE (Tris-borate-EDTA) buffer, and electrophoresed at 70 V for 150 min. The gel was stained in ethidium bromide solution (2 µL Etbr/100 mL in 1X TBE buffer) for 40 min and visualized under UV using a Bio Doc Image Analysis System with Uvisoft analysis package (Cambrige, UK).

RAPD data were recorded as for the presence (1) of a band and for its absence (0) and were transferred to a binary matrix. Only reproducible bands were scored. For distance matrix analysis of the RAPD data, the WINDIST software was used. The dendogram was constructed on the basis of this matrix by UPGMA (unweighted pair group method using arithmetic average), using the PHYLIP 3.5c software program (Felsenstein, 1993).

RESULTS

We used a total of 34 decamer oligonucleotide primers to determine genetic relationships between 26 selected promising mulberry genotypes (22 *M. alba*, 3 *M. nigra* and 1 *M. rubra*), and the results of RAPD analysis are summarized in Tables 1 and 2 and Figures 1 and 2.

As indicated in Table 1, the 34 RAPD primers generated a total of 367 RAPD bands and among them 330 RAPD bands were found to be polymorphic, resulting in 89.9% polymorphism. The number of RAPD products varied from 7 to 14 (Table 1). The primers OPW1,

OPBB15 and OPBD17 gave the highest number of RAPD products (14), while OPY6 and OPBB04 gave the lowest number of RAPD products (7). The level of polymorphism varied from 55.6% (OPW8) to 100.0% (OPA12, OPW6, OPH19, OPB8, OPW11, OPW18, OPBA06, OPBA08, OPBB04, and OPBB07). An average of 9.7 polymorphic RAPD bands was recorded. Figure 1 displays the RAPD pattern generated by OPA4 from genomic DNA of 26 mulberry genotypes (*Morus* spp).

Table 1. Primers employed with the number of random amplified polymorphic DNA (RAPD) markers obtained, their sequence, and size of the fragments.

Primer code	Sequence of primer 5'→3'	Size (bp) min-max	Total number of bands	Total number of RAPD products per primer	Polymorphism level (%)
OPW1	CTCAGTGTCC	250-2500	14	13	92.9%
OPW4	CAGAAGCGGA	350-2000	11	9	81.8%
OPW5	GGCGGATAAG	290-2000	12	11	91.7%
OPW6	AGGCCCGATG	350-2000	13	13	100.0%
OPW7	CTGGACGTCA	350-1500	10	9	90.0%
OPW8	GACTGCCTCT	300-1500	9	5	55.6%
OPW11	CTGATGCGTG	400-1600	11	11	100.0%
OPW17	GTCCTGGGTT	600-1500	10	9	90.0%
OPW18	TTCAGGGCAC	500-2500	10	10	100.0%
OPA4	AATCGGGCTG	400-4000	11	9	81.8%
OPA12	TCGGCGATAG	450-2000	10	10	100.0%
OPA13	CAGCACCCAC	450-2800	11	10	90.9%
OPH16	TCTCAGCTGG	400-2000	10	8	80.0%
OPH19	CTGACCAGCC	400-3000	12	12	100.0%
OPY1	GTGGCATCTC	350-1500	10	9	90.0%
OPY6	AAGGCTCACC	500-2500	7	6	85.7%
OPY8	AGGCAGAGCA	350-1150	9	8	88.9%
OPY11	AGACGATGGG	150-2000	13	11	84.6%
OPY13	GGGTCTCGGT	300-1250	8	7	87.5%
OPY15	AGTCGCCCTT	480-3000	8	6	75.0%
OPY16	GGGCCAATGT	300-1400	10	9	90.0%
OPB8	GTCCACACGG	280-2000	12	12	100.0%
OPB10	CTGCTGGGAC	400-1450	10	8	80.0%
OPBA03	GTGCGAGAAC	230-1200	11	9	81.8%
OPBA06	GGACGACCGT	250-1700	12	12	100.0%
OPBA08	CCACAGCCGA	250-1000	11	11	100.0%
OPBB04	ACCAGGTCAC	350-1000	7	7	100.0%
OPBB07	GAAGGCTGGG	360-2400	12	12	100.0%
OPBB11	TGCGGGTTCC	240-1050	11	10	90.9%
OPBB13	CTTCGGTGTG	200-1000	12	10	83.3%
OPBB15	AAGTGCCCTG	350-1500	14	13	92.6%
OPBC08	GGTCTTCCCT	400-1200	10	8	80.0%
OPBD07	GAGCTGGTCC	380-1400	12	11	91.7%
OPBD17	GTTCGCTCCC	100-1300	14	12	85.7%
Total			367	330	
Avarage				9.7	89.9%

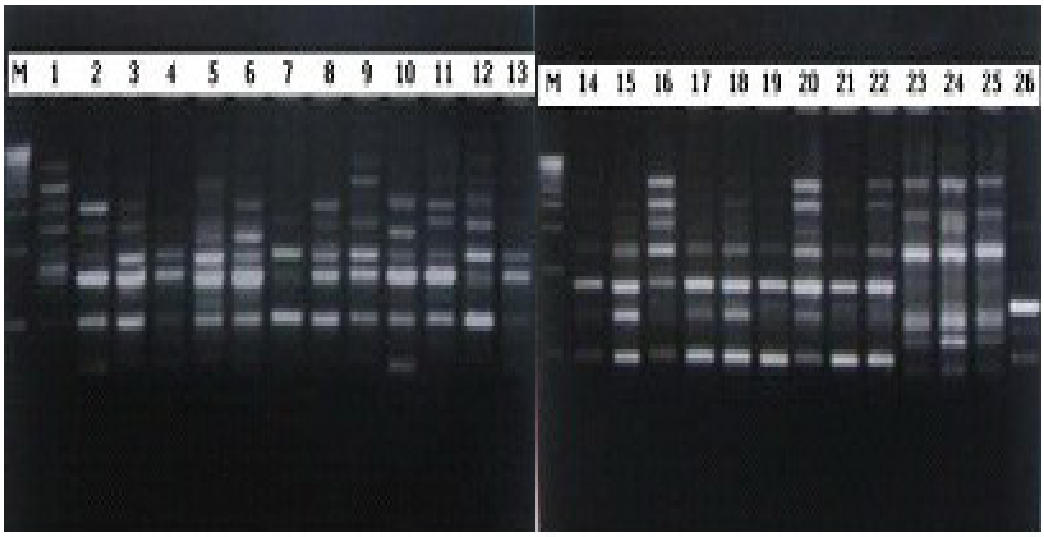

Figure 1. RAPD bands obtained by amplification with primer OPA4 (M = marker, *lane 1* = Oltu34, *lane 2* = Oltu3, *lane 3* = Oltu32, *lane 4* = Olur1, *lane 5* = Olur87, *lane 6* = Olur88, *lane 7* = Olur91, *lane 8* = Olur92, *lane 9* = Olur23, *lane 10* = Olur45, *lane 11* = Olur7, *lane 12* = Olur49, *lane 13* = Olur52, *lane 14* = Olur55, *lane 15* = Olur57, *lane 16* = Olur53, *lane 17* = Oltu8, *lane 18* = Oltu12, *lane 19* = Oltu13, *lane 20* = Oltu14, *lane 21* = Oltu20, *lane 22* = Oltu21, *lane 23* = Oltu58, *lane 24* = Olur90, *lane 25* = Olur8, *lane 26* = Oltu18).

As shown in Table 2, the highest genetic similarity (0.80) was between Oltu58 (*M. nigra*) and Olur90 (*M. nigra*), whereas the lowest genetic similarity (0.36) was between Oltu3 (*M. alba*) and Oltu18 (*M. rubra*).

The dendogram (Figure 2) shows genetic relationships among the mulberry genotypes. In this dendogram, 26 mulberry genotypes grouped into four major clusters, indicating greater genetic diversity among them. The first group includes Oltu58, Olur8 and Olur90, genotypes that all belong to *M. nigra*. The second group includes Oltu34, Oltu32, Olur1, Olur87, Olur88, Olur91, Olur92, Olur23, Olur45, Olur7, Olur49, Olur52, Olur55, Olur57, Olur53, Oltu8, Oltu12, Oltu13, Oltu14, Oltu20, and Oltu21 (all of *M. alba*). In the dendogram, the third group consists of only Oltu3 (*M. alba*) and the last group includes only Oltu18 (*M. rubra*), which is very distant from the other genotypes.

DISCUSSION

As indicated in the Results section, genetic similarity varied from 0.36 to 0.80. These results indicate that the genotypes Oltu58 (*M. nigra*) and Olur90 (*M. nigra*) are genetically closer than other mulberry genotypes. A previous study showed that among mulberry species, the highest genetic variability was among *M. alba* and that in contrast the lowest genetic variability was among *M. nigra* genotypes (Kafkas et al., 2008). Our study also supports the idea that *M. alba* genotypes has higher variability.

According to the results, there is a high genetic diversity between and within mulberry species. These results also support the idea that mulberry genotypes used in this region

Table 2. Similarity matrix values based on RAPD data between 26 mulberry genotypes (1 - Oltu34, 2 - Oltu3, 3 - Oltu32, 4 - Olur1, 5 - Olur87, 6 - Olur88, 7 - Olur91, 8 - Olur92, 9 - Olur23, 10 - Olur45, 11 - Olur7, 12 - Olur49, 13 - Olur52, 14 - Olur55, 15 - Olur57, 16 - Olur53, 17 - Oltu8, 18 - Oltu12, 19 - Oltu13, 20 - Oltu14, 21 - Oltu20, 22 - Oltu21, 23 - Oltu58, 24 - Olur90, 25 - Olur8, 26 - Oltu18).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1	1.00																									
2	0.55	1.00																								
3	0.65	0.53	1.00																							
4	0.53	0.50	0.63	1.00																						
5	0.63	0.46	0.62	0.57	1.00																					
6	0.60	0.53	0.63	0.57	0.63	1.00																				
7	0.57	0.49	0.57	0.56	0.53	0.60	1.00																			
8	0.61	0.56	0.65	0.54	0.56	0.70	0.61	1.00																		
9	0.57	0.48	0.57	0.50	0.52	0.58	0.62	0.63	1.00																	
10	0.53	0.52	0.58	0.54	0.49	0.60	0.54	0.64	0.57	1.00																
11	0.50	0.50	0.55	0.49	0.47	0.57	0.50	0.58	0.54	0.58	1.00															
12	0.58	0.50	0.59	0.49	0.60	0.60	0.55	0.63	0.60	0.61	0.56	1.00														
13	0.50	0.51	0.51	0.44	0.50	0.55	0.58	0.59	0.61	0.55	0.56	0.71	1.00													
14	0.53	0.42	0.48	0.47	0.51	0.48	0.53	0.50	0.53	0.44	0.46	0.51	0.48	1.00												
15	0.59	0.48	0.54	0.49	0.52	0.52	0.58	0.52	0.56	0.46	0.43	0.52	0.46	0.64	1.00											
16	0.65	0.46	0.60	0.55	0.61	0.62	0.58	0.59	0.55	0.53	0.51	0.59	0.51	0.55	0.63	1.00										
17	0.62	0.46	0.58	0.49	0.58	0.56	0.59	0.54	0.58	0.51	0.48	0.58	0.57	0.58	0.68	0.73	1.00									
18	0.56	0.47	0.56	0.48	0.52	0.51	0.54	0.52	0.55	0.53	0.47	0.52	0.53	0.54	0.57	0.62	0.72	1.00								
19	0.49	0.38	0.46	0.45	0.49	0.46	0.51	0.47	0.53	0.46	0.39	0.48	0.50	0.48	0.51	0.53	0.61	0.54	1.00							
20	0.58	0.45	0.53	0.47	0.56	0.54	0.55	0.55	0.54	0.51	0.46	0.61	0.57	0.58	0.60	0.66	0.70	0.62	0.61	1.00						
21	0.52	0.41	0.48	0.44	0.51	0.47	0.50	0.48	0.50	0.45	0.44	0.51	0.50	0.56	0.53	0.57	0.67	0.65	0.61	0.64	1.00					
22	0.55	0.45	0.49	0.46	0.56	0.50	0.55	0.52	0.53	0.49	0.53	0.58	0.50	0.56	0.50	0.63	0.59	0.55	0.53	0.58	0.62	1.00				
23	0.46	0.41	0.53	0.44	0.47	0.52	0.48	0.52	0.47	0.46	0.46	0.50	0.47	0.46	0.45	0.51	0.53	0.47	0.43	0.50	0.49	0.47	1.00			
24	0.48	0.41	0.52	0.44	0.46	0.51	0.48	0.51	0.47	0.47	0.48	0.52	0.48	0.47	0.47	0.51	0.56	0.49	0.43	0.50	0.49	0.49	0.80	1.00		
25	0.43	0.41	0.47	0.40	0.42	0.48	0.48	0.45	0.45	0.41	0.49	0.47	0.50	0.44	0.47	0.50	0.54	0.47	0.45	0.45	0.46	0.47	0.69	0.78	1.00	
26	0.41	0.36	0.38	0.38	0.39	0.42	0.39	0.44	0.45	0.41	0.40	0.43	0.41	0.40	0.39	0.41	0.40	0.39	0.42	0.39	0.39	0.40	0.36	0.41	0.40	1.00

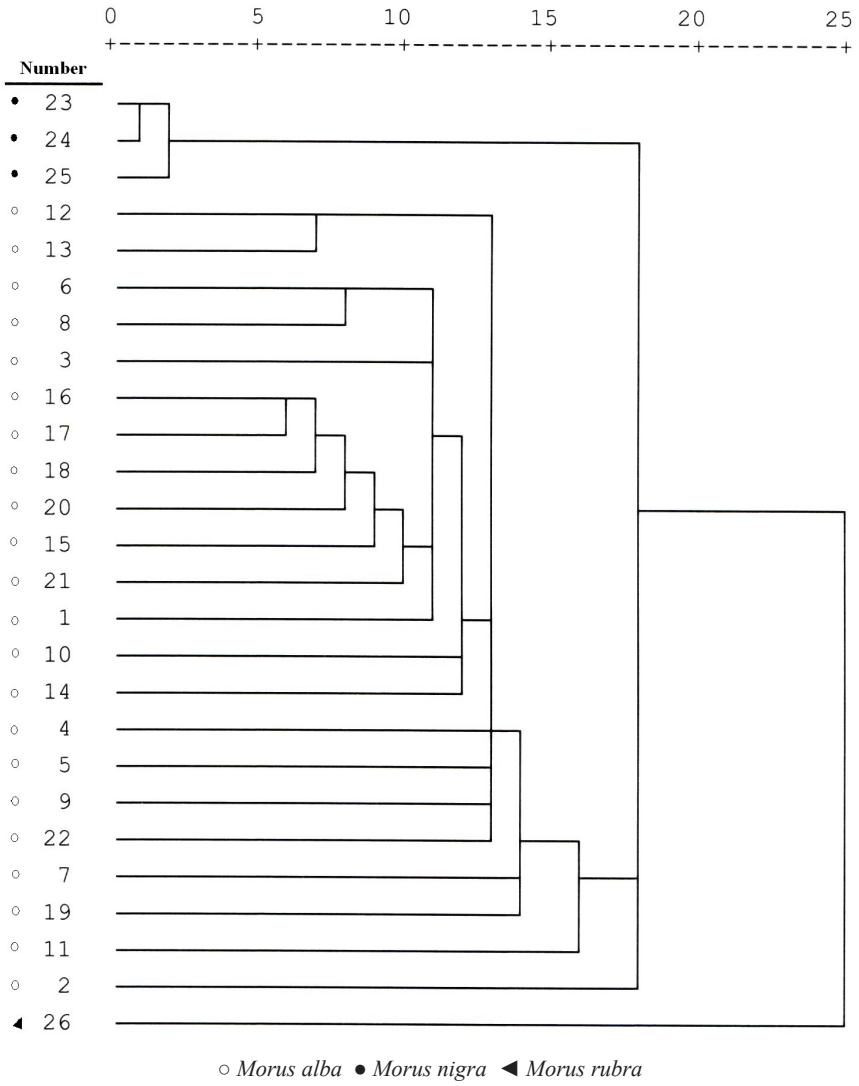

Figure 2. UPGMA dendogram showing the relationships of mulberries (1 - Oltu34, 2 - Oltu3, 3 - Oltu32, 4 - Olur1, 5 - Olur87, 6 - Olur88, 7 - Olur91, 8 - Olur92, 9 - Olur23, 10 - Olur45, 11 - Olur7, 12 - Olur49, 13 - Olur52, 14 - Olur55, 15 - Olur57, 16 - Olur53, 17 - Oltu8, 18 - Oltu12, 19 - Oltu13, 20 - Oltu14, 21 - Oltu20, 22 - Oltu21, 23 - Oltu58, 24 - Olur90, 25 - Olur8, 26 - Oltu18.

previously selected by humans among seed-propagated materials and by mutations may have occurred during the long cultivation period and may also share this high genetic diversity. On the other hand, we can also conclude that RAPD analysis can be useful and the least expensive molecular technique for grouping and characterizing mulberry genotypes (*Morus* spp). Our study shows that there is a very high polymorphism rate in the mulberry germplasm. The high level of genetic variability detected within the mulberry germplasm

collected in the Erzurum region could be attributed to either the distant geographical or genetic origin. On the other hand, an average similarity ratio of 0.594 was recorded for the mulberry genotypes, suggesting that the genome may differ in genotypes. These results also indicate the potential use of these genotypes, particularly the more distant ones, for future breeding programs.

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REFERENCES

Awasthi AK, Nagaraja GM, Naik GV, Kanginakudru S, et al. (2004). Genetic diversity and relationships in mulberry (genus *Morus*) as revealed by RAPD and ISSR marker assays. *BMC Genet.* 5: 1.

Ercisli S (2004). A short review of the fruit germplasm resources of Turkey. *Genet. Resour. Crop Evol.* 51: 419-435.

Ercisli S and Orhan E (2007). Chemical composition of white (*Morus alba*), red (*Morus rubra*) and black (*Morus nigra*) mulberry fruits. *Food Chem.* 103: 1380-1384.

Ercisli S and Orhan E (2008). Some physico-chemical characteristics of black mulberry (*Morus nigra* L.) genotypes from Northeast Anatolia region of Turkey. *Sci. Hort.* 116: 41-46.

Felsenstein J (1993). PHYLIP (Phylogeny Inference Package) User Manual. University of Washington, Seattle.

Kafkas S, Özen M, Doğan, Özcan B, et al. (2008). Molecular characterization of mulberry accessions in Turkey by AFLP markers. *J. Am. Soc. Hort. Sci.* 133: 593-597.

Kar PK, Srivastava PP, Awasthi AK and Awasthi SR (2008). Genetic variability and association of ISSR markers with some biochemical traits in mulberry (*Morus* spp.) genetic resources available in India. *Tree Genet. Genomes* 4: 75-83.

Lin RC, Ding ZS, Li LB and Kuang TY (2001). A rapid and efficient DNA minipreparation suitable for screening transgenic plants. *Plant Mol. Biol. Rep.* 19: 379a-379e.

Vijayan K (2004). Genetic relationships of Japanese and Indian mulberry (*Morus* spp.) genotypes revealed by DNA fingerprinting. *Plant Syst. Evol.* 243: 221-232.

Vijayan K and Chatterjee SN (2003). SSR profiling of Indian cultivars of mulberry (*Morus* spp.) and its relevance to breeding programs. *Euphytica* 131: 53-63.

Vijayan K, Srivastava PP and Awasthi AK (2004a). Analysis of phylogenetic relationship among five mulberry (*Morus*) species using molecular markers. *Genome* 47: 439-448.

Vijayan K, Awasthi AK, Srivastava PP and Saratchandra B (2004b). Genetic analysis of Indian mulberry varieties through molecular markers. *Hereditas* 141: 8-14.

Vijayan K, Kar PK, Tikader A, Srivastava PP, et al. (2004c). Molecular evaluation of genetic variability in wild populations of mulberry (*Morus serrata* Roxb.). *Plant Breed.* 123: 568-572.

Zhao W, Zhou Z, Miao X, Wang S, et al. (2006). Genetic relatedness among cultivated and wild mulberry (Moraceae: Morus) as revealed by inter-simple sequence repeat analysis in China. *Can. J. Plant Sci.* 86: 251-257.

Zhao W, Wang Y, Chen T, Jia G, et al. (2007). Genetic structure of mulberry from different ecotypes revealed by ISSRs in China: an implications for conservation of local mulberry varieties. *Sci. Hort.* 155: 47-55.

