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Full Length Research Paper

Molecular characterization of mulberry germplasm from Eastern Anatolia

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Mulberry has an important quality for both horticultural production and sericulture in Turkey. Anatolia has an extensive potential for mulberry. On the other hand, these mullberry germplasm has not been adequately determined and relatedness among them has not been properly investigated. A total of 47 mullberry genotypes; 20, 14, 5, 4 and 4 from Erzincan, Elazığ, Diyarbakır, Malatya and Mardin provinces, respectively, were comparatively investigated in order to determine their relationships by 55 RAPD markers obtained from 6 primers (Operon OPA12, OPA13, OPAF04, OPF07, OPG02 and OPG03) and their relatedness were determined.

Key words: Characterization, molecular, mulberry, RAPD.

INTRODUCTION

Mulberry is a perennial fruit species belongs to the genus *Morus* of the family Moraceae, Magnoliophyta division, Magnoliopsida class, Urticales order (Anonymous, 2006). The origin of mulberry is Asia (Awasthi et al., 2004). The mulberry is not a very large tree and only grows to about 16 m in height; its erect and thick stem is cylindirical; its bark is cracked and is grey-brown colors. Its leaves in general have a heart-shaped base and a pointed tip; the indented leaves are alternately arranged, simple, often lobed, serrated on the margin and upper part is darker green. Mulberry leaves are ecologically important as the only food source of the silkworm. Mulberry trees are either dioecious or monoecious and sometimes will transform from one sex to another. The flowers are held on short, green, pendulous, nondescript catkins that emerge in the axils of the current season's growth and on spurs on older wood. They are wind pollinated and some cultivars will set fruit without any pollination (Anonymous, 2009)

The most important mulberry species are *Morus alba* (white mulberry), *Morus australis* (Chinese mulberry), *Morus indica* (Indian mulberry), *Morus microphylla* (Texas mulberry), *Morus nigra* (Black mulberry), *Morus rubra* (Red-purple mulberry) and *Morus serrata* (Himalian mulberry) (Tutin, 1996; Vijayan et al., 2004).

The mulberry plants are deciduous and are produced for their fruit and leaves in all parts of Turkey (Anonymous, 2005). The species *M. alba*, *M. nigra* and *M. rubra* are common in Turkey (Ercisli, 2004; Ercisli and Orhan, 2007; Özgen et al., 2009). The main mulberry production areas of Turkey are Black Sea Region and Eastern and Central Anatolian Regions. Total mulberry production is about 55 000 t and Erzincan, the leading producer province, counts for 5 793 t of it followed by Malatya (5 501 t) and Elazig (4 770 t) provinces (Anonymous, 2003).

Morphological markers are few and might have epistatic effects. Isozyme markers are also few in numbers and may also be affected by environment and post-translational modification and the use of them is very restricted. Therefore, molecular DNA marker analyses which are not affected by environment have been suggested for the determination of genetic similarity among genotypes (Gilbert et al., 1999). DNA markers such as RFLP, RAPD, SCAR, AFLP, SSR and ISSR have been advantageous by being large in number and not affected by the environment, especially in fingerprinting, marker assisted selection and genome mapping (Tanksley et al., 1989; Waugh and Powel, 1992; Rafalski and Tingey, 1993;

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Lee, 1995; Vos et al., 1995; Winter and Kahl, 1995; Yıldırım and Kandemir, 2001). Molecular marker technology has been employed in plant germplasm collection and breeding studies (Lee, 1995; Winter ve Kahl, 1995; Duvick, 1996; Gilbert et al., 1999). By reducing their expenses and increasing their applicability, molecular markers will definitely be the most important component of the plant breeding and will ease and fasten the procedures.

In the protection of plant germplasm, the determination of available variation and development of effective protection methods are imperative (Hodgkin et al., 2001). Molecular DNA marker methods have been increasingly employed in genetic studies of fruit species as well as vegetable species (Badenes and Parfitt, 1998; Ağaoğlu and Ergül, 1999; Göçmen et al., 1999a; Göçmen et al., 1999b; Polat et al., 1999; Cansian and Echeverrigaray, 2000; Leitao, et al., 2000; Li and Quiros, 2000). In addition to the molecular studies in grapes (Ağaoğlu ve Ergül 1999), apples (Zhou ve Li, 2000), apricots (Hormaza, 2001; Hurtado et al., 2002; Hagen et al., 2001), sweet cherries (Tavaud et al., 2001), almonds (Martins et al., 2001), pistachios (Badenes ve Parfitt, 1998), loquats (Polat et al., 1999) and lemons (Göçmen et al., 1999a), there have been a few molecular studies in mulberries in the world and Turkey (Bhattacharya and Ranade, 2001; Awasthi et al., 2004; Vijayan et al., 2004; Banerjee et al., 2007; Orhan et al., 2007; Kafkas et al., 2008; Kar et al., 2008; Zhao et al., 2009).

There is high possibility of a rich genetic diversity in local genotypes of mulberry due to the natural mutations, cross pollination and propogation techniques. Selections among various genotypes should be done in order to save and protect the local mulberry genotypes and to facilitate from their properties in breeding programs. Erzincan, Elazig, Malatya, Mardin and Diyarbakir provinces of Eastern Anatolia Region in Turkey have rich mulberry populations. In the present study, we employed molecular RAPD method, which is comparatively an easy and cheap molecular method, to define genetic similarity among some mulberry genotypes collected in these proviences.

MATERIAL AND METHODS

Plant material

A total of 47 mullberry genotypes; 20, 14, 5, 4, and 4 from Erzincan (genotypes D-), Elazığ (genotypes DE-), Diyarbakır (genotypes DD), Malatya (genotypes DM-), and Mardin (genotypes DMR-) obtained from the different provinces, respectively, were sampled for molecular investigation in order to determine the potential and diversity of these regions.

DNA extraction

Genomic DNA was extracted from young leaf tissues employing a modified CTAB procedure with PVP (Doyle and Doyle, 1987; Orhan et al., 2007). DNA was quantified by Biotech UV 1101 photometer.

RAPD amplification

The six 10-mer primers from Operon Technologies (A12, A13, AF04, F07, G02, and G03) were chosen from preliminary studies. The optimized reaction contained 10X Buffer 1.5 µl, dNTPs (10 mM) 1.8 µl, magnesium chloride (25 mM) 1.2 µl, primer (100 µM) 0.1 µl, Taq DNA Polymerase (Fermentas) (5unit) 0.2 µl, water 9.4 µl sample DNA 1.3 µl (100 ng/ µl) in a 15-µl final volume. DNA reactions were performed in a Model 212-1CE thermal cycler (Lab-Line Instruments Inc.). After 2 min of heating at 94°C, amplifications were performed under the following regime: 2 cycles of 30 s at 95°C, 1 minute 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; a final extension reaction of 5 min at 72°C (Orhan et al., 2007) was added. Reactions were replicated at least twice to control reproducibility of patterns. After amplification, PCR products were analyzed in 1.5% agarose gels in 1X TAE at 100 V using a Model 192 horizontal gel electrophoresis system (BIO-RAD) for 2.5 h, stained with ethidium bromide and photographed by the gel documentation analysis system (Syngene, UK).

A presence(1)/absence(0) binary data matrix obtained from scoring polymorphic RAPD bands was used to calculate Jaccard similarity coefficients (Jaccard 1908) and to estimate the molecular genetic diversity among mulberry genotypes (Bhattacharya and Ranade, 2001; Awasthi et al., 2004; Vijayan et al., 2004) (Figure 1). The unweighted pair-group method using arithmetic average (UPGMA) cluster analysis, and the resulting dendrograms and multidimensional scalings (MDS) were performed on the genetic distance matrices using the computer program NTSYpc version 2.02k (Rohlf 1997; Labate, 2000).

RESULTS AND DISCUSSION

Mulberry is a very important plant species for both fruit and silkworm productions in Turkey (Anonymous, 2005; Özgen et al., 2009). Turkey has a large genetic potential and diversity for mulberry (Orhan et al., 2007; Kafkas et al., 2008). Moreover, mulberry is widely produced and utilized in many ways in Erzincan and neighbouring proviences (Elazığ, Malatya, Diyarbakir and Mardin) for a long time. The mentioned provinces have a large but not sufficiently studied mulberry potential. With this study, employing molecular RAPD data, which is comparatively an easy and cheap method, the genetic similarity among some mulberry genotypes collected in these proviences were determined.

Erzincan, Malatya and Elazığ provinces are the leading mulberry producers in Turkey. Total mulberry production is about 55 000 t and Erzincan, the leading producer province, counts for 5 793 t of it followed by Malatya (5 501 t) and Elaziğ (4 770 t) proviences (Anonymous, 2003).

The six primers, which were previosly known to be polymorphic, generated a total of 55 (86%) polymorphic RAPD bands (Table 1). Jaccard similarity coefficients were used to estimate the molecular genetic diversity among mulberry genotypes. The dendrogram and 2D scaling based on Jaccard distance values were formed (Figures 1 and 2).

The results revealed that there were no similar mulberry genotypes in all the ones studied. Based on the molecular Jaccard matrix, the most similar genotypes

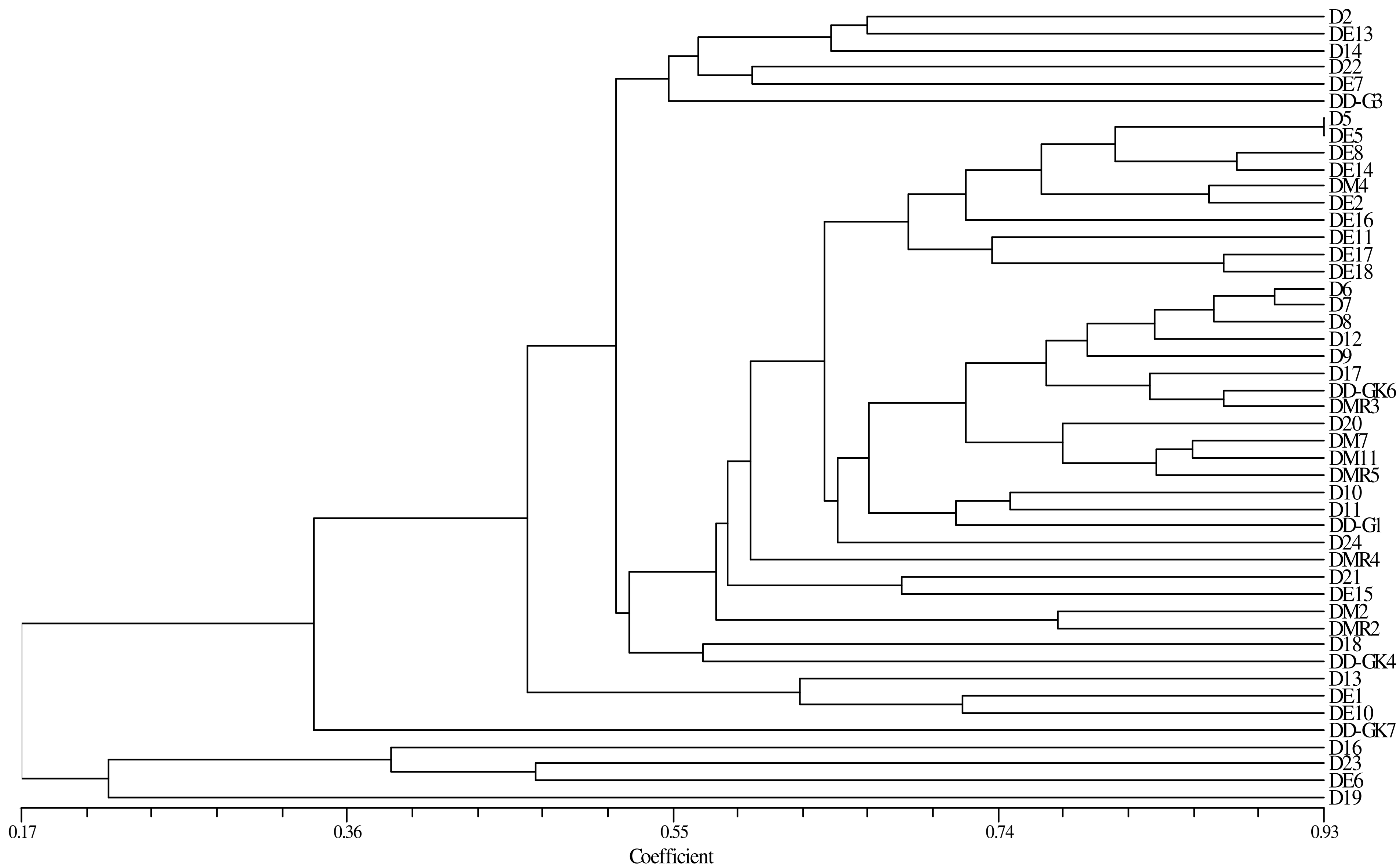

Figure 1. Associations among mulberry genotypes revealed by UPGMA clustering analysis on the basis of the molecular Jaccard distance values. A total of 47 mullberry genotypes, 20, 14, 5, 4 and 4 from Erzincan (genotypes D-), Elazığ (genotypes DE-), Diyarbakır (genotypes DD-), Malatya (genotypes DM-) and Mardin (genotypes DMR-) provinces, respectively, were sampled for molecular investigation in order to identify the potential and diversity of these regions.

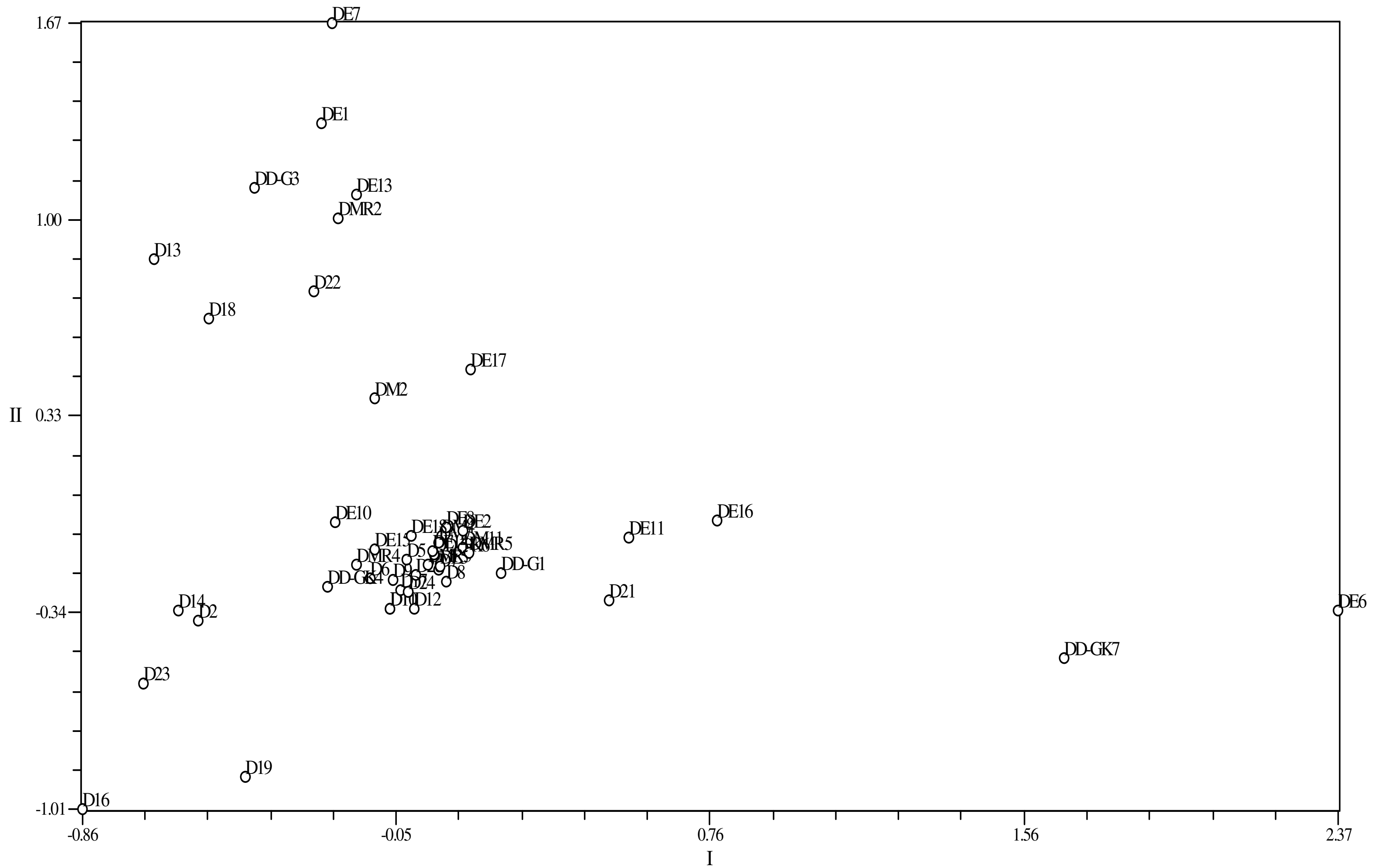

Figure 2. Associations among mulberry genotypes revealed by 2D scaling analysis on the basis of the molecular Jaccard distance values.

(0.93) were D5 (a genotype from Erzincan) and DE5 (a genotype from Elazig) followed by D6 and D7 (two geno-types from Erzincan) (0.90). The most distant genotypes were also determined. Among the evaluated genotypes, the most distinct ones were D16, D19, and D23 from Erzincan's genotypes, DE6 from Elazig's genotypes and DD-GK7 from Diyarbakir's genotypes.

The molecular mulberry studies in the world and

Table 1. Polymorphisms detected at 55 loci by using 6 RAPD primers.

Primers	Polymorphic band numbers
A12	10
A13	9
AF4	9
F07	8
G02	10
G03	9

Turkey have increased (Bhattacharya and Ranade, 2001; Awasthi et al., 2004; Vijayan et al., 2004; Orhan et al., 2007; Kafkas et al., 2008; Zhao et al., 2009). Bhattacharya and Ranade (2001) determined the genetic similarity among nine mulberry varieties in India with 23 RAPD and 3 DAMD primers. These researchers found that 133 (74%) RAPD bands and 21 (91 %) DAMD bands were polymorphic. Awasthi et al. (2004) employed the RAPD and ISSR techniques in the 15 wild and cultivated forms of mulberries. These researchers found 128 polymorphic RAPD bands from 19 primers and 93 polymorphic ISSR bands from 4 primers and stated that both RAPD and ISSR techniques could be successfully used in mulberry characterization studies. Vijayan et al. (2004) studied 19 mulberry genotypes belonging to different species with 15 RAPD primers and 15 ISSR primers and determined that 86% of the RAPD bands and 78% of the ISSR bands were polymorphic. Orhan et al. (2007) worked on the 15 white mulberry genotypes selected from Oltu town of Erzurum province in Turkey. These researchers determined the genetic relatedness of these mulberry genotypes with 101 RAPD markers obtained from 16 primers and stated that the genetically distinct genotypes could be utilized in the future breeding studies.

Banerjee et al. (2007) studied the genetic relationships of mulberry (*Morus* spp.) germplasm in India using 14 morphometric traits. These researchers observed wide variation for all the traits among the genetic divergence of 25 mullbery genotypes fprm varied agroclimatic conditions of India

Kafkas et al., (2008) revealed the genetic relationships of mulberry accessions in Turkey using Amplified Fragment Length Polymorphism (AFLP) markers. These researchers screened the 43 mulberry accessions using AFLP techniques, with 8 primer combinations. They found 337 (80.5%) polymorphic bands. Their study indicated that *M. nigra* and *M. rubra* are molecularly distinct from *M. alba* and their results also propose that *M. nigra* accessions having a low level of morphological variation are molecularly similar. These researchers screened the 18 mulberry accessions using ISSR techniques and protein and sugar content.

Kar et al. (2008) analysed the genetic relationships of mulberry accessions in India using Inter Simple Sequence Repeats (ISSR) markers and some biochemical traits. They found 84 (100%) polymorphic bands from 14 primers and the genetic diversity recorded among the mulberry accessions had an average of 0.263 ± 0.094.

Zhao et al. (2009) studied the genetic relationships of mulberry (*Morus* L.) germplasm in China using sequence-related amplified polymorphism (SRAP) markers. These researchers screened the 23 mulberry accessions using SRAP techniques, with 12 primer combinations selected for their reproducibility and polymorphism. They found 59 (71.1%) polymorphic bands and their revealed genetic similarity coefficient ranged from 0.6905 to 0.9524, with an average of 0.8330. Their results from cluster analysis were in general agreement with our morphologic classification.

In the present study, the six primers, which were previosly known to be polymorphic, generated a total of 55 polymorphic RAPD bands in the 47 mulberry genotypes. It was noted that there were no very similar ones among the studied genotypes. Based on the molecular Jaccard matrix, the most similar genotypes (0.93) were D5 (a genotype from Erzincan) and DE5 (a genotype from Elazig) followed by D6 and D7 (two genotypes from Erzincan) (0.90). Relationships among genotypes were best visualized by comparing their clustering and MDS charts. According to the molecular dendrograms and 2D scaling, the most distant genotypes were also determined. Of all evaluated genotypes, the most distinct ones were D16, D19 and D23 from Erzincan's genotypes, DE6 from Elazig's genotypes and DD-GK7 from Diyarbakir's genotypes, which are most probably introduced from the other regions.

The genetic variation among the studied mulberry genotypes was very high. Although most genotypes were in the same grouping, some genotypes collected from the same provinces generally tended to be grouped such as two groups of Erzincan's genotypes ((D13, D18 and D22) (D2, D14, D16, D19 and D23)) and a group of Elazig genotypes (DE1, DE7, DE11, DE13, DE16 and DE17).

In conclusuion, the genetic similarities among the genotypes grown in the same region were generally found close because they had been reproduced from the similar genotypes. On the other hand, different adapted genotypes from the same region could be the introductions from various other regions. It is thougtht that there could be hopeful genotypes in the studied region where a substantional amount of genetic variation has been existed and found in the present study.

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