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Genetic Variability Based on Randomly Amplified Polymorphic DNA in Mistletoe Fig (*Ficus deltoidea* Jack) Collected from Peninsular Malaysia

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ABSTRACT : *Ficus deltoidea* Jack is an important and popular medicinal plant species found in the Malaysia. Plants are being collected and used based on morphology and authentication to prevent adulteration is not in practice. In this study, twenty-six accessions of *F. deltoidea* Jack were collected from Kelantan and Terengganu states of Peninsular Malaysia to examine their genetic similarities and differences using randomly amplified polymorphic DNA (RAPD) technique. Out of 20 arbitrary primers, two primers (*D-10* and *D-11*) were selected which produced reliable DNA polymorphism. *D-10* and *D-11* primers generated 138 RAPD bands ranging from 250 bp to 3000 bp. Ninety-nine of them were polymorphic loci (72%) and thirty-nine were non-polymorphic loci (28%). A total of 56 bands with polymorphic loci were amplified with primer D-10 and analyzed by cluster analysis and UPGMA to present a dendrogram depicting the degree of genetic relationship among 26 accessions. Eight RAPD markers were sequenced to determine their identity. RAPD analysis showed the genetic diversity among 26 accessions of *F. deltoidea* Jack. The RAPD profile and RAPD marker sequences reported in this paper could be used in plant and/or plant material authentication. This study also suggested that RAPD can be a useful technique to study DNA polymorphism in *F. deltoidea* Jack.

Keywords : DNA marker, DNA polymorphism, Mas cotek, RAPD-PCR analysis

INTRODUCTION

Ficus is a large genus in the family Moraceae. It contains about 750 species and most of that are found in tropical and subtropical region (Grison et al., 2002). All plants in the genus are woody, ranging from trees and shrubs to climbers. The phloem fibers of *Ficus* species are good substitutes for hemp. Fruits of some species are edible or used medicinally. Many *Ficus* species are hosts of *Laccifer lacca* Kerr, a scale insect that secrets a resinous substance (Grison et al., 2002). *Ficus deltoidea* Jack is one of the *Ficus* species which is mainly distributed in Peninsular Malaysia, Philippines, Thailand, Sumatra, Java, Borneo (Sarawak, Sabah, East-Kalimantan), Celebes, and Moluccas (tropical Asia) (USDA, 2007). It is an important and popular medicinal plant in Malaysia. Locally, in Malay language it is called as '*Mas Cotek*'. It is cultivated as a houseplant for its leaves. It is believed that it is the only *Ficus* species that can produce fruits when cultivated indoors (McKenny and Lineberger, 2002). Six varieties of *F. deltoidea* namely, *augustifolia*, *bilobata*, *trengganuensis*, *kunstleri*, *intermedia*, and *motleyana* are available and known in Malaysia (Kamaruddin and Latiff, 2002).

The leaves of *F. deltoidea* are being used by almost all indigenous people throughout Malaysia. Based on traditional knowledge, women uses *F. deltoidea* leaves as medicine after childbirth (Daily Express, 2000). Roots and leaves are also used in Malay traditional medicine to treat fever whereas leaf alone is usually used to relieve headache (Kamaruddin and Latiff, 2002; Oukabli et al., 2003; Sonibare,

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2004). Traditionally, leaves are used in treatment of lung related diseases, as an aphrodisiac and to maintain youthful appearance (Kamaruddin and Latiff, 2002; Starr et al., 2003).

The leaves of this plant are thick, dark green in color, deltoid in shape, rounded at the apex, tapering at the base and upper side shows black, rusty or golden spots (Starr et al., 2003). Leaves are also used in preparation of herbal tea and it has been commercialized in Malaysia. However currently, plants are being collected from their wild habitats or nurseries based on their morphological traits and authentication to prevent adulteration are not in practice. Thus, accurate verification of cultivar identity is important to prevent adulteration, to confirm raw material, and to protect intellectual property rights (IPR). Arbitrary-primed DNA markers can be very useful for genetic fingerprinting and precise plant identification (Waldron et al., 2002). In this study we have analyzed *F. deltoidea* Jack plant accessions (26) available in our collection by using randomly amplified polymorphic DNA (RAPD) technique. This paper reports the findings of the RAPD analysis and randomly isolated RAPD markers sequences for *F. deltoidea* Jack.

Table 1. Accession numbers, varieties, and collection locations of *F. deltoidea* Jack in this study

Accession	Location*	Variety
FDT01	T	*F. deltoidea* Jack var. *bilobata*
FDT02	T	*F. deltoidea* Jack var. *trengganuensis*
FDT03	T	*F. deltoidea* Jack var. *trengganuensis*
FDT04	T	*F. deltoidea* Jack var. *angustifolia*
FDT05	T	*F. deltoidea* Jack var. *motleyana*
FDT06	T	*F. deltoidea* Jack var. *trengganuensis*
FDK07	K	*F. deltoidea* Jack var. *trengganuensis*
FDT08	T	*F. deltoidea* Jack var. *angustifolia*
FDT09	T	*F. deltoidea* Jack var. *bilobata*
FDK10	K	*F. deltoidea* Jack var. *bilobata*
FDK11	K	*F. deltoidea* Jack var. *angustifolia*
FDT12	T	*F. deltoidea* Jack var. *trengganuensis*
FDT13	T	*F. deltoidea* Jack var. *intermedia*
FDT14	T	*F. deltoidea* Jack var. *motleyana*
FDT15	T	*F. deltoidea* Jack var. *trengganuensis*
FDT16	T	*F. deltoidea* Jack var. *trengganuensis*
FDT17	T	*F. deltoidea* Jack var. *bilobata*
FDT18	T	*F. deltoidea* Jack var. *bilobata*
FDT19	T	*F. deltoidea* Jack var. *bilobata*
FDT20	T	*F. deltoidea* Jack var. *trengganuensis*
FDT21	T	*F. deltoidea* Jack var. *trengganuensis*
FDT22	T	*F. deltoidea* Jack var. *kunstleri*
FDT23	T	*F. deltoidea* Jack var. *trengganuensis*
FDT24	T	*F. deltoidea* Jack var. *trengganuensis*
FDT25	T	*F. deltoidea* Jack var. *moyleyana*
FDT26	T	*F. deltoidea* Jack var. *kunstleri*

*T, Terengganu (Malaysia); K, Kelantan (Malaysia)

MATERIALS AND METHOD

Twenty six accessions of *F. deltoidea* Jack were collected randomly from Kelantan and Terengganu states of Malaysia (Table 1). The collected plant accessions are being maintained at Melaka Institute of Biotechnology, Melaka (Malaysia).

Young leaves were washed in 70% ethanol for 5 minute and then in sterile deionized water for 2 minute to avoid the surface contamination. After being air-dried, leaves samples from all accession were ground separately into powder with the help of liquid nitrogen using mortar-pestle. Total genomic DNA was isolated using a method described by Sambrook et al. (1989) with minor modifications. The genomic DNA samples were purified by phenol/chloroform/isoamyl-alcohol extractions (thrice), followed by precipitation with isopropanol and sodium acetate. After centrifugation, genomic DNA pellets were washed with 70% ethanol and dried DNA pellets were dissolved in TE buffer and stored at -20℃ until used.

Twenty decamer oligonucleotide primers (Kit D) were purchased from Proligo (Colorado, USA). RAPD reactions for each sample were carried out in a total volume of 25 μl consisting of 25 ng template genomic DNA, 1X PCR buffer, 4 mM $MgCl_2$, 0.4 mM dNTP mix, 2.5 U of Taq polymerase and 0.6 μM primer. The amplification was performed in a programmable thermal cycler (Bio-Rad). After hot start (95.0℃ for 3 min), 50 PCR cycles were as follows; 95.0℃ for 30 sec, 38.5℃ for 1 min, and 74.0℃ for 1 min. A final step of extension was carried out at 72.0℃ for 10 min. Fifteen μl of RAPD-PCR products were analyzed by electrophoresis in 1% agarose gel and stained with ethidium bromide.

Randomly selected RAPD fragments were excised from the agarose gel and purified using Qiagen Gel Extraction Kit (Qiagen, Germany). Purified RAPD DNA fragments

were cloned in PCR cloning vector, pGEM®-T Easy (Promega), and harbored in the *E. coli* strain DH5-α. Plasmid DNA was isolated using plasmid DNA purification Kit (Promega). After confirmation of insert and its size, purified plasmid DNA was used in sequencing of cloned RAPD fragments. Sequences of RAPD fragments were compared with other organism in plant database of NCBI. Nucleotide sequences of RAPD markers were aligned using CLUSTAL W (Myers and Miller, 1988).

RAPD bands (generated by D-10 random primer) were scored '1' for the presence or '0' for the absence of band. Pair-wise distances (similarity matrices) were computed based on Jaccard's coefficient of similarity, using NTSYS-pc version 2.0 software. Dendrogram was constructed using unweighted pair group method with arithmetic averages (UPGMA) cluster analysis using NTYSYS-PC version 2.0.

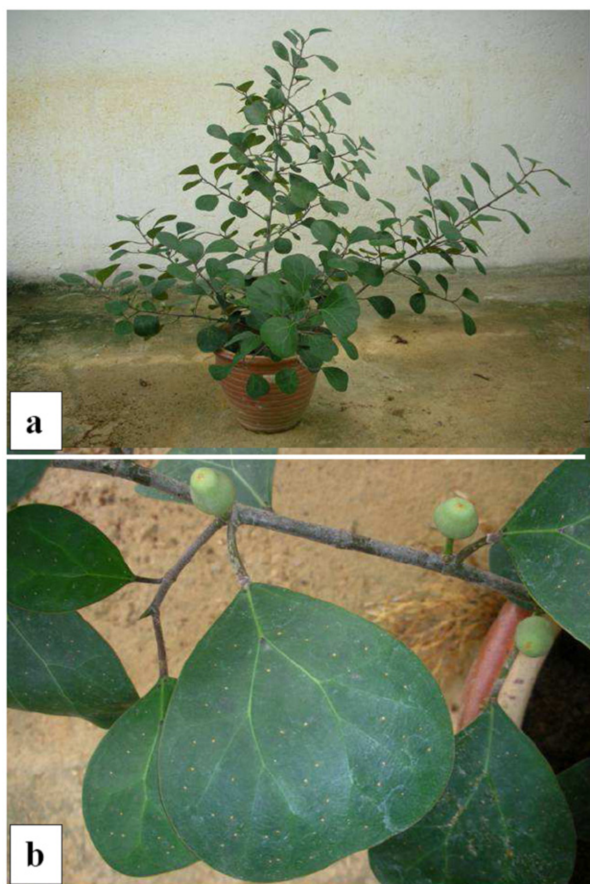

Fig. 1. Morphology of *F. deltoidea* Jack plant. (a) Fruits bearing *F. deltoidea* Jack plant; (b) closer view of leaves and fruits.

RESULTS AND DISCUSSION

Authentic identification of medicinal plants is important for quality control and in detecting adulteration to protect interests of growers and end consumers. It is also necessary for plant breeders to ensure protection of IPR. The traditional method for identifying plant species by morphological characters is now being replaced by DNA profiling techniques because of some limitations of morphological data (Virk et al., 1995). RAPD technique-based DNA profiling has been used for the analysis of phylogenetic relationship (Millan et al., 1996), rational designing of breeding programs, genetic diversity and precise identification of duplicates within the large germplasm populations (Virk et al., 1995), and management of genetic resources (Bretting and Widrelechner, 1995). Evidently, RAPD technique is a rapid and sensitive, which can be used to estimate relationship between closely, and more distantly related species and group.

High quality genomic DNA was prepared from 26 accessions of *F. deltoidea* Jack (Fig. 1). The primer screening step resulted in 2 decamer primers, D-10 and D-11 which detected DNA polymorphisms. The RAPD-PCR amplification profiles of total genomic DNA from twenty six accessions using two random primers (D-10&11) produced 138 discrete bands ranging in size from 0.25-3.0 kb, out of which 99 were polymorphic (Table 2).

As shown in Fig. 2 (a&b) the RAPD band patterns were

Table 2. Total number of amplified DNA fragments and number of polymorphic fragments generated by PCR using selected random primers

Name of primer	Sequence of primers	Total no. of amplified products (No. of DNA bands)	No. of polymorphic products [DNA bands]	RAPD fragment size range (kb)
D-10	5'-GGTCTACACC-3'	70	56	0.25-3.0
D-11	5'-AGCGCCATTG-3'	68	43	0.25-3.0

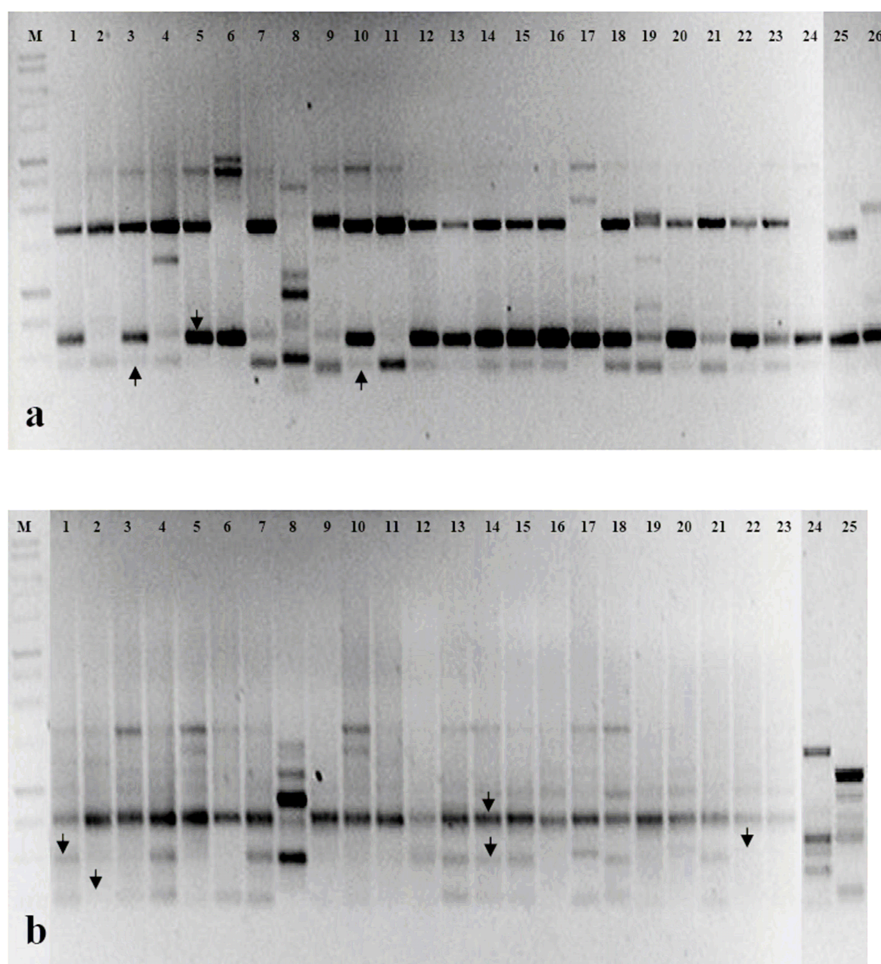

Fig. 2. RAPD profile of 26 accessions of *F. deltoidea* Jack collected from Kelantan and Terengganu states of Malaysia. Lane M, 1 kb ladder; lane 1-26, FDT01-FDT26 accession numbers listed in Table 1. (a) RAPD bands amplified by primer D-10; (b) RAPD bands amplified by primer D-11. Arrows indicate cloned and sequenced RAPD fragments (lane no 26 is not shown).

generated by primer D-10 and D-11 respectively. To observe the genetic diversity and relatedness within and between six varieties of *F. deltoidea* Jack, RAPD comparative analysis was carried out for 26 accessions using RAPD profile generated by selected primer, D-10 (Fig. 2a).

Both polymorphic and non-polymorphic RAPD bands generated by primer D-10 were analyzed using NTYSYS-PC software version 2.0. It divided 26 accessions into 4 major big clusters (Fig. 3 and Table 3). Cluster 1 was the biggest comprised of 20 accessions. Under Major Cluster 1, there were 2 minor clusters. Minor Cluster 1a composed of 8 accessions (FDT01, FDT13, FDT20, FDT26, FDT22, FDT03, FDT05, and FDK10) with the average value J=0.67, while Minor Cluster 1b composed of 12 accessions (FDT02, FDK07, FDT09, FDT23, FDT21, FDT12, FDT18, FDT14, FDT16, FDT15, FDT04, and FDT19) with the average value J=0.62. The differences value between these two minor clusters is 0.05. It indicates close genetic relatedness between 2 minor clusters because the distance value is quite low. In Minor Cluster 1a, almost identical banding patterns were observed in all accessions in group 1 and group 2. It could be interpreted that these accessions share the same genetic information and hence shows the similar banding patterns. In Minor Cluster 1b, group 3 is an out-group within this cluster, group 4 has similar banding patterns and group 5 has closely related but indicates different genetic information. Major Cluster 2 contains only one accession, FDK11 and this accession

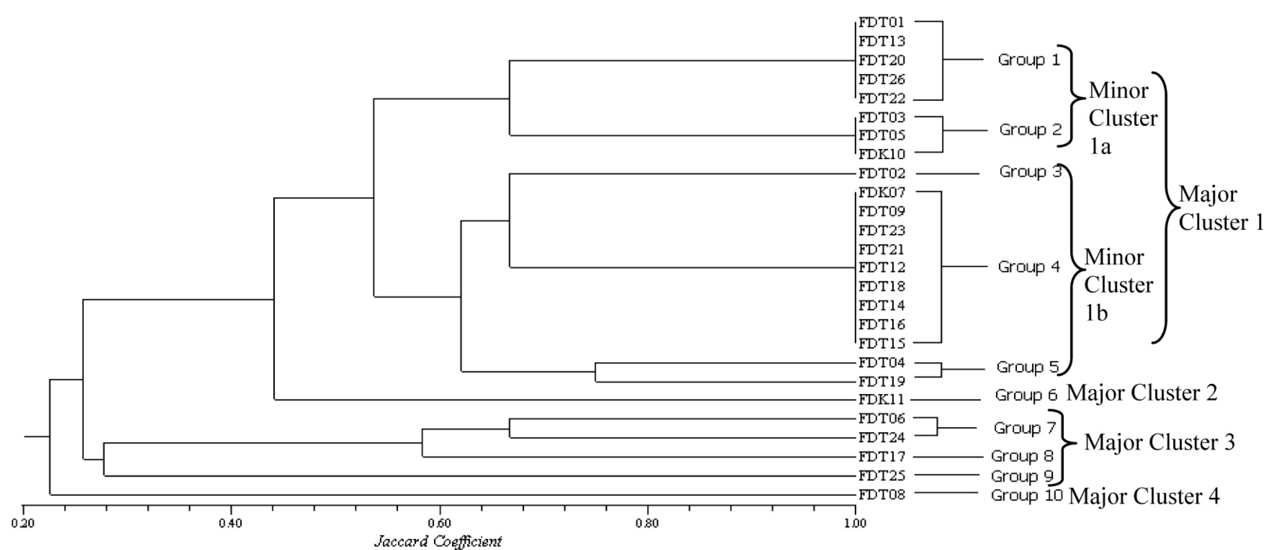

Fig. 3. Cluster analysis of *F. deltoidea* Jack accessions based on RAPD.

Table 3. Major cluster groups of *F. deltoidea* Jack accessions based on similarity matrix generated by random primers

Cluster	Accessions
1	FDT01, FDT13, FDT20, FDT26, FDT22, FDT03, FDT05, FDK10, FDT02,FDK07, FDT09, FDT23, FDT21, FDT12, FDT18, FDT14, FDT16, FTD15, FDT04, FDT19
2	FDK11
3	FDT06, FDT24, FDT17, FDT25
4	FDT08

is an out-group. Major Cluster 3 contains 4 accessions that belong to group 7, 8 and 9. Two accessions, FDT06 and FDT24 in group 7 are closely related to each other, both represent var. *trengganuensis* but displays genetic diversity based on RAPD analysis.

Interestingly, accession No FDT24 shows a unique band amplified by random primer, D-10. This band could be useful in diagnosing FDT24 accession. This may delegate an identity and can be used in the development of ‘molecular ID’ for this accession. Group 8 and 9 indicate they are related but not very closely. Major Cluster 4 contains an accession, FDT08 which is placed in at base of the other groups. Interestingly, this FDT08 is very far from other accessions due to the different banding patterns and hence shows its own group to the lowest cluster. Accessions, FDT04, FDT08 and FDK11 represent var. *angustifolia* but do not show identical RAPD profile generated by both (D-10 and D-11) primers. Hence, it can be used in precise identification of respective accessions.

Reproducible results (from triplicate) were obtained in this study using specific primer-template DNA combination. Extreme care was taken not to alter any experimental parameters. Though, there are no other molecular data to support the present classification, it is clear that identification purely based on morphology cannot correctly designate different accessions of the same species. Such types of discrepancies were also reported in rice (Virk et al., 1995). Therefore, it is suggested that more number of markers be analyzed using a large number of primers and other sensitive techniques like AFLP endowed with high resolution. We expect that *F. deltoidea* plant accessions, presently grouped into different clusters may be grouped in the same fashion. It is also assumed that the high level of genetic variation observed within the segregants, makes them less useful for specific identification.

The similarity matrix obtained using Jaccard coefficient

is shown in Table 4. Similarity coefficient was ranged from 0.1428571 to 1.0000000 in 26 accessions of *F. deltoidea* tested in this study. The RAPD amplification products (DNA bands) generated by primers, D-10 and D-11, can be classified into two groups: variable (polymorphic) and constant (non-polymorphic). Non-polymorphic RAPD fragments can be used to identify species/members of a genus exclusively if the fragment is a unique polymorphism in a genus (Williams et al., 1990). Similarly, RAPD fragment polymorphic at the species level will operationally identify members of a given species if the fragment is constant among all members of the species, species specific bands or characters (Welsh and McCelland, 1990).

Eight RAPD fragments that are shown by arrows in Fig. 2 represent both polymorphic and non polymorphic DNA bands. These eight bands were cloned and sequenced. DNA sequences of sequenced RAPD fragments were trimmed, processed, analyzed and deposited in the NCBI nucleotide sequence database, GenBank. Table 5 shows the Genbank accession numbers of deposited RAPD markers developed for *F. deltoidea*.

Table 4. Similarity matrix of 26 accessions of *F. deltoidea* Jack based on RAPD profile. The number 1-26 in this table represents FDT01-FDT26 accessions that are listed in Table 1

	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	1.0000000																									
2	0.3333333	1.0000000																								
3	0.6666667	0.2500000	1.0000000																							
4	0.2500000	0.6666667	0.2000000	1.0000000																						
5	0.6666667	0.2500000	1.0000000	0.2000000	1.0000000																					
6	0.2500000	0.0000000	0.5000000	0.0000000	0.5000000	1.0000000																				
7	0.6666667	0.6666667	0.5000000	0.5000000	0.5000000	0.2000000	1.0000000																			
8	0.1666667	0.1666667	0.1428571	0.1428571	0.1428571	0.1428571	0.3333333	1.0000000																		
9	0.6666667	0.6666667	0.5000000	0.5000000	0.5000000	0.2000000	1.0000000	0.3333333	1.0000000																	
10	0.6666667	0.2500000	1.0000000	0.2000000	1.0000000	0.5000000	0.5000000	0.1428571	0.5000000	1.0000000																
11	0.2500000	0.6666667	0.5000000	0.5000000	0.5000000	0.2000000	0.5000000	0.1428571	0.5000000	0.5000000	1.0000000															
12	0.6666667	0.6666667	0.5000000	0.5000000	0.5000000	0.2000000	1.0000000	0.3333333	1.0000000	0.5000000	0.5000000	1.0000000														
13	1.0000000	0.3333333	0.6666667	0.2500000	0.6666667	0.2500000	0.6666667	0.1666667	0.6666667	0.6666667	0.2500000	0.6666667	1.0000000													
14	0.6666667	0.6666667	0.5000000	0.5000000	0.5000000	0.2000000	1.0000000	0.3333333	1.0000000	0.5000000	0.5000000	1.0000000	0.6666667	1.0000000												
15	0.6666667	0.6666667	0.5000000	0.5000000	0.5000000	0.2000000	1.0000000	0.3333333	1.0000000	0.5000000	0.5000000	1.0000000	0.6666667	1.0000000	1.0000000											
16	0.6666667	0.6666667	0.5000000	0.5000000	0.5000000	0.2000000	1.0000000	0.3333333	1.0000000	0.5000000	0.5000000	1.0000000	0.6666667	1.0000000	1.0000000	1.0000000										
17	0.2500000	0.0000000	0.5000000	0.0000000	0.5000000	0.5000000	0.2000000	0.1428571	0.2000000	0.5000000	0.2000000	0.2000000	0.2500000	0.2000000	0.2000000	0.2000000	1.0000000									
18	0.6666667	0.6666667	0.5000000	0.5000000	0.5000000	0.2000000	1.0000000	0.3333333	1.0000000	0.5000000	0.5000000	1.0000000	0.6666667	1.0000000	1.0000000	1.0000000	0.2000000	1.0000000								
19	0.5000000	0.5000000	0.4000000	0.7500000	0.4000000	0.1666667	0.7500000	0.2857143	0.7500000	0.4000000	0.4000000	0.7500000	0.5000000	0.7500000	0.7500000	0.7500000	0.1666667	0.7500000	1.0000000							
20	1.0000000	0.3333333	0.6666667	0.2500000	0.6666667	0.2500000	0.6666667	0.1666667	0.6666667	0.6666667	0.2500000	0.6666667	1.0000000	0.6666667	0.6666667	0.6666667	0.2500000	0.6666667	0.5000000	1.0000000						
21	0.6666667	0.6666667	0.5000000	0.5000000	0.5000000	0.2000000	1.0000000	0.3333333	1.0000000	0.5000000	0.5000000	1.0000000	0.6666667	1.0000000	1.0000000	1.0000000	0.2000000	1.0000000	0.7500000	0.6666667	1.0000000					
22	1.0000000	0.3333333	0.6666667	0.2500000	0.6666667	0.2500000	0.6666667	0.1666667	0.6666667	0.6666667	0.2500000	0.6666667	1.0000000	0.6666667	0.6666667	0.6666667	0.2500000	0.6666667	0.5000000	1.0000000	0.6666667	1.0000000				
23	0.6666667	0.6666667	0.5000000	0.5000000	0.5000000	0.2000000	1.0000000	0.3333333	1.0000000	0.5000000	0.5000000	1.0000000	0.6666667	1.0000000	1.0000000	1.0000000	0.2000000	1.0000000	0.7500000	0.6666667	1.0000000	0.6666667	1.0000000			
24	0.3333333	0.0000000	0.6666667	0.0000000	0.6666667	0.6666667	0.2500000	0.1666667	0.2500000	0.6666667	0.2500000	0.2500000	0.3333333	0.2500000	0.2500000	0.2500000	0.6666667	0.2500000	0.2000000	0.3333333	0.2500000	0.3333333	0.2500000	1.0000000		
25	0.3333333	0.0000000	0.2500000	0.2500000	0.2500000	0.2500000	0.2500000	0.1666667	0.2500000	0.2500000	0.0000000	0.2500000	0.3333333	0.2500000	0.2500000	0.2500000	0.2500000	0.2500000	0.5000000	0.3333333	0.2500000	0.3333333	0.2500000	0.3333333	1.0000000	
26	1.0000000	0.3333333	0.6666667	0.2500000	0.6666667	0.2500000	0.6666667	0.1666667	0.6666667	0.6666667	0.2500000	0.6666667	1.0000000	0.6666667	0.6666667	0.6666667	0.2500000	0.6666667	0.5000000	1.0000000	0.6666667	1.0000000	0.6666667	0.3333333	0.3333333	1.0000000

Table 5. *Ficus deltoidea* Jack RAPD markers, their length and GenBank accession numbers

Name of variety	Accession No	Primer used to generate RAPD marker	RAPD fragment Polymorphic (P) / non polymorphic (N)	Clone ID	Sequence length (bp)	GenBank accession number
F. deltoidea Jack var. *bilobata*	FDT01	D-11	*N*	D11-1-2	558	EF029044
F. deltoidea Jack var. *trengganuensis*	FDT02	D-11	*P*	D11-2-1	300	EF029042
F. deltoidea Jack var. *trengganuensis*	FDT03	D-10	*P*	F6B	427	DQ825506
F. deltoidea Jack var. *motleyana*	FDT05	D-10	*N*	F5-1	610	EF029045
F. deltoidea Jack var. *bilobata*	FDK10	D-10	*P*	F10-3	400	EF029047
F. deltoidea Jack var. *motleyana*	FDT14	D-11	*N*	D11-3-1	599	EF029039
F. deltoidea Jack var. *motleyana*	FDT14	D-11	*P*	D11-5-2	507	EF029041
F. deltoidea Jack var. *kunstleri*	FDT22	D-11	*N*	D11-6-3	558	EF029046

Analysis of all eight RAPD marker sequences by nucleotide-nucleotide blast (BLASTN), and BLASTX using NCBI database gave no hits (Wilbur and Lipman, 1983). Most interestingly, no significant similarities were found when 8 nucleotide sequences (Table 5) blasted individually against *Arabidopsis thaliana* genome database.

Previous research shows that repetitive DNA has often been occurred and detected in RAPD fragments (Paran and Michaelmore, 1993). RAPD comparative analysis relies on the RAPD fragment size and not on the nucleotide sequence of the respective fragments. RAPD fragment size is considered as a dependable indicator of homology (Rieseberg, 1996). However, it is known that DNA sequence analysis provides the most specific and specific method for detecting identity (Franca et al., 2002). Therefore, to find out the identity similarity % at nucleotide level for the same sized RAPD fragments, DNA sequence of D11-1-2 (Genbank accession No EF029044) was compared with DNA sequence of D11-6-3 (Genbank accession No EF029046) using CLUSTALW. Both RAPD fragments are generated by same primer, D-11. D11-1-2 sequence is from accession, FDT01 and represents *F. deltoidea* Jack var. *bilobata*. Whereas, D11-6-3 RAPD sequence is obtained from accession, FDT22 that represents *F. deltoidea* Jack var. *kunstleri*. As shown in Fig. 4, the nucleotide sequence alignment of aforementioned sequences by CLUSTALW showed 1278 as alignment score.

When RAPD fragments that looks visibly of equal size from *F. deltoidea* Jack var. *kunstleri* (Genbank accession No EF029046), *F. deltoidea* Jack var. *motleyana* (Genbank accession No EF029041) and *F. deltoidea* Jack var. *bilobata* (EF029044) were compared by CLUSTALW showed 2768 as alignment score for three sequences. Whereas nucleotide-nucleotide blast (BLASTN) analysis showed that *F. deltoidea* Jack var. *kunstleri* (Genbank accession No EF029046) and *F. deltoidea* Jack var. *bilobata* (Genbank accession No EF029044) share 99% identity at nucleotide level. However, RAPD marker sequence from *F. deltoidea* Jack var. *motleyana* (Genbank accession No EF029041) does not show significant identity with RAPD markers that are of equal size (Genbank accession Nos EF029044; EF029046). The size of the RAPD fragment (marker) visibly appears the same; nevertheless nucleotide sequence analysis reveals the differences which could be used in precise identification of *bilobata*, *motleyana*, and *kunstleri* varieties. In addition, RAPD markers, D11-3-1 and F5-1 (Genbank accession Nos EF029039, EF029045) sequence which are generated from non-polymorphic band should help in identification of *F. deltoidea* Jack var. *motleyana*.

RAPD markers (nuclear DNA markers) are inherited in a Mendelian manner, and essentially all DNA markers have no effect on the phenotype because RAPD markers are reflections of the natural variation present in the DNA sequence (Kumar, 1999). In essence, the RAPD method used in this study displayed appreciable intra-population variation or molecular polymorphism, which pre-existed in varieties and accessions studied. In spite of their morphological identity, substantial polymorphism was observed within varieties under study.

Despite the consideration that the per cent genome surveyed by different primers remains extremely less, the extent of polymorphism was found to be high. The study revealed that though the decamer primers are very small in comparison to the large genome of *F. deltoidea* Jack, they produced appreciable amplicons, sufficient to demarcate all accessions in our collection. The dendrogram also established genetic relatedness within and between six varieties and quantum of changes that occurred in the genome in the process of evolution.

CONCLUSIONS

In summary, the results from this study indicate that the RAPD technique can be used as a reliable, simple, easy to handle, inexpensive and elegant attractive useful tool in molecular diagnosis of *F. deltoidea* Jack. Thus RAPD proved to be useful in molecular profiling of 26 different accessions of *F. deltoidea* Jack. Study also demonstrates that RAPD fragments that appear identical based on DNA band size shows deviation at nucleotide level. RAPD marker sequences could be used to develop sequence-based markers and the reasonable diversity observed in

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D11-1-2      AGCGCCATTGCC-TATTAGTTGGCCTTTCGAGTCAAACGATACTTGAATTCGTTCTCC-- 57
D11-6-3      AGCGCCATTGTTATATCAGAAATTTTTGGCAGATCACTAGGACTCCATTTCAGACGCCAT 60
             **********   *** **      **   **   *     ***  * ***   * **

D11-1-2      TCTCGACC--GGACAACGTGCACTCG--TTTCAGTAGGGTTGCTCTTCTTTTTCCATCCT 113
D11-6-3      TCTCAACGAAGGACCACCGTTTTTCGGACTTCCACAGTGTCTCTTAGCACCCACCACACA 120
             **** **   **** **      ***   ***   ** **   **   *     ***  *

D11-1-2      CCTCGGCATTT--AATCTGT-----CAGTGTTGGTCCTTTTGGGTTTTCCAACATGAGAT 166
D11-6-3      TCCGGTAAGTTGTAATGTATACGTATAATGCTGAGCGTTTCGGATTTCTTAAAATTTTTT 180
              *  *  * **  *** * *      * ** **  * *** ** ***   ** **    *

D11-1-2      TGGGTCAACG--TGGTCTCGGCGTTCTCGTTCGAGATT-GTG-AATCAATCTATCCGACA 222
D11-6-3      GGCTTCTGTGAATAACCTATTCGGGTTAGGGTGGGATTTGTGTAATTTTTTTAAGGGAAA 240
              *  **   *  *   **   **   * *   * **** *** ***   * **   ** *

D11-1-2      AAACACAAAATCTCAGTATAATCCTAAAATTTACCTAACAACGGACATAATCTCACCTCT 282
D11-6-3      AACCAATAGACTGTTAT-TAATGATTGTATGTAAATAAGAGGTGAGATTATGTCCGTTGT 299
             ** **  * *      * ****  *   ** **  *** *   ** ** ** **   * *

D11-1-2      TATTTACATACAATCATTA-ATAACAGTCTATTGGTTTTTCCCTTAAAAAAATTACACAA 341
D11-6-3      TAGGTAAATTTTAGGATTATACTGAGATTTTGTGTTTTGTCGGATAGATTGATT-CACAA 358
             **  ** **   *  **** *      * *  ** *** **   ** *   *** *****

D11-1-2      ATCCCACCCTAACCCGAATAGGTTATTCACAGAAGCCAAAAAATTTTAAGAAATCCGAAA 401
D11-6-3      -TCTCGAACGAGAACGCCGAGACCA--CGTTGACCCAATCTCATGTTGGAAAACCCAAAA 415
              ** *   * *   **   **   *  *   **  * *    ** **   *** ** ***

D11-1-2      CGCTCAGCATTATACGTATACATTACAACTTACCGGATGTGTGGTGGGCGCTAAGAGACA 461
D11-6-3      GGACCAACACTG-----ACAGATTAAA--TGCCGAGGAGGATGGAAAAAGAAGAGCAACG 468
              *  ** ** *      * * **** *  *  *  *  *  ***     *   **  **

D11-1-2      CTGTGGAAGTCCGAAAAACGGTGGTCCTTCGTTGAGAATGGCGTCTGAAATGGAGTCCTA 521
D11-6-3      CTACTGAAA--CGAGTGCACGTTGTCA--GGTCGAGA--GGAGAACGAATTCAAGTATCG 522
             **   ***   ***      ** ***    ** ****  ** *   *** *  ***

D11-1-2      GTGATCTGCCAAAAATTTCTGATATAACAATGGCGCT 558
D11-6-3      TTTGACTCGAAAGGCCAACTAATA-GGCAATGGCGCT 558
              *   **   **      ** ***   **********
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Fig. 4. Comparative DNA sequence analysis of non-polymorphic RAPD markers using CLUSTALW. D11-1-2 and D11-6-3 are clone IDs of RAPD markers stated in table 5. * indicates the nucleotides that are identical in both sequences in the alignment; Gaps, marked as '-' were introduced to maximize the alignment of nucleotides sequences compared; underlined nucleotide sequence shows the location of D-11 primer.

this study can be applied in rational designing of *F. deltoidea* Jack breeding programs (Powell et al., 1996). Nevertheless, more studies are needed to understand genetic diversity in *F. deltoidea* Jack.

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