Genetic Analysis of Abnormal Fruiting in Tissue Culture-Derived Trees of Date Palm 'Barhy' Grown in Saudi Arabia

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Abstract

Tissue culture via embryogenesis has been extensively applied for the large-scale micropropagation of some elite cultivars of date palm, such as 'Barhy'. Since this method requires high levels of growth regulators (auxins 2,4-D) that are known to cause mutations, and several subcultures to multiply embryogenic callus, cultures are prone to somaclonal variations. Considering the economic loss, Random Amplified Polymorphic DNA (RAPD) analysis was performed on 30 abnormal and normal fruit bearing tissue cultured (TC) 'Barhy' trees and 5 offshoot-derived 'Barhy' trees to resolve genetic variation between the two phenotypes and to tag markers for the early selection of true-to-type plants. Clear amplified DNA products were detected with 25 RAPD primers, but only four of them produced a few reproducible and distinguishable polymorphic bands that indicated some genetic variation. Most of the offshoot and TC-derived plants showed low levels of genetic variation. Only 3 out of 30 TC-derived plants showed a significant level of genetic variation with both offshoot-derived and TC-derived plants. Due to the low levels of genetic variation among the abnormal and normal fruit bearing TC-plants, no marker was identified that could differentiate between the normal and abnormal fruit bearing phenotypes. The results indicated that abnormal fruit development in TC-derived 'Barhy' may be attributed to epigenetic changes occurring during tissue culture stages and not to any major changes in the genome.

INTRODUCTION

Traditionally, date palm (*Phoenix dactylifera* L.), is propagated from offshoots, but their limited availability and low survival rate are the main constraints. Somatic embryogenesis is therefore being applied extensively as an alternative for large-scale propagation of true-to-type plants of some elite date palm cultivars (Tisserat, 1979; Bhansali et al., 1988; Zaid and de Wet, 1999; Al-Khalifah, 2000; Al-Khayri, 2003; Al-Khalifah et al., 2004). Since this method requires high levels of growth hormones (auxins 2,4-D) and several long subcultures to multiply embryogenic callus and/or the embryoids, cultures are more prone to somaclonal variation due to genetic and/or epigenetic changes during the process (Kaeppler et al., 2000; Kunert et al., 2003).

Plant growth hormones like 2,4-D are known to cause mutations. In tissue cultured (TC) plants of 'Barhy' that were introduced in Saudi Arabia during 1992 and later, physiological disorders like failure in fruit set, leaves with wide leaflets, variegated leaves, deformed and seedless fruits, and formation of multi-carpel fruitlets (local name: Shees) may be attributed to somaclonal variation (McCubbin et al., 2000; Al-Wasel, 2000; Djerbi, 2000). Some of these disorders are reversible and are believed to be due to epigenetic changes (Cohen et al., 2004; Gurevich et al., 2005). In other cases, it is hard to differentiate between permanent genetic changes and epigenetic changes (Cullis et al., 1999; Sala et al., 1999). Epigenetic changes are expressed under stress conditions, possibly due to DNA methylation, DNA amplification and/or activation of transposable elements (Brar and Jain, 1998; Kaeppler et al., 2000). DNA methylation is reported to

increase with the increase in concentration of auxin (2,4-D) and to decrease with the increase in concentration of kinetin (LoSchiavo et al., 1989).

A few molecular techniques have been used on tissue cultured date palms, aimed at detecting specific markers related to abnormal phenotypic traits. Molecular markers like isozymes (Saker et al., 2000; Azeqour et al., 2002), RAPD (Saker et al., 2000), and AFLP (Gurevich et al., 2005) have been used but did not reveal any variation between true-to-types and off-types that were phenotypically indistinguishable at their early stages of growth. Variation was detected only in those plantlets that showed phenotypic differences at early stages of tissue culture.

In the present study, Random Amplified Polymorphic DNA (RAPD) analysis between normal and abnormal fruit bearing TC and offshoot-derived 'Barhy' trees, was performed to resolve genetic variation between true-to-types and off-types and to tag markers for the early selection of true-to-type plants.

MATERIALS AND METHODS

Plant Material

Young leaf samples from 30 tissue culture-derived and 5 offshoot-derived 'Barhy' trees were collected from two different orchards of Al-Qassim area, Saudi Arabia. Offshoot-derived plants were selected from a large population that was raised from different 'Barhy' plant sources. All TC-derived 'Barhy' trees originated from the same source plants. They were both normal fruiting and abnormal, multi-carpel (Shees) fruitlet producing trees (Table 1).

Total Genomic DNA Extraction and Amplification

Total genomic DNA was extracted using the minipreparation method of Dellaporta et al. (1983). Concentrations of the different samples of DNA were determined by using a fluorometer (Hoefer DyNA Quant 200; Pharmacia Biotech.). The stock DNAs were diluted with TE buffer to make a working solution of 10 ηg μl^{-1} DNA.

A total of 25 RAPD primers of A, B, C and D series (OPERON Technologies, CA, USA) were used for DNA amplification (Table 2). All the selected primers were among the 37 prescreened primers for date palm DNA amplification in our laboratory (Askari et al., 2003). PCR amplification reactions, electrophoresis of the amplified RAPD fragments and documentation by the Gel Documentation System (Bio Rad) were performed using the procedures described by Al-Khalifah and Askari (2003). The molecular weights of the fragments were estimated by comparison with standard size markers (100 base pair Ladder and Kilo Base DNA Marker, Amersham Pharmacia Biotech.).

Genetic Analysis

The 25 selected amplification profiles were compared with each other by using 'Diversity Data Base' (Bio Rad) software. The data was applied to estimate the genomic similarity between the plants on the basis of number of shared amplified fragments (Nei, 1978; Nei and Li, 1979). Cluster analysis by the unweighted paired group of arithmetic means (UPGMA) was also performed and a dendrogram was constructed by Dice coefficient method with the help of 'Diversity Data Base' (Bio Rad) software.

RESULTS

Clear amplified DNA products were detected with all 25 primers but only four of them gave a few reproducible and distinguishable polymorphic bands, indicating some genetic variation (Fig. 1). Similarity values for the reciprocal pairs of samples ranged from 0.409 to 0.986 (Table 3). For most of the pairs, these values were higher than 0.75, suggesting close genomic similarities between the samples.

Genetic Similarities between Offshoot-Derived Plants

The 5 offshoot-derived plants (B21–25) showed very close genomic similarity with a range of 0.829 to 0.950 Nei and Li's coefficients in the similarity matrix (Table 3).

Cluster analysis by unweighted paired group method of arithmetic mean (UPGMA) also showed close clustering of the offshoot-derived plants indicating their true-to-type nature. Among the 5 offshoot-derived plants maximum similarity (0.950) was observed between plants B22 and B23 (Fig. 2). The plant B21 was closely related to plant B24 with a second highest value of 0.933 in the similarity matrix. The plant B25 showed the lowest similarity to plant B23 (0.829).

Genetic Similarities among the Thirty Tissue Culture-Derived Plants

Of the 30 TC-derived plants only 3 (A1, A13 and A14) showed low genomic similarity (0.409–0.476) to normal fruit bearing TC plants B19 and B20. The remainder of the TC plants showed more than 60% similarity. The TC-derived plant A1 in general showed a minimum degree of similarity to all other TC plants ranging from 0.419 to 0.756. It showed minimum genomic similarities with TC plants B19 (0.419) and B20 (0.476), while maximum similarities with A13 (0.756), B1 (0.727) and A14 (0.711). The TC plant A13 also showed low levels of similarities with TC plants B19 (0.409) and B20 (0.419) and maximum similarities with A14 (0.957) and B1 (0.821). Similarly, TC plant A14 showed low levels of similarities with B19 (0.455) and B20 (0.419) and maximum similarities with B1 (0.821) and A5 (0.815). Data in the similarity matrix and the phylogenetic tree indicated that TC plants A1, A13 and A14 were genomically distant from the two normal fruit bearing TC plants B19 and B20 (0.409–0.476), while with the abnormal fruit bearing TC plants were 60% similar genomically.

A pair-wise genomic similarity between the TC plants, excluding the pairing of A1, A13 and A14 with B19 and B20, showed a close relationship with a range of 0.607–0.986. Maximum similarity was observed between TC plants B12 and B13 (0.986). The second highest value of 0.97 was observed between 5 pairs of TC plants A2-B7, A3-B12, A6-B13, A15-B4 and B15-B18. Two pairs of TC plants A5-B1 and B14-B17 showed the third highest genomic similarity value (0.96).

Genetic Similarities between Tissue Culture and Offshoot-Derived Plants

Of the 30 tissue culture-derived plants 27 showed more than 61% genomic similarity with offshoot-derived plants. The genomic similarity ranged from 0.615 to 0.976 in the Nei and Li's similarity matrix, indicating their true-to-type genomic nature. Only 3 out of 30 TC plants (A1, A13 and A14) showed a low level of similarity with offshoot-derived plants (0.455–0.478). These plants also produced more than 60% multicarpel fruitlets (Shees).

DISCUSSION

Although DNA polymorphism was very low, some genetic variation was detected both in offshoot-derived and in TC-derived plants of 'Barhy'. Corniquel and Mercier (1994) also detected some genetic variation between different plants of 'Barhy' by applying RAPD and RFLP techniques. Saker et al. (2000) detected a low level of somaclonal variation in young TC-derived plantlets by using RAPD. They have reported genetic variation in only 4% of the 70 analyzed plantlets. Recently, Gurevich et al. (2005) applied AFLP on abnormal and normal fruit bearing plants of 'Barhy' that originated from both tissue culture and offshoots, and detected a low level of genetic variation among the plants but they did not detect any DNA marker linked to this abnormal fruit trait. In the present study a very low level of genetic variation was detected among both the 5 offshoot-derived and the 30 abnormal and normal fruit bearing TC-derived plants. The randomly selected samples from the offshoot-derived population showed a range of 82–90% similarity, leaving the chance of a low level of variation within the population. These variations are because they originated from different mother plants.

A pair-wise genomic similarity estimates between the TC plants excluding the pairing of A1, A13 and A14 with B19 and B20, showed a close genomic similarity among the TC plants with a range of 0.607–0.986. All plants that showed low levels of genetic similarity to B19 and B20 were abnormal fruit bearing trees. This indicates that at least to

some extent the abnormal fruit bearing off-types can be detected from the normal plants through RAPD analysis.

For most of the other pairs, their similarity values were higher than 0.75, suggesting close genomic similarities among TC plants irrespective of their off-type phenotype. It is therefore suggested that the abnormal fruit bearing traits in most of the TC plants are not only due to genomic changes but also due to some epigenetic changes that might have occurred during tissue culture stages. Reverting of most of the abnormal fruit bearing TC plants to normal, within a span of at least 10 years (Cohen et al., 2004), and in some cases an increase in the abnormal fruit percentage, strongly support epigenetic effects that are expressed under stress conditions. The stress conditions may cause DNA methylation, DNA amplification and/or activation of transposable elements in the plants (Hirochika et al., 1996).

Due to the low level of genetic variation among the abnormal and normal fruit bearing TC-plants, no consistent marker could be identified between the two phenotypes except for the low level of similarity observed between three abnormal and two normal plants. Gurevich et al. (2005) were unable to detect any specific AFLP marker or banding pattern which could differentiate between normal and abnormal fruit bearing 'Barhy' plants. Limited population size and the use of a low number of primers may be two of the reasons for not being able to detect linked markers specific to this trait. New advanced molecular techniques like DNA microarray technology may help to resolve the genetic diversity and detection of markers linked to this trait (Kunert et al., 2002).

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Tables

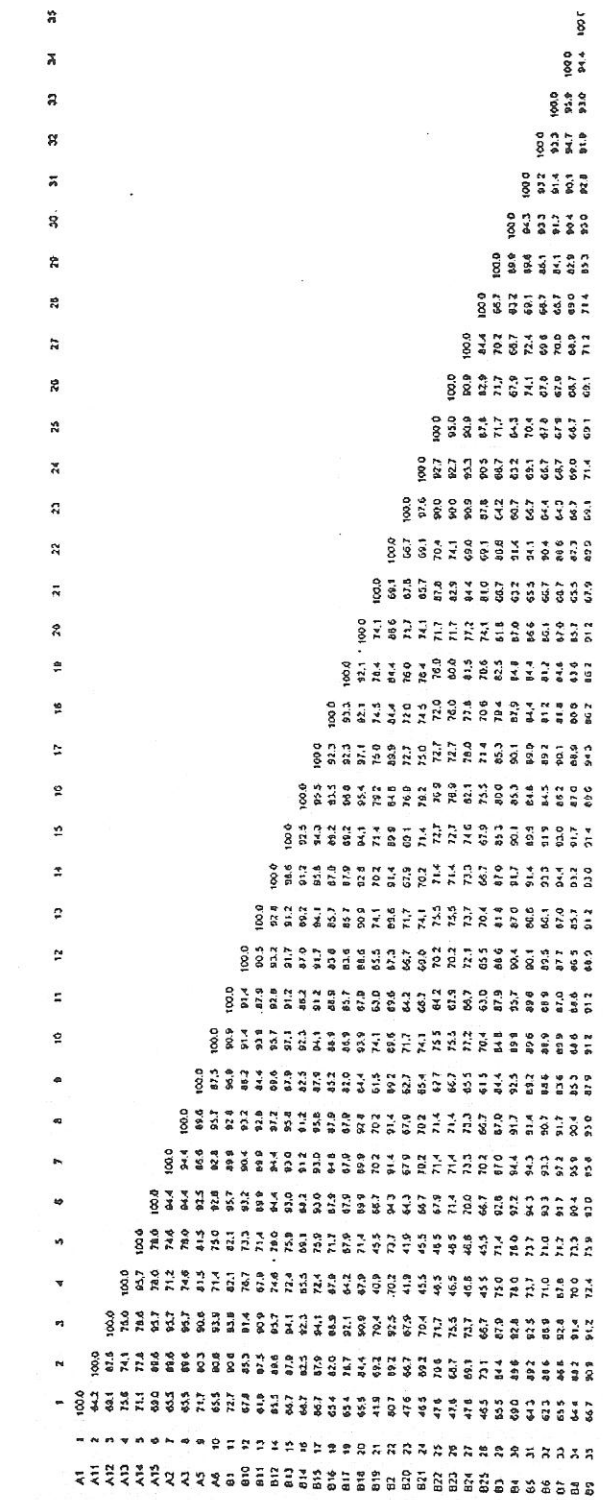
Table 1. List of 35 plants originated from tissue culture and or offshoots that were used in this study.

Accession #	Mode of cultivation	Phenotype
A1-A3	Tissue culture	Multiple carpel fruitlets 80%
A5, A6, A11-A14	Tissue culture	Multiple carpel fruitlets 60%
A15	Tissue culture	Low fruit set
B1-B18	Tissue culture	Multiple carpel fruitlets 50%
B19-B20	Tissue culture	Normal
B21–25	Offshoot	Normal

Table 2. List of 25 RAPD primers with total number of bands and number of polymorphic bands produced in each profile of 35 genotypes of 'Barhy'.

RAPD primers	Total amplified fragments	Polymorphic fragments	RAPD primers	Total amplified fragments	Polymorphic fragments
OP A01	08	0	OP B14	12	0
OP A04	06	0	OP B15	07	0
OP A07	09	0	OP B16	11	0
OP A10	13	0	OP B19	08	0
OP A11	11	0	OP B20	09	0
OP A12	15	1	OP C01	10	0
OP A15	09	0	OP C10	13	0
OP A17	10	2	OP C19	11	1
OP B01	18	2	OP D05	07	0
OP B08	08	0	OP D08	08	0
OP B11	14	0	OP D11	08	0
OP B13	11	0	OP D15	10	0
			OP D20	11	0

Table 3. Similarity matrix for Nei and Li's coefficients of 35 'Barhy' genotypes obtained from RAPD markers.



Figures

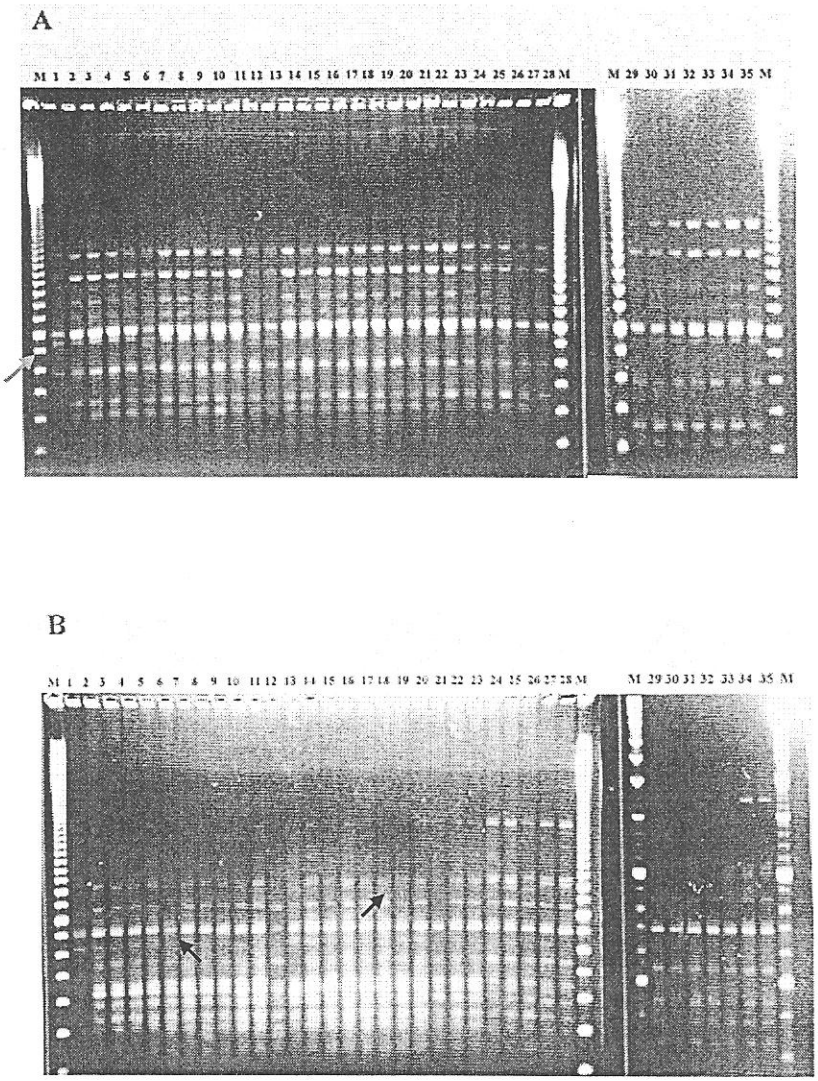


Fig. 1. RAPD profiles of 30-tissue culture and 5 offshoot-derived plants of 'Barhy' using primers OPA12 (A) and OPB01 (B). "Lanes 1–14: A1-A3, A5, A6, A11-A15 and lanes 15–35: B1-B25. M: Molecular size marker".

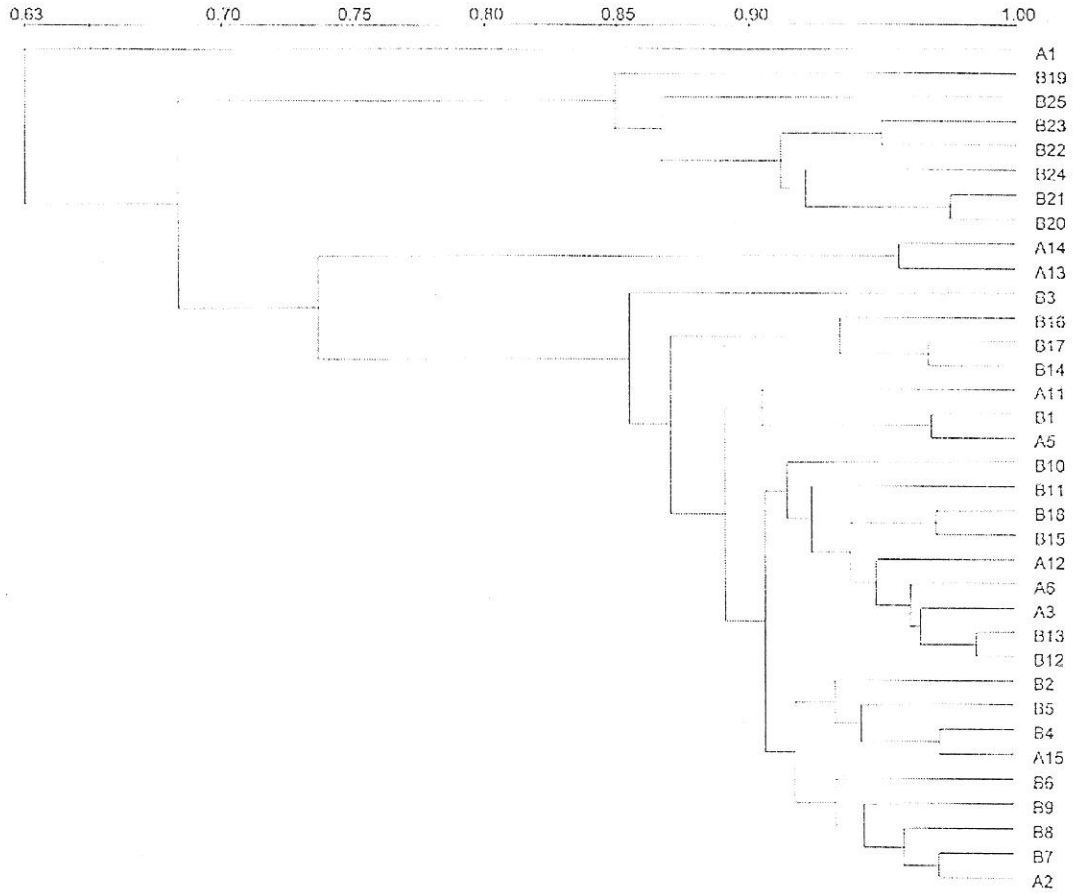


Fig. 2. A dendrogram of phylogenetic relationships among the 35 genotypes of 'Barhy' based on Nei and Li's similarity coefficient generated from 25 RAPD profiles.