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Genetic Stability in Date Palm Micropropagation

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Abstract: Application of plants derived tissue culture is depended on genetic stability of them. The purpose of present study was the studying of genetic stability in date palm (Var. Berhi) micropropagation. In this study 3 enzyme systems of PRX, SHD and ACP were used by electrofocusing method on polyacrylamide gel. The result of this experiment showed that, only in ACP system, differences were not observed and micropropagated plants of this variety were similar to each other as well as their original plant but in two enzyme systems of PRX and SHD showed polymorphism and the number of bands and their location on the gel were different between original and micropropagated plant.

Key words: Isozyme, genetic stability, micropropagation, date palm

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is one of the most important trees in tropical and subtropical regions of Iran. Berhi is one of the best date palm cultivars. This cultivar is traditionally propagated by offshoots. The number of offshoots is relatively few. Recently tissue culture is used for rapid propagation of date palm. But application of micropropagated plants is depended on genetic stability of them. Isozymes have applied for different purposes in research about date palm, for example these markers are used to distinguishing between cultivars (Torres and Tisserat, 1980), to plant breeding (Azam-Ali *et al.*, 2006), to identification of cultivars (Stegemann *et al.*, 1987), to screen for Bayoud-resistant cultivars (Baaziz and Saaidi, 1988; Baaziz, 1989; Baaziz *et al.*, 1994; Bendiab *et al.*, 1993), to detect off-types plant from micropropagation (Salman *et al.*, 1988; Saker *et al.*, 2000), to carry out population studies (Bendiab *et al.*, 1998), to biochemical classification of date palm cultivars (Al-Jibouri and Adham, 1990) and to studying of genetic variation in date palm cultivars (Bennaceur *et al.*, 1991) or genetic diversity among coconut genotypes (Geethalakshmi *et al.*, 2005).

In this study, three enzyme systems were used for studying of micropropagated date palm (Var. Berhi) and comparison of them with original plants from offshoot.

MATERIALS AND METHODS

This study was carried out at the Department of Horticulture Science in the College of Agriculture, Shahid Chamran University during 2001-2002.

Plant material: Three palms each of three different type date palm plant (Var. Berhi) were studied:

- A: Micropropagated date palm plants (from England)
- B: Original date palm plants (propagated by offshoot)
- C: Micropropagated date palm plants (from Iran)

All samples were received from date palm research institute genebank of Ahwaz. Mature leaflet tissue of plants was found to be the best for enzyme extraction.

Each sample was repeated with 3 replication to check the appearance of the isozyme banding pattern.

Extraction: 0.5 g from each sample was chopped in to small pieces, then samples were extracted in 1.5 mL of extraction buffer containing 20 sucrose, 3% Poly Ethylene Glycole (PEG), 1.5% Bovine Serum Albumin (BSA), 0.8% Poly Vinyl Polypyrrolidone (PVPP) and 0.01 M Dithio Threitol (DTT). The extracts were centrifuged at 5000 rpm for 20 min at 4°C. The clear extract were used for enzyme electrophoresis.

Electrophoresis: In this study, Isoelectric Focusing (IEF) with ultra thin poly acrylamid gel (0.25 mm thickness) was used. The composition of gel was consisted of 79% glycerol (20%), 10.2% monomers (41.5% w/v with 1 part bis acrylamid and 32.2 part acrylamid), 3.9% ampholytes (ampholin: 3.5-5, 5-7 and 7-9 in equal volumes 1:1:1), 6.8% ammonium persulfate (10 mg mL⁻¹) and 0.1 Tetramethyl ethylene diamine (TEMED).

Electrophoresis was performed in two stages: (1) Prefocusing: 700 V, 23 mA, 9 W, 2°C until 30 min and (2) Focusing: 3500 V, 23 mA, 9 W, 2°C until 80 min. After prefocusing stage, the samples were loaded as amount of 27 µL for acid phosphatase and shikimate dehydrogenase and 20 µL for peroxidase system. Then focusing step was performed until final voltage of 3500 V. In this study, three enzymic systems were stained. After drying of gels in oven, isozyme banding pattern were studied.

RESULTS AND DISCUSSION

In the last decades many scientists studied the production using tissue culture technique in different methodology. The genetic fidelity of the tissue cultured clones are examined through isozymic patterns (Gupta and Varshney, 1999). An isozymic profile, which is very stable during the growth of cultured tissue and is not affected by the environmental factors, should be considered as a marker (Chatterjee and Prakash, 1993).

In the present study the isozyme banding pattern of PRX, SHD and ACP, were studied. The number of bands (isozymes) in each of enzymatic system (Table 1) and location of bands on gels were showed (Fig. 1-3).

The Number of isozymes in two enzymatic systems namely PRX and ACP was similar in all plants (original and micropropagated plants), but in SHD system, the number of isozymes ranged from 10 bands in C to 13 bands in A and B.

Peroxidase (PRX): The number of isozymes was similar for original (B) and micropropagated plants (A, C) but the location of 2 bands on the gel were not similar between them and polymorphism observed between original and micropropagated plants (Fig. 1).

Polymorphism in other plant systems were found and reported by other workers. Isozymic profiles of different micropropagated clones of *Cordyline terminalis* were assessed for their genetic stability. Ten clones were tested for six isozymes. A few showed variation with respect to the banding pattern in esterase and superoxide dismutase (Ray *et al.*, 2006). ADH and EST patterns of micropropagated rice plants showed polymorphisms compared with plants of the original varieties (Medina *et al.*, 2004).

Shikimat dehydrogenase (SHD): The diagrammatic representation of isozyme banding of SHD showed 13 bands, which 10 bands were appeared in all plants and 3 bands only were observed in A and B.

Table 1: Frequency of isozyme bands in different sample of date palm (Var. Berhi)

Enzyme system	No. of bands		
	A	B	C
PRX	10	10	10
ACP	13	13	13
SHD	13	13	10

A: Micropropagated date palm plants (from England), B: Original date palm plants (propagated by offshoot), C: Micropropagated date palm plants (from Iran)

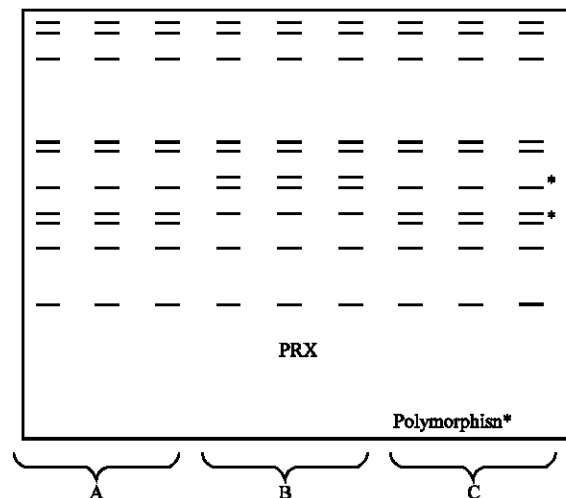


Fig. 1: Diagrammatic representation of PRX for A: Micropropagated date palm plants (from England), B: Original date palm plants (propagated by offshoot) and C: Micropropagated date palm plants (from Iran)

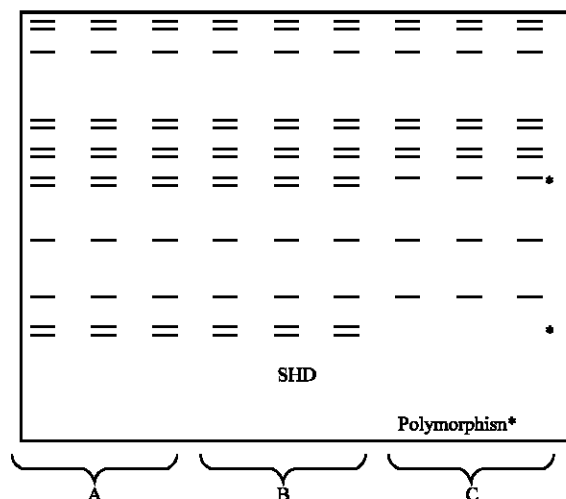


Fig. 2: Diagrammatic representation of SHD for A: Micropropagated date palm plants (from England), B: Original date palm plants (propagated by offshoot) and C: Micropropagated date palm plants (from Iran)

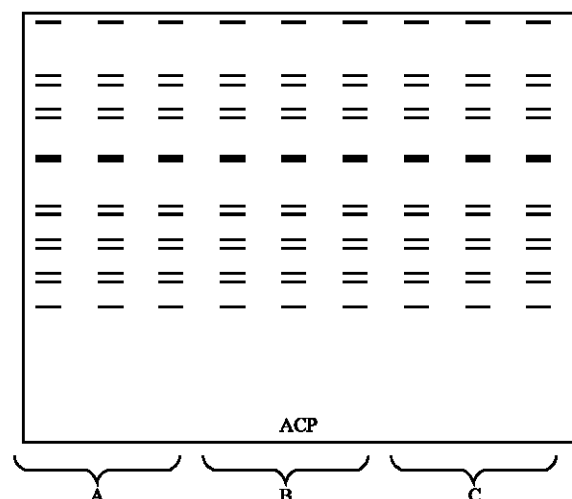


Fig. 3: Diagrammatic representation of ACP for A: Micropropagated date palm plants (from England), B: Original date palm plants (propagated by offshoot) and C: Micropropagated date palm plants (from Iran)

In the present study, 2 enzymatic systems of PRX and SHD were found to be polymorphic. Earlier worker reported that out of 600 regenerants of *Triticum aestivum*, 13 plants revealed isozyme variation in ADH patterns (Davies *et al.*, 1986).

Acid phosphatase (ACP): In diagrammatic representation of isozyme banding of ACP activity, 13 bands were observed in all plant samples.

Based on result in this investigation, only in ACP system differences were not observed between original and micropropagated plants of Berhi variety, with micropropagated plants of this variety were similar to each other as well as their original plant. In this investigation repeated sampling of Berhi cultivar showed the same set of isozyme banding pattern. Similar observation have been reported for other date palm cultivars (Al-Jibour and Adham, 1990). Earlier researchers have reported similar observation in several cultivar of date palm. For example the regenerated plants of four date palm cultivars derived from callus culture showed similar banding patterns within cultivars for EST, GOT and cytosolic LAP on polyacrylamide electrophoresis (Salman *et al.*, 1988). This result was confirmed by analyzing PER, POD and GOT. Similar banding patterns for PER and GOT were detected in all analyzed plants (Saker *et al.*, 2000).

We also found changes in PRX and SHD profiles of the micropropagated plants. As it is well known that isozyme plants may vary with development and differentiation stages (Scandalios, 1974), we compared leaf

tissues at the same developmental stage from the two different types of plant material (original and micropropagated plants). So, we could suggest that the differences distinguished had a epigenetics basis. The polymorphisms of PRX and SHD patterns possibly occurred. Such variants might have caused such a variation either through triggering the expression on specific gene which result in fewer number of isozyme (missing band on the gel) (Salman *et al.*, 1988), or because of changes due to post translational modifications (Acquaah, 1992). We believe that the polymorphism found in the *in vitro* micropropagated plants could be only a variation during the in vitro procedure. On the other hand, the different banding pattern was observed in the PRX and SHD systems may be explained on the fact that a possible genetic modification has been taken place in few cell on the culture medium.

According to the result obtained, we suggest that this experiment should be carry out with more isozyme systems and other molecular markers such as DNA markers.

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