



Electrophoretic analysis of three selected isoenzymes of date palm pollen grains

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Abstract. The electrophoretic patterns of soluble protein, non-specific esterase (EST), glutamate oxaloacetate transaminase (GOT), and glutamate dehydrogenase (GDH) were studied in extracts of pollen grains from local cultivars of date palm (*Phoenix dactylifera* L.). Each pollen extract contained several EST and GOT isoenzymes, and qualitative variations exist between some cultivars. Three isoenzymes of GDH are present in each pollen extract, however, there was no electrophoretic variation present between cultivars.

Key words: Glutamate oxaloacetate transaminase; Glutamate dehydrogenase; Non-specific esterase; *Phoenix dactylifera*.

Introduction

In higher plants, pollen grains differ in their fertilization ability (Currah, 1983; Pfahler, 1965; Pfahler, 1967). Also, breeding studies on several plant species showed that fertilization ability of pollen depends on pollen genotypes (Currah, 1983; Pfahler, 1967; Mulcahy, 1974).

Date palm, which is a cross pollinated crop adapted to arid environments, has been the subject of several studies to improve fruit quality and production (Higazy *et al.*, 1982; Nixon, 1926; Nixon, 1927; Ream and Furr, 1970). Although, it has been demonstrated that there is a positive association between pollen type and fruit quality and production (Higazy *et al.*, 1982; Nixon, 1926; Nixon, 1927), little progress was made in solving breeding and taxonomic problems in this species. This is due in part to the lack of genetic information. Therefore, if progress is to be made in solving such problems, a genetic analysis is required.

An attempt has been made to use morphological characteristics of pollen grains as markers for genetic

analysis (Basha *et al.*, 1988). However the potential value of these characteristics is limited. There are only few phenotypic markers, and it is difficult in estimating the degree of genetic variability by phenotypic characteristics. Moreover, the phenotype is often appreciably affected by variation in the environment.

Recent studies in the fields of population genetics, breeding, evolutionary genetics, and systematics have concentrated on the use of protein and isoenzyme patterns as markers (Gates and Boulter, 1979; Hamrick and Mitton, 1979). The use of gel electrophoretic technique makes it possible to investigate genetic differences and similarities between and among species.

Materials and Methods

Pollen grains were collected in the flowering season of 1990 from spadices of male local cultivars (Table 1). The spadices were supplied by College of Agriculture or were obtained from the market. Pollen grains were prepared as described previously (Al-Helal, 1989), and were stored at -8°C.

Extraction

Soluble protein was extracted by homogenizing 20 mg of pollen grains in a 1 ml, 0.1 M chilled tris/HCl buffer, pH 6.8, containing 20% (w/v) sucrose in a polyethylene tube, for 16 hours at 4°C. The crude extract was centrifuged at 4000 rpm for 5 min. The supernatant was frozen at -8°C and then was freeze-dried and dissolved in 400 μ l of extraction buffer.

Electrophoresis

The method of electrophoresis was described previously (Al-Helal, 1988).

Staining

Gels were stained for GOT, EST and GDH as described by Brewbaker *et al.* (1968) with some modifica-

tions.

Gels used for non-specific esterase (EST, E.C. 3.1.1.2) were incubated in 100 ml of 0.2 M sodium acetate buffer, pH 5.0, containing 40 mg α -naphthyl acetate and 100 mg fast blue B salt.

Reaction mixture for glutamate-oxaloacetate transaminase (GOT, E.C. 2.6.1.1) was 100 ml, 0.2 M sodium acetate buffer, pH 5.0, containing 500 mg L-aspartic acid, 70 mg α -ketoglutaric acid, 10 mg pyridoxal-5-phosphate and 300 mg fast blue B salt.

The reaction mixture for glutamate dehydrogenase (GDH, E.C. 1.4.1.2) was 75 ml, 0.1 M tris/HCl buffer, pH 9.0, containing 40 mg β -nicotinamide adenine dinucleotide (β -NAD), 2 mg phenazine methosulphate, 35 mg nitroblue tetrazolium and 300 mg sodium glutamate.

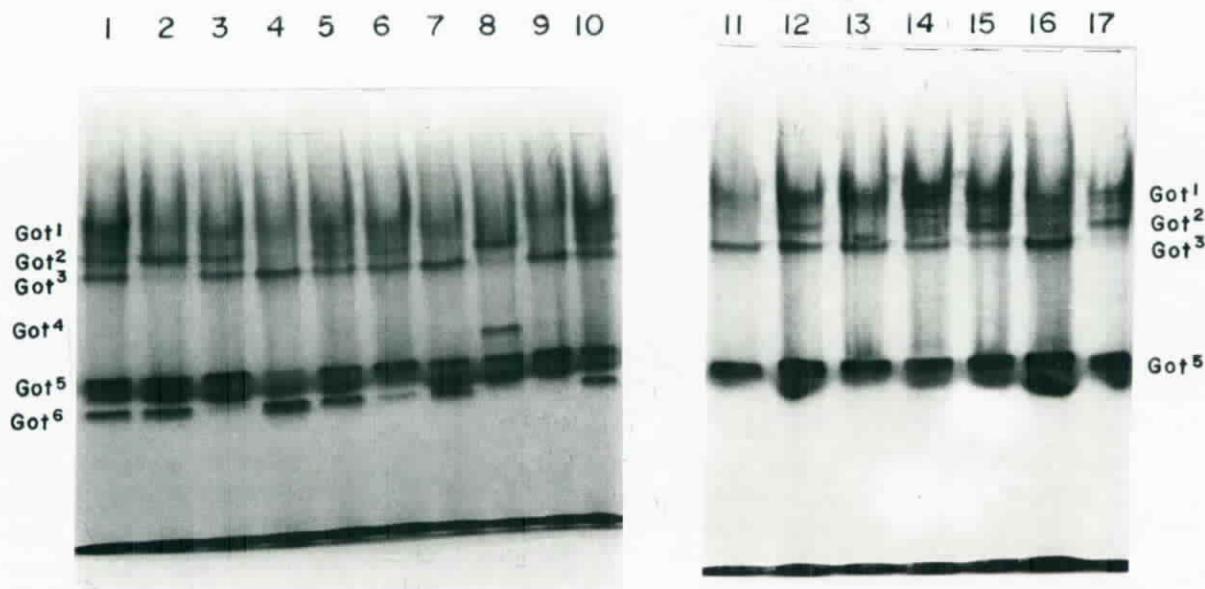


Fig. 1a. Photographic representation of GOT zymogram patterns from date palm pollen extracts. Sample numbers correspond with those given in Table 1.

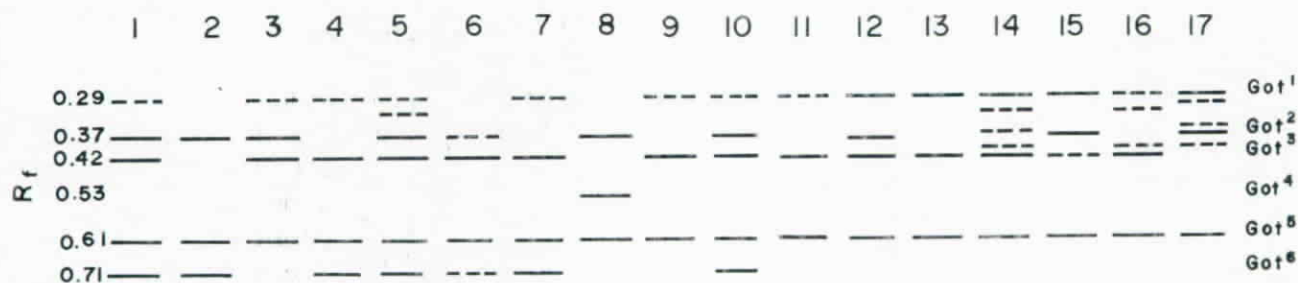


Fig. 1b. Diagrammatic representation of GOT zymogram patterns from date palm pollen extracts. Sample numbers corresponded with those given in Table 1. Dashed lines represent faint bands.

All gels were incubated in the reaction mixture for 1/2 hour at 25°C and were destained and photographed as described in Al-Helal (1988).

The extraction and the electrophoresis for each enzyme were repeated twice, and the results were identical.

Results

GOT Patterns

A pink zone (Rf 0.63) appeared immediately in each extract as the gels were immersed in the reaction mixture for either GOT or EST. This band appears to be a non-specific component that stained with fast blue

B salt. This zone is useful for comparing band relative mobility.

In all pollen grain extracts studied, GOT zymograms resolved into a total of 6 distinct electrophoretic variants in major band and into 4 variants in minor bands (Fig. 1). The number of isoenzymes present in each extract ranged from 3 to 6 based on number of bands resolved.

The slowest moving major band (Rf 0.29, classified as GOT¹ in Fig. 1) appeared to be common in all extracts. However there were differences in its staining intensity between cultivars. In some cases it was barely resolved on the gel. Therefore it cannot be used as a cultivar marker.

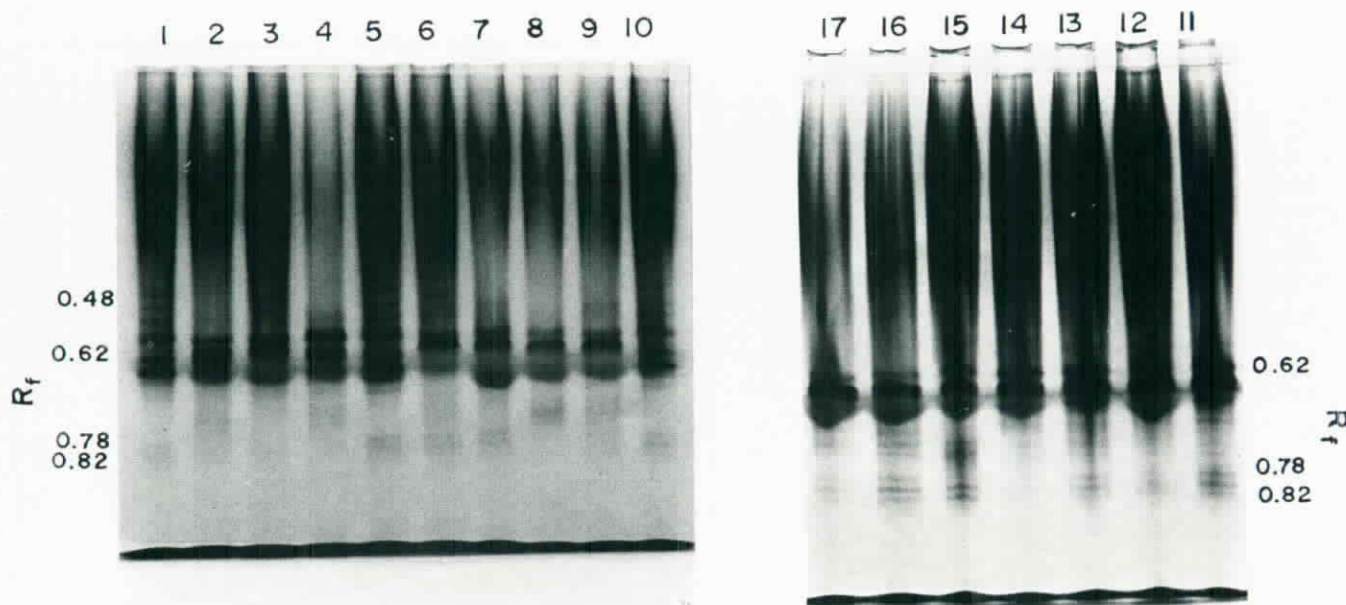


Fig. 2a. Photographic representation of EST zymogram patterns from date palm pollen extracts. Details as in Fig. 1a.

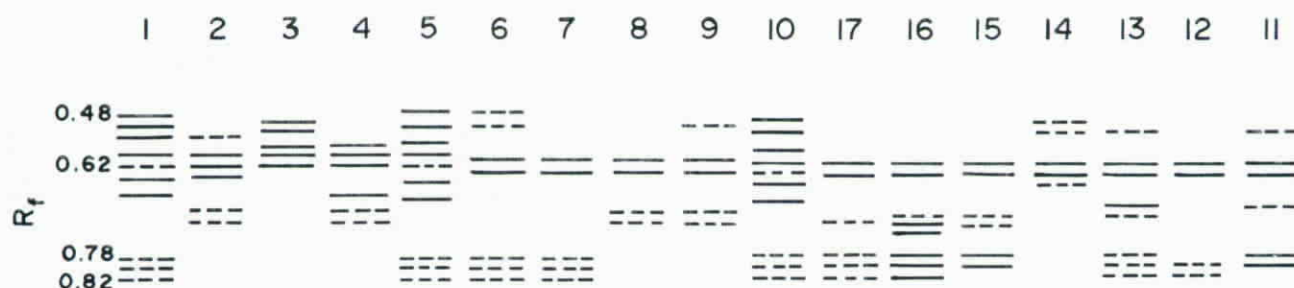


Fig. 2b. Diagrammatic representation of EST zymogram patterns from date palm pollen extracts. Details as in Fig. 1b.

Table 1. *Date palm cultivars studied and their sources*

Code number	Cultivar name	Source
1	Sakari (12/11) like	College of Agriculture
2	Unknown	obtained
3	Sari-like	As no. 1
4	Unknown	from tree in the university campus
5	Sakari (11/11)-like	As no. 1
6	Sagri-like	As no. 1
7	Unknown	As no. 2
8	Unknown	As no. 2
9	Unknown	As no. 2
10	Unknown	As no. 1
11	Unknown	As no. 1
12	Salag-like	As no. 1
13	Unknown	As no. 2
14	Maktomi-like	As no. 1
15	Matomi-like	As no. 1
16	Unknown	As no. 2
17	Dokani-like	As no. 1

On the other hand, the other major bands (Rf. 0.37, 0.42, 0.61 and 0.71; classified as GOT², GOT³, GOT⁵, and GOT⁶) are suitable as cultivar markers. The fourth major component (Rf 0.53, classified as GOT⁴) only detected in cv. 8, appears to be useful for identifying this cultivar.

Cultivars 4, 8, 9, and 17 can be identified by GOT patterns. On the basis of the banding patterns of the major component of GOT, cvs. 1, 5, 6, and 10 appear to be electrophoretically identical, but cv. 6 could be distinguished by the low intensity of GOT⁶. Similarly cvs. 2 and 7 were electrophoretically identical. Cultivars 3, 12 and 15 were electrophoretically identical on the basis of the patterns of the major GOT bands, but cv. 15 had several minor bands. Also, cvs. 9, 11, 13, 14 and 16 appeared to be identical on the basis of the banding of the major components, but cv. 14 had several minor bands.

EST Patterns

The activities of EST were resolved into a total of at least 14 variants, and the number of bands present in each extract range from 3 to 10. Some components had low enzyme activity and therefore can be hardly seen in the photograph (Fig. 2).

As shown in the figure there were quantitative and qualitative differences between cultivars in EST zymograms. Cultivars 2 and 7 which were identical in the

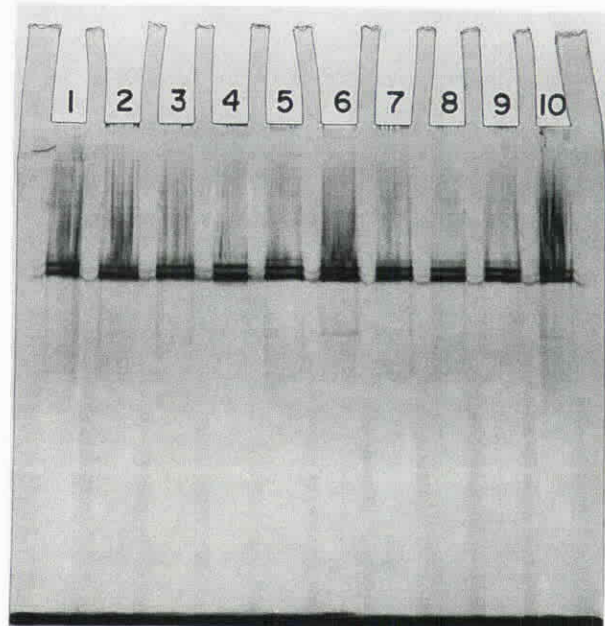


Fig. 3. Photographic representation of GDH zymogram patterns from date palm pollen extracts. Details as in Fig. 1a.

banding patterns of the major component of GOT, differed in EST banding patterns. Also, cvs. 3, 12 and 15 could be identified on the basis of EST patterns. Similarly, cv. 6 differed in EST patterns from cvs. 1, 5 and 10.

GDH Patterns

GDH was resolved into 3 distinct electrophoretic bands in each extract. As shown in Fig. 3, the different cultivars studied were identical in GDH banding patterns.

Discussion

It is clear from the results that GOT isoenzymes occurred in two regions, the first region consisted of 1 to 3 major isoenzymes (classified in Fig. 1 as GOT¹, GOT², and GOT³), while the second region contained one or two isoenzymes (GOT⁵ and GOT⁶). In addition, another isoenzyme (GOT⁴) was detected in cv. 8 only. This might indicate that this cultivar is a mutant, and it is useful for breeding investigations. However, the presence of GOT isoenzymes in two regions is similar to what has been reported for leaf extracts of different male and female cultivars of date palm (Ream and

Furr, 1970). The authors suggested that the isoenzymes of the first region might be dimeric coded by two alleles, and the isoenzymes of the second region might be monomeric coded by two alleles. The results of the present study appear to support their conclusion. Also, Al-Jibouri (1988) analyzed GOT patterns of pollen grains of Iraqi varieties and showed its activity in two regions.

As shown in this investigation, GDH activity consists of 3 isoenzymes in one region with no variation present between cultivars. This might suggest that GDH is a dimeric and no allelic variation was found for this isoenzymes in the date palm pollen grain extracts studied.

As shown in the results, some samples cannot be distinguished on the basis of GOT and EST patterns (cvs. 1, 5 and 10). This might indicate that these samples are genetically identical, and further isoenzyme analysis is required for a reliable conclusion. Also, the samples might be from the same cultivar, cvs. 1 and 5 are sakri but were given different code numbers where they were supplied, and cv. 8 is unknown obtained from the same source.

In conclusion, the results demonstrate the usefulness of date palm pollen isoenzymes for investigating genetic variation. This can aid in solving breeding problems, such as studying the *in vivo* pollen competition.

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棗椰子花粉之三種同功異構酶分析

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本報告利用當地栽培種之棗椰子花粉，進行可溶性蛋白質，酯酶，麩草轉氨酶及麩胺酸脫氫酶之同功異構酶之電泳分析。每一花粉抽取液中含有數種酯酶及麩草轉氨酶之異構酶，且電泳帶型質之變異，存在於一些栽培種。花粉麩胺酸脫氫酶一般具有三條電泳帶，品種間沒有變異。