

Amylase isoenzymes and protein of date palm (*Phoenix dactylifera* L.) fruit

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Abstract. Amylase isoenzymes of fruit extracts of thirteen date palm cultivars were separated on polyacrylamide gel electrophoresis (PAGE). Each extract resolved into two bands, one component had α -amylase activity and the other component possessed β -amylase activity. Two electrophoretic variants of α -amylase were observed and each cultivar displayed one of the two variants. No electrophoretic variant was observed for β -amylase. The protein zymogram patterns of fruit extracts were studied on SDS-PAGE under both reducing and non-reducing conditions. The protein patterns appeared to be cultivar-specific.

Key words: α -Amylase variants; β -Amylase; Date palm fruit; *Phoenix dactylifera* L.; Protein patterns.

Introduction

Date palm (*Phoenix dactylifera* L.) is the most economically important crop grown in the Kingdom of Saudi Arabia, which is considered to be one of the leading countries in date production. There are over 400 different species have been reported in Saudi Arabia (Hussien and Al-Zeid, 1974).

Physical characteristics and chemical composition of fruit of various cultivars grown in Saudi Arabia have been reported by several investigators (Saad *et al.*, 1986; Hussein and El-Zeid, 1975; Moustafa and Hussien, 1977). All these investigators showed that ripen fruits contain high percentage of sugars and their level vary among different cultivars. Protein content appeared to be low in fruit (Saway *et al.*, 1983; Saad *et al.*, 1988). However, there is limited information concerning enzymatic

changes associated with fruit ripening. Mutlak (1984) reported an increase in polyphenolase (Polyphenol oxidase, O-diphenol: O₂ oxidoreductase) activity during ripening and this increase is associated with darkening of tamar.

According to Barret (1973), nothing is known about date genetics. Torres and Tisserat (1980) used the zymogram patterns of five isoenzymes in leaf extracts for genetic studies and they revealed the important use of isoenzymes as a gene marker to aid breeding programs.

This work was initiated to investigate the possible use of protein and isoenzyme patterns of date palm fruit for genetic studies and as cultivar marker, since they have been used widely for such proposal among plants (Gates and Boulter, 1979; Al-Helal, 1985; Natarella and Sink, 1975; Lundkvst and Rudin, 1977; Torres *et al.*, 1978).

Materials and Methods

Date palm fruits of the following cultivars (Shashi, Bent Saif, Zelah, Khlas from Riyadh, Khlas from Al-Hassa, Makfari, Ruzaz, Sokari; Borhi, Khadri, Monif, Ubas, Saka, and one is unknown) were obtained commercially.

Enzyme Preparation

The enzyme was extracted by homogenizing 1 g of fruit in 4 ml 0.075 M, pH 7.25 chilled sodium phosphate buffer containing 20% (w/v) sucrose in a polyethylene tube, overnight at 4°C. The crude extract was centrifuged at 4,000 rpm for 5 min and the supernatant was used for electrophoretic analysis.

Protein Extraction

As described for enzyme preparation except that 0.2 M pH 6.8 chilled Tris-HCl buffer containing 20% (w/v) sucrose and 2% (w/v) sodium dodecyl sulphate (SDS) was used as extraction buffer. Under reducing conditions a 4 μ l of 2-mercaptoethanol was added to each 100 μ l extract.

Electrophoresis

Discontinuous, vertical polyacrylamide gel electrophoresis (PAGE) was performed according to the method of Davis (1963) using LKB equipment. Development was carried out at 10°C at constant current (25 mA).

The SDS gel contained 1% (w/v) SDS and the developing buffer contained 0.1% (w/v) SDS.

Staining

Gels used for amylase and phosphorylase study were incubated 2 h at 25°C in solution of 1% (w/v) soluble starch containing either 0.1 M phosphate buffer pH 6.0 or 0.1 M acetate buffer pH 5.0. The gels were washed with acidified water and were stained in acidified iodine-

potassium iodine solution according to the method of Brewbaker *et al.* (1968).

Gels used for R-enzyme study were incubated 2 h at 25°C in solution of 1% (w/v) amylopectin containing 0.1 M acetate buffer pH 5.0 and were stained as above.

Protein bands were made visible by staining in Coomassie Blue dissolved in ethanol:acetic acid:water (100:15:85) and destaining was carried out in a mixture of the same solvent.

Effect of EDTA on Amylase Activity

The polyacrylamide gel, following development, was incubated in 5 mM ethylenediamine tetraacetic acid (EDTA) for 20 min, at room temperature and was stained for amylase as usual.

Effect of Heat on Amylase Activity

To the clear supernatant, CaCl₂ was added to give a final concentration of 5 mM. Then the extract was incubated in water bath at 70°C for 15 min, and was used for electrophoretic analysis.

Results

Amylase activity was studied in extracts of ripen fruits of 13 date palm cultivars. The amylase isoenzymes were separated on 7.5% PAGE. The results showed that each extract consisted of two bands, one fast migrating component (Rf 0.88), had low level of amylase activity, required large volume of extract (150 μ l of 1:4 ratio between tissue and extracting buffer) to be resolved on the gel, appeared pink in colour on the gel and black on the photograph and when larger volume of extract was used (400 μ l or more) it appeared white in colour (classified as amyl² on Figs. 1, 2 and 3). The other component was slower in mobility, had high level of amylase activity (therefore, it appeared as broad band on the gel especially

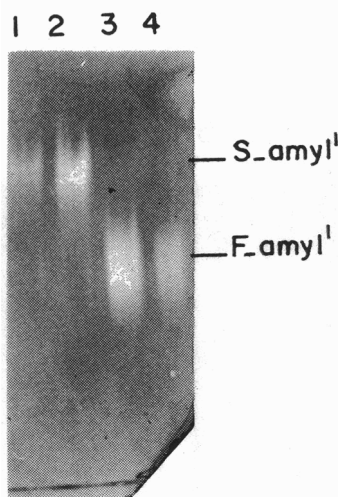


Fig. 1. Zymogram patterns of date palm fruit amylase on 7.5% PAGE. Gel was incubated in starch solution free of phosphate. Track: 1-Zelah; 2-Shashi; 3-Khlas from Al-Hassa; 4-Ruzaz. Note: Sample loaded was 30 μ l.

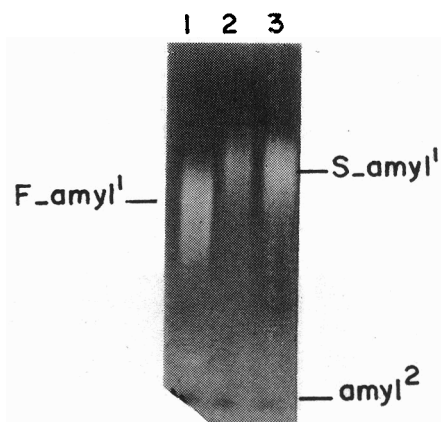


Fig. 2. Zymogram patterns of date palm fruit amylase on 7.5% PAGE. Assay solution as in Fig. 1. Track: 1-Khlas from Al-Hassa; 2-Zelah; 3-Shashi. Note: Sample loaded was 150 μ l.

when large volume of extract was used) and appeared white in colour on the gel (classified as amyl^1 on Figs. 1 and 2).

Amylase activity was studied in extract from unripen fruit of one of the cultivar [Zelah]. No band of amylase activity was detected. Also no band of amylase activity was detected in seed extract.

Effect of EDTA and Heat on Amylase Activity

Amyl^1 lost its activity when the gel was incubated in EDTA solution prior to enzyme assay, while amyl^2 retained its activity (Fig. 3A).

The activities of amyl^1 and amyl^2 were retained when the extracts, which contained CaCl_2 , was incubated in water bath at 70°C for 15 min (Fig. 3B).

These treatments indicate that amyl^1 is α -amylase while amyl^2 is probably β -amylase but it is heat stable.

The Isoenzymes of Amylase

Two isoenzymes of α -amylase were observed in the extracts of the cultivars studied and only one variant occurred in each cultivar. The variants were classified as S-amiyl^1 for the less anodic variant (the slower migrating component, R_f 0.32) and F-amiyl^1 for the faster migrating component (R_f 0.54, see Fig. 1). The

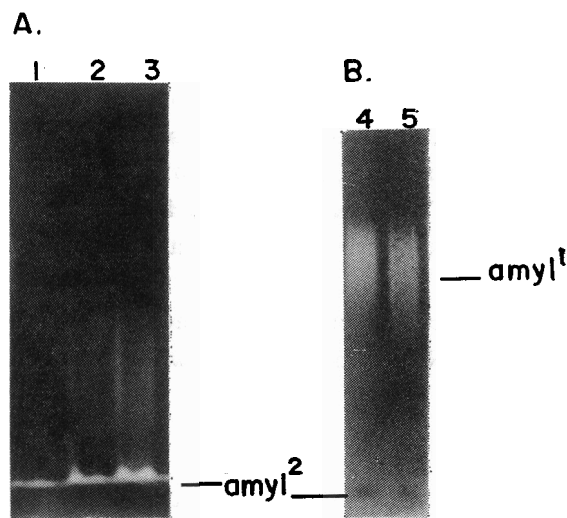


Fig. 3. Zymogram patterns of date palm fruit amylase on 7.5% PAGE. Assay solution as in Fig. 1. A. Gel was incubated in 5 mM EDTA solution for 20 min prior to enzyme assay. B. Extract containing CaCl_2 was incubated at 70°C for 15 min prior to electrophoresis. Track: 1-Shashi (400 μ l); 2-Zelah (400 μ l); 3-Khadri (500 μ l); 4-Makfari (150 μ l); 5-Borhi (150 μ l). Note: Numbers between parentheses are sample loaded.

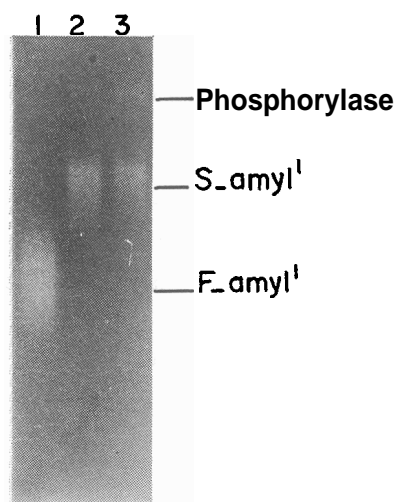


Fig. 4. Zymogram patterns of date palm fruit amylase and phosphorylase on 7.5% PAGE. Gel was incubated in starch solution containing phosphate. Track: 1-Khlas from Al-Hassa; 2-Zelah; 3-Shashi. Note: Sample loaded was 30 μ l.

ratio was 4:9 (F-amylase¹: S-amylase¹).

No variant was observed for amylase².

Phosphorylase and R-enzyme

When the gel, after electrophoresis, was incubated in starch solution containing phosphate buffer, no additional bands were observed on the gel except in extract of one cultivar (Shashi). This band (Rf 0.14) represented phosphorylase activity. Compare Fig. 1 and Fig. 4.

When amylopectin was used as substrates no band of R-enzyme activity was observed in any extract.

Protein

The soluble protein of extracts of ripen fruits of ten cultivars was separated on 17% PAGE under dissociating conditions under both non-reducing conditions and reducing conditions.

The results in Fig. 5 and Fig. 6 showed that each extract contained limited number of major bands and several minor bands. Each extract

showed qualitatively and quantitatively distinct protein pattern and no common band was

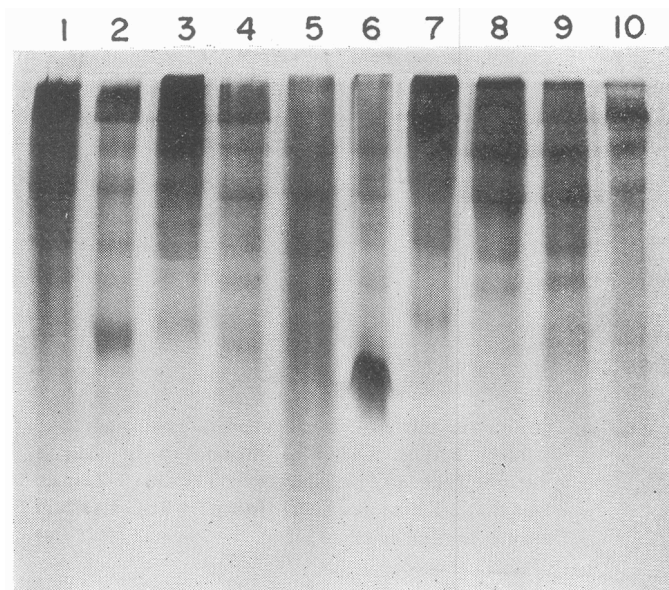


Fig. 5. Zymogram patterns of date palm fruit protein on 17% SDS-PAGE, under non-reducing condition. Track: 1-Bent Saif; 2-Monif; 3-Ruzaz; 4-Makfari; 5-Shashi; 6-Zelah; 7-Khlas from Al-Hassa; 8-Solrari; 9-Khlas from Riyadh; 10-Sakai. Note: Sample was 100 μ l.

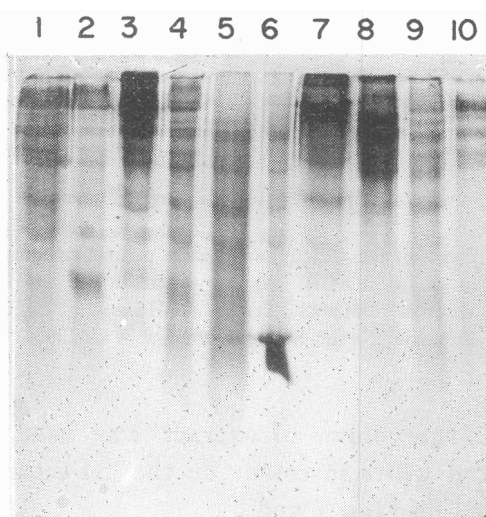


Fig. 6. Zymogram patterns of date palm fruit protein on 17% SDS-PAGE, under reducing condition. Track: 1-Bent Saif; 2-Monif; 3-Ruzaz; 4-Makfari; 5-Shashi; 6-Zelah; 7-Khlas from Al-Hassa; 8-Sokari; 9-Khlas from Riyadh; 10-Sakai. Note: Sample loaded was 100 μ l.

observed for all cultivars studied. The zymogram patterns under non-reducing conditions appeared to be similar to that under reducing condition since no obvious differences could be detected.

Discussion

The ripen fruit of date palm had one band of α -amylase activity. The identification of this component as α -amylase was based on its inactivation by EDTA and it was heat stable in the presence of Ca^{2+} . Among several properties, heat lability and inactivation by EDTA, were used by several workers as typical distinguishing for α -amylase on the gel (Miintz, 1977; Yomo and Varner, 1973; Tanaka and Akazawa, 1970).

The data indicated the presence of multiple forms of α -amylase in date palm. Similar results have been reported for several plant species (Jacobsen *et al.*, 1970; Müntz, 1977; Adams *et al.*, 1981; Callis and Ho, 1983; Bilderback, 1971; Yomo and Varner, 1973; Tanaka and Akazawa, 1970).

α -Amylase of date palm appears to be coded by two alleles, one coding for the fast moving band and the other coding for the slower migrating band. Allelic variation has been reported for amylase (Chao and Scandalios, 1971; Al-Helal, 1985).

The fast migrating component (Rf 0.88) appeared to be β -amylase, although it was heat stable, because it retained full activated when the gel treated with EDTA. The results showed that no electrophoretic variants was present for this band in the 13 cultivars studied, so it can be assumed that no allelic variation exist for the gene coding for this isoenzyme.

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棗椰子 (*Phoenix dactylifera* L.) 果實的澱粉同功酶及蛋白質

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取13種棗椰子的栽培品種，其果實萃取液經聚丙烯胺電泳 (PAGE) 分離出澱粉同功酶。每一萃取液皆解析得二帶，一具有 α -澱粉酶活性，另一則呈 β -澱粉酶活性。其中 α -澱粉酶可觀察到二個電泳變異，但每種萃取液只呈現其中一種；而 β -澱粉酶却無觀察到電泳變異。果實萃取液在還原 (reducing) 和非還原 (non-reducing) 情況下，跑 SDS-PAGE時，其蛋白質圖譜 (zymogram patterns) 呈現栽培品種之專殊性。