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Identification of Date Palm Male Cultivars by the Electrophoretic Pattern of Pollen Soluble Proteins

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ABSTRACT. The soluble proteins of pollen grains from date palm cultivars, Sakaie, Seleg, Maktumy, Khashkar, Shagra-El-Qassim, Succari, Meneifi, Sefri, Khalas, Nabut Zamel, Nabut Sif, Khashram, Barhi, Dehcini and Khudari were separated by slab gel electrophoresis. The pollen protein patterns were representative to the cultivars. Soluble proteins of pollen grains were separated into 31 protein bands, but only, 7 to 9 bands were found in each cultivar. High degrees of similarities were found among Maktumy and Khudari (75%). Succari and Khashram (67%) and Nabut Zamil and Khashram (67%). Khashkar was totally different from Sakaei and Maktumy, while Maktumy was different only from Khashram. The similarities among other cultivars varied considerably as this ranged from 11 to 60%.

Introduction

An accurate identification of date palm (*Phoenix dactylifera* L.) male cultivars is of a great importance for date breeders and growers. In Saudi Arabia, identification of male cultivars mostly depends upon information obtained from local farmers. Therefore, the local names of cultivars vary among locations in the country.

Scanning electron microscopy of pollen grains was used to identify date palm male cultivars (Shaheen 1983). The ultrastructure of pollen grains varied among cultivars. The morphology of date palm leaves also was utilized for identification of certain male cultivars in the central region of Saudi Arabia (Shaheen *et al.* 1986).

Electrophoresis profiles of seed proteins and pollen grain proteins are increasingly being utilized as a new approach for species identification and as useful tool for tracing back the evaluation of various groups of plants (Boulter *et al.* 1967, El-Meleigi 1985, Johanson and Hall 1966, and Menk *et al.* 1973).

The purpose of this work is to use the electrophoretic pattern of pollen soluble proteins for identification of date palm male cultivars.

Material and Methods

Pollen grains of date palm cultivars: "Sakaie", "Seleg", "Maktumy", "Khashkar", "Shagra-El-Qassim", "Succari", "Meneifi", "Sefri", "Khalas", "Nabut Zamel", Nabut-Sif", "Khashram", "Barhi", "Dcheini" and "Khudari" were collected during the flowering season of 1986 from different areas of the central region of Saudi Arabia. The collected pollens were freeze dried and stored in sealed vials at 5°C until used for extraction of soluble proteins.

Total soluble proteins of freeze dried pollens were extracted by mixing 10 mg pollen powder with 10 ml solution of 0.1 M Tris HCl buffer (pH 8) containing 0.5 M sucrose and 0.1% (W/v) ascorbic acid. The extract was stored for 2 hrs at 4°C, then centrifuged at 30,000 g for 20 min. The supernatants were utilized for protein analysis (El-Meleigi *et al.* 1987).

Soluble proteins of pollen grains were separated in a discontinuous polyacrylamide-sodium dodecyl sulfate (SDS) gel electrophoresis as described by Conejero and Scmancik (1977) and El-Meleigi *et al.* (1987). Protein solutions were boiled for 4 min with equal volume of 0.08 M Tris-HCl buffer (pH 8) containing 1.8% SDS, 4.4% 2-Mercaptoethanol (MCE) and 3% glycerol. Three layers of gel (1.5 mm thickness) were used per slab, namely, 14% acrylamide, resolving gel (bottom), 6.3% acrylamide, spacer gel (middle) and top layer staking gel, 4% acrylamide. The sample size of soluble proteins applied to gel electrophoresis was 100 µl.

The electrophoresis was run for 2.5-3 hrs at 20 m.a. per slab gel at 4°C. The buffer solution (pH 9.3) was composed of Tris (20.2 g), glycine (4.3 g), SDS (0.7 g) in 700 ml H20. A vertical electrophoresis LKB 2001 was used.

Gels were fixed overnight in a solution of 12.5% (W/v) Trichloroacetic acid (TCA), 25% (v/v) isopropanol and stained for 3 hrs in commassie-brilliant blue R-250 and destained in water (Mourer 1971). Slab gels were dried in LKB 2003 gel dryer and scanned in LKB 2202 ultrascan laser densitometer.

The following formula was used to compare soluble proteins spectra of any two cultivars (Vaughan 1975).

$$\text{Percent similarity} = \frac{\text{No. of similar band pairs}}{\text{No. of different bands} + \text{No. of similar bands}} \times 100$$

Results

The profiles of pollen soluble proteins of various date palm cultivars separated by slab gel electrophoresis were presented in Fig. 1. Each cultivar possessed a unique protein profile. The differences among cultivars were observed in the position and heights of peaks.

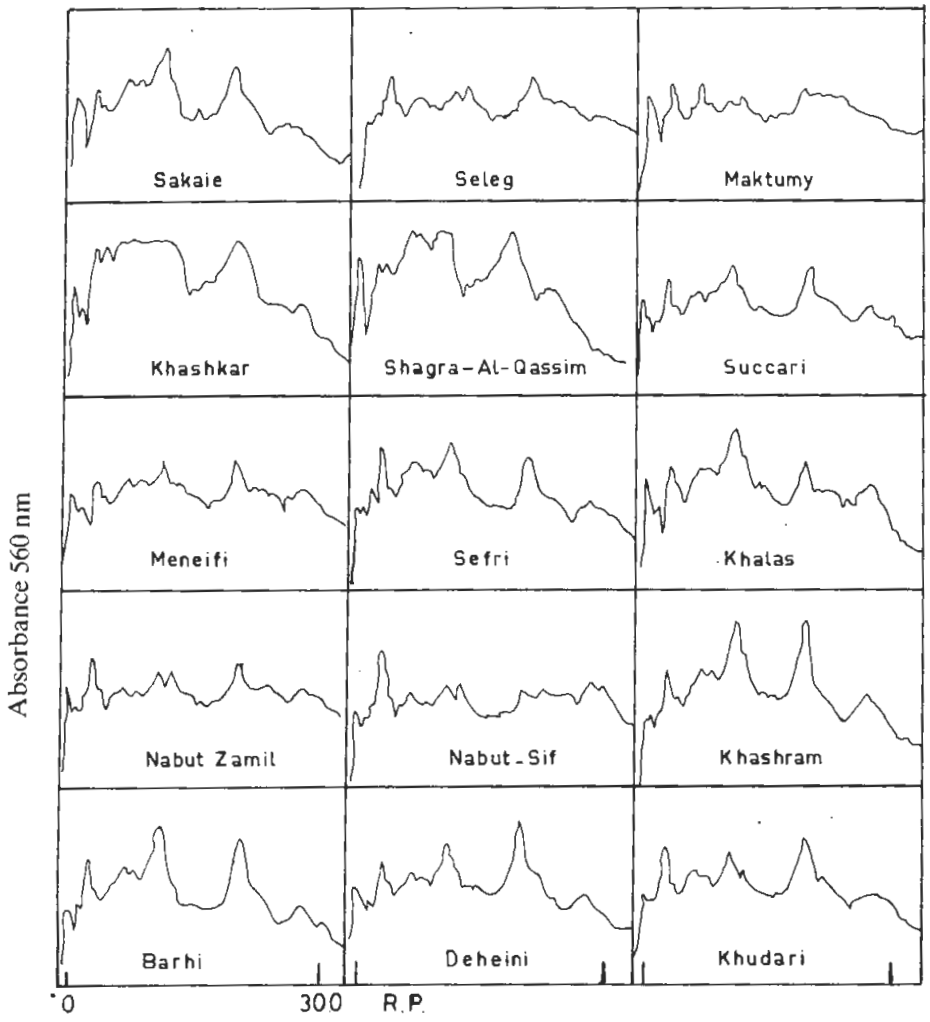

FIG. 1. Densitometer scanning of slab gel electrophoretic patterns of soluble proteins separated from pollen grains of fifteen date palm cultivars. Vertical axis represent intensity of protein bands while horizontal axis represent mobility of protein bands from origin at left side.

The illustrated diagram of protein bands of various cultivars (Fig. 2) showed that pollen of each cultivar contain 7 to 9 protein bands. However, the mobility of various bands varied among cultivars and protein bands were found in 31 locations.

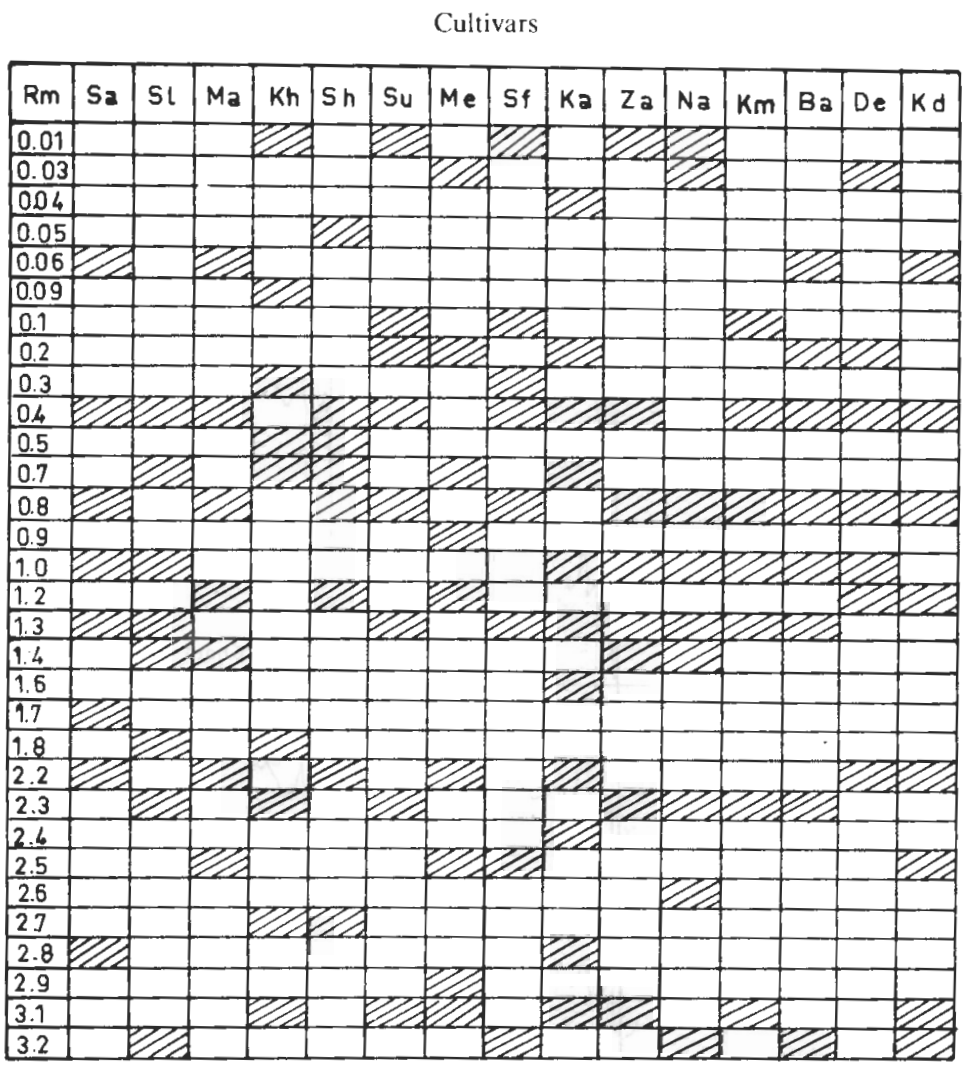

FIG. 2. Diagramatic illustration of electrophoretic pattern pollen grains soluble protein bands of date palm cultivars, Sakaie (Sa), Seleg (Sl), Makturny (Ma), Khashkar (Kh), Shagra-El-Qassim (Sh), Succari (Su), Meneifi (Me), Sefri (Sf), Khalas (Ka), Nabut Zamil (Za) Nabut-Sif (Na), Khashram (Km), Barhi (Ba), Deheini (De), and Khudari (Kd). Rm represent the ratio of distance travelled by the band from the top of running gel to the distance travelled by the tracking dye.

The percentage similarities between each two pairs of cultivars varied from 0 to 75% (Table 1). The highest degrees of similarities were found between Maktumy and Khudari (75%), Succari and Khashram (67%), Nabut Zamel and Khashram (67%), Succari and Khashram (67%), Succari and Nabut Zamel (60%), Nabut Zamil and Nabut-Sif (55%), Nabut Sif and Barhi (50%) and Meneifi and Deheini (50%). The similarities among other cultivars were less than 50% and Khashkar was completely different from both Sakaie and Maktumy in soluble protein patterns. Also, Maktumy was totally dissimilar of Khashram cultivar in protein patterns of pollen grains (Table 1).

TABLE 1. Similarities among date palm male cultivars according to the electrophoretic pattern of their pollen grain proteins (%).

Cultivars	Saleg	Mak	Khash	Shag	Succ.	Men.	Sef.	Kha.	Zam.	Nab.	Khas	Barh.	Deh.	Khudari
1. Sakaie	23	36	0	27	30	8	20	39	36	25	36	46	36	36
2. Seleg		15	21	20	23	13	20	27	31	39	36	31	23	15
3. Maktumy			0	40	15	23	23	19	33	15	0	25	36	75
4. Khashkar				21	21	13	13	11	7	13	15	14	7	17
5. Shagra-El-Qassim					14	21	14	20	14	7	15	15	40	36
6. Succari						13	46	27	60	23	67	46	25	23
7. Menifi							15	25	6	6	7	6	50	31
8. Sefri								12	25	31	36	25	15	25
9. Khalas									27	11	29	36	29	19
10. Nabut Zamel										55	67	33	25	25
11. Nabut-Sif											33	50	31	14
12. Khashram												50	27	23
13. Barhi													36	33
14. Deheini														36

Discussion

The electrophoretic patterns of soluble protein of seeds and leaf enzymes were successfully used for identification of wheat cultivars (Johanson and Hall 1966, Menk *et al.* 1973), bean (Boulter *et al.* 1967), brassica species (Vaughan 1975), barley cultivars (El-Meleigi 1985), and pomegranate cultivars (El-Meleigi *et al.* 1987). Our results had also shown that the electrophoretic patterns of pollen could be successfully used for identification of date palm cultivars.

Date palm male cultivars examined in this study were distinct from each others at different degrees. The high similarities among some cultivars i.e. Maktumy and Khudari might represent close genetic background.

The morphological characteristics of similar and dissimilar cultivars should be investigated in order to verify the results obtained by electrophoretic identification of *pollen* proteins.

The use of a reliable tool such as electrophoresis identification of date palm cultivars would help in naming and screening date palm cultivars across the country for scientific purposes and breeding programs. Total dependence on local names of date

palm cultivars creates serious confusion in results obtained by date palm researchers in various parts of Saudi Arabia. Therefore, further work on use of biochemical techniques as well as morphological and histological studies should be conducted for the purpose of accurate identification of date palm cultivars.

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التمييز بين ذكور بعض أصناف نخيل البلح بواسطة الالكتروفوريسز للبروتينات الذائبة في حبوب اللقاح

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المستخلص . تم فصل البروتينات الذاتية من حبوب لقاح ذكور نخيل البلح ، أصناف صقعي ، سلج ، مكتومي ، خشكار ، شقراء القصيم ، سكري ، منيفي ، صفري ، خلاص ، نبوت سيف ، خشرم ، برحي ، دهيني وخضري بواسطة الالكتروفوريسز .

كانت أنماط البروتينات المفصولة ممثلة للأصناف المستخلصة منها . ورغم أن عملية الفصل نتج عنها إحدى وثلاثون خط طيفي (حزمة) إلا أن عدد الحزم المفصولة لكل صنف كانت تتراوح بين ٧، ٩ فقط . ولقد ظهر أن هناك تشابه كبير بين صنفي مكتومي وخضري بنسبة وصلت إلى ٧٥٪ ، سكري وخشرم بنسبة ٦٧٪ وكذلك نبوت سيف وخشرم بنسبة ٦٧٪ أيضًا . وتبين أن الصنف خشكار مختلف كلية عن صنفي الصقعي والمكتومي ، بينما ظهر أن الصنف مكتومي اختلف عن صنف الخشرم . هذا وقد تراوحت أوجه الشبه بين الأصناف الأخرى بنسب مختلفة تقع بين ١١-٦٠٪ .