ISOZYMES POLYMORPHISM AND PEROXIDASE ACTIVITY OF IRANIAN DATE PALM CULTIVARS

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ABSTRACT

To obtained of good and stable markers, other than morphological for identification of Iranian date palm cultivars, isozyme polymorphism of peroxidase (POX), acid phosphatase (ACP), shikimate DH (SKD) and isocitrate DH (ICD) were study using IEF method for 40 cultivars. Also peroxidase activity as bayoud disease resistance marker was determined for 20 date palm cultivars. The results showed that ACP with 21 bands had the highest number of bands where POX, SKH and ICD had 18, 17 and 16 bands, respectively. The rate of polymorphism for all enzymes was 79% and approximately all cultivars were classified only by POX, ACP and SKH enzymes. It was appears that Suweidani, Firsi, Bureim, Khasab and Chibchab, which Attention are less commercial, had low level of POX activity.

INTRODUCTION

The date palm (*Phoenix dactylifera* L.) has considerable role in economy of several countries of the world which located in arid regions. On the basis of FAO reports in 1998, Iran had maximum of date production, export and area cultivation of date palm in the world, and Four hundreds date cultivars were reported from Iran (Dawson, 1964, 1982). Generally, identification of this cultivars was only based on vegetative and reproductive morphological characters. These markers have less important because they 1) limited in number, 2) dependant to phenological stages or environmental

conditions, 3) can not be use throughout the year (reproductive characters) and 4) long life of the date palm. Successful application of Isozymes electrophoresis to date palm cultivars identification was reported by several investigators (Tisserat and Torres, 1979/80, Baaziz and Saaidi, 1988, Al-Jibouri, 1989/90, Bennaceur, 1991 and Booij *et al* 1995). These markers have several advantageous because are less caused by environmental conditions, co dominant, without epistatic and pleotropic effects and interpretable as genand loci. In this study isozyme polymorphism and peroxidase activity were used as biochemical markers for resolution between cultivars. The peroxidase activity also believed to related to bayoud disease resistance in date palm (Baaziz, 1988, 1989, 1996). This disease is caused by *Fusarium oxysporium* and was not reported from Iran.

MATERIALS AND METHODS

Plant material

All samples in this study which consisted of 40 important economical date palm cultivars were taken from Date Palm Research Institute Gene bank of Ahwaz (collected by late Dawson). All cultivars (5 cultivars, Ghanami, Khukri, Wardi, Sumismi and Nar kharuk helow were male), tested for isozyme polymorphism, but only 20 cultivars were selected for determination of their POX activity. Immediately after taken of samples, they put on ice until extraction.

Extraction

Extraction for both isozyme and peroxidase activity was performed by use of pieces of full mature green leaves. For isozyme extraction , weight 0.5 g leaf from each cultivars and cut in to small pieces then crushed in mortar with 1 nl of extraction buffer consisting of 20% sucrose, 3% PEG, 0.8% PVPP , 1.5% BSA , 0.01 M DTT, 0.001 M EDTA and 0.35 M thioglycolic acid sodium salt (EDTA and thioglycolic acid were excepted for peroxidase extraction buffer). Extracts were then centrifuged at 5000 rpm for 10 minute in cold temperature (4 °C). The supernant was poured into epindorf tubs and stored in -70 ° C until used.

Extraction for peroxidase activity was performed according to Baaziz (1987) with few modification in two steps 1) crused 1.5 gr leaf in 3 ml → All CC is ml acetone- water solution (5 parts acetone and 1 part water) and then freeze- dried, 2) the powder was homogenized in 5CC tris-Hcl 0.1 M

pH=7.5 contain 10% V/V glycerol and 10% W/W PVPP, followed by centrifuging at 10000 rpm for 25 minute. All steps were performed in cold temperature (4 °C). The cleared samples were then stored in -70 ° C until used.

Electrophoresis

IEF with ultra thin polyacrylamide gel (0.25 mm thickness) was used in this study. Gel composition based on volume percentage consisted of 10.2% monomers (46 %W/V with 1 part BIS and 32.2 part acrylamide), 79 % glycerol (20%), 3.9% ampholytes (Pharmacia producted ampholins: 3.5-5, 5-7 and 7-9 in equal volumes 1:1:1), 0.01 % TEMED and 6.8 % ammonium per sulfate (10 mg/ml).Before TEMED and ammonium persulfateis added, the gel mixture was degassed carefully. Aspartic acid 0.04 M and NaOH 1.3 M were used as anolyte (+) and catholyte (-) buffers respectively. Prefocusing was performed in two steps: 1) 30 minute at final voltage equal 700 V and 2) after change the anode strip with fresh one, prefocusing continued for 15 minute at final voltage equal 1200 V. 28 μ L from each samples were loaded on 2 pieces of filter paper (0.5 ×1 cm^2) at 0.5 cm from anode. Focusing was performed in 80 minute at final voltage equal 3500 V.20-30 minute after loading, samples would absorbed by gel, so, we can remove the filter papers. Current and power were constant at all over the run (2 mA and 0.82 W per one cm of gel width). The distance between electrodes was 9 cm. In this study 7 enzyme systems, peroxidase (POX), shikimate DH (SKH), acid phosphatase (ACP), isocitrate DH (ICD), estrase (EST), glutamate oxaloacetate transaminase (GOT)and endopeptidase (ENDO) were tested. Visualization of POX and EST isozymes were performed according to Shaw and Prasad (1970), SKH according to Wendle (1989) ACP and GOT, according to Al-Jibouri (1988) and ICD and END according to Vallegos (1983). After drying gels, they scan with densitometer (Helena 24-process) and evaluate number of bands and Their intensities.

Protein concentration determination

In this study Lowry method with bovine serum albumin as standard was used for determination of protein concentration in tissues of green leaves of date palm cultivars.

Peroxidase activity determination

POX activity was determined by guaiacol as substrate, according to Baaziz (1987). The rate of absorbance per minute of color development was measured at 4 minute in 470 nm by spectrophotometer. The results were explained as units for total peroxidase activity and units mg $^{-1}$ protein for specific peroxidase activity.

RESULTS

Isozymes polymorphism

Only four enzyme systems consist of POX, ACP, SKH and ICD were found to be had good resolution but the other enzymes showed non interpretable bands. ACP with 21 bands, had highest number of band than other systems. The strong dark brown bands specially appear in basic pH_s to just cathode pole (Figure 1). Four bands were observed in all cultivars, so the rate of polymorphism for this enzyme was 81%.For POX 18, red bands were observed from anode to cathode (Figure 1). Three bands were observed in all cultivars, so rate of polymorphism for this enzyme was 83.3%. Seventeen blue bands were visualized for SKH, which all bands were appear in Zerec cultivar. Most of all bands were located between pH 5-8 (Figure 1). Two bands were observed in all cultivars, so this enzyme had 88.2% polymorphism rate. For ICD, 16 blue bands were observed, approximately between pH 5 to 7 (Figure 1), which 6 bands from those were observed in all cultivars and rate of polymorphism for this enzyme was 62.5%.

Peroxidase activity

Peroxidase activity was obtained for all 20 cultivars assayed. Shekkar with 663.9 Uhad highest level and Chebchab with 184.2 U had lowest level of total POX activity (Table 1), and the highest and lowest levels of specific POX activity with 86.7 U mg $^{-1}$ and 18.3 U mg $^{-1}$ protein, belong to Ashgar and Khasab cultivars, respectively.

Discussion

As described in results, the rate of polymorphism for each enzyme system was high, and this rate for all enzyme systems was 79%. Therefore

Figure 1. Isozymes of isocitrate DH(ICD),acid phosphatase(ACP)
Shikmate DH(SKH) and peroxidase (POX) which separated by
IEF in 3.5--9 For Iranian date palm cultivars.

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Table 1

protien concentration and peroxidase activity in leaves of 20 female date palm cultivars of Iran.

Cultivar	Protien concentration		Peroxidase activity	
	mg/mL	mg /g*	Total A. (Units)	Specific A. (U/mg)**
Shakar	2.672	8.9	663.9	74.6
Ista-amran	4.173	13.91	578.1	41.6
Ashgar	1.892	6.3	546.3	86.7
Hadal	2.288	7.62	536	70.3
Gantar	2.534	8.47	514.6	60.7
Ishaq	2.172	7.24	513.8	71
Khadrawi	4.077	13.59	498.7	36.7
Deiri	4.366	14.55	495.5	34
Diqal zard	2.152	7.17	491.6	68.6
Hamrawi	3.148	10.49	472.5	45
Hallawi	2.927	9.75	466.1	47.8
Berhee	2.206	7.35	403.4	54.9
Suweidani	3.956	13.18	381.2	28.9
Zahidi	2.878	9.59	371.6	38.7
Jozi	1.767	5.89	344.6	58.5
Diqal surkh	3.348	11.16	327.9	29.4
Firsi (Fersi)	2.823	9.41	260.5	27.7
Khasab	4.209	14.03	256.5	18.3
Bureim(Breim)	2.857	9.53	232.7	24.4
Chibchab	1.913	6.37	184.2	28.9

*mg protien per gr fresh weght of leaf

** Units per mg protien

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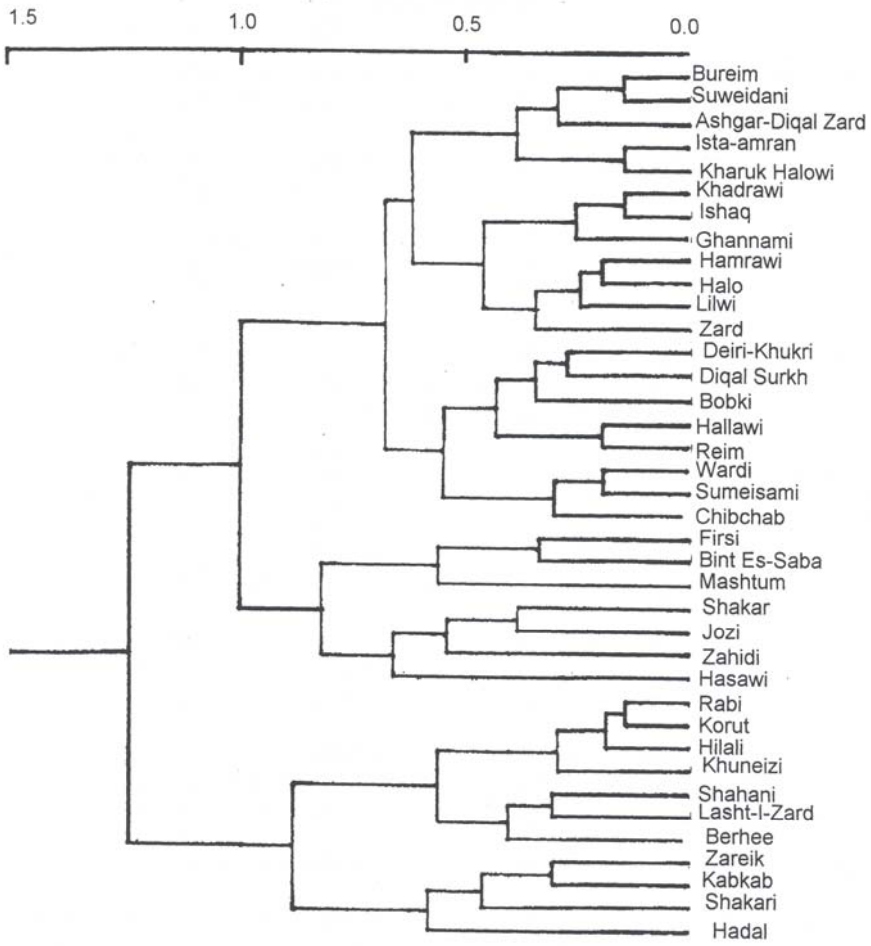
the isozyme markers exhibited in date palm and almost all cultivars (except Ashgar, Degol zard, Khuki and Dayri) were classified only by 3 enzyme systems consist of POX, ACP and SKH (Figure 2). Tisserat and Torees (1979/80) were reported that the date palm isozymes consist of alcohol DH (ADH), EST, GOT, phosphogluco-isomerase (PGI) and phosphogluco mutase (PGM), had 88% polymorphic loci and also Bennaceure *et al* (1991) were reported that date palm isozymes belong to ADH, aspartate amino transaminase (AAT), ACP, ENDO leucine amino peptidase (LAP) and PGM had good polymorphism and percentage of polymorphic loci was high (71.4 to 100). However there are no differences were observed among male and female.

Results obtained from POX activity as showed in Table 1, appear that the important commercial date palm cultivars which have considerable export and area cultivation such as Ista-amran, Gantaar, Khadrawi, Deiri, Berhi, showed medium to high level of peroxidase activity; but the less important cultivars such as Chibchab, Khasaab, Digal surkh and Firsi showed the lowest level of POX activity and no correlation observed between level of POX activity and dry weight and thickness of leaves (data not showed). Baaziz (1989) reported the correlation between POX activity and resistance to bayoud disease in date palm. He pointed out that Bou-Skri and Bou-Feggous cultivars from Morocco which sensitive to bayoud disease, had the lowest level of specific peroxidase activity (39.44 and 21.45 Umg^{-1} respectively), and Bou – Sthammi Noir which was resistance to this disease had highest level of POX activity (215.45).

According to the results obtained here, we expect that those cultivars which showed low level of POX activity, have relatively low resistance to bayoud disease, Therefore more field and laboratory studies are needed to describe this.



Figur 2: Dendrogram obtined from cluster analysis of datas belong to peroxidase, acid phosphatase and shikimate DH isozymes in 40 date palm cultivars.

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