Euphytica **65**: 23–32, 1993.

Correlation of isoenzyme polymorphism and Bayoud-disease resistance in date palm cultivars and progeny

K. Bendiab[1], M. Baaziz[1], Z. Brakez[1] & My.H. Sedra[2]
[1] *Laboratoire de Biochimie et Biologie Moléculaire des Plantes, Université Cadi Ayyad, Faculté des Sciences, B.P. S 15, Marrakech, Morocco;* [2] *Laboratoire de Phytopathologie, Centre Régional du Haouz Pré-Sahara, INRA, B.P. 533, Marrakech, Morocco*

Received 1 April 1992; accepted 4 November 1992

Key words: Phoenix dactylifera, date palm, cultivars, fusariosis, variation, inheritance, isoenzymes, *Fusarium oxysporum* f. sp. *albedinis*

Summary

Seedlings of date palm (*Phoenix dactylifera* L.), obtained from seven cultivars crossed with two males, were analyzed by polyacrylamide gel electrophoresis for esterase (EST), glutamate oxaloacetate transaminase (GOT), endopeptidase (ENP) and alcohol dehydrogenase (ADH) polymorphisms. Eleven, eight, five and two phenotypes were revealed for the enzymes tested, respectively. Seedlings of F_1 populations derived from Bayoud (*Fusarium*)-resistant and low fruit quality cultivars were characterized by a high electrophoretic polymorphism, when compared with progenies of Bayoud-susceptible and high fruit quality cultivars. In almost all cases, the most frequent electrophoretic phenotypes scored for each enzyme in different F_1 populations, were similar to those of the corresponding parent cultivars. Heterozygous phenotypes have been found for, at least, 3 loci; *Got-2, Est-1* and *Enp,* indicating that such loci could be used to screen for hybrid seedlings. The expected Mendelian segregation of allozymes has been observed for these 3 loci, in many F_1 populations. It seems that progenies of Bayoud-resistant cultivars are characterized by a high level of electrophoretic polymorphism. The estimation of this index and the search for genetic linkage with segregating allozymes, may be biochemical criteria useful as an aid in distinguishing date palm seedling populations derived from Bayoud-resistant cultivars and suitable for breeding programs.

Introduction

The date palm (*Phoenix dactylifera* L.) is a long lived dioecious monocotyledon, which is cultivated for food, fuel, fibre and shelter. The species is slow flowering and fruiting and it is difficult to distinguish the date producing female trees from the male trees before the first flowering, when the plants are about 5 years old. In addition to slow growth, the date palm in North Africa also suffers from a vascular fusariosis, called Bayoud disease, where the causal agent is an imperfect fungus: *Fusarium oxysporum* f. sp. *albedinis* (Pereau-Leroy, 1958; Louvet & Toutain, 1973). Date palms are generally propagated by separating and independently establishing the offshoots produced by an individual tree. This method maintains the genetic integrity of cultivars but does not increase the genetic diversity between individuals (Pereau-Leroy, 1958; Saaidi, 1979). Selecting seedlings with high genetic potential in breeding programme requires the development of tests to assess purity, distinguish cultivars and identify hybrid plants in a hybridization programme.

The use of electrophoresis to estimate certain genetic characteristics of plant populations has become widespread since many years. In general, cross-pollinating species give rise to heterogeneous progenies. In this case, electrophoretic techniques could be used as an aid to characterize cultivars for differences in frequency of electrophoretic patterns. So, identification can be considered as seed (or/and seedling) sample identification rather than cultivar identification (Ramirez & Pisabarro, 1985). Many plant cultivars have been characterized by isoenzyme variations. Recent examples are: bermudagrass (Dabo et al., 1990), kiwifruit (Messina et al., 1991) and strawberry (Nehra et al., 1991). In date palm (*Phoenix dactylifera* L.), electrophoretic analyses have been carried out using the water-soluble proteins extracted from fruits of 8 cultivars collected in Saudi Arabia and one cultivar from Iraq (Stegemann et al., 1987). Thus, native proteins showed characteristic molecular weights in the range of 20000, 22000, 24000 and 27000 and differentiation by isoelectric points either in the range pH 4–5 or pH 8–9. In addition, Al-Helal (1988) showed that date protein patterns of 13 cultivars appeared to be specific and that each cultivar displayed one of two α-amylase variants. Using seeds of the date palm cultivar 'Medjool', Chandra-Sekhar & De Mason (1988) demonstrated that electrophoretic protein profiles differed in respect to seed tissues. Generally, isoenzyme studies have been carried out using leaf material. Thus, genetic control of many isoenzymes was determined using several seedling populations of known parents and starch gel electrophoresis technique (Torres & Tisserat, 1980). On the basis of esterase phenotypes, 10 Moroccan cultivars of date palm have been grouped into four sets and more phenotypes have been revealed, when compared with former studies (Baaziz & Saaidi, 1988). Finally, Bennaceur et al. (1991), using 31 Algerian date palm cultivars demonstrated, with 7 enzyme systems, that genetic variability is greater in the cultivars in the western regions than in the eastern regions. An identification key was devised and nearly 65% of the cultivars were identified from 5 enzyme systems.

In other cases, correlations between electrophoretic polymorphisms of several enzymes and some other genetic traits have been studied. Thus isoenzymes were used as an aid to design a progeny test to select ponderosa pine trees which produce seedlings with a superior ability to survive on degraded coal-mine spoils (Woods et al., 1984), isoenzyme variation of *Chenopodium album* L. indicated a reduced variation in triazine herbicide resistant populations (Gasquez & Compoint, 1981; Warwick & Marriage, 1982) and similar results were obtained by Warwick & Black (1986) in *Amaranthus retroflexus* L.

On the basis of esterase, endopeptidase, glutamate oxaloacetate transaminase and alcohol dehydrogenase isoenzyme variations, the present study was conducted to give a preliminary comparison of seven lots of date palm seedlings obtained from seven crosses using two male lines and seven cultivars as females. Good cultivars are generally characterized by their fruit quality and the level of resistance of Bayoud disease. Our objective was to determine the usefulness of isoenzymes to identify F_1 plant populations which could be used to replace diseased date palm groves.

Materials and methods

Plant material. Seeds of date palm (*Phoenix dactylifera*) were collected in Zagora (South Morocco) from seven cultivars with differences in resistance to Bayoud disease and fruit quality as described by Pereau-Leroy (1958) and Saaidi (1979). Seed lots and hence plant lots 1–7 were obtained from the following cultivars (one tree per cultivar): Iklane (IKL), Bou-Sthammi noire (BSTN), Sair Layalet (SLY), Bou-Slikhene (BSL), Bou-Skri (BSK), Jihel (JHL) and Aguellid (AGL). The three first cultivars (Bayoud-resistant) and BSL (incompletely resistant) were pollinated with a resistant male (M-2, INRA). The last three cultivars (Bayoud-susceptible) were pollinated with a susceptible male (M-1, INRA). The principal characters of each cultivar are summarized in Table 1. Seeds were germinated, then planted in 250 m^3 pots (10 plants per pot) filled with Marrakech soil (South Morocco) and grown, from September, in a date

palm hot-house. When the plants had 2 leaves, 4 cm long (0.24 ± 0.01 g) samples of leaves were taken from plants of each lot. Leaf samples of the seven female cultivar parents and the two male lines were collected from the intermediate circumference of the palm crowns. Plant material was stored at − 40° C until later use.

Enzyme extractions. Seedling leaves were ground in a cooled mortar in 1 ml 'TAMET buffer', pH 7.0, composed of 0.5 M Tris, 0.3 M ascorbic acid, 2% 2-mercaptoethanol, 0.01 M EDTA, 2.8% Triton X-100 and 10% (w/w) insoluble PVP. The homogenate was centrifuged at 9000 g for 7 min. If not used immediately, the supernatant (TAMET buffer extract) was frozen at − 40° C for later enzyme electrophoretic analysis.

Electrophoresis and staining gels for enzyme activity. Electrophoresis was performed on 11% polyacrylamide gels, using two buffer systems. Tris-Glycine system (pH 8.3) previously described by Baaziz & Saaidi (1988) was useful in separating esterase and glutamate oxaloacetate transaminase isoenzymes. Tris-Borate system (pH 8.1), containing 0.89 M Tris, 0.89 M boric acid and 0.025 M EDTA, was more efficient in resolving endopeptidase and alcohol dehydrogenase isoenzymes. Electrophoresis was carried out at 170 V for 4–5 hours. Esterases were stained by using 1% α-naphthylacetate as substrate. Glutamate oxaloacetate transaminase was assayed by incubating gels for 15 min in 0.25 M Tris-HCl buffer, pH 7.2, containing 0.026 M aspartic acid and 0.007 M α-ketoglutaric acid. Gels were rinsed with distilled water and incubated, again, for 20 min, in the same buffer containing 0.2% (w/v) Fast blue BB. The staining mixture for endopeptidase contained 0.08% (w/v) α N-Benzoyl-DL-Arginine-β-Naphthylamide (BANA) as substrate. The alcohol dehydrogenase assay consisted of 100 ml 0.02 M Tris-HCl buffer, pH 7.2, 30 mg NAD, 20 mg NBT, 4 mg PMS and 1 ml ethanol. In all cases, gels were washed, fixed in 7% acetic acid and immediately photographed.

Analysis of the data. The banding patterns of the four enzymes were treated as phenotypic characters of each individual. Phenotypic polymorphism for each enzyme and plant lot was estimated using the P_j index utilized by Kahler et al. (1980), Al Mouemar & Gasquez (1983) and Warwick & Black (1986):

$$P_j = \sum_{i=1}^{n} P_i (1 - P_i) = 1 - \sum_{i=1}^{n} P_i^2,$$

where P_i is the frequency of the i th phenotype and n is the number of phenotypes observed per enzyme for each plant lot. The weighted average amount of phenotypic polymorphism ($\bar{P}$) over the observed enzymes is given by:

$$\bar{P} = \sum_{j=1}^{K} (1/N_j)P_j / \sum_{j=1}^{K} 1/N_j),$$

where N_j is the total number of phenotypes observed per j th enzyme for K enzymes.

Results

The date palm experimental station of Zagora contains many Moroccan cultivars, where the principal morphological characters used for their identification are mainly those of the fruit (Pereau-Leroy, 1958; Saaidi, 1979). The geographical distributions of the seven cultivars used in this study are given in Table 1. Cultivars which produce fruits of high quality are susceptible to Bayoud disease.

In order to provide a preliminary evaluation of genetic variation in the seven F_1 populations of date palms and their corresponding parent cultivars, electrophoretic analysis of leaf enzymes was carried out with the use of the TAMET extracts. Diagrammatic representations of electrophoretic patterns observed among seedlings are given in Fig. 1, where eight (A-H), five (A-E), eleven (A-K) and two (A-B) electrophoretic phenotypes have been distinguished among the seven plant lots, for glutamate oxaloacetate transaminase (Got), endopeptidase (Enp), esterase (Est) and alcohol dehydrogenase (Adh), respectively. The phenotypes observed were characterized by the relative mobility (R_f) of each band, when using 11% polyacrylamide gels. Alcohol dehydrogenase migrated at R_f value 0.15. The Got set of isoenzymes had R_f values of 0.18 to

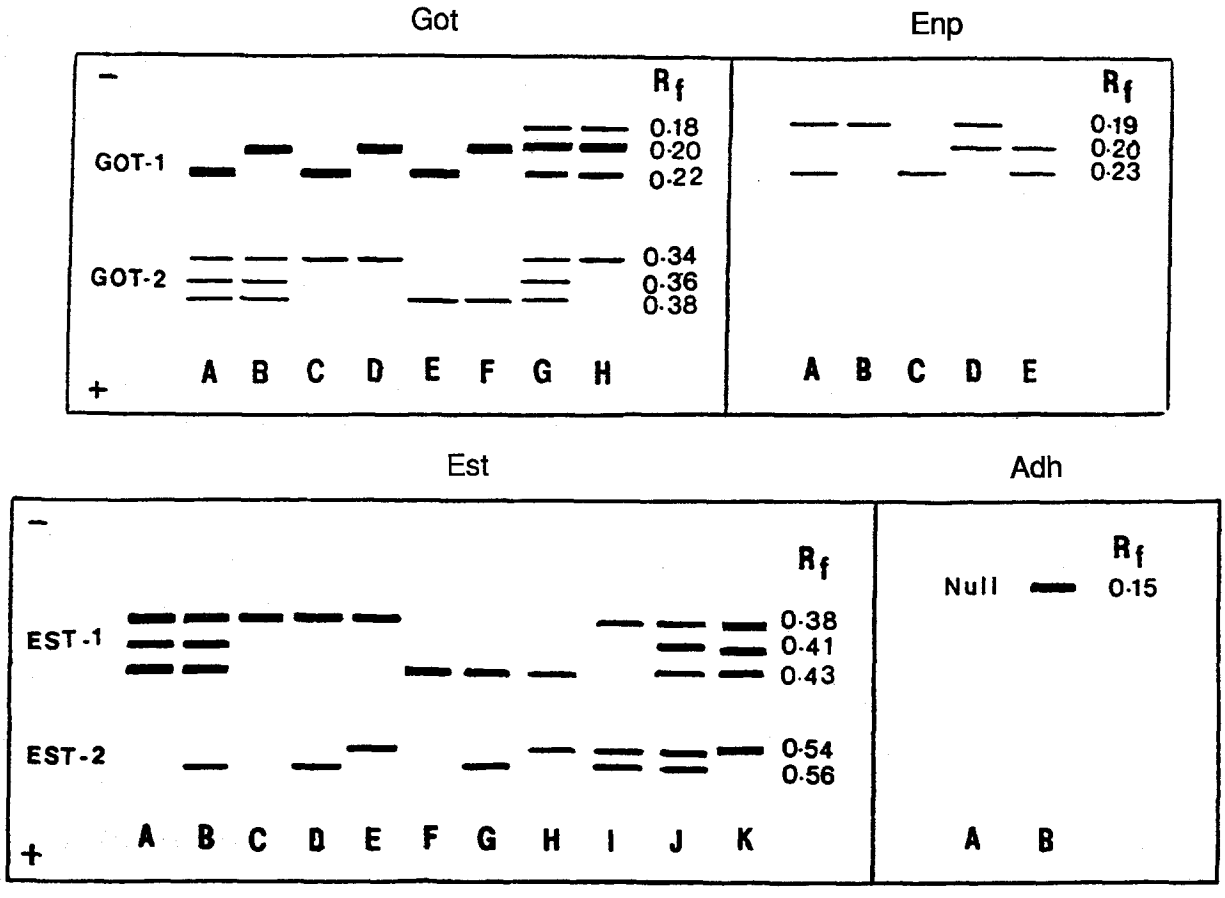

Fig. 1. Diagrammatic representation of isoenzyme patterns (A-K) observed for glutamate oxaloacetate transaminase (Got), endopeptidase (Enp), esterase (Est) and alcohol dehydrogenase (Adh) in date palm seedlings derived from seven cultivars. R_f values are indicated for each band of activity. The abbreviations at the left correspond to the names of glutamate oxaloacetate transaminase and esterase sets.

0.22 and 0.34 to 0.38 for GOT-1 and GOT-2, respectively. The set of endopeptidase isoenzymes had three R_f values; 0.19, 0.20 and 0.23. The set of esterase isoenzymes had R_f values of 0.38 to 0.43 and 0.54 to 0.56 for Est-1 and Est-2, respectively. No differences, in respect of plant age, have been detected during the period covering this study (data not shown).

Electrophoretic phenotypes are determined for each enzyme system. For Got, phenotypes with

Table 1. Characteristics and geographical locations of seven fructifying date palm cultivars in south Morocco

Cultivar	Fruit characters	Comportment to Bayoud	Principal location
Iklane (IKL)	Soft date, lower quality	Resistant	Draa, Bani-est
Bou-Sthammi noire (BSTN)	Small and soft date, medium quality	Resistant	Saghro, Draa
Sair-Layalet (SLY)	Date of medium size and quality	Resistant	Bani-ouest
Bou-Slikhene (BSL)	Small date, medium quality	Incompletely resistant	Tafilalet
Bou-Skri (BSK)	Dry date, high quality	Susceptible	Anti-Atlas
Jihel (JHL)	Half-dry date, high quality	Susceptible	Saghro, Bani
Aguellid (AGL)	Long date, high quality	Susceptible	Draa

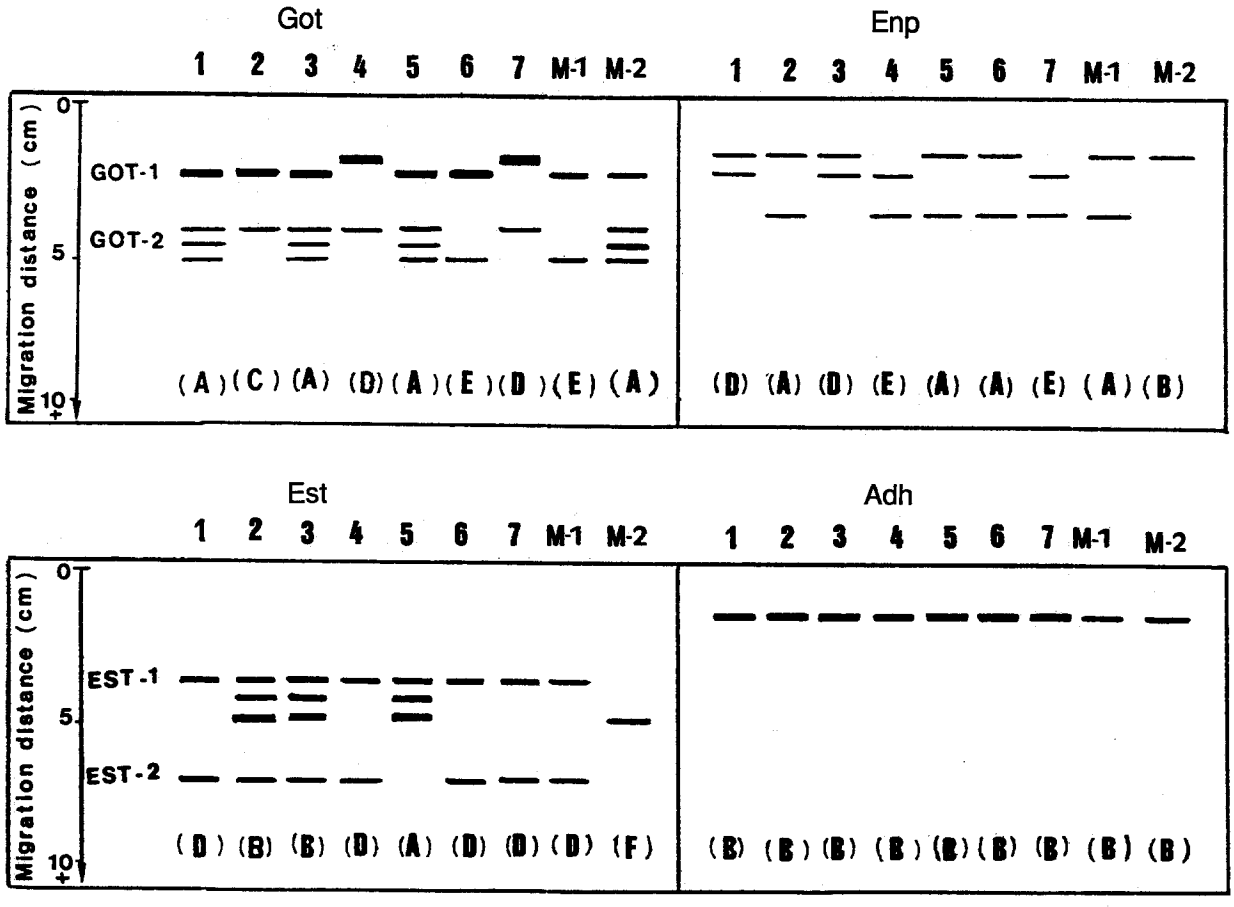

Fig. 2. Zymograms of glutamate oxaloacetate transaminase (Got), endopeptidase (Enp), esterase (Est) and alcohol dehydrogenase (Adh) in leaf extracts of date palm cultivars: lane 1, cv. IKL; lane 2, cv. BSTN; lane 3, cv. SLY; lane 4, cv. BSL; lane 5, cv. BSK; lane 6, cv. JHL and lane 7, cv. AGL. Date palm males are M-1 and M-2. The abbreviations at the left correspond to the names of glutamate oxaloacetate transaminase and esterase sets. Letters in parentheses refer to the corresponding phenotypes reported in Fig. 1.

maximum bands are G (6 bands) and A, B and H (4 bands). The highest numbers of Est bands has been found in phenotypes J (5 bands) and B,K (4 bands). Esterase phenotype F is characterized by only one band. Endopeptidase zymograms exhibited phenotypes with one (B,C) and two (A,D,E) bands. ADH phenotypes A and B correspond to the absence or presence of activity (one band), respectively. Zymograms of the four enzyme systems were analyzed, for the female parent cultivars and the male lines (Fig. 2). The Bayoud-resistant cultivars (IKL, BSTN, SLY and BSL) were characterized by a relatively high levels of heterozygotic characters. Thus, the triple banded patterns of Got-2 and Est-1 which correspond to the expression of heterodimeric molecules (due to the presence of heterozygotes) as well as the homodimeric molecules, were found in cultivars IKL, BSTN and SLY. In contrast, the Bayoud-susceptible cultivar, BSK was always characterized by the absence of Est-2 activity. Furthermore, the slow migrating doublet of endopeptidase, found in IKL and SLY, was absent from all Bayoud-susceptible cultivars analyzed. No differences between resistant and susceptible cultivars were found using ADH zymograms. Frequencies of isoenzyme phenotypes within each lot or population of date palm seedlings were analyzed (Table 2). The least diversity for Est and Got was shown by lots 6 and 7, both in paucity of patterns and in the predominance of one pattern, such as esterase phenotype D, characterized by one band at each of the Est-1 and Est-2 loci.

While all F_1 populations derived from field Bayoud-resistant parents exhibited the esterase pattern B (one and three bands at Est-2 and Est-1 loci, respectively). In contrast to the relative uniformity of seedlings from lots 5,6 and especially 7, GOT enzyme was been found to be more electrophoretically diversified in plant lots 1, 2, 3 and 4, that had been obtained by crossing male M-2 with IKL, BSTN, SLY and BSL, respectively. Electrophoretic patterns E and A of GOT were, to some extent, more frequent in the progenies of high fruit quality and Bayoud-susceptible cultivars. Thus, plant lot 7, obtained from germinated seeds of AGL cultivar crossed with the male M-1, was characterized by only the phenotype A (one and three bands at GOT-1 and GOT-2 sets, respectively). Got phenotypes G and H are present only in the progeny of BSL cultivar (plant lot 4). The frequency of endopeptidase phenotypes is related to the seedling sources, since the double-banded patterns D and A are largely represented in the F_1 populations from IKL and BSL, respectively. ENP pattern E is a phenotype that has been encountered only in the F_1 plant population from AGL (lot 7). All F_1 plant populations 5,6 and 7, obtained from crosses of Bayoud-susceptible cultivars and the

Table 2. Frequencies of isoenzyme phenotypes for esterase (Est), glutamate oxaloacetate transaminase (Got), endopeptidase (Enp) and alcohol dehydrogenase (Adh) in seven F_1 populations of date palm seedlings (seedling lots) obtained from seven different cultivars crossed with two males

Enzyme	Phenotype[a]	Seedling lots						
		lot 1 (52)	lot 2 (54)	lot 3 (56)	lot 4 (52)	lot 5 (53)	lot 6 (65)	lot 7 (56)
Est	A	0.30	–	0.02	0.18	0.16	–	–
	B	0.30	0.24	0.29	0.24	0.22	–	–
	C	–	–	–	0.16	0.10	–	0.17
	D	–	–	0.08	0.31	0.12	0.53	0.50
	E	–	–	–	0.07	0.04	0.32	0.23
	F	0.18	–	–	–	0.02	–	–
	G	0.20	0.30	0.36	–	0.06	–	–
	H	0.02	0.28	0.06	–	0.14	–	–
	I	–	–	–	–	–	0.15	0.10
	J	–	–	0.02	0.04	–	–	–
	K	–	0.18	0.17	–	0.14	–	–
Got	A	0.50	0.36	0.43	0.58	0.46	–	1.00
	B	–	0.22	–	0.17	–	–	–
	C	0.23	0.40	0.24	0.15	0.10	–	–
	D	0.02	0.02	0.02	0.06	–	–	–
	E	0.21	–	0.31	–	0.44	0.85	–
	F	0.04	–	–	–	–	0.15	–
	G	–	–	–	0.02	–	–	–
	H	–	–	–	0.02	–	–	–
Enp	A	–	0.50	0.05	0.64	0.51	0.44	0.25
	B	0.26	0.50	0.36	0.02	0.21	0.37	–
	C	–	–	0.04	0.02	0.28	0.19	0.20
	D	0.74	–	0.55	0.32	–	–	0.21
	E	–	–	–	–	–	–	0.34
Adh	A	0.32	0.17	0.34	0.42	0.15	0.19	0.09
	B	0.68	0.83	0.66	0.58	0.85	0.81	0.91

[a] See Fig. 1 for a correspondence of these letters.

Table 3. Isoenzyme phenotypes of glutamate oxaloacetate transaminase (Got), esterase (Est), endopeptidase (Enp) and alcohol dehydrogenase (Adh) scored in date palm cultivars as females (F) and male lines (M) and compared with the two most frequent phenotypes of seven corresponding F_1 populations (F_1)

Enzyme		Seedling lots						
		1	2	3	4	5	6	7
Got	M	A	A	A	A	E	E	E
	F	A	C	A	D	A	E	D
	F_1	(A, C)	(C, A)	(A, E)	(A, B)	(A, E)	(E, F)	(A)
Est	M	F	F	F	F	D	D	D
	F	D	B	B	D	A	D	D
	F_1	(A, B)	(G, H)	(G, B)	(D, B)	(B, A)	(D, E)	(D, E)
Enp	M	B	B	B	B	A	A	A
	F	D	A	D	E	A	A	E
	F_1	(D, B)	(A, B)	(D, B)	(A, D)	(A, C)	(A, B)	(E, A)
Adh	M	B	B	B	B	B	B	B
	F	B	B	B	B	B	B	B
	F_1	(B, A)	(B, A)	(B, A)	(B, A)	(B, A)	(B, A)	(B, A)

male M-1, contained with high frequency, the alcohol dehydrogenase phenotype B. Phenotype A (absence of enzyme activity) was found to be more present in seedling lots 1, 3 and 4.

When compared with adult cultivars, date palm seedling lots were found to be generally, similar to their parental cultivar sources on the basis of their phenotype frequency (Table 3). For instance, the progeny of JHL (seedling lot 6) was identified by the most frequent phenotypes E, D, A and B for glutamate oxaloacetate transaminase, esterase, endopeptidase and alcohol dehydrogenase, respectively. The parent source (JHL) showed the same phenotypes for each enzyme. Except BSL, the two most frequent phenotypes of each enzyme in a seedling lot correspond, in about 90% cases, to the phenotype of the parent cultivar. Maximum phenotype similarity was found using endopeptidase and alcohol dehydrogenase. In each seedling lot measures the amount of phenotypic polymorphism in each enzyme separately (P_j) and together ($\bar{P}$) for each of the F_1 plant populations (plant lots), are reported in Table 4. These calculations are based on frequency data for the enzyme phenotypes presented in Table 2. Based on the polymorphism values for each enzyme (P_j), great differences between plant lots could be observed depending on the kind of enzyme used. For example, Got enzyme is monomorphic in the progeny of AGL cultivar (plant lot 7). However, this enzyme is very polymorphic in F_1 populations obtained by crossing the male M-2 with the Bayoud-resistant cultivars IKL, BSTN and SLY and the incompletely resistant cultivar, BSL that correspond, respectively, to plant lots 1, 2, 3 and 4. Enp was the only enzyme that exhibited more polymorphism in the F_1 populations from crosses of Bayoud-susceptible cultivars, since P_j index interval for these populations

Table 4. Amount of phenotypic polymorphism in esterase (Est), glutamate oxaloacetate transaminase (Got), endopeptidase (Enp) and alcohol dehydrogenase (Adh) enzymes separately (P_j) and together ($\bar{P}$) for each of F_1 populations (plant lots) of date palm seedlings corresponding to different parent sources

Plant lot	Phenotypic polymorphism (P_j)				$\bar{P}$
	Est	Got	Enp	Adh	
1	0.75	0.65	0.38	0.43	0.48
2	0.74	0.66	0.50	0.28	0.42
3	0.74	0.66	0.56	0.45	0.53
4	0.78	0.61	0.49	0.49	0.53
5	0.85	0.58	0.62	0.25	0.43
6	0.59	0.25	0.63	0.31	0.40
7	0.66	0.00	0.74	0.16	0.31

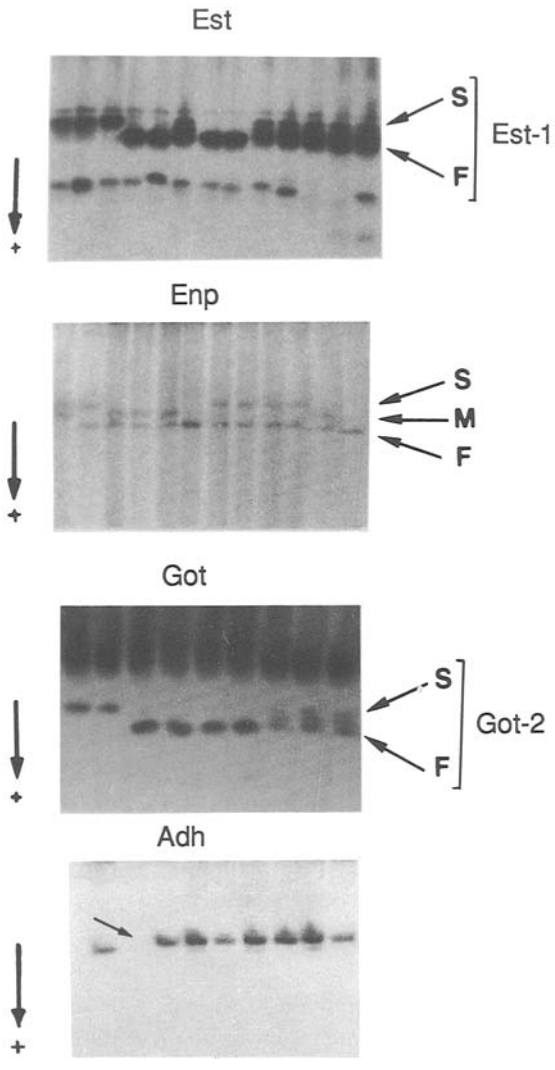

Fig. 3. Electrophoretic phenotypes of esterase (Est), endopeptidase (Enp), glutamate oxaloacetate transaminase (Got) and alcohol dehydrogenase (Adh) revealed in leaf extracts of date palm seedlings. Heterozygous characters are shown at Est-1, Enp and Got-2 sets. Letters S, M and F refer to bands with slow, medium and fast mobility, respectively. The arrow on Adh zymogram shows null activity.

(lots 5, 6 and 7) was 0.62 to 0.74, while the variation was between 0.38 to 0.56 in the F_1 populations from crosses of Bayoud-resistant cultivars. The amount of phenotypic polymorphism over all observed enzymes for plant lot 7 ($\bar{P} = 0.31$) is nearly half those obtained for the seedling lots 3 and 4. Generally, phenotypic polymorphism varied in the interval of $\bar{P}$ values 0.31 to 0.43 for the F_1 plant populations from crosses of Bayoud-susceptible and high fruit quality cultivars and 0.42 to 0.53 for populations obtained from crosses of Bayoud-resistant and lower quality date cultivars. The variation observed in each of the isoenzyme systems studied can be attributed to different alleles at particular loci. Got-2 and Est-1 loci may correspond to two polymorphic loci, with two alleles (F and S) and coding for a dimeric molecule (Fig. 3). Enp phenotypes may result from the expression of one locus with three alleles (F, M and S) and coding for a monomeric enzyme. For Got-2 and Est-1 loci, genotypes SS, FF and SF correspond to phenotypes with single slow, single fast and triple-banded patterns, respectively. Enp genotypes SF, SM and MF refer to double-banded patterns composed of bands with slow (S), fast (F) or medium (M) mobilities. Differences in phenotypic frequencies between seedling lots are attributed, in part, to allelic frequencies at Enp, Est-1 and Got-2 loci. So, progenies of Bayoud-susceptible cultivars are characterized by the disappearance or the impoverishment of alleles M, F and S of the three loci, respectively (Table 5). Accordingly, allozyme equilibrium typifying resistance could be disrupted. Concerning GOT-2 locus,

Table 5. Frequencies of allozymes at Enp, Est-1 and Got-2 loci of endopeptidase, esterase and glutamate oxaloacetate transaminase, respectively, scored in seven F_1 populations of date palm seedlings

Locus[a] & allele	F_1 population[b]						
	1 (52)	2 (54)	3 (56)	4 (52)	5 (53)	6 (65)	7 (56)
Enp							
F	0.00	0.25	0.06	0.34	0.53	0.41	0.50
M	0.37	0.00	0.28	0.16	0.00	0.00	0.27
S	0.63	0.75	0.66	0.50	0.47	0.59	0.23
Est-1							
F	0.70	0.79	0.67	0.23	0.48	0.00	0.00
S	0.30	0.21	0.33	0.77	0.52	1.00	1.00
Got-2							
F	0.50	0.29	0.52	0.39	0.67	1.00	0.50
S	0.50	0.71	0.48	0.61	0.33	0.00	0.50

[a] See Fig. 3 for a correspondence of these letters.
[b] Number in parentheses refers to sample size analyzed.

normal Mendelian segregation of genotypes was observed in 5 F_1 populations (lots 1, 2, 3, 6 and 7). For example, the cross of male M-2 (genotype FS) with cv. IKL (genotype FS) gave rise to the expected ratio 1:2:1 (SS:FS:FF). The cultivars JHL (genotype FF) and AGL (genotype SS) allow the formation of F_1 plants with only the genotypes FF and FS, respectively, when crossed with male M-1 (genotype FF). Three F_1 populations (lots 3, 6 and 7) showed the expected segregation for EST-1 locus. The cultivars JHL and AGL, exhibiting SS genotypes, gave rise to only the genotype SS in the corresponding F_1 populations, when pollinated with male M-1 (genotype SS). Four F_1 populations (lots 2, 5, 6 and 7) exhibited the expected ratio of segregation for Enp locus. For example, BSTN (genotype FS) crossed with male M-2 (genotype SS) give rise to F_1 plants with genotypes SS and SF in the expected ratio 1:1.

Discussion

Creation of Bayoud-resistant and high date quality cultivars of date palm remains largely open to chance, since information on the genetics of these characteristics is not available. Nevertheless, the abundance of good quality khalts (fructifying plants derived from seedlings) in the regions where the best cultivars (high fruit quality producing varieties) have predominated and the frequency of these khalts in active Bayoud foci (Saaidi, 1979), suggest that quality and resistance are transmissible independently and can be combined in an individual tree. Since the expression of these traits depends upon a range of factors including the growth stage and the effect of the environment, linkage of resistance and fruit quality genes with molecular markers would provide a powerful tool for screening the progenies. Based on the total enzyme banding patterns obtained for the seven F_1 plant populations (plant lots), differences have been noted between the corresponding phenotypic polymorphisms. With regard to the high polymorphism in seedlings of Bayoud-resistant cultivars, the presence in resistant varieties of dimers at Est-1 and Got-2 sets (appearance of single slow, single fast and triple banded patterns) contributes to increased number of possible electrophoretic phenotypes in the resulting progenies. Thus, phenotypes B and A, typified by three bands at Est-1 and Got-2 sets, respectively, predominated in date palm seedlings derived from Bayoud-resistant cultivars pollinated with male M-2. This result suggests that a great number of plants within these F_1 populations presented heterozygotic characters. Heterozygous phenotypes have been found at 3 loci (Got-2, Est-1 and Enp) indicating that such loci could be used to screen for the percentage of hybrid seedlings obtained. Such kinds of phenotypes have been used to demonstrate the hybrid status of *Spartina anglica* (Poaceae) (Guenegou et al., 1988), verification of interspecific plum X apricot hybrids (Byrne & Littleton, 1989) and the selection of somatic hybrids between *Lycopersicon esculentum* and *Lycopersicon peruvianum* (Wijbrandi et al., 1990). Similarity between the most frequent electrophoretic patterns of an F_1 population and the pattern of its corresponding cultivar, might be used, in some situations, to identify a parent cultivar and then, to decide the usefulness of the seedling lot in a breeding program. Thus hybrid plants allowing the association of different alleles could be obtained. In date palm, since higher fruit quality cultivars are generally, more susceptible to Bayoud disease, it appears that during their domestication, selection and clonal propagation there has been some loss in both disease resistance and isoenzyme diversity.

The expected Mendelian segregation of allozymes observed for Got-2, Est-1 and Enp loci in many F_1 populations could be of interest to develop a linkage map for *Phoenix dactylifera* and to tag genes controlling commercially important characters. Thus linkage of rust resistance genes from wild barley with the isozyme loci Est-2, Acp 3 and Dip 2 has been shown by Feuerstein et al. (1990). An association was demonstrated between the malate dehydrogenase locus and the sex determining genes in *Asparagus officinalis* L. (Maestri et al., 1991). In our studies, the possibility that at least some of the isoenzyme loci are linked to genes controlling the resistance to Bayoud disease is of considerable interest and further testing is currently in progress.

Acknowledgements

This research was supported by a grant from the International Foundation for Science, Stockholm, Sweden, No. D/1359-2 and in part by the Third World Academy of Sciences, Trieste, Italy. We thank Dr. H. Faraj (INRA, Morocco) for cooperation in supplying seed collections and Dr. O. Bouab and Dean A. Mokhlisse (Université Cadi Ayyad, Marrakech, Morocco) for continuous encouragements.

References

Al-Helal, A.A., 1988. Amylase isoenzymes and protein of date palm (*Phoenix dactylifera* L.). Bot. Bull. Academia Sinica 29: 239–244.

Al-Mouemar, A. & J. Gasquez, 1983. Environmental conditions and isozyme polymorphism in *Chenopodium album* L. Weed Res. 23: 141–149.

Baaziz, M. & M. Saaidi, 1988. Preliminary identification of date palm cultivars by esterase isoenzymes and peroxidase activities. Can. J. Bot. 66: 89–93.

Bennaceur, C., C. Lanaud, M.H. Chevallier & N. Bounaga, 1991. Genetic diversity of the date palm (*Phoenix dactylifera* L.) from Algeria revealed by enzyme markers. Plant Breed. 107: 56–69.

Byrne, D.H. & T.G. Littleton, 1989. Interspecific hybrid verification of Plum × Apricot hybrids via isozyme analyses. HortScience 24: 132–134.

Chandra-Sekhar, K.N.C. & D.A. De Mason, 1988. Quantitative ultrastructure and protein composition of date palm (*Phoenix dactylifera* L.) seeds: a comparative study of endosperm vs. embryo. Amer. J. Bot. 75: 323–329.

Dabo, S.M., C.M. Taliaferro & E.D. Mitchell, 1990. Bermudagrass cultivar identification by use of isoenzyme electrophoretic patterns. Euphytica 51: 25–31.

Feuerstein, E., A.H.D. Brown & J.J. Burdon, 1990. Linkage of rust resistance genes from wild barley (*Hordeum spontaneum*) with isozyme markers. Plant Breed. 104: 318–324.

Gasquez, J. & J.P. Compoint, 1981. Isoenzymatic variation in populations of *Chenopodium album* L. resistant and susceptible to triazines. Agro-Ecosystem 7: 1–10.

Guenegou, M.C., J. Citharel & J.E. Levasseur, 1988. The hybrid status of *Spartina anglica* (*Poaceae*). Enzymatic analysis of the species and the presumed parents. Can. J. Bot. 66: 1830–1833.

Kahler, A.L., R.W. Allard, A.M. Krzakow, C.G. Wehrhahn & E. Nevo, 1980. Associations between isozyme phenotypes and environment in the slender wild oat (*Avena barbata*). Theor. Appl. Genet. 56: 31–47.

Louvet, J. & G. Toutain, 1973. Recherche sur les fusarioses. VIII. Nouvelles observations sur la fusariose du palmier dattier et précisions concernant la lutte. Ann. Phytopathol. 5: 35–52.

Maestri, E., F.M. Restivo, G.P. Marziani Longo, A. Falavigna & F. Tassi, 1991. Isozyme gene markers in the dioecious species *Asparagus officinalis* L. Theor. Appl. Genet. 81: 613–618.

Messina, R., R. Testolin & M. Morgante, 1991. Isozymes for cultivar identification in kiwifruit. HortScience 26: 899–902.

Nehra, N.S., K.K. Kartha & C. Stushnoff, 1991. Isozymes as markers for identification of tissue culture and greenhouse-grown strawberry cultivars. Can. J. Plant Sci. 71: 1195–1201.

Pereau-Leroy, P., 1958. Le palmier dattier au Maroc. Ministère d'Agriculture, Service de la Recherche Agronomique, Maroc et Institut Français de Recherche Fruitière Outre-Mer, Institut des Fruits et Agrumes Coloniaux.

Ramirez, L. & G. Pisabarro, 1985. Isozyme electrophoretic patterns as a tool to characterize and classify rye (*Secale cereale* L.) seed samples. Euphytica 34: 793–799.

Saaidi, M., 1979. Contribution à la lutte contre le Bayoud, fusariose vasculaire du palmier dattier. Thèse de Doctorat d'Université, Université de Dijon.

Stegemann, H., A.M.R. Afify & K.R.F. Hussein, 1987. Identification of date (*Phoenix dactylifera* L.) cultivars by protein patterns. Phytochem. 26: 149–153.

Torres, A.M. & B. Tisserat, 1980. Leaf isozymes as genetic markers in date palms. Amer. J. Bot. 67: 161–167.

Warwick, S.I. & P.B. Marriage, 1982. Geographical variation in populations of *Chenopodium album* resistant and susceptible to atrazine. I. Between and within-population variation in growth and response to atrazine. Can. J. Bot. 60: 483–493.

Warwick, S.I. & L.D. Black, 1986. Electrophoretic variation in triazine-resistant and susceptible populations of *Amaranthus retroflexus* L. New Phytol. 104: 661–670.

Wijbrandi, W., W. Van Capelle, C.J. Hanhart, E.P. Van Loenen Martinet-Schuringa & M. Koornneef, 1990. Selection and characterization of somatic hybrids between *Lycopersicon esculentum* and *Lycopersicon peruvianum*. Plant Sci. 70: 197–208.

Woods, J.H., G.M. Black & F.W. Allendorf, 1984. Using isozyme analysis to aid in selecting genetically superior Ponderosa pine for coal-mine spoil reclamation. Northwest Sci. 58: 262–268.