Biochemical characterization of date-palm cultivars using isozyme markers

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INTRODUCTION

Date-palm *(Phoenix dactylifera* L.) is an arborescent monocotyledon, with separate male and female trees. It is cultivated in arid areas for food and many other commercial purposes. However, its cultivation is difficult for many reasons: the species, like other long-lived perennials, is slow to flower and fruit, and it is difficult to determine the sex of the trees before the first flowering, when they are about 5 years of age. Finally, date-palm shows a slow clonal propagation *via* offshoots, which are produced in a limited number by a tree during its life. Date-palm is of great socio-economic importance in the south of Tunisia were about 10% of population depend directly or indirectly on date-palm and undercovered cultures [1]. On the other hand, recent investigation on date-palm genetic resources revealed the high diversity of Tunisian palm groves. Thus, 250 cultivars (cvs.) have been reported, but only 120 of them were characterized on the basis of some morphological traits especially those of fruit (shape, weight, consistency, color, etc.) [2]. However, most of these criteria could easily be modified by the environmental conditions. Therefore, many problems have arisen concerning cultivars nomenclature. Different vernacular names may refer to the same cultivar and, conversely, different cultivars in different regions may have the same name. On the other hand, it is sometimes very difficult to distinguish ‘khalts’ (female tree derived from seedling) from traditional cultivars, since both can produce fruits of similar quality. Hence, the lack of a practical key for cultivar identification as well as the long life cycle of the tree, prompted the need to establish appropriate methods for identification.

Additional Key words: *Phoenix dactylifera* L., isozyme polymorphism, cultivar identification.

Isozymes represent biochemical markers which are successfully used as a possible alternative or complementary method for characterization of crop plant cultivars [3,4,5].

In this paper we present the results of the study of enzyme polymorphism in 29 date-palm cultivars using starch and polyacrylamide gel electrophoresis. The enzyme systems corresponded to glutamate oxaloacetate transaminase (GOT), phosphoglucomutase (PGM), shikimate dehydrogenase (SDH) and phosphoglucoisomerase (PGI). The isozyme profiles obtained were used to establish an identification key of the analyzed date-palm cultivars.

MATERIALS AND METHODS

Twenty nine date-palm cultivars were collected from three date growing regions in Tunisian namely Gabès, Nefzaoua and Djerid (Table 1). Leaflets from each cultivar were excised and stored in liquid nitrogen (LN) for later enzyme electrophoresis. Enzymes were extracted from 1g of leaves in 2.5 ml of extraction buffer [6, with slight modifications]. A prior crushing of leaflet material in LN made the extraction easier. The homogenate was centrifuged at 20 000 xg at 4^{o}C for 40 min. Supernatant were collected for immediate electrophoresis, or stored in -80^{o}C.

Starch and polyacrylamide gel electrophoresis was carried out using a modifications of the procedures described in [7 and 8] respectively. Staining mixtures and gel fixation followed those described in [9]. Cultivar identification was achieved according to Bennaceur et al. [10].

RESULTS

In a previous paper, we reported the mode of genetic inheritance of the four studied enzyme systems in date-palm [11]. Thus, it has been established that, five polymorphic loci were resolved namely Got-1, Got-2, Pgm, Sdh and Pgi-2. The representative zymograms of each enzyme system are illustrated in figure1. The corresponding genotypes for each cultivar are given in table 2.

3.1. Zymograms description

GOT isozymes are the most polymorphic, since two zones of enzyme activity named Got-l and Got-2, and seven different banding patterns designated A1-A7 were revealed (fig 1). The A1 profile characterized by three bands at each zone, corresponding to heterozygotic individuals for a dimeric molecule. Similarly for PGI were two zones of enzyme activity named Pgi-l and Pgi-2, and 3 different profiles classified D1-D3 were observed (fig.1). The common band at the Pgi-l may be related to chloroplastic gene according to Gotlieb [12] , while the triple-banded pattern at the D2 profile indicated the dimeric enzyme expressed at the locus Pgi-2.

For the PGM, one zone of enzyme activity and five different profiles designed B1-B5 were scored (fig 1). The double-banded pattern at the B1 profile suggested a heterozygote individuals for a monomeric structure. Finally, SDH zymograms show one zone of enzymatic activity and four different banding patterns classified C1-C4 (fig 1). As for the PGM isozyme, SDH seems to be of monomeric structure.

3.2. Cultivar identification

A total of 19 different profiles were detected in the 29 cultivars using the four enzyme systems. These produced 28 multilocus genotypes. A dichotomous key was effected by hierarchically grading the enzyme with the greater number of genotypes observed (GOT, PGM, SDH and PGI). Individuals were then sorted and those of identical genotype were grouped (fig.2). Thus, 27(93.3%) of the 29 cultivars were identified uniquely. GOI' genotype A6 was unique to the cv. Hlawi originated from Iraq. Surprisingly, cultivars ‘Boufeggous’ and ‘Fhal kseba’ show a similar multilocus genotype in spite of their different origin and their distinctiveness regarding several morphological and fruiting traits [2].

DISCUSSION AND CONCLUSIONS

In order to evaluate date-palm genetic stock in Tunisia, we have initiated this work using four enzyme systems and 29 cultivars collected throughout their areas of distribution in the south of the country. Results of electrophoretic analysis revealed five polymorphic loci at the four. enzyme system studied, with twelve different alleles : 3 for each Pgm and

Sdh loci and 2 for. each Got-1, Got-2 and Pgi-2 loci. These results are partly in agreement with those described by Torres and Tisserat [6] and Bennaceur et al. [10]. However, our procedures revealed additional polymorphism in I'GM locus and further precision concerning the GOT isozymes structure in date-palm [11].

On the other hand, isozyme polymorphism revealed, allowed us to establish a practical key for cultivars identification. From the isozyme genotypes of GOT, PGM, SDH and PGI enzymes, we can accurately distinguish 27 of the 29 cultivars studied (93.3%) which represents a high percentage of discrimination.

The analysis of other enzyme markers or the combination between isozyme identification and the traditionally method based on fruit characteristics should allow us to distinguish the remaining cultivars.

Isozyme analysis can be considered as useful tool for cultivar identification since it is reliable, rapid and can provide identification at an early stage in the date-palm life cycle. Therefore, this technique can be used to identify vegetatively propagated offshoots and tissue culture originated seedlings. It may also used to identify the mixed population that is essential for breeding programs.

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Table 1. List of the date-palm cultivar analyzed. DG9 and T17 are two males.

	Region		
	Gabès	Nefzaoua	Djerid
Cultivar	Lemsi	Fermla	Ammari
	Rouchdi	Horra	Lagou
	Kenta	Deglet nour	Bser hlou
	Bouhattam	Fhal kseba	Ftimi
	Smiti	Rakli	Tazerzit noire
	Aguiwa	Sfiri	Tazerzit jaune
	Grin ghzal	Tofli	Kentichi
	Denga	Kechdou ahmar	Hlawi
			Boufeggous
			Deglet bey
			Ghars
			DG9
			T17

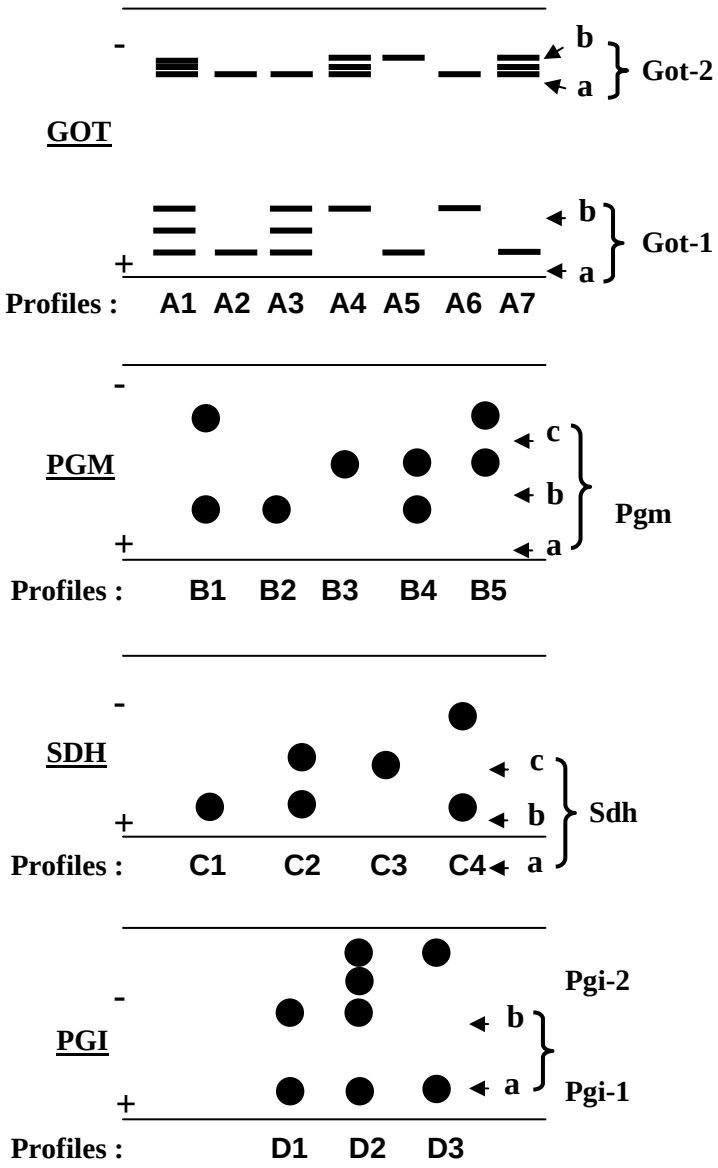

Figure 1. zymograms of glutamate oxaloacetate transaminase (GOT), phosphoglucomutase (PGM), shikimate dehydrogenase (SDH) and phosphoglucoisomerase (PGI) revealed in leaf extracts of the date-palm cultivars analyzed. Five polymorphic loci were resolved namely: Got-1, Got-2, Pgm, Sdh and Pgi-2. The direction of migration is toward the bottom of figure. Letters a, b and c refer to alleles designation. Arrows at the right of figure indicate the relative position of each allele.

Table 2. Multiloci genotypes of the date-palm cultivars analyzed.

Cultivar	Sdh	Pgm	Pgi-2	Got-1/Got-2
Lemsi	aa	aa	aa	ab/ab
Rouchdi	aa	ab	ab	ab/aa
Kenta	ab	ac	ab	aa/aa
Bouhattam	ab	aa	aa	ab/aa
Smiti	ac	ac	aa	aa/aa
Aguiwa	ac	ab	ab	ab/aa
Grin ghzal	ac	ab	bb	ab/ab
Denga	ab	ac	aa	ab/aa
Fermla	ab	aa	ab	ab/aa
Horra	aa	bc	ab	aa/ab
Deglat nour	aa	ab	ab	aa/ab
Fhal kseba	ab	ab	ab	ab/ab
Rakli	aa	ab	ab	ab/ab
Sfiri	aa	bb	ab	ab/aa
Tofli	ab	ab	aa	aa/aa
Kechdou ahmar	aa	ab	aa	ab/ab
Ammari	ab	ac	bb	aa/aa
Lagou	ab	aa	bb	aa/ab
Bser hlou	ac	aa	ab	ab/aa
Ftimi	aa	bb	ab	aa/bb
Tazerzit noire	bb	ab	aa	bb/ab
Tazerzit jaune	ab	ac	ab	ab/aa
Kentichi	bb	ab	ab	bb/ab
Hlawi	bb	bb	bb	bb/aa
Boufeggous	ab	ab	ab	ab/ab
Deglat bey	aa	bc	ab	aa/aa
Ghars	aa	aa	ab	ab/aa
DG 9	aa	bc	aa	aa/bb
T 17	ab	ac	bb	aa/ab

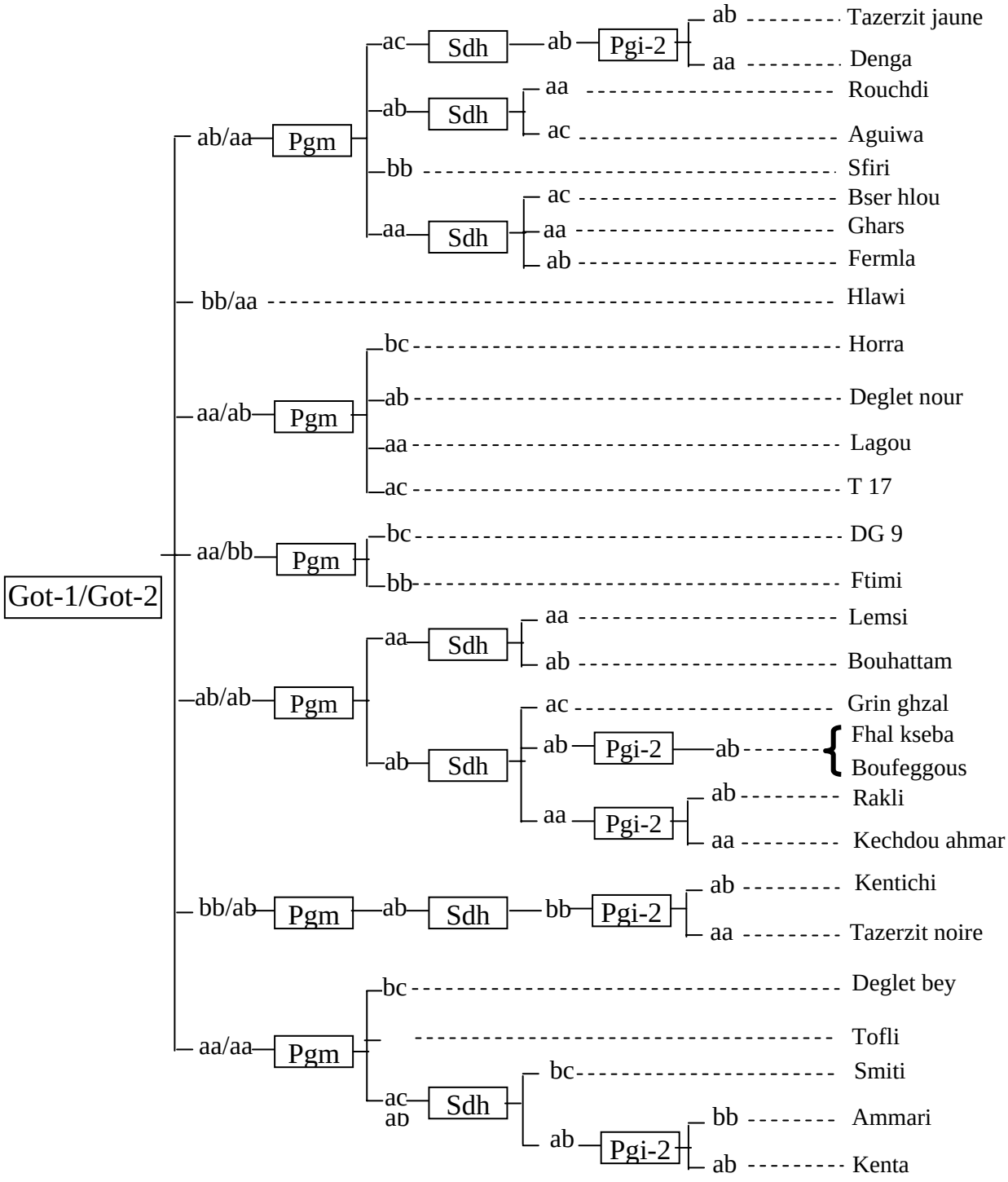

Figure 2. Identification key of the 29 date-palm cultivars based on their 5-locus genotypes.