GENETIC VARIATION IN *PHOENIX CANARIENSIS* AND *P. DACTYLIFERA* (ARECACEAE) POPULATIONS OF GRAN CANARIA USING ISOZYME ELECTROPHORESIS

By P.A. SOSA [1], N. BOUZA [1], M.A. CABRERA-PÉREZ [2] & A. LUQUE [1]

With 1 figure and 2 tables

ABSTRACT. The paper describes the preliminary results obtained using electrophoresis techniques to characterise genetically two *Phoenix* species present in the Canary Islands. Electrophoretic techniques were used to asses the degree of genetic variation and genetic differentiation among the Canarian palm (*Phoenix canariensis*) and the date palm (*Phoenix dactylifera*) from Gran Canaria (Canary Islands). 24 putative alleles (from 12 loci) were interpreted from the isozyme banding patterns. The genetic variation, measured as polymorphism, number mean alleles per locus and heterozygosity, was higher for *Phoenix canariensis* populations. Three loci (SOD-2, EST-2 and PGM-1) were exclusive of the canarian palm populations, indicating that they can be used as diagnostic loci to differentiate both species.

INTRODUCTION

The Canarian palm (*Phoenix canariensis* Chabaud) is an endemic species distributed in the Canary Islands (HANSEN & SUNDING 1993). The date palm (*Phoenix dactylifera* L.) a widespread species has been recently introduced in the archipelago, probably from North Africa (KUNKEL 1992). At this time *P. dactylifera* is found mainly in the eastern islands (HANSEN & SUNDING 1993). Both species are very similar morphologically, and probably very closely related, although the canarian palm has a bushier crown (more leaves), darker green leaves, a thicker trunk, with no off-shoots and smaller fruits, called Tamaras.

Hybridisation between both species has been reported (KUNKEL & KUNKEL 1974), this could pose problems with regard to the conservation of the Canarian genotype.

1 Departamento de Biologia. Campus Universitario de Tafira. Universidad de Las Palmas de Gran Canaria. 35017 Las Palmas. Canary Islands. Spain.

2 Vivero Forestal de Tafira. Viceconsejería de Medio Ambiente. Gobierno Autónomo de Canarias. 35017 Las Palmas. Canary Islands. Spain.

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Isozyme gel electrophoresis is a well established and widely used technique for the study of genetic variation at the molecular level; its limitations and biases, as well as applications and advantages, have been described at length (SOLTIS & SOLTIS 1989). Electrophoresis data may be helpful in defining species because isozymes are relatively unaffected by the environment. Thus they may be useful with plants that are phenotypically plastic and difficult to identify on the basis of morphology. *Phoenix canariensis* and *P. dactylifera* are indistinguishable when they are young, and therefore, isozymes could be a powerful method as taxonomic markers to distinguish them.

Material and Methods

Sampling.

Two populations of *Phoenix canariensis* (CHABAUD) and one population from *P. dactylifera* (L.) from Gran Canaria were examined. Sampling sites are shown in Fig. 1. Pieces of healthy leaflet from adult individuals (between 22 to 34) were transported to the laboratory in an ice box. In the laboratory the samples were carefully cleaned cut and stored at-80 °C. They were ground with mortar and pestle in liquid nitrogen until the sample was reduced a fine powder, before the addition (1:3 w/v) of the grinding buffer (0.1 M Tris pH 7.4 with 0.014 M Ascorbic Acid, 2mM EDTA, 1% Tween-80, 0.1 M 2-Mercaptoethanol, 0.013 M ClK and 3% PVPP). The tissue-buffer mix was allowed to thaw and the crude extracts were absorbed onto paper wicks (Whatman N° 3; 1 x 0.3 cm) which were inserted vertically into the starch gel. Individuals from different populations and species were run together on the same gel to determine whether corresponding electromorphs has similar mobility.

Fig. 1 - Map of Gran Canaria (Canary Islands) showing location of sampling sites. 1: Tafira Alta; 2: Jinamar; 3: San Lorenzo.

Electrophoresis

The methodology for electrophoresis followed the methods reported previously (SOSA & GARCÍA-REINA 1992). Seven enzymatic systems were studied by starch gel electrophoresis: Acid phosphatase (ACP); Esterase (EST); Glucose-6-Phosphate dehydrogenase (G6PDH); Malic Enzyme (Me); Phosphoglucose Isomerase (PGI); Phosphoglucose Mutase (PGM) and Superoxide Dismutase (SOD). Staining mixtures for G6PDH consisted of 100 mg Glucose 6 phosphate, 10 mg NADP, 100 mg $MgCl_2$, 3 ml 1% MTT, 0.5 ml 1% PMS, 10 ml 1 M Tris-HCl pH 8.0 and 90 ml H_2O. All systems migrated anodally.

Data analyses

Allelic frequencies were inferred directly from isozyme phenotypes and knowledge of protein quaternary structures. The proportion of polymorphic loci (P), mean number of alleles per locus (A/L) and heterozygosity (H) were calculated to determinate the amount of genetic variability for each population.

Results

Other enzymatic systems were tested: Malate dehydrogenase (MDH), Glutamate oxalacetate transaminase (GOT), NADH-Diaphorase (DIA), isocitrate dehydrogenase (IDH) and alkaline phosphatase (ALKPH) but they could not be used because of poor resolution and/or unreliable results.

An important loss of enzyme activity was detected when the samples were stored at -18°C. Only those leaves stored at -80°C kept their activity for a long time.

A total of 24 alleles for 12 loci was inferred from the electromorph patterns (Table 1). Four loci (G6PDH, Me, PGM-3 and PGI-1) were monomorphic for the same allele in all populations from both species. However, differences could be seen in other loci and alleles. ACP-1 and PGM-2 were polymorphic only for *Phoenix canariensis* populations, whereas were fixed for *P. dactylifera*. Three loci (SOD-2, EST-2 and PGM-1) were not detected in *Phoenix dactylifera* population (Table 1).

Table 2 summarises the genetic variability in *Phoenix canariensis* and *P. dactylifera* populations. Twelve loci were identified for the canarian palms populations, which seven were polymorphic. The Jinamar population displayed a higher genetic variability than Tafira Alta population for number mean alleles per locus and heterozygosity. Same percentage of polymorphic loci was found for both *Phoenix canariensis* populations (58.3%). *P. dactylifera* population exhibited less genetic variability than P. *canariensis* for all the parameters used (Table 2).

Discussion

The present paper describes the preliminary results obtained using electrophoresis

techniques to characterise and identify genetically two *Phoenix* species present in the Canary Islands. These techniques provide the best estimate of genetic variation and are generally considered to be most meaningful at and below congeneric species level (KEPHART 1990). Thus, electrophoretic differentiation can provide an important alternative measure of evolutionary divergence from that of morphological differentiation.

TABLE 1 - Gene frequencies of *Phoenix canariensis* and *P. dactylifera* detected in Gran Canaria populations

Especies	*Phoenix canariensis*		*P. dactylifera*
Population	Tafira Alta	Jinamar	San Lorenzo
Locus			
ACP-1a	0.93	0,67	1
ACP-1b	0,07	0,33	0
EST-1a	0,38	0,41	0
EST-1b	0,62	0,59	0,25
EST-1c	0	0	0,75
EST-2a	0,81	0,95	0
EST-2b	0,13	0,05	0
EST-2c	0,06	0	0
G6PDH	1	1	1
Me	1	1	1
PGI-1	1	1	1
PGI-2a	0	0,17	0,33
PGI-2b	0,5	0,33	0,13
PGI-2c	0	0,17	0,33
PGI-2d	0,5	0,33	0,21
PGM-1a	0,3	0,56	0
PGM-1b	0,7	0,33	0
PGM-1c	0	0,11	0
PGM-2a	0,3	0,22	1
PGM-2b	0,7	0,78	0
PGM-3	1	1	1
SOD-1a	0,5	0,5	0,5
SOD-1b	0,5	0,5	0,5
SOD-2	1	1	0

Although one cannot draw conclusions with certainty until more individuals and populations of both species have been examined, the presents results are in agreement with

previous studies (TORRES & TISSERAT 1980). Thus, the level of genetic variation found in both species is well within the range reported for most plants (HAMRICK 1989). The proportion of polymorphic loci found in *Phoenix* populations ranged from 0.58 (for the *P. canariensis* populations) to 0.33 (for the *P. dactylifera* population). It is generally considered that genetic variation is lower in restricted species than in their widespread congeners, although many endemic plant species maintain relatively high levels of genetic variation (SOLTIS & SOLTIS 1989). This seems to be the case for *P. canariensis* which have a higher genetic variation than *P. dactylifera* which is a widespread species.

TABLE 2 - Genetic variation of *Phoenix canariensis* and *P. dactylifera* populations from Gran Canaria. N: Number of loci; NPL: Number of polymorphic loci; P: Polymorphism; A/L: Number mean alleles per locus; H: Heterozygosity

Species	Population	N	NPL	P	A/L	H
P. canariensis	Tafira Alta	12	7	58.3	1.67	0.230
	Jinamar	12	7	58.3	1.83	0.263
P. dactylifera	S. Lorenzo	9	3	33.3	1.55	0.177

Each species of *Phoenix* seems to show a distinctive pattern of enzyme bands. SOD-2, EST-2 and PGM-1 loci appear only in the Canarian palm populations (Table 2), indicating that they can be considered as diagnostic loci between both species. If this tendency is confirmed, the isozyme techniques can be used as molecular markers in the formulation of *Phoenix canariensis* management strategies.

Although further studies are necessary, including the characterisation of hybrids between both species, we hope that this study further emphasises the potential of electrophoretic techniques for *Phoenix* systematic and evolution.

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