Malate dehydrogenase isozyme patterns in cladophylls of a *Opuntia ficus-indica* Mill. (Cactaceae) clonal population

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ABSTRACT. Malate dehydrogenase (MDH) isozymes were used as biochemical markers to discriminate and cluster cladophylls of plants of one clonal population of the prickly pear, *Opuntia ficus-indica* (Cactaceae). The isozyme electrophoretic patterns obtained with MDH provided 8 isozymes and 5 different electrophoretic phenotypes. Similarity in cladophylls was estimated using Jaccard's coefficient. This clonal population studied was founded by only one propagule, and after 50 years, it is likely to have been formed by asexual and sexual propagules. Since that differential expression of MDH isozymes could play a significant role in overall plant cell metabolism, we suggest that the cladophylls of prickly pear that were clustered together showing identity or higher similarity are specially a suitable source for industrial procedures of industrial extraction of commercial interest compounds because a same extraction protocols can be most quickly and easily standardized using genetically uniform materials. Electrophoretic patterns of MDH isozymes can be used as an effective tool for previously determine the genetic similarity in the cladophylls of *O. ficus-indica* plants.

Key words: isozymes, malate dehydrogenase, prickly pear, polymorphism, genetic similarity.

RESUMO. Padrões de isozimas de malato desidrogenase em população clonal nos cladófilos de *Opuntia ficus-indica* Mill (Cactaceae). Isozimas de malato desidrogenase (MDH) foram usadas como marcadores moleculares para discriminar e agrupar cladófilos de plantas de uma população clonal de cactus da espécie *Opuntia ficus-indica* (Cactaceae), conhecida como palma. O padrão eletroforético obtido revelou 8 isozimas MDH e 5 fenótipos eletroforéticos diferentes. A similaridade entre os cladófilos foi estimada usando o coeficiente de similaridade de Jaccard. Essa população clonal estudada foi fundada por somente um propágulo e, após 50 anos, parece ser formada por propágulos assexuais e sexuais. Uma vez que a expressão diferencial de isozimas MDH pode ter um papel significante no metabolismo das células da planta, sugerimos que os cladófilos de palma que foram agrupados com os mais altos valores de similaridade são os mais adequados para serem utilizados em procedimentos de extração industrial de compostos de interesse comercial, porque um mesmo protocolo de extração pode ser mais rapidamente e facilmente padronizado quando se utiliza material geneticamente uniforme. O padrão eletroforético das isozimas MDH pode ser usado como uma ferramenta efetiva para uma análise prévia da similaridade genética entre os cladófilos das plantas de *O. ficus-indica*.

Palavras-chave: isozimas, malato desidrogenase, palma, polimorfismo, similaridade genética.

Introduction

Among the major applications of isozymes, they are indicated as the most frequently applied technique to plant genetic resources management (Esquinas, 1981; Simpson and Withers, 1986; Bretting and Widrlechner, 1995). The malate dehydrogenase isozymes, particularly, has been reported as effective tool for characterizing species of *Opuntia* (prickly pear cactus), using both cladodes and pollen tissues (Chessa *et al.*, 1997). Despite lower genetic variability detected for Italian cultivars and ecotypes of *O. ficus-indica* (Uzun, 1997) the malate dehydrogenase isozymes revealed considerable variability and were specially used for the genotype identification of *Opuntia* species and varieties from Italian germplasm (Chessa *et al*., 1997).

Opuntia ficus-indica is one of the cactus species extensively cultivated for its fruits and cladodes (Russel and Felker, 1987; Nobel, 1988). The cladodes and fruits are well accepted by the animals and have high digestibility (Boza *et al.*, 1995). Recent

studies have reported the prickly pear juice characteristics for industrial applications (Gurrieri *et al*., 2000; Turker *et al*., 2001). The enzymes obtained from plant extract and fruit also have industrial interest (Teixeira *et al.*, 2000).

The objective of the present study is to analyse the electrophoretic patterns of malate dehydrogenase isozymes to verify the genetic similarity in plants of one population of *O. ficus-indica* which was obtained from only one cladode by vegetative propagation and have been naturally maintained for 50 years in the rural region of *Marumbi*, a district of *Jandaia do Sul*, northwestern region of the State of *Paraná* (PR), Brazil (BR). In this population that have been conserved for 50 years the ramets are spatially aggregated (in the form of a only large clump) and it's possible to observe several plants growing from axilary buds in old cladodes that fell on the ground. This *O. ficus-indica* population have been considered as a clonal population and the cladodes of these plants are utilized as source of mucilage extraction for industrial applications.

Material and methods

Samples of 27 young cladophylls (or pads) measuring 6-8 cm length were randomly collected from the *O. ficus-indica* cactus plants; the distance from the samples of cladophylls ranged from 2-3 m. Electrophoretic evaluations were carried out on pieces (6-10 mm) of young cladophylls. The cladophyll pieces were individually homogenized with a glass rod in an microcentrifuge tube (Eppendorf) using 60 µl of 1.0 M phosphate buffer, pH 7.0, containing 5% PVP-40, 0.01 M DTT (dithiothreitol), 10 mM sodium metabisulfite, 50 mM ascorbic acid, 1.0 mM EDTA, and 0.5% β-mercaptoethanol solution (Resende *et al.*, 2000). After homogenization, the samples were centrifuged at 45.000 x g for 30 minutes at 4 °C in a Sorval 3K-30 centrifuge.

The malate dehydrogenase isozymes (MDH; EC 1.1.1.37) were analyzed using starch gels (14%) electrophoresis prepared in 0.0155 M Tris, 0.0043 M citric acid, pH 7.0, and the electrode buffer consisted of 0.155 M Tris and 0.043 M citric acid, pH 7.0 (Machado *et al.*, 1993). Electrophoresis was carried out at 4 °C for 14-15 h, at 2.5 V/cm of gel. MDH isozymes were visualized with a staining solution containing 15 ml 0.1 M Tris-HCl buffer, pH 8.6, 200 mg malic acid, 1 ml NAD (β-nicotinamide adenine dinucleotide; 10 mg/ml), 0.5 ml MTT (thiazolyl blue; 5 mg/ml), and 0.5 ml PMS (phenazine methosulfate; 5 mg/ml), as reported for MDH isozymes of the *Cereus peruvianus* cactus species (Machado *et al.*, 1993).

Data were analyzed by comparing the MDH patterns on the basis of the presence or absence of each isozyme. The similarity between cladophylls was calculated using Jaccard's coefficient, and UPGMA cluster analysis was performed using the NTSYS-pc sofware (Rohlf, 1989).

Results

The MDH enzyme system revealed four anodal regions with 8 isozymes showing distinct mobility in cladophylls of *O. ficus-indica*. The fastest anodal region can be considered to be the cytoplasmatic MDH isozyme in aggrement with data previously reported for *O. ficus-indica* (Mukerji and Ting, 1969) and for most vegetables (Newton, 1983). The MDH-2, MDH-7, and MDH-8 isozymes were observed in all *O. ficus-indica* cladophylls while the MDH-1, MDH-3, MDH-4, MDH-5, and MDH-6 isozymes showed differential pattern of expression (Figure 1A). The slower region (MDH-1, MDH-2, and MDH-3 isozymes) seems to be the MDH found in microbodies (peroxisomes) and MDH-4, MDH-5, and MDH-6 isozymes (the region of intermediate activity) should be of mitochondrial origin (Rees, 1990). The mitochondrial MDH isozyme phenotype formed by three regularly spaced bands, with the intermediate band frequently being more intensely stained than the outer bands, seems to be a typical pattern that would be expected for random binding of peptide chains produced by two distinct genes coding for dimeric enzymes (Richardson *et al.*, 1986; Pasteur *et al.*, 1988). In *O. ficus-indica*, the differential expression of mitochondrial and microbodies MDH isozymes yielded 5 different MDH electrophoretic phenotypes (Figure 1B). The proportion of cladophylls showing mitochondrial MDH isozymes absent was 25.9% (11.1 and 14.8% showing phenotypes A and B) and the proportion of cladophylls showing C, D, and E phenotypes was 37.1%, 22.2%, and 14.8%, respectively (Figure 1B).

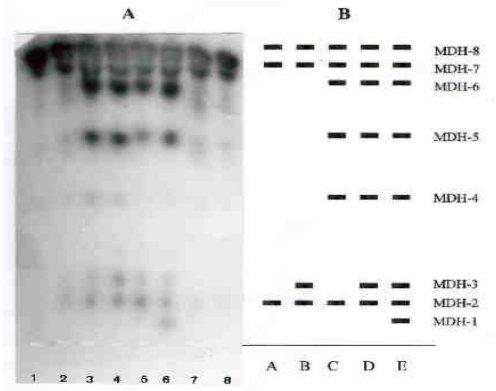

Figure 1. Malate dehydrogenase isozyme phenotypes of *Opuntia ficus-indica* on starch gel, pH 7.0 observed in young cladophylls of the Marumbi population plants (A: samples 1-8). In B the five different MDH isozyme phenotypes detected in Marumbi population

The relationship among the 27 cladophylls of the *O. ficus-indica* (M1-M27) was estimated from MDH electrophoretic phenotypes using Jaccard's coefficient of similarity (Table 1). A dendrogram produced by cluster analysis showed that the most of cladophylls (75%) were clustered in 5 groups showing similarity 80.3% (Figure 2).

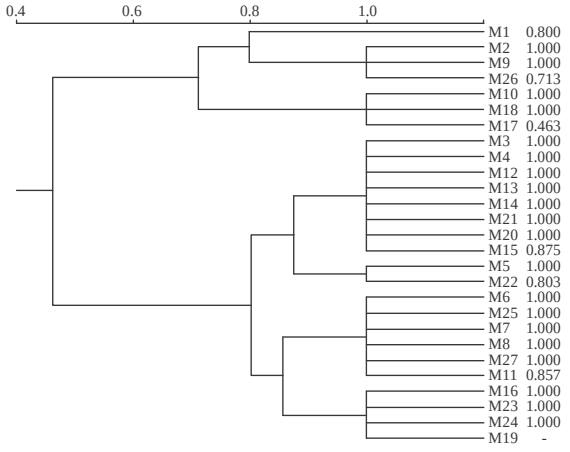

Figure 2. Dendrogram representing the relationship between the 27 plants of the Marumbi population (M1-M27) based on UPMGA cluster analysis of the malate dehydrogenase isozymes using Jaccard´s similarity coefficient.

Discussion

Although the *O. ficus-indica* cladophylls have showed similar appearance and measure, we have found 5 different banding patterns for MDH isozymes. The Marumbi population studied was founded by only one propagule and after 50 years its likely to have been formed by propagules showing different MDH isozyme patterns. According to Parker (1979), if mutation, migration, selection and sexual recruitment are absent in a clonal population, the genotype fixation should be expected occur.

Despite the large number of seeds produced, very few seedlings arising from sexual reproduction were observed in many other *Opuntia* species (Manjuano *et al.*, 1996), but in the Marumbi population of *O. ficus-indica,* that also produces a large quantity of fruits, the outcrossing among individuals seems to be occurring. The seeds showed a low germination rate (1%; unpublished results) but if thousands of seeds are disseminated yearly, then, some viable seedlings would be expected to arise in this population. *O. ficus-indica* seems regularly produce sexual progeny as a reproductive alternative to vegetative reproduction. Thus, it is possible that the individual variations observed in the clonal *O. ficus-indica* population of Marumbi could be attributed to outcrossing, gene flow recombination, or mutation. Gene flow recombination, outcrossing, mutation and polyphyletic origins have been reported as sources of genetic variation in clonal populations (Ellstrand and Roose, 1987). If a small amount of gene flow and/or mutation adds new clones to a population from time to time, clonal variation may be maintained. Gene flow occurring among adjacent populations with different introduction histories or founded by different propagules may be of major importance for maintaining the MDH variability in *O. ficus-indica.*

Table 1. Jaccard's coefficient of similarity of the Marumbi population of *Opuntia ficus-indica* (M1-M27). The coefficients were calculated from malate dehydrogenase isozymes data for 5 polymorphic bands

M1	1.000
M2	0.8001.000
M3	0.6250.5001.000
M4	0.6250.5001.0001.000
M5	0.5000.3750.8750.8751.000
M6	0.3750.4290.7500.7500.8571.000
M7	0.3750.4290.7500.7500.8571.0001.000
M8	0.3750.4290.7500.7500.8571.0001.0001.000
M9	0.8001.0000.5000.5000.3750.4290.4290.4291.000
M10	0.6000.7500.3750.3750.4290.5000.5000.5000.7501.000
M11	0.3750.4290.7500.7500.8571.0001.0001.0000.4290.5001.000
M12	0.6250.5001.0001.0000.8750.7500.7500.7500.5000.3750.7501.000
M13	0.6250.5001.0001.0000.8750.7500.7500.7500.5000.3750.7501.0001.000
M14	0.6250.5001.0001.0000.8750.7500.7500.7500.5000.3750.7501.0001.0001.000
M15	0.6250.5001.0001.0000.8750.7500.7500.7500.5000.3750.7501.0001.0001.0001.000
M16	0.5000.5710.8750.8750.7500.8570.8570.8570.5710.4290.8570.8750.8750.8750.8751.000
M17	0.6000.7500.3750.3750.4290.5000.5000.5000.7501.0000.5000.3750.3750.3750.3750.4291.000
M18	0.6000.7500.3750.3750.4290.5000.5000.5000.7501.0000.5000.3750.3750.3750.3750.4291.0001.000
M19	0.5000.5710.8750.8750.7500.8570.8570.8570.5710.4290.8570.8750.8750.8750.8751.0000.4290.4291.000
M20	0.6250.5001.0001.0000.8750.7500.7500.7500.5000.3750.7501.0001.0001.0001.0000.8750.3750.3750.8751.000
M21	0.6250.5001.0001.0000.8750.7500.7500.7500.5000.3750.7501.0001.0001.0001.0000.8750.3750.3750.8751.0001.000
M22	0.5000.3750.8750.8751.0000.8570.8570.8570.3750.4290.8570.8750.8750.8750.8750.7500.4290.4290.7500.8750.8751.000
M23	0.5000.5710.8750.8750.7500.8570.8570.8570.5710.4290.8570.8750.8750.8750.8751.0000.4290.4291.0000.8750.8750.7501.000
M24	0.5000.5710.8750.8750.7500.8570.8570.8570.5710.4290.8570.8750.8750.8750.8751.0000.4290.4291.0000.8750.8750.7501.0001.000
M25	0.3750.4290.7500.7500.8571.0001.0001.0000.4290.5001.0000.7500.7500.7500.7500.8750.5000.5000.8570.7500.7500.8570.8570.8571.000
M26	0.8001.0000.5000.5000.3750.4290.4290.4291.0000.7500.4290.5000.5000.5000.5000.5710.7500.7500.5710.5000.5000.3750.5710.5710.429 1.000
M27	0.3750.4290.7500.7500.8571.0001.0001.0000.4290.5001.0000.7500.7500.7500.7500.8570.5000.5000.8570.7500.7500.8570.8570.8571.000 0.429 1.000
	M1 M2 M3 M4 M5 M6 M7 M8 M9 M10 M11 M12 M13 M14 M15 M16 M17 M18 M19 M20 M21 M22 M23 M24 M25 M26 M27

The individual variations observed in *O. ficus-indica* cladophylls could also be attributed to somatic mutations occurring in the different cladodes of this species. Mutation in the somatic tissues was proposed to justify the change in expression of parthenocarpy in *O. ficus-indica*. Seeded fruits developed only from one branch that bore flowers with small ovules, whereas on other branches of the same plants, flowers with large ovules and seedless fruits developed (Weiss *et al.*, 1993).

The different MDH isozyme electrophoretic phenotypes in the *O. ficus-indica* cladophylls can be considered to reflect the differential expression of genetic information and regulation of differential activity of the mitochondrial and microbodies MDH genes. Mitochondrial MDH isozyme is very closely associated with the citric acid cycle in plants, is confined to the mitochondrial matrix, and catalyzes the oxidative decarboxylation of malate *in vivo*. The citric acid is the dominant component of carbon metabolism in the mitochondria of higher plants and the citric acid cycle provides reducing equivalents to the electron chain for ATP synthesis and also provides numerous substrates for biosynthetic reactions in the cytoplasm (Douce and Neuburger, 1990). Thus, mitochondrial MDH isozymes (present or absent in cladophylls of *O. ficus-indica*) could play a significant role in overall plant cell metabolism.

The present study shows a differential expression of MDH isozymes in clonal population of *O. ficus-indica* whose cladophylls are utilized as source of mucilage extraction for industrial applications. Since that differential expression of MDH isozymes could play a significant role in overall plant cell metabolism we suggest that the cladophylls of prickly pear that were clustered together showing identity or high similarity (87.5 and 85.7% similarity) are specially a suitable source for industrial procedures of extraction of the interest compounds because a same extraction protocols can be most quickly and easily standardized using genetically uniform materials. Electrophoretic patterns of MDH isozymes can be used as an effective tool for previously determine the genetic similarity between the cladophylls of *O. ficus-indica* plants. On the other hand, the cladophylls of prickly pears showing a distant relationship are recommended for implementation of conservation and breeding programs through vegetative multiplication and seed collection in genebanks of the different genotypes. Populations with high levels of polymorphic isozymes should be the focal point of conservation biologists for capturing much of the genetic variation of the species.

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