II. GENÉTICA E MELHORAMENTO DE PLANTAS

INHERITANCE OF MALATE DEHYDROGENASE IN WILD PEPPER [1]

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ABSTRACT

Leaf extracts from wild pepper (*Capsicum flexuosum* Sendt) were analysed for the presence of malate dehydrogenase (E.C. 1.1.1.37; MDH) isozymes using starch gel electrophoresis. Seven phenotypes for MDH isozymes were observed in the genitors. Genetic analysis in F_1 progenies revealed five loci coding for MDH. Isozyme banding patterns of hybrids indicated that MDH-3 and MDH-4 genes code for monomeric enzymes, while MDH-5 for a dimeric isoform. In MDH-2 loci, one particular F_1 progeny showed a significant deviation from the expected isozyme pattern. It is possible that other genes are controlling the expression of MDH-2 in pepper. Also, there are two alleles coding for MDH-2 isozyme. On the other hand, MDH-1 was monomorphic for all genotypes used in the experiment.

Index terms: wild pepper, *Capsicum flexuosum*, malate dehydrogenase, inheritance.

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RESUMO

HERANÇA DA MALATO DESIDROGENASE EM PIMENTA-SILVESTRE

Extratos de folhas de pimenta silvestre (*Capsicum flexuosum* Sendt) foram analisados para a presença do sistema isoenzimático malato desidrogenase (E.C. 1.1.1.37; MDH), usando a técnica eletroforese em gel de amido hidrolisado. Sete fenótipos de malato desidrogenase foram observados entre os genitores. As análises de segregação em progênies F_1 revelaram que cinco locos gênicos estavam envolvidos na codificação de MDH. Os padrões de bandeamento dos híbridos indicaram que os genes MDH-3 e MDH-4 codificavam para enzimas monoméricas, enquanto o MDH-5, para uma isoforma dimérica. Para o loco MDH-2, detectou-se desvio significativo para proporção de segregação esperada. Outros genes podem estar controlando a expressão de MDH-2 em pimenta. Como nos outros locos MDH, detectaram-se dois alelos codificando para MDH-2. Por outro lado, o MDH-1 foi monomórfico para todos os genótipos avaliados no experimento.

Termos de indexação: pimenta-silvestre, *Capsicum flexuosum*, malato desidrogenase, herança.

1. INTRODUCTION

The genus *Capsicum* is constituted by five domesticated species: *Capsicum annuum*, *C. baccatum*, *C. chinense*, *C. frutescens* and *C. pubescens*. In addition to them, there are approximately thirty known wild species in the same genus (Pickersgill, 1969; Eshbaugh, 1980). Belonging to this group of wild species is *C. flexuosum*, which has strong pungency due to high content of alkaloids in the fruits (Teixeira, 1995). This particular species is widely distributed in some countries of South America, especially in Argentina, Paraguay and Brazil (Casali & Couto, 1984). Despite the importance of the genus *Capsicum* as source of condiments and for potential medicinal uses, little is known about the genetic variability, in particular among the wild species (McLeod et al., 1979, 1983; Teixeira, 1996).

For years agronomic and morphological characteristics have been used to differenciate genetic diversity in plants. However, techniques like isozymes and DNA analysis are widely used in the investigation of genetic diversity and evolution of plants (Alfenas et al., 1991; Tanksley & Orton, 1983). Preliminary isozyme assays with malate dehydrogenase (E.C.1.1.1.37; MDH) showed that high polymorphism can be observed in *Capsicum*. Arulsekar et al. (1986) studying the genetics of malate dehydrogenase in peach observed complex pattern of isozymes, since isoforms were associated with mitochondria, microbodies or cytosol. This fact did allow complete separation of isoforms by electrophoresis; furthermore, the enzyme has catalytic activity as monomer, dimmer and tetrameric forms which may difficult a complete study of MDH inheritance in plants (Pasteur et al., 1988).

Multiplicity of enzymes is due to genetic causes by multiple loci and/or multiple alleles. In addition, structural also non-structural gene loci have been identified which determine post-transcriptional or post-translational modifications of isozymes (Breitenbach-Dorfer & Geburek, 1995). Such genetic alterations of isozymes have been reported for malate dehydrogenase in vascular plants, such as *Lycopersicon pimpinelifolium* (Rick et al., 1979), *Zea mays* (Goodmam et al., 1980) and *Sorghum* sp (Doebley et al., 1986). In the intraspecific *Capsicum annuum* crosses, 9% of the loci deviated from the expected ratios, compared to 22% in the interspecific backcrosses and 50% in the F_2 (Zamir & Tadmor, 1986).

This paper reports investigations of two generations of *C. flexuosum* plants for the study of the inheritance of malate dehydrogenase isozymes.

2. MATERIAL AND METHODS

Capsicum flexuosum, accession number LL-1952 from Banco de Germoplasma de Hortaliças da Universidade Federal de Viçosa (BGH/UFV) were used in crosses and MDH isozyme analysis. The chi-square test (χ^2) was used to test the results.

Young leaves of plants grown in greenhouse were homogenized in buffer containing 0,1 Tris-HCl buffer (pH), 40% PVP and 0,1% 2-mercaptoethanol. A sample of the extract was applied to a 12% slab hydrolyzed starch gel (Sigma) in Tris-citric acid buffer as described by Shaw and Prasad (1970). Gel was equilibrated for 30 min at 150 Volts before sample application and 300 Volts applied during electrophoretic running. The gel was revealed in the reaction buffer containing 15 mg NAD^+, 1 mg phenyl methyl sulfonate, 15 mg distilled water, 7.5 ml 0.5 M Tris-HCl (pH 7.1), 5 ml 0.01 M L-malate (pH 7.0) and 2.5 ml 0.1 M NaCN. The patterns of MDH bands were scored in the first 30 min of reaction.

3. RESULTS AND DISCUSSION

There were distinct patterns of MDH isozymes for the seven genitors of *Capsicum flexuosum* tested (Figure 1). Based on χ^2 test from progenies F_1, MDH isozymes showed codominance expression and five loci seemed to be involved on gene segregation (Table 1). In addition to that, three loci appeared as polymorphic with two alleles. The MDH-1 isozyme form did not show variation among the lines of *Capsicum flexuosum* (Figure 1), which it might be classified as monomorphic locus. This fast isoform may represent the MDH isozyme found in mitochondria and it is well conserved throughout of several plant species as reported by Tanksley & Orton (1983).

Crosses between strains G (heterozygous) and C (homozygous) showed that MDH-5 may be controlled by a single gene (Table 1). This gene seems to

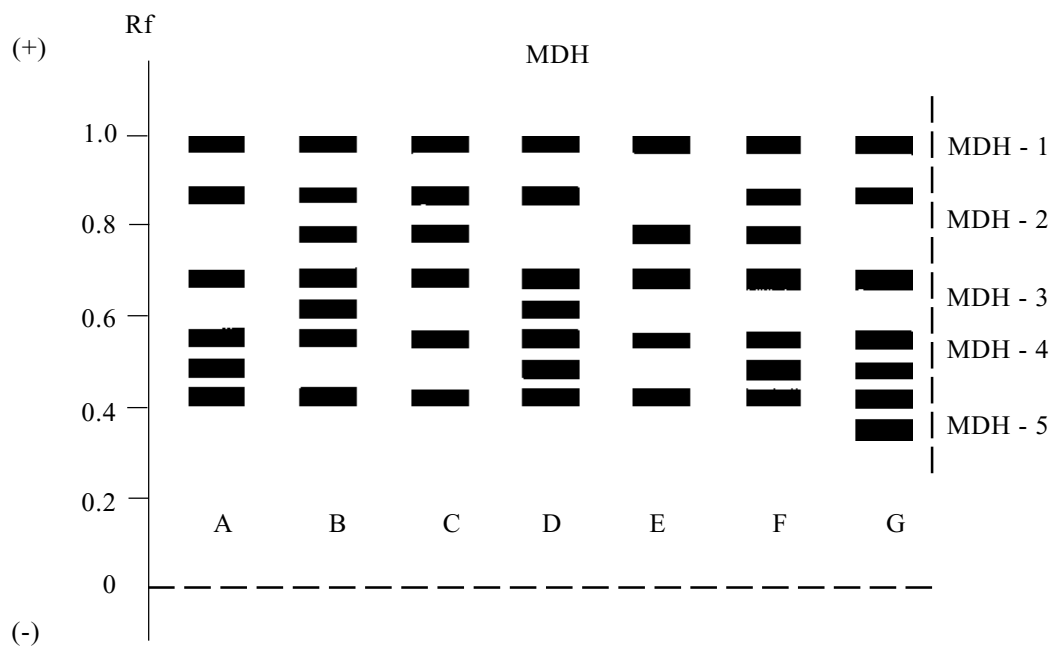

Figure 1. Zymogram pattern (A to G) for malate dehydrogenase isozymes in wild pepper

code for a MDH isozyme of tetrameric structure. However, the banding pattern observed for MDH-5 may indicate different polymeric subunits of protein structure, representing the dimeric isoform (Figure 1). Similar conclusions were obtained by Arulsekar et al. (1986) studying the segregation of MDH in 290 cultivars of peach. Hock (1973) observed that malate dehydrogenase located in microbodies had slower migration in gels when compared to those located in other subcellular structures. This fact suggests that MDH-5 codes for isozymes which are confined in cell microbodies.

The deviation observed for the MDH-2 locus by the χ^2 test showed that the proportion of segregation was not random (Table 1). It is possible that the expression of MDH-2 is modified by other genes.

Table 1. Chi-square analysis for the inheritance of MDH in wild pepper

Loci	Cross type	Progeny			Deviation		Heterogeneity	
					χ^2(df)[1]	P	χ^2(df)	P
MDH-1		aa	ab	bb				
	aa X aa	500	-	-	-	-	-	-
MDH-2		aa	ab	bb				
	aa X aa	23	4	-	-	-	-	-
	aa X bb	-	176	4	-	-	-	-
	ab X bb	-	73	51	3,903(1)	0,01-0,05	0,359(3)	0,80-0,90
	ab X ab	23	55	20	1,653(2)	0,30-0,50	1,410(3)	0,70-0,80
	aa X ab	24	47	-	3,725 (1)	0,05-0,10	7,831(4)	0,01-0,05
MDH-3		aa	ab	bb				
	aa X aa	172	3	-	-	-	-	-
	aa X ab	135	145	-	0,357(1)	0,50-0,70	1,321(8)	> 0,95
	ab X ab	10	24	11	0,055(2)	> 0,95	-	-
MDH-4		aa	ab	bb				
	aa X aa	138	-	-	-	-	-	-
	ab X ab	10	25	11	0,392(2)	0,80-0,90	0,291(1)	0,50-0,70
	ab X aa	160	156	-	0,051(1)	0,80-0,90	3,050(10)	> 0,95
MDH-5		aa	ab	bb				
	aa X aa	457	-	-	-	-	-	-
	aa X ab	22	21	-	0,023(1)	0,80-0,90	1,920(3)	0,50-0,80

[1] df: degrees of freedom.

The data, showed that there is homogeneity among the analyzed segregations for MDH-2, as confirmed by the acceptance of null hypothesis. There are various causes for the non-Mendelian segregation, which may be produced by factors affecting all steps from chromosome disjunction through seed maturation.

Several studies have demonstrated that the distortion of segregation for isozyme and other molecular markers may be produced by lethal genes, or other linked loci, which affect the viability of the markers (Zamir & Tadmor, 1986; Bradshaw & Stettler, 1994). Certation or competition among pollen grains (Hornaza & Herrero, 1992), self-incompatibility mechanisms (Savolainen et al., 1992), or presence of segregation distorters (Lyttle, 1991) may account for other mechanisms involved in distortion of segregation.

Malate dehydrogenase activity for bands MDH-3 and MDH-4 seems to comprise two distinct genes, which give as final products two different monomeric enzymes or alternatively one monomeric and another superposed dimer. This supposition was based on the distribution of MDH loci for progenies (Table 1). But, the presence of a dimeric isozyme may not exist, since only a faint band for the dimer was present in heterozygous plants. This supposition was confirmed by the χ^2 at P = 0.05 (Table 1). Thus MDH-3 and MDH-4 seems to code for two monomers as final product.

Previous works have shown that malate dehydrogenase for *Capsicum* genus are coded by only two loci (McLeod et al., 1979, 1983), however it was not included any *Capsicum flexuosum* in those studies. The most feasible hypothesis for the existence of five MDH loci in *C. flexuosum* may resist in gene duplication occurred during evolution or due to pos translational changes in the polypeptides composition. Further studies are required in order to establish the subcellular location of MDH isozymes as well as for the control of their expression.

4. CONCLUSIONS

1. There are five loci coding for MDH in *C. flexuosum*.

2. The loci MDH-1 can be considered as monomorphic.

3. The loci MDH-2 showed distortion from Mendelian segregation.

4. Loci MDH-3 and MDH-4 code for a monomeric isoform.

5. Loci MDH-5 code for isoform that form a dimer isozyme.

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