

A study of biochemical markers of Kale (*Brassica oleracea* var. *acephala*) clones resistant to aphids (*Brevicoryne brassicae*)

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ABSTRACT

Seven kale clones (*Brassica oleracea* var. *acephala*), assessed for resistance to aphids (*Brevicoryne brassicae*) by Paula *et al.* (*An. Soc. Entomol. Bras.* 24: 99-104, 1995) underwent isozyme analysis using Esterase (EST), Acid Phosphatase (ACP), Glutamate dehydrogenase (GDH), Glutamate Oxaloacetate Transaminase (GOT), Leucine Aminopeptidase (LAP), Malate Dehydrogenase (MDH) and Peroxidase (PO) systems to identify resistant genotypes. EST, the only monomorphic enzyme was inefficient for discriminating between genotypes, whereas ACP, because of its accentuated polymorphisms, allowed the separation of five out of seven of the clones. No association between the ACP loci and resistance or susceptibility to plant aphid was established. However, ACP using standard 5, and GOT using standard 9, identified the Venus clone, one of the most resistant to the pest. The other systems were not efficient in separating clones or in identifying resistance, because of the coincidence of the isozyme patterns.

INTRODUCTION

The ideal choice in the fight against insect pests is the use of resistant varieties as the insect populations can be reduced below the economic damage level without disturbing or polluting the ecosystem (Lara *et al.*, 1979).

Morphological, agronomic and physiological traits have traditionally been used to identify resistant phenotypes. However, these methods are limited because the recessive alleles, most useful as markers, can be deleterious when in homozygous condition and, also, because epistatic and/or pleiotropic effects can

significantly limit the number of markers in a genetic stock (Barros, 1991).

Biochemical markers, such as isozymes, are more reliable for genotypic screening. They quantify the genetic variability at a level closer to the DNA, and also allow the identification of the heterozygote, since the alleles at each loci are co-dominant (Adams, 1983; Alfenas *et al.*, 1991). According to Hancock and Iezzoni (1988), the greatest advantage of isozymes as genetic markers is the possibility of identification of the genotypes in the seedling stage, allowing early selection of the desirable individuals.

MATERIAL AND METHODS

Resistance to aphids was first assessed in clones of seven kale varieties (Table I). A Completely Randomized Design experiment with six replications

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was carried out. In each replication, 10 aphids were put in a 250 ml plastic pot containing a kale leaf, which was replaced every three days. The number of surviving aphids was counted daily throughout the experiment and these dates were recorded and used to calculate the average life expectancy of the insect, using the methodology of Rabinovich (1978).

These clones were analyzed for seven isozyme systems: EST, ACP, GDH, GOT, LAP, MDH and PO. Electrophoresis in hydrolysed potato starch gels (Sigma) at 12% was used to obtain the isozyme bands. The standard procedure of Alfnas *et al.* (1991) was used.

Samples from the meristem of lateral shoots of ten five-month-old plants of each clone were collected for electrophoresis. They were immediately squashed in solution containing 0.6 g sodium phosphate dibasic (0.034 M), 7 g sucrose (0.2 M), 2.56 g polyvinyl pyrrolidone (2.56%), 100 g L-ascorbic acid (5.7 mM), 50 g dithiothreitol (3 mM), 100 g diethyldithiocarbamate (5.8 mM), 50 g sodium bisulfite (2.6 mM), 50 mg sodium borate (2.5 mM), 0.2 ml 2-mercaptoethanol (0.2%), 1 g polyethylene glycol (1%) and 100 ml distilled water (Alfnas *et al.*, 1991). After paper filtering, the extract was absorbed with "wicks" (strips of 10 x 4 mm Whatman 3 MM chromatographic paper) and applied to the gels. Shaw and Prasad's (1970) methods for gel/electrode buffer and staining were used for the PO and MDH enzymes, while the Soltis *et al.* (1983) method was used on the other enzymes.

In order to read the bands, the gels were dried by the "Embroidery Frame Method" (Alfnas *et al.*, 1991), then they were laid over the surface of a diaphanoscope and the route of the bands was measured using a ruler.

RESULTS AND DISCUSSION

Table I shows the data from a trial for kale resistance to aphids attack. Figure 1 shows the isozyme band patterns, based on the polymorphisms found. Interclonal variability was absent only for the EST system. The PO and ACP systems were the most polymorphic, showing five isozyme patterns each.

Table I - Life expectancy of *Brevicoryne brassicae* in Kale clones.

Clones	Life expectancy (days) ¹
Roxa	13.11 a
Talo Roxo	09.03 b
Crista-de-galinha	08.25 b
Sobejo-de-galinha	07.77 b
Portuguesa	06.56 c
Vênus (Joenes)	04.38 c
Manteiga	03.16 c

¹The means followed by the same letter do not differ significantly at the 5% level of probability by the Scott-Knott test. Source: Paula *et al.* (1995).

Table II describes the patterns obtained for the six polymorphic isozymes. The Manteiga clone, the most resistant to aphids, had patterns very similar to those of other clones, for the ACP, GDH, GOT, LAP and MDH systems, rendering them of little use as markers. Genotype identification through the PO system was even more difficult, because of the intraclonal variation found in the Manteiga clone, which displayed patterns 19 and 20. However, the intraclonal polymorphism for

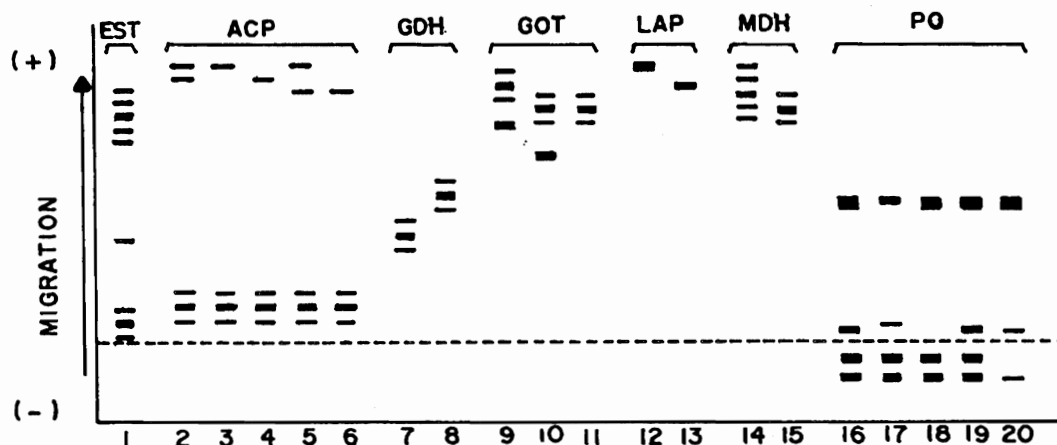


Figure 1 - Isozyme Patterns of Esterase (EST), Acid Phosphatase (ACP), Glutamate Dehydrogenase (GDH), Glutamate Oxaloacetate Transaminase (GOT), Leucine Aminopeptidase (LAP), Malate Dehydrogenase (MDH) and Peroxidase (PO) in the electrophoresis of kale clones.

the isoperoxydases was different from the other clones, allowing the biochemical discrimination of the pest-resistant Manteiga genome, based on patterns 19 and 20.

Table II - Isozyme Patterns of Acid Phosphatase (ACP), Glutamate Dehydrogenase (GDH), Glutamate Oxaloacetate Transaminase (GOT), Leucine Aminopeptidase (LAP), Malate Dehydrogenase (MDH) and Peroxidase (PO) in kale clones.

Clones	Enzymatic patterns					
	ACP	GDH	GOT	LAP	MDH	PO
Crista-de-galinha	3	7	10	13	15	18
Manteiga	6	7	11	12	14	19-20
Portuguesa	4	7	11	12	14	16
Roxa	2	7	11	12	15	16
Sobejo-de-galinha	3	7	11	12	14	16
Talo Roxo	6	8	11	12	15	17
Vênus (Joenes)	5	7	9	12	15	17

The intraclonal variation is due to the fact that PO is a multienzymatic complex that acts in various catalytic reactions (Alfnas *et al.*, 1991). This probably results in phenotypic variation.

Isozyme identification of the Venus clone, classified as resistant (Table I), was possible using the GOT and ACP systems, with patterns 9 and 5, respectively. The great variability of ACP, which allowed the discrimination of 5/7 of the clones, characterized this system as promising for genotype identification in kale. The identification of the Portuguesa clone, based on pattern 4, was also done by this enzyme. However, similarities were found in the other clones in the other isozyme systems assessed (Figure 1). The Roxa clone could only be identified using ACP pattern 2, as similarities were found when the other systems were used.

Although the ACP system was efficient in discriminating the genotype, there appeared to be no relation between resistance or susceptibility to *B. brassicae* and the isozyme coding loci. Locus 1, of greater migration, present in the Roxa clone, was also found in the resistant clones (Venus genotype), while locus 2, of intermediate migration, was absent in Roxa and showed great activity in medium and high resistance clones such as Talo Roxo and Manteiga. Locus 3, of least migration, behaved in a monomorphic way (Figure 2). There was no consistent relation between these isozymes and susceptibility or resistance of kale to aphids.

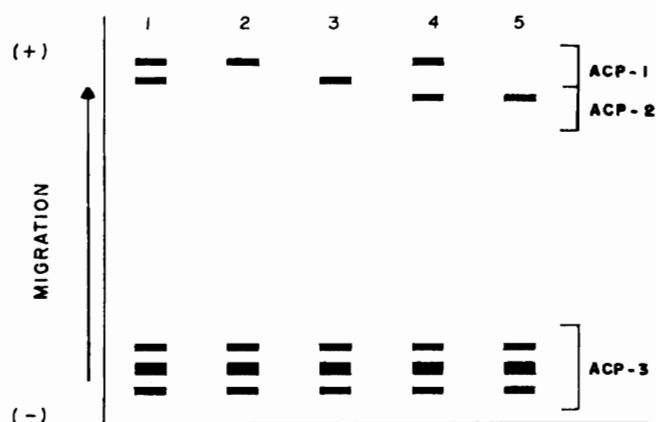


Figure 2 - Isozyme Patterns of Phosphatase Acid (ACP) in the electrophoresis of kale clones (*).

(*): 1 (Roxa); 2 (Crista-de-galinha and Sobejo-de-galinha); 3 (Portuguesa); 4 (Vênus); and, 5 (Manteiga and Talo Roxo).

RESUMO

Sete clones de couve-comum (*Brassica oleracea* var. *acephala*), avaliados quanto a resistência a pulgão (*Brevicoryne brassicae*) por Paula *et al.* (*An. Soc. Entomol. Brasil.* 24: 1995), foram submetidos à análise isozimática, utilizando-se os sistemas Esterase (EST), Fosfatase Ácida (ACP), Glutamato Desidrogenase (GDH), Glutamato Oxaloacetato Transaminase (GOT), Leucina Aminopeptidase (LAP), Malato Desidrogenase (MDH) e Peroxidase (PO), para a identificação de genótipos resistentes. A EST, única enzima monomórfica, foi ineficiente na discriminação genotípica, enquanto a ACP, pelo acentuado polimorfismo, permitiu a separação de 71% dos clones, revelando-se um potente marcador bioquímico. Entretanto, não foi constatada associação entre locos da ACP e resistência ou susceptibilidade a pulgão. Contudo, a ACP, pelo padrão 5, e GOT, pelo padrão 9, identificaram o clone Vênus, caracterizado como dos mais resistentes à praga. Os demais sistemas não foram eficientes na separação de clones ou na identificação de resistência, devido à coincidência dos padrões isozimáticos encontrados.

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