

Effects of host resistance on the isozymatic patterns of *Bipolaris sorokiniana* (Dematiaceae, Moniliales)

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ABSTRACT

Nine isolates of *Bipolaris sorokiniana* were inoculated on three cultivars of wheat plants (susceptible, moderately resistant, resistant). Eight days after the inoculation, the isolates were recovered (27 isolates) and the following isozymatic patterns were analyzed: esterase, alkaline phosphatase, acid phosphatase, malate dehydrogenase, and superoxide dismutase. The esterase system was the most polymorphic, and the isolates recovered from the susceptible cultivar showed the highest variability. This is evidence that this cultivar exerts low selection pressure on the pathogen.

INTRODUCTION

Bipolaris sorokiniana Shoemaker (syn.: *Helminthosporium sativum*, *H. sorokinianum*, *Drechslera sorokiniana*), anamorph of *Cochliobolus sativus*, is pathogenic to wheat and other gramineous hosts. It shows a high morphological variability in culture media and it also varies in virulence (Mehta, 1981).

This fungal species is haploid and its genetics is still unknown. Very few isoenzymatic studies have been conducted on this species. Populations of *B. sorokiniana*, isolated from different wheat cultivars, showed isoesterasic polymorphism (Matsumura, 1991), and, based on the esterase patterns, it is possible to identify the wheat cultivar from which the fungus was isolated. Variability of the esterase, malate dehydrogenase, and acid phosphatase patterns on *B. sorokiniana* isolates obtained from seeds of one wheat cultivar without any relation with the host origin was also observed by Valim-Labres (1995).

The objective of the present study was to determine the effect of the host cultivar on the expression of the isoenzymatic genotypes of *B. sorokiniana* isolates.

MATERIAL AND METHODS

Nine isolates were grown on sorghum seeds following a method described by Mehta (1981). The conidial suspension was adjusted to 1×10^4 spores/ml and sprayed on wheat seedlings of the cultivars BH 1146 (original host), IAC-5 Maringá, and BR 8, classified as moderately resistant, susceptible, and resistant to *B. sorokiniana*, respectively. Eight days after inoculation, the fungus was re-isolated (originating 27 isolates).

All samples for electrophoretic analysis were obtained from culture grown on PDA media (potato dextrose agar) for seven days at a temperature of $25 \pm 1^\circ\text{C}$ and a 12-h daylength. Polyacrylamide gel electrophoresis was carried out with homogenized extracts of fungal propagules (35 ± 1 mg micelia, conidiophores, conidia). The homogenization was done in 60 μl of buffer (Scandalios 1969, Roose and Gottlieb, 1976) and 40 μl of bromophenol blue.

The new isolates were tested by α and β esterase (EST), acid phosphatase (ACP), alkaline phosphatase (ALP), malate dehydrogenase (MDH), and superoxide dismutase (SOD). The analyses were carried out in polyacrylamide gel 6% by horizontal electrophoresis, with Scandalios (1969) buffers for the first three systems, and with Roose and Gottlieb (1976) buffers for MDH and SOD. The enzymes were developed according to the following protocols: Scandalios (1969) for EST and ACP, Vallejos (1983) for ALP, Brewer and Sing (1970) for MDH and SOD. The relative migrations (RM) of the molecules were calculated in relation to the bromophenol line.

RESULTS

The isolates showed polymorphism in the EST, MDH, and ACP systems. The SOD and ALP systems were monomorphic.

Esterase

The 27 isolates showed three monomorphic (C, E, F) and six polymorphic zones, one of which had two bands (Table I). There was polymorphism within the same isolate recovered from different hosts. Isolate 03/92 showed four polymorphic zones, with three new zones of slow migration in isolate 03/92-IAC. The lowest polymorphism, with only one zone, was shown by the isolate 15/92. All the isolates recovered from the wheat cultivar BR 8 showed the band RM 0.97, while all the isolates recovered from the cultivar BH 1146 did not show the band RM 0.80. Considering the polymorphism among isolates within the same cultivar, it was observed that the isolates from cultivar BH 1146 showed five zones, with two polymorphic zones, and four

phenotypes (Figure 1); the isolates from the cultivar IAC-5 showed nine zones, with five polymorphic zones, and seven phenotypes (Figure 2); the isolates from the cultivar BR 8 showed six zones, with only one polymorphic, and three phenotypes (Figure 3).

Malate dehydrogenase

The 27 isolates showed five zones in MDH, but only isolate 05/92 showed different phenotypes (Table II).

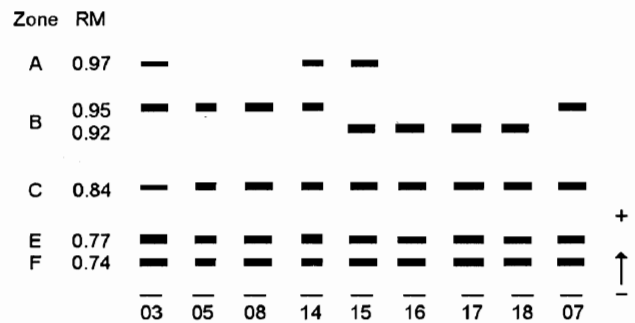


Figure 1 - Isoesterase zymogram of *Bipolaris sorokiniana* isolates recovered from the wheat cultivar BH 1146.

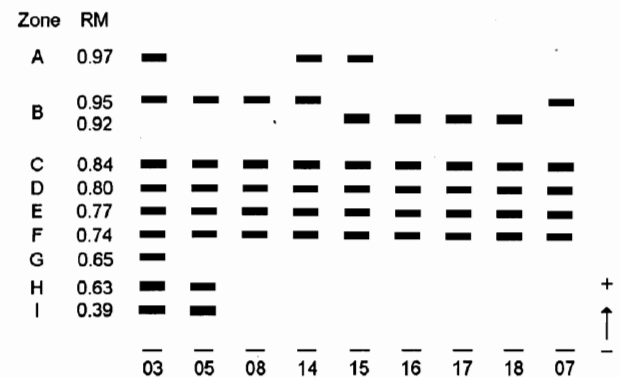


Figure 2 - Isoesterase zymogram of *Bipolaris sorokiniana* isolates recovered from the wheat cultivar IAC-5.

Table I - Isoesterasic patterns for isolates of *Bipolaris sorokiniana* recovered from the wheat cultivars BH 1146 (bh), IAC-5 (iac), and BR 8 (br).

Zone	Band RM	Isolates																										
		03/92			05/92			08/92			14/92			15/92			16/92			17/92			18/92			07/93		
		bh	iac	br	bh	iac	br	bh	iac	br	bh	iac	br	bh	iac	br	bh	iac	br	bh	iac	br	bh	iac	br	bh	iac	br
A	0.97	+	+	+	-	-	+	-	-	+	+	+	+	+	+	+	-	-	+	-	-	+	-	-	+	-	-	+
B	0.95	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
	0.92	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
C	0.84	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D	0.80	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+
E	0.77	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
F	0.74	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
G	0.65	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H	0.63	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I	0.39	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+ = Presence of band; - = absence of band.

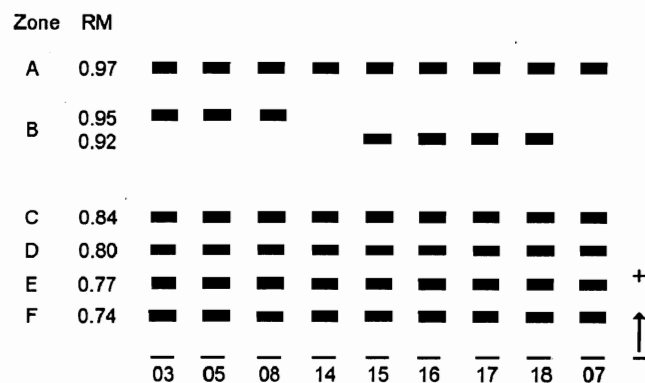


Figure 3 - Isoesterase zymogram of *Bipolaris sorokiniana* isolates recovered from the wheat cultivar BR 8.

Acid phosphatase

The isolates showed two monomorphic and one polymorphic zones (Table III). Such polymorphism was observed among the isolates and within the same isolate. The isolates of IAC-5 cultivar were monomorphic in this system (Table III).

DISCUSSION

The greatest polymorphism in EST, showed by the isolates recovered from cultivar IAC-5, suggests that this cultivar had little influence on the expression of the

pathogen genotype. As this fungus is haploid and heterokaryotic and the cultivar is susceptible, it expressed several pathogen genotypes, resulting in polymorphism in five zones among the isolates. The cultivar BR 8, classified as highly resistant, exerted some selection pressure, allowing the expression of only two genotypes, with variation in one zone. The cultivar BH 1146, moderately resistant, expressed of four genotypes, with alteration in two zones.

Matsumura (1991), analyzing the isoesterasic pattern of 30 isolates of *Bipolaris sorokiniana* recovered from the wheat cultivars IAC-5 and BR 4, observed differences in bands for the isolates from the two cultivars, with the presence of some bands in one group and its absence in the other. The author also observed that the isolates were more similar within the group than between groups, which may be evidence that the host plant influences the expression of the pathogen genotype.

In the case of malate dehydrogenase, polymorphism within the same isolate occurred only for the isolate 5. Staples and Stahmann (1963), working with *Uromyces phaseoli* in bean leaves, suggest that the host affects the number and form of the malate dehydrogenase of the pathogen during the infective process. On the other hand, the pathogen affects the enzymatic synthesis in the host by intensifying some enzymes and restraining others.

Table II - Malate dehydrogenase patterns for isolates of *Bipolaris sorokiniana* recovered from the wheat cultivars BH 1146 (bh), IAC-5 (iac), and BR 8 (br).

Band	Isolates																													
	03/92			05/92			08/92			14/92			15/92			16/92			17/92			18/92			07/93					
RM	bh	iac	br	bh	iac	br	bh	iac	br	bh	iac	br	bh	iac	br	bh	iac	br	bh	iac	br	bh	iac	br	bh	iac	br	bh	iac	br
0.35	+	+	+	-	+	+	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+
0.22	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.08	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table III - Acid phosphatase patterns for isolates of *Bipolaris sorokiniana* recovered from the wheat cultivars BH 1146 (bh), IAC-5 (iac), and BR 8 (br).

Band	Isolates																													
	03/92			05/92			08/92			14/92			15/92			16/92			17/92			18/92			07/93					
RM	bh	iac	br	bh	iac	br	bh	iac	br	bh	iac	br	bh	iac	br	bh	iac	br	bh	iac	br	bh	iac	br	bh	iac	br	bh	iac	br
0.64	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.43	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-0.14	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	-	+	-	+	+	+	-	+	+	-	+	+

We did not find, in the literature review, references related to the participation of acid phosphatase, superoxide dismutase, and alkaline phosphatase in the infection process.

The enzymatic action of host infection by several fungal species was reviewed by Schäfer, in 1994. Pascholati *et al.* (1993), using an esterase inhibitor in conidia of *Colletotrichum graminicola*, prevented the infection of corn plants because the adhesion of the conidia was inhibited due to the lack of esterase. Nicholson *et al.* (1972), using histochemical methods, showed the presence of esterase during the spore germination and formation of appressoria of *Venturia inaequalis*. Deising *et al.* (1992) and McKeen (1974) reported the involvement of these enzymes in the infection processes by *Uromyces vicia-fabae* and *Botrytis cinerea*, respectively.

According to Flor's theory there is (1956), for each gene for virulence in the pathogen one gene for resistance in the host. As the esterases are involved in the infection process, the expression of different genotypes for the same isolate in distinct host cultivars is evidence of the host pressure on the pathogen genotype.

The esterases are an enzymatic system susceptible to environmental changes since their action is restricted to the peripheral metabolism of the fungi, different from the other systems from which the products are originated and utilized intracellularly (Gillespie and Kojima, 1968). It is possible that the number of genes involved is higher in the esterase system than in those systems of internal metabolism where the products are much more uniform. As the esterases have a large number of genes that code for different molecules with the same function, the corresponding host genes may neutralize some, but not all genes of the pathogens. The susceptible host cultivar may have lower number of complementary genes, which allows the expression of various genotypes of esterase in the pathogen. The opposite may occur with a resistant host and its interaction with the pathogen.

Malate dehydrogenase and superoxide dismutase are enzymes of internal metabolism and therefore are less susceptible to environmental changes. Acid phosphatase and alkaline phosphatase, although belonging to the external metabolism, do not seem to be directly involved with the infection process and, therefore, the activation of a defence mechanism at this level is not necessary.

It is also possible to infer that the effect of the host cultivar on the isoenzymatic pattern of the pathogen is related to the enzyme function in the infection process and the resistance of the cultivar is related

to the number of host genes, corresponding to the genes of the enzymes involved in the process.

ACKNOWLEDGMENTS

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RESUMO

Nove isolados de *Bipolaris sorokiniana* foram inoculados em três cultivares de trigo (suscetível, moderadamente resistente e resistente). Oito dias após a inoculação, os isolados foram recuperados (27 isolados) e os seguintes sistemas isoenzimáticos foram analisados: esterase, fosfatase alcalina, fosfatase ácida, malato desidrogenase e superóxido dismutase. O sistema de esterase foi o mais polimórfico e os isolados recuperados da cultivar suscetível apresentaram a maior variabilidade, indicando que esta cultivar exerce pouca pressão seletiva sobre o patógeno.

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