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RELATIONSHIP BETWEEN ISOENZYME BANDING PATTERNS AND DISEASE RESISTANCE TO ASHY STEM BLIGHT IN BEAN (*Phaseolus vulgaris L.*).

FAYED A. H.; E.M.I. MAHGOUB *; M.I. GHONIM. ** and H.M. Kamel*

***Genetics Dept., Faculty of Agriculture, Zagazig University**

****Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt.**

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ABSTRACT: Thirty bean (*Phaseolus vulgaris L.*) genotypes were evaluated for their reactions to infection by *Macrophomina phaseolina* (Tassi) Goid. under greenhouse conditions. Most of tested genotypes were susceptible except Nebraska and Royal Nel that are resistant, while, Morgan, Mexico 309, Sigma, and EMY were intermediate. The studies were based on crosses between the resistant genotype Nebraska and the susceptible genotypes (Giza 6 and S1) and the intermediate genotype Morgan. Isozymes banding pattern for the four parents used in the crosses, F1 and F2 populations from leaf tissues showed 20 different peroxidase or esterase anodal bands. The number and concentration of peroxidase and esterase isoenzymes increased owing to *M. phaseolina* infection. These changes in number and concentration of peroxidase and esterase isoenzymes were correlated with infection by *M. phaseolina* showing that, peroxidase and esterase activity had increased by fungal infection. These results suggest that peroxidase and esterase may play a role in the active defense mechanism of the plant and could be considered positive biochemical markers for *M. phaseolina* resistance.

INTRODUCTION

Ashy stem blight of common beans (*Phaseolus vulgaris* L.) incited by the fungus *M. phaseolina* causes considerable damage in bean production areas that are characterized by high temperature and drought conditions. *M. phaseolina* has a wide host range and geographic distribution and also a major pathogen of many crops including

sorghum, cotton, soybean, chickpea, sunflower, corn, cowpea, and peanut (Schwartz, 1989 and Mihail 1992). Miklas and Beaver (1994) reported that field resistance to *M. phaseolina* is controlled by more than one gene. Olaya *et al.*, (1996) indicated that two dominant complementary genes (Mp-1 and Mp-2) conditioned resistance of bean accession BAT 477 to *M. phaseolina*. However, a direct relationship between Mp-1 and Mp-2 and field resistance is exhibited by BAT 477. The number and concentration of peroxidase isoenzymes in bean hypocotyls increased in response to *R. solani* infection. A highly pathogenic isolate from okra (*Hibiscus esculentus L.*) stimulated isoenzyme induction more quickly than a weakly pathogenic one from cotton (Wasfy *et al.*, 1984). Wagih (1991) reported that both qualitative and quantitative changes in peroxidase after abrasion and PMV infection were demonstrated in *Phaseolus vulgaris* following localized virus infection and he also reported that no qualitative or quantitative changes in esterase isoenzymes were observed in primary bean leaf tissues following PMV-infection.

Esterases had a large number of bands and were represented by at least 10 loci in Maize, (MacDonald and Brewbaker 1972) and at least 8 loci in pepper (Mcleod *et al.* 1979), finally 18 isoesterase loci in pea (Guirgis *et al.* 2000).

The aim of the present work was to screen some bean genotypes to infection by *M. phaseolina*. In addition, peroxidase and esterase isozyme banding patterns of parents, F1 and F2 generations were studied in relation to fungal infection with *M. phaseolina*.

MATERIALS AND METHODS

1. Isolation and identification of the causal organism:

Isolation from naturally infected stems of bean plants were collected from Sharkia, Ismailia and Giza governorates. Plants were washed thoroughly with running tap water, then cut into small portions and surface sterilized by immersing in 0.1% mercuric chloride solution for 2 minutes, then passed in distilled sterilized water and dried between two sterilized filter papers and transferred to sterilized Petri dishes containing Potato Dextrose

Agar medium and incubated at 25°C for 7 days.

The isolated fungi were examined microscopically 3-7 days later and identified according to Clements and Chear, (1957) and Gilman (1957). All fungi isolates were purified using hyphal tip technique. Confirmation of the identification was kindly carried out by Plant Pathology Research Institute, Agric. Res. Center, Giza, Egypt.

Dry Sclerotia of *M. phaseolina* were produced in a liquid medium containing 10g peptone, 15g dextrose, 0.25g $MgSO_47H_2O$ and 0.5g K_2HPO_4 in one liter of water. After two-week incubation at 30°C, mycelial mats with abundant sclerotia were homogenized in a mixer with distilled water, centrifuged, washed once and then dried for 48 hrs. (Abawi and Pastor-Corrales 1989).

2. Pathogenicity test:

Three isolated of *M. phaseolina* were collected previously; i.e. Giza, Sharkia and Ismailia governorates; and individually tested for their pathogenicity on susceptible genotype Giza 6 under greenhouse conditions. Pots were sterilized by immersing them in 5% formalin solution for 15 minutes and left to dry for 7 days and the clay-sandy soil used for planting were sterilized by 5% formalin solution for 3 weeks and left to allow formaldhyde evaporation. Sclerotia were mixed thoroughly in sterilized soil at a rate of 2g sclerotia /kg of soil. About 2-3 cm layer of the infested soil used for the three-tested isolates of *M. phaseolina* placed on top of bean seeds planted in pots (10-cm in diameter) arranged in four replicates, forming a layer over seeds. The pots were then incubated in a greenhouse at 20-33°C and 35-80% relative humidity (Pastor-Corrales and Abawi 1988 and Abawi and Pastor-Corrales 1989). Percentages of pre, post emergence damping off, and survived plants were recorded 15, 30 and 60 days after planting, respectively.

3. Screening of bean genotypes to ashy stem blight:

Thirty genotypes namely; Giza 3, Giza 6, S1, Bronco, Xera, Paulista, Nebraska, Serpo, Helda, Fasol (Flageolet), BARc-RR-9, BelJersy-RR-10, BelMioDok-RR-8, 4-11853 (BelDakMi-RMRI), Mexico 309, Morgan, Primel, Hab 460, Hab 464, Diacol, RC628, Tucam, Deul, Julta, Kylian, Royal Nel, Coby, EMY, Nerina and Sigma were used. These genotypes

were obtained from Central Administration of seeds (CAS), Vegetable Research Department, Agricultural Research Center, to screen their susceptibility to ashy stem blight disease. Four surface sterilized seeds of each genotype per pot were planted in sterilized pots (10 cm in diameter) under artificial inoculation and four replicates were employed for each treatment. Four pots containing sterilized soil were used as control. Percentages of pre, post emergence damping-off, survived plants and disease severity rating were recorded after 15, 30 and 60 days after planting, respectively.

4. Disease assessment:

Disease severity ratings were recorded after 60 days of plantation using a pretransformed scale from 1 to 9 similar to the severity scale (Centro Internacinal de Agricultura Tropical (CIAT) (Schoonhoven and Pastor-Corrales 1987). Disease severity rating (DSR) 1-3 resistant, 3.1-6.0 intermediate and 6.1-9.0 susceptible.

5. Crosses between selected genotypes under green house condition:

Four genotypes (Nebraska, Morgan, Giza 6, and S1) were selected from thirty genotypes and crossed. Nebraska was used as resistant female parent, Morgan as intermediate parent, Giza 6 and S1 as susceptible parents were employed as males. F1 seeds were planted to obtain F2 seeds. The parental seeds and their F1 and F2 seeds were sown in pots under infection and un-infection conditions by *M. phaseolina*. The investigation was carried out at the greenhouse, El- Kassassin Horticultural Research Station during 2000-2003.

6. Peroxidase and Esterase assay in leaves of parents, F_1 and F_2 generations plants:

a. Enzyme extraction: After three weeks, leave samples (5g) of each three infected and non-infected plants of each parent and F1 were taken randomly, while F2 plants were taken from resistant and susceptible plants. Samples were crushed directly in an ice-cold (0-4°C)/M tris extraction buffer, pH 7.8. The enzyme extraction buffer and procedures were applied according to Tanksely and Orton (1983).

b. Gel preparation: sample loading and electrophoresis: For separating gels, a 15% and 17.5% discontinuous non- dissociating, polyacrylamide gel mixture, using a stock of 30% acrylamide was loaded in 20 × 20 cm. Bio-Rad BROTEN-II Vertical Slab Cell.

Sample volumes with equal protein concentrations were used for loading in gels after determining the concentration of protein in all samples according to Bradford (1976). The buffers used in sample loading and electrophoretic conditions were described by Guirgis *et al*., (1996).

c. Staining and data collection: for detection of isoperoxidases, gels were incubated for 30 minutes on 30°C in 0.01% benzidine hydrochloride in 1M sodium acetate solution of pH 4.7, few drops of 30% hydrogen peroxid were added immediately (Guikema and Sherman. 1980).

As for esterase isozyme bands detection, Kahler and Allars (1970) procedures were applied with some modification suggested by Tanksely and Rick (1980). The gels were incubated in a freshly prepared mixture of α and β naphthyl acetate and 0.1% fast blue RR salt. Then, gels were kept in the staining solution in a dark cabinet at 30°C for 1-2 hrs. The traveled distances by isozyme bands were recorded directly using a UVP fluorescent transilluminator pattern and photographed.

7. Statistical analysis:

The data were statistically analyzed using the analysis of variance procedure for completely randomized experimental design. Treatment means were compared using Least Significant Difference (L.S.D) according to Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

1. Isolation, identification and pathogensis:

Three isolates were isolated from stems collected from different locations (Giza, Sharkia and Ismailia governorates) and identified as *Macrophomina phaseolina* (Tassi) Goid. the causal organism of ashy stem blight disease of bean (Mp1, Mp2 and Mp3) respectively.

The pathogenicity test was carried out using the three isolates of *M. phaseolina*. Data shown in Table (1) indicated that, all the tested isolates were pathogenic to Giza 6 bean genotype. The isolate Mp1, which isolated from bean stems at Giza governorate, had a superior effect on Giza 6 and was the most pathogenic isolate since; it caused 56.25, 25.0, and 18.75% of pre, post emergence damping-off and survived plants, respectively followed by isolate Mp2, which isolated from Sharkia governorate, its analogous values, were 31.25, 12.50, and 56.25 % of

Table (1): Pathogenicity test of three isolates of *M. phaseolina* tested on susceptible genotype (Giza 6) after 15, 30 and 60 days from sowing under greenhouse conditions.

M. phaseolina isolates	%of emergence damping-off		%of Survived plants (60 days)
	Pre (15 days)	Post (30 days)	
Giza isolates (Mp1)	56.25	25.0	18.75
Sharkia isolates (Mp2)	31.25	12.50	56.25
Ismailia isolates (Mp3)	18.75	0.00	81.25
Control	0.00	0.00	100.0
$LSD_{0.05}$	18.14	11.54	32.82

pre, post emergence damping-off and survived plants, respectively. Isolate Mp3, which isolated from Ismailia governorate, was less pathogenic isolate then exhibited 18.75, 0.0, and 81.25 % of pre, post emergence damping-off and survived plants, respectively. Control treatment did not score any data for all the tested parameters and gave 100% survived plants. Generally, the isolate Mp1 of *M. phaseolina* was more effective than the other tested isolates so that, it was chosen for further studies.

2. Reaction of bean genotypes to *M. phaseolina* under greenhouse conditions:

The reactions of the 30 genotypes were carried out to evaluate susceptibility to infection with *M. phaseolina* (isolate Mp1) under artificial infection. Data in Table (2) indicated that, Nebraska and Royal Nel genotypes were resistant while Kylian, EMY, Mexico 309, Sigma, and Morgan were classified as intermediate genotypes. Giza 6, S1, Diacoal, Serpo, and Xera were classified as susceptible. Nebraska genotype recorded 6.25, 0.00, 93.75 % and 1.8 of pre, post emergence damping-off, survived plants and diseases severity rating, respectively. Morgan genotype recorded 37.50, 12.50, 50.00% and 5.0 of pre, post emergence damping-off, survived plants and diseases severity rating, respectively. The susceptible parents S1 and Giza 6 were recorded 50.0, 25.0, 31.25%, 7.0 and 50.0, 37.5, 12.5 %, 8 of pre, post emergence, survived plants and diseases severity rating, respectively.

Table (2): Percentage of pre, post emergence damping off, survived plants and disease severity rating of 30 bean genotypes under artificial infection with *M. phaseolina* in greenhouse.

Genotypes	% Of emergence damping-off		% Of survived plants (60 days)	DSR (1-9)
	Pre (15 days)	Post (30 days)		
Nebraska	6.25	0.00	93.75	1.8
Royal Neal	12.5	0.00	87.5	2.5
Kylian	31.25	0.00	68.75	3.5
Mexico 309	37.50	0.00	62.50	4.0
4-11853(BelDakMi-RMR1)	31.25	12.50	56.25	4.5
Fasol (Flageolet)	31.25	12.50	56.25	4.5
Sigma	31.25	12.50	56.25	4.8
EMY	31.25	12.50	56.25	4.8
Morgan	37.50	12.50	50.00	5.0
Julta	50.00	6.25	43.75	5.8
Bronco	56.25	6.25	37.50	6.0
Paulista	43.75	18.75	37.50	6.0
Bel MioDok-RR-8	43.75	18.75	37.50	6.0
Deul	43.75	12.50	43.75	6.3
Hab 464	50.00	18.75	31.25	6.5
Tucam	43.75	18.75	37.50	6.5
Coby	56.25	6.25	37.50	6.8
S1	50.00	25.0	31.25	7.0
Bel Jersy-RR-10	62.50	12.50	25.00	7.0
BARc-RR-9	50.00	18.75	31.25	7.0
Primel	62.50	12.50	25.00	7.0
Helda	62.50	18.75	18.75	7.5
Nerina	62.50	18.75	18.75	7.5
RC 628	62.50	18.75	18.75	7.8
Giza 6	50.00	37.5	12.50	8.0
Hab 460	56.25	31.25	12.50	8.0
Diacol	75.00	12.50	12.50	8.5
Giza 3	62.50	31.25	6.25	8.8
Serpo	43.75	50.00	6.25	8.8
Xera	93.75	0.00	6.25	8.8
L.S.D. $_{0.05}$	38.47	27.19	34.03	1.08

3. Peroxidase isozyme polymorphism and fungi infection with *M. phaseolina*:

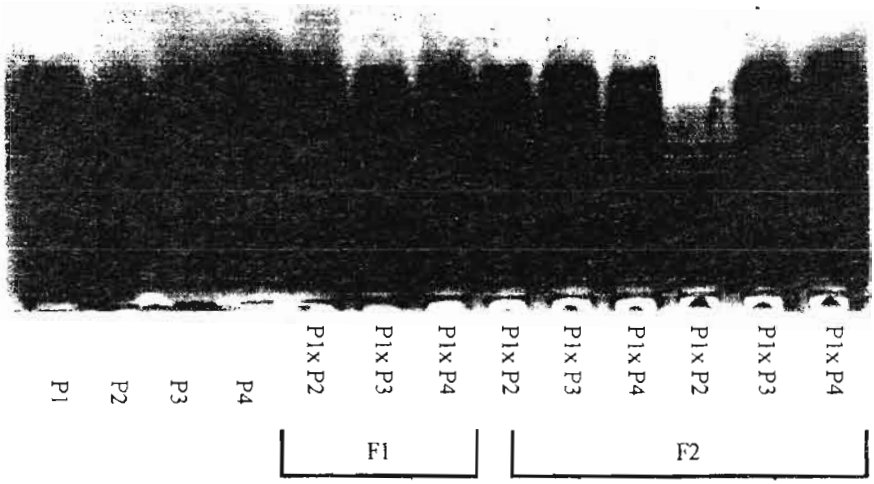
The zymograms of isoperoxidase banding pattern for four parental, F1 and F2 populations in leaf of un-infested plants showed 20 peroxidase anodal bands (Photograph 1, Figure 1 and Table 3). The fast migrating band has the position of 4.1 cm anodal to the origin appeared to be active in all leaf tissues of parental, F1 and F2 populations except the F2 cross (P1×P2). This suggests that this band might be controlled by the presence (+) versus absence or null (N) alleles. Such polymorphism was described by Staples and Stahmann (1964) and Sako and Stahmann (1972) in beans and Reddy and Stahmam (1975) in peas.

In addition, the zymogram of anodal banding patterns showed that the intensities of the bands were condensed respectively, especially in F1 and F2 generations. The zymogram of isoperoxidase demonstrated that, the anodal variant at the position 1.5 cm anodal to the origin was found to be predominant in all screened leaf tissues of parental genotypes and their F1 and F2 generations except P4 and F2 cross (P1×P3). This means that this variant is dominant in all leaf tissues of beans, exhibiting a sort of specificity. Such tissue specificity for isoperoxidase appeared to have a considerable role in expressing variability of different molecular forms of this enzyme. In this regard, Upodhya and Jonice Yee (1968) reported that, the stages of development at which isozymes appear or disappear related primarily to the "turning on" or depression of loci controlling or modifying synthesis of isozymes.

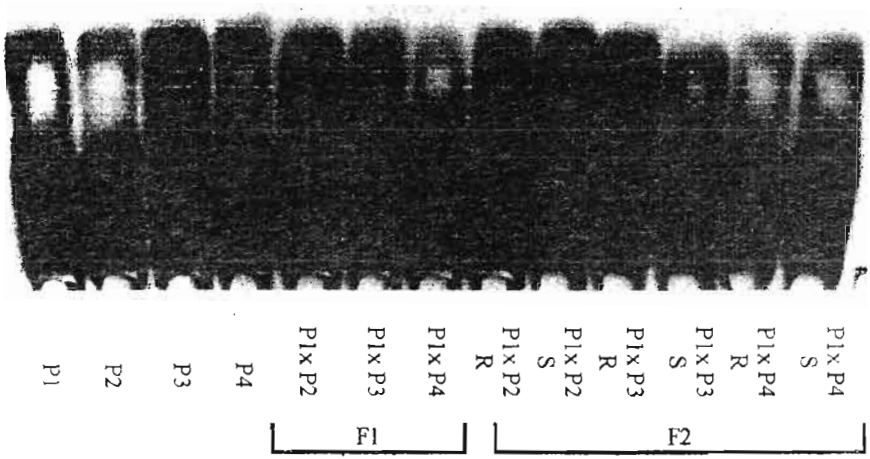
The electrophoretic banding pattern for peroxidase isozymes in infected plants of four parental genotypes and their F1 and F2 generations; presented in Photograph (2), figure (2) and Table (4); showed 23 peroxidase anodal bands in most entries.

These zymogram showed that the intensity of the bands increased after fungal infection especially in bands had the positions of 0.5, 0.7, 0.9, 2.5, 2.7 and 2.9 in parental genotypes and their F1, F2 generations.

These changes in number and concentration of peroxidase isoenzymes might correlate with infection by *M. phaseolina* suggesting that peroxidase activity had increased by fungal infection



Photograph (1): Polyacrylamide gels for Peroxidase isozymes in leaf tissue extracts of four parental genotypes, F1 and F2 generations of beans before inoculation with *M. phaseolina*



Photograph (2): Polyacrylamide gels for Peroxidase isozymes in leaf tissue extracts of four parental genotypes, F1 and F2 generations of beans after inoculation with *M. phaseolina*

P1= Nebraska P2= Giza 6 R= Resistant

P3= S1 P4= Morgan S= Susceptible

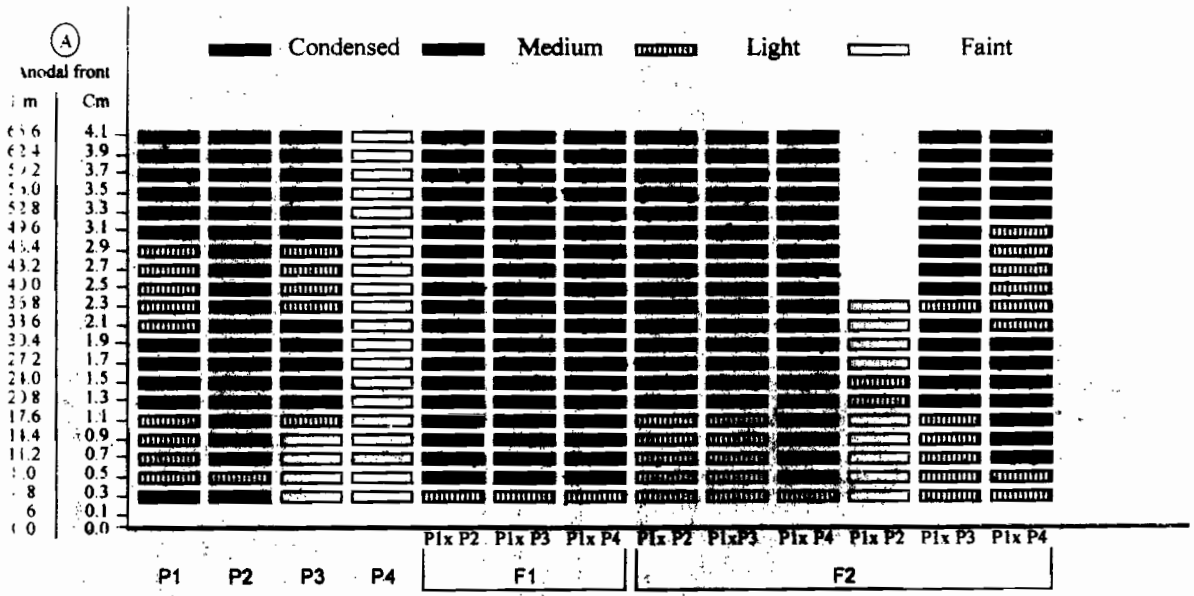


Fig1: Schemotic representation showing the migration distance and relative mobility (Rm) of Peroxidase electrophoretic bands observed in leaf tissue extracts of four parental genotypes and their F1 and F2 generations of beans before inoculation with *M. phaseolina*

P1= Nebraska P2= Giza 6

P3= S1 P4= Morgan

Table (3): Isozyme expression of peroxidase banding patterns represented as presence (+) or absence (-) of different isozyme variants in leaf tissue extracts of bean before inoculation with *M. phaseolina*.

Bands No.	Migration distance (cm)	P1	P2	P3	P4	F1			F2					
						P1×P2	P1×P3	P1×P4	P1×P2	P1×P3	P1×P4	P1×P2	P1×P3	P1×P4
1	4.1	+	+	+	+	+	+	+	+	+	+	-	+	+
2	3.9	+	+	+	+	+	+	+	+	+	+	-	+	+
3	3.7	+	+	+	+	+	+	+	+	+	+	-	+	+
4	3.5	+	+	+	+	+	+	+	+	+	+	-	+	+
5	3.3	+	+	+	+	+	+	+	+	+	+	-	+	+
6	3.1	+	+	+	+	+	+	+	+	+	+	-	+	+
7	2.9	+	+	+	+	+	+	+	+	+	+	-	+	+
8	2.7	+	+	+	+	+	+	+	+	+	+	-	+	+
9	2.5	+	+	+	+	+	+	+	+	+	+	-	+	+
10	2.3	+	+	+	+	+	+	+	+	+	+	+	+	+
11	2.1	+	+	+	+	+	+	+	+	+	+	+	+	+
12	1.9	+	+	+	+	+	+	+	+	+	+	+	+	+
13	1.7	+	+	+	+	+	+	+	+	+	+	+	+	+
14	1.5	+	+	+	+	+	+	+	+	+	+	+	+	+
15	1.3	+	+	+	+	+	+	+	+	+	+	+	+	+
16	1.1	+	+	+	+	+	+	+	+	+	+	+	+	+
17	0.9	+	+	+	+	+	+	+	+	+	+	+	+	+
18	0.7	+	+	+	+	+	+	+	+	+	+	+	+	+
19	0.5	+	+	+	+	+	+	+	+	+	+	+	+	+
20	0.3	+	+	+	+	+	+	+	+	+	+	+	+	+

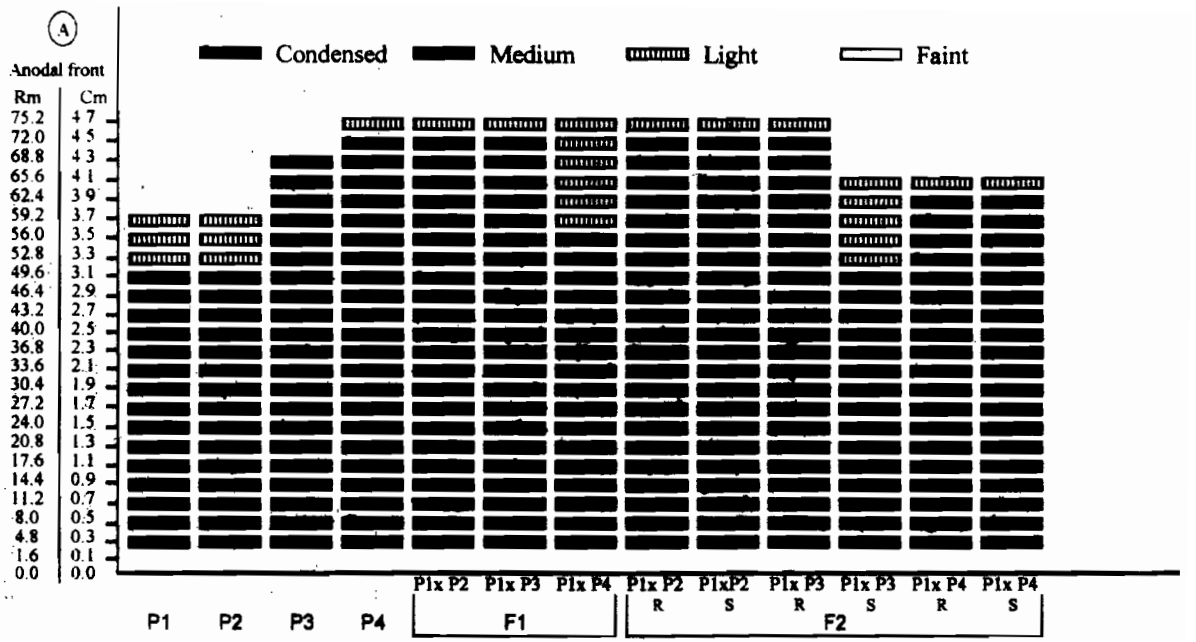


Fig2: Schemotic representation showing the migration distance and relative mobility (Rm) of Peroxidase electrophoretic bands observed in leaf tissue extracts of four parental genotypes and their F1 and F2 generations of beans after inoculation with *M. phaseolina*

P1= Nebraska P2= Giza 6 R= Resistant

P3= S1 P4= Morgan S= Susceptible

Table (4): Isozyme expression of peroxidase banding patterns represented as presence (+) or absence (-) of different isozyme variants in leaf tissue extracts of bean after inoculation with *M. phaseolina.*

Bands No.	Migration distance cm))	P1	P2	P3	P4	F1			F2					
						P1×P2	P1×P3	P1×P4	P1×P2		P1×P3		P1×P4	
									R	S	R	S	R	S
1	4.7	-	-	-	+	+	+	+	+	+	+	-	-	-
2	4.5	-	-	-	+	+	+	+	+	+	+	-	-	-
3	4.3	-	-	+	+	+	+	+	+	+	+	-	-	-
4	4.1	-	-	+	+	+	+	+	+	+	+	+	+	+
5	3.9	-	-	+	+	+	+	+	+	+	+	+	+	+
6	3.7	+	+	+	+	+	+	+	+	+	+	+	+	+
7	3.5	+	+	+	+	+	+	+	+	+	+	+	+	+
8	3.3	+	+	+	+	+	+	+	+	+	+	+	+	+
9	3.1	+	+	+	+	+	+	+	+	+	+	+	+	+
10	2.9	+	+	+	+	+	+	+	+	+	+	+	+	+
11	2.7	+	+	+	+	+	+	+	+	+	+	+	+	+
12	2.5	+	+	+	+	+	+	+	+	+	+	+	+	+
13	2.3	+	+	+	+	+	+	+	+	+	+	+	+	+
14	2.1	+	+	+	+	+	+	+	+	+	+	+	+	+
15	1.9	+	+	+	+	+	+	+	+	+	+	+	+	+
16	1.7	+	+	+	+	+	+	+	+	+	+	+	+	+
17	1.5	+	+	+	+	+	+	+	+	+	+	+	+	+
18	1.3	+	+	+	+	+	+	+	+	+	+	+	+	+
19	1.1	+	+	+	+	+	+	+	+	+	+	+	+	+
20	0.9	+	+	+	+	+	+	+	+	+	+	+	+	+
21	0.7	+	+	+	+	+	+	+	+	+	+	+	+	+
22	0.5	+	+	+	+	+	+	+	+	+	+	+	+	+
23	0.3	+	+	+	+	+	+	+	+	+	+	+	+	+

These also mean that, peroxidase might play a role in the active defense mechanism of the plant and could be considered as positive biochemical markers for *M. phaseolina* resistance. These conclusions were found correlated by Wagih (1991) and Wasfy *et al.* (1984) in bean. The increase is generally of a quantities nature affecting the entirely new isozyme bands have occurred Hislop and Stahmann, (1971).

4. Esterase isozyme polymorphism and fungal infection with *M. phaseolina*:

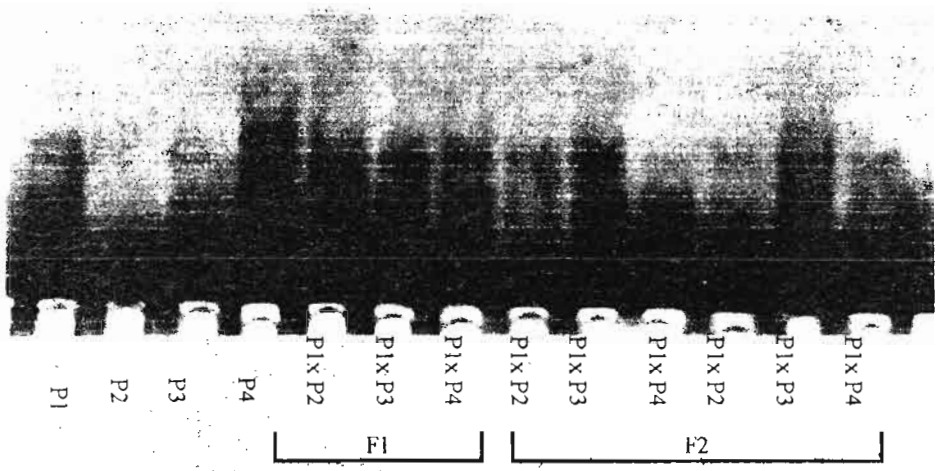
The electrophoretic banding pattern for esterase isozymes in un infested plants of four parental genotypes and their F1 and F2 generations are presented in Photograph (3), figure (3) and Table (5). These esterase-banding patterns showed 20 esterase anodal molecular forms which were not expressed in all studied genotypes.

The electrophoretogram of esterase isozymes showed that, the variant at the position 4.8 cm anodal to the origin was the fastest band while the variant at the position 0.1 was the slowest band. The anodal band at the position 4.8 cm. to the origin could be assigned as presence (+) and absence (N) alleles might be involved in controlling its expression in leaf tissues of bean (Table 5).. The variants at the positions 4.6 and 4.4 cm to the origin were found to be active in P4 and F1, crosses (P1×P2) and (P1×P4). While the anodal band at the position, 4.2 cm to the origin was active in P4, all F1 and F2 generations except F2 of the cross (P1×P2). These bands might be controlled by two alleles.

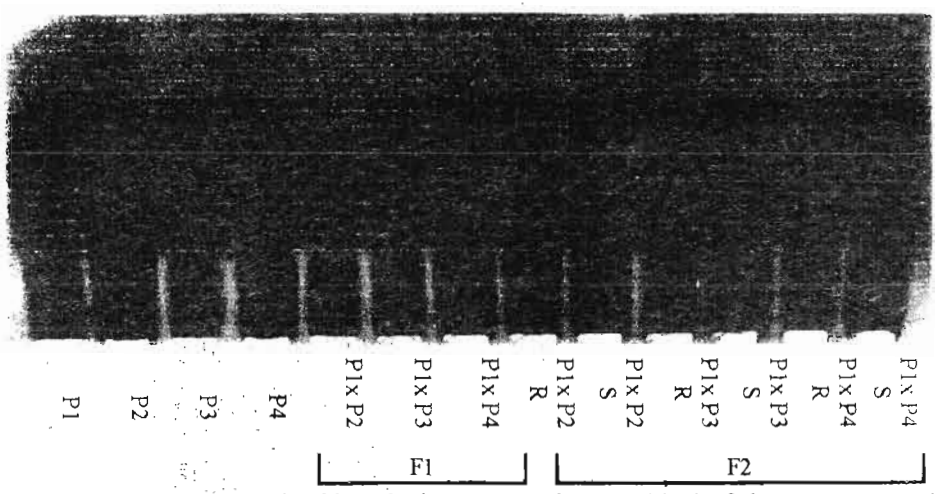
The variant at the position 4.0 cm anodal to the origin was found to be active in all screened leaf tissues of parental, F1 and F2 generations, this mean that, this bands is dominate.

A cluster of three esterase isozymes, which had the positions 3.8, 3.6 and 3.4 cm anodal to the origin were found to be predominant in all screened leaf tissues extracts for the parental, F1 and F2 genotypes (Photograph 3, figure 3 and Table 5). The isozyme intensity of these three-banded cluster were similar in parental genotypes and F1 hybrids, but varied in F2 populations.

The isozyme variants at the positions 3.2, 3.0, 2.8, 2.6, 2.4 till 2.0 cm to the origin were condensed in their intensities especially in P1, P2 and F1 hybrids of bean, but differed in F2 populations.



Photograph (3): Polyacrylamide gels for Esterase isozymes in leaf tissue extracts of four parental genotypes, F1 and F2 generations of beans before inoculation with *M. phaseolina*



Photograph (4): Polyacrylamide gels for Esterase isozymes in leaf tissue extracts of four parental genotypes, F1 and F2 generations of beans after inoculation with *M. phaseolina*

P1= Nebraska P2= Giza 6 R= Resistant

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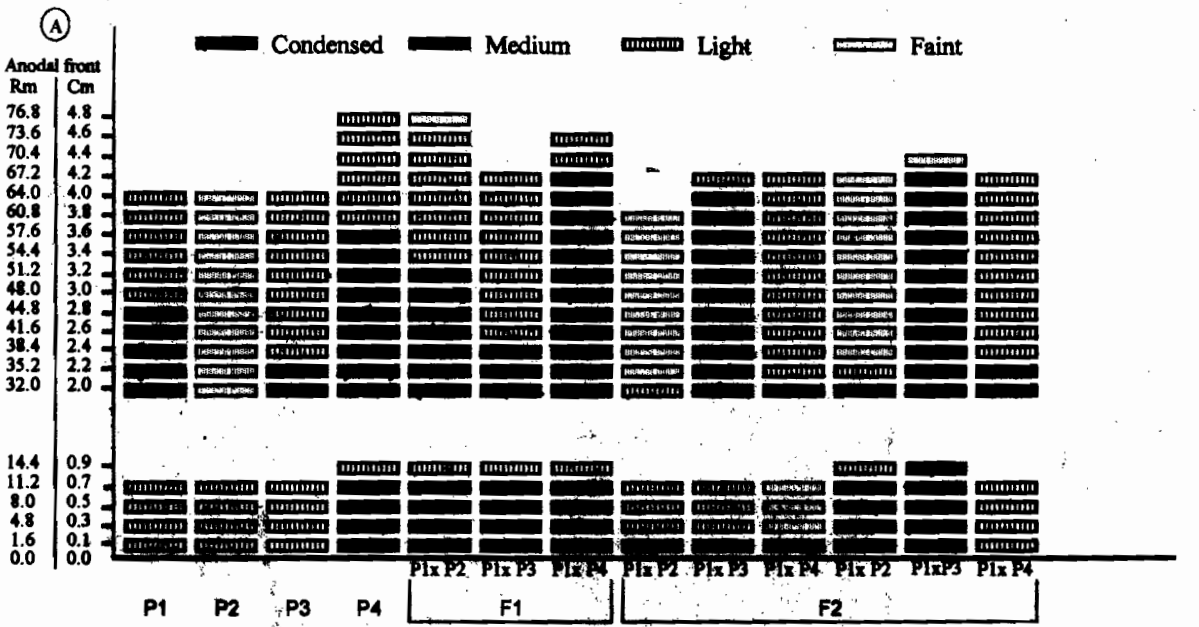

Fig3: Schemotic representation showing the migration distance and relative mobility (Rm) of Esterase electrophoretic bands observed in leaf tissue extracts of four parental genotypes and their F1 and F2 generations of beans before inoculation with *M. phaseolina*

P1= Nebraska P2= Giza 6

P3= S1 P4= Morgan

Table (5): Isozyme expression of esterase banding patterns represented as presence (+) or absence (-) of different isozyme variants in leaf tissue extracts of bean before inoculation with *M. phaseolina*.

Bands No.	Migration distance (cm)	P1	P2	P3	P4	F1			F2					
						P1×P2	P1×P3	P1×P4	P1×P2	P1×P3	P1×P4	P1×P2	P1×P3	P1×P4
1	4.8	-	-	-	+	+	-	-	-	-	-	-	-	-
2	4.6	-	-	-	+	+	-	+	-	-	-	-	-	-
3	4.4	-	-	-	+	+	-	+	-	-	-	-	+	-
4	4.2	-	-	-	+	+	+	+	-	+	+	+	+	+
5	4.0	+	+	+	+	+	+	+	-	+	+	+	+	+
6	3.8	+	+	+	+	+	+	+	+	+	+	+	+	+
7	3.6	+	+	+	+	+	+	+	+	+	+	+	+	+
8	3.4	+	+	+	+	+	+	+	+	+	+	+	+	+
9	3.2	+	+	+	+	+	+	+	+	+	+	+	+	+
10	3.0	+	+	+	+	+	+	+	+	+	+	+	+	+
11	2.8	+	+	+	+	+	+	+	+	+	+	+	+	+
12	2.6	+	+	+	+	+	+	+	+	+	+	+	+	+
13	2.4	+	+	+	+	+	+	+	+	+	+	+	+	+
14	2.2	+	+	+	+	+	+	+	+	+	+	+	+	+
15	2.0	+	+	+	+	+	+	+	+	+	+	+	+	+
16	0.9	-	-	-	+	+	+	+	-	-	-	+	+	-
17	0.7	+	+	+	+	+	+	+	+	+	+	+	+	+
18	0.5	+	+	+	+	+	+	+	+	+	+	+	+	+
19	0.3	+	+	+	+	+	+	+	+	+	+	+	+	+
20	0.1	+	+	+	+	+	+	+	+	+	+	+	+	+

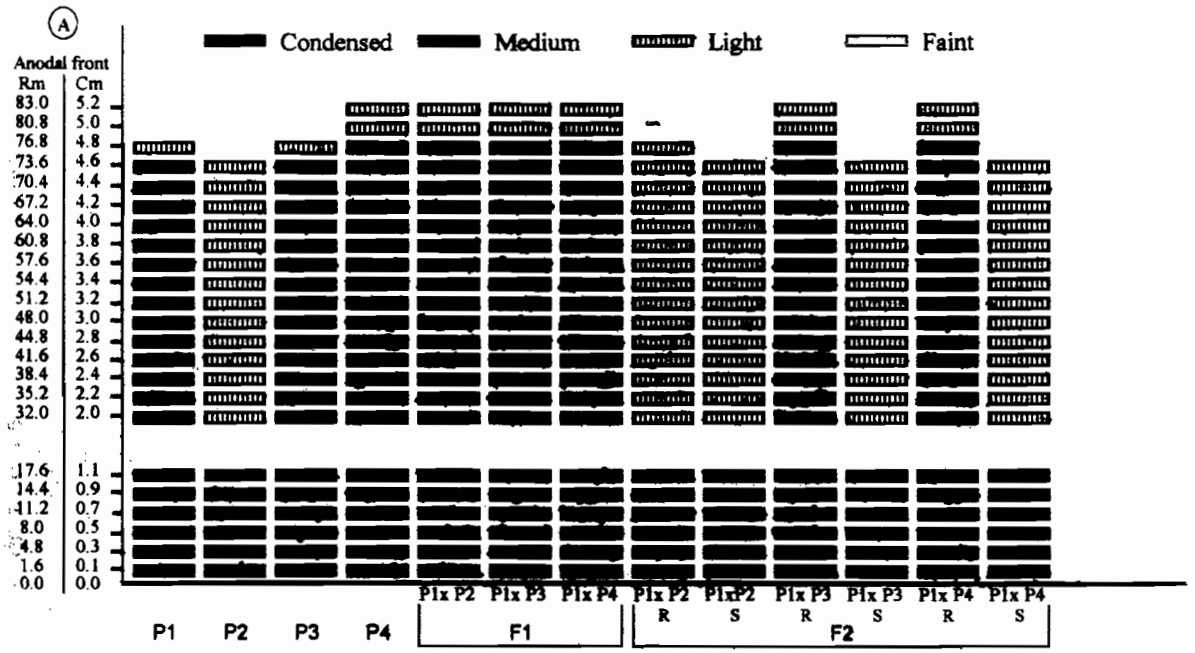

Fig4: Schemotic representation showing the migration distance and relative mobility (Rm) of Esterase electrophoretic bands observed in leaf tissue extracts of four parental genotypes and their F1 and F2 generations of beans after inoculation with *M. phaseolina*

P1= Nebraska P2= Giza 6 R= Resistant

P3= S1 P4= Morgan S= Susceptible

Table (6): Isozyme expression of esterase banding patterns represented as presence (+) or absence (-) of different isozyme variants in leaf tissue extracts of bean after inoculation with *M. phaseolina*.

Bands No.	Migration distance (cm)	P1	P2	P3	P4	F1			F2					
						P1×P2	P1×P3	P1×P4	P1×P2		P1×P3		P1×P4	
									R	S	R	S	R	S
1	5.2	-	-	-	+	+	+	+	-	-	+	-	+	-
2	5.0	-	-	-	+	+	+	+	-	-	+	-	+	-
3	4.8	+	-	+	+	+	+	+	+	-	+	-	+	-
4	4.6	+	+	+	+	+	+	+	+	+	+	+	+	+
5	4.4	+	+	+	+	+	+	+	+	+	+	+	+	+
6	4.2	+	+	+	+	+	+	+	+	+	+	+	+	+
7	4.0	+	+	+	+	+	+	+	+	+	+	+	+	+
8	3.8	+	+	+	+	+	+	+	+	+	+	+	+	+
9	3.6	+	+	+	+	+	+	+	+	+	+	+	+	+
10	3.4	+	+	+	+	+	+	+	+	+	+	+	+	+
11	3.2	+	+	+	+	+	+	+	+	+	+	+	+	+
12	3.0	+	+	+	+	+	+	+	+	+	+	+	+	+
13	2.8	+	+	+	+	+	+	+	+	+	+	+	+	+
14	2.6	+	+	+	+	+	+	+	+	+	+	+	+	+
15	2.4	+	+	+	+	+	+	+	+	+	+	+	+	+
16	2.2	+	+	+	+	+	+	+	+	+	+	+	+	+
17	2.0	+	+	+	+	+	+	+	+	+	+	+	+	+
18	1.1	+	+	+	+	+	+	+	+	+	+	+	+	+
19	0.9	+	+	+	+	+	+	+	+	+	+	+	+	+
20	0.7	+	+	+	+	+	+	+	+	+	+	+	+	+
21	0.5	+	+	+	+	+	+	+	+	+	+	+	+	+
22	0.3	+	+	+	+	+	+	+	+	+	+	+	+	+
23	0.1	+	+	+	+	+	+	+	+	+	+	+	+	+

The electrophoretic banding pattern for Esterase isozymes in infected plants of four parental genotypes and their F1 and F2 generations are presented in Photograph (4), figure (4) and Table (6). The electrophoretogram of esterase in the screened leaf tissues showed that, the variant at the position 5.2 cm anodal to the origin was found the fast band while the variant at the position 0.1 cm was the slowest band. The number and concentration of esterase bands increased after infection with *M. phaseolina.* Twenty three bands were found after infection obtained among P4, F1, F2 cross (P1× $P3_R$) and (P1× $P4_R$).

The variants that appeared after inoculation by *M. phaseolina,* might mean that, fungal inoculation induces many elicitors during and after the time course of fungal development. These elicitors affect different metabolic reactions causing the appearance of the isoesterase variant of the position 5.2 cm anodal to the origin. Also, the electrophoretogram of isoesterase leaf tissues of parental genotypes and their F1 and F2 generations showed the appearance of the variant band at the position 1.1 cm anodal to the origin. Meanwhile, the zymogram of isoesterase showed that, the intensities of anodal bands at the bands had positions 0.1,0.3, 0.5, 0.7, 0.9 and 1.1 were condensed after the inoculation with *M. phaseolina.* This clearly indicated that, esterase may play a role in the active defense mechanism of the plant and could be positive biochemical markers for *M. phaseolina* resistance. Similar conclusion was reported by Roby *et al.* (1985) and Zhang and Punja (1994) who reported that the appearance or disappearance of different isoesterase molecular forms might be as a result after fungal inoculation, suggesting that a group of elicitors might be induced thereby affect the regulation mechanisms of isoesterase loci during the time course of infection and /or interfere with many metabolic pathways causing a transitory inhibition of some molecular forms of esterase through different elicitor induced during fungal infection.

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العلاقة بين نمازج حزم المشابهات الانزيمية ومقاومة مرض لفحة الساق الرمادية في الفاصوليا

احمد حسن فايد*، السيد محمود ابراهيم محجوب*، مجدي ابراهيم غنيم**، هشام محمد كامل*

*قسم الوراثة-كلية الزراعة-جامعة الزقازيق

** معهد بحوث أمراض النباتات -مركز البحوث الزراعية-الجيزة

تـم تقيـيم ٣٠ طـراز جينـي لنبات الفاصوليا للإصابة بمرض لفحة الساق الرمادية والمسـبب له فطـر ماكروفومينا فاصيولينا تحت ظروف الصوبة وكانت معظم الطرز الجينية حساسة للإصابة بدرجات مختلفة فيما عدا الطرزين الجينيين نبرسكا وروبال نيل أظهرا مقاومة للفطـر بيـنما الطرز الجينية مورجان و ميكسكو ٣٠٩وسجما و أمي أظهروا مقاومة متوسطة للفطر. وقد اعتمدت هذه الدراسة علي التهجين بين الأب المقاوم نبرسكا وكلاً من مورجان كأب

متوســـط المقاومـــة و حيزة ٦ و S1 كأباء حساسة.أظهر التفريد الكهربي للأنزيم البيروكسديز والأســـتراز وجود ٢٠ مشابهاً انزيمياً لكل منهم قبل الإصابة بالفطر وبعد الإصابة زادت كثافة المشابهات الانزيمية وكذلك العدد إلي ٢٣ مشابهاً انزيمياً لكلا الانزيمين .التغيير في عدد وكثافة المشـــابهات الأنزيمية لكل من أنزيمي البيروكسديز و الأستراز بعد الإصابة بفطر ماكروفومينا فاصيولينا يشير الي دور كلاهما في ميكانيكية مقاومة النبات للفطر.