PEROXIDASE ISOZYME (S) AS A BIOCHEMICAL MARKER FOR CHOCOLATE SPOT DISEASE RESISTANCE GENE (S) IN FABA BEAN (*VICIA FABA* L.)

By
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ABSTRACT

The present study was conducted to test if the peroxidase isozyme could be used as a biochemical genetic marker associated with the chocolate spot disease (*Botrytis fabae*) resistance gene(s) in faba bean (*Vicia faba*). Peroxidase isozymes were extracted from the leaves of two faba bean cultivars showing different response to the disease, i.e. Giza 402 (susceptible) and Giza 461 (resistant), and their F_1, F_2 crosses in two directions. The test was made under artificial infection with two isolates of the causal pathogen in the greenhouse.

The results showed differences in number, migration and density of bands between the two cultivated varieties and their F_1 plants. Also, the artificial infection with the pathogen induced an additional band with a high molecular weight than those of the control (uninfected), parents and F_1 plants.

Out of the F_2 segregants, the peroxidase isozymes were extracted from 120 plants following the artificial infection with the two *Botrytis fabae* isolates spore suspensions of both the forward and the reciprocal crosses. The majority of the susceptible F_2 plants exhibited higher intensity of the band (No. 3) as well as, Giza 402 cultivar and F_1 plants descended from H_{12} Giza 461 (♂) x Giza 402 (♀).

The majority of F_2 resistant plants exhibited lower activity of this isozyme.

INTRODUCTION

Faba bean (*Vicia faba* L.) is one of the oldest food crops grown by man and it is used as a source of protein for both human and animals. It has a major role in crop rotation for reducing production cost by increasing soil nitrogen *via* symbiotic nitrogen fixation.

There are several diseases that cause serious damage to this crop and cause heavy losses in both yield and seed quality. These losses in Egypt were estimated to be more than 55% (Mohamed, 1982). The chocolate spot disease caused by *Botrytis fabae* was estimated to reduce the leaf area and decrease the total yield from 27% (Williams, 1975) up to 38.5% (Hanounik, 1981).

Many authors reported that the increase in peroxidase activity may be one of the plant defense systems against infection by the pathogen (Hammerchmid *et al.*, 1982; Bashad *et al.*, 1987 and Kozlowska and Krzywanski, 1989). Moreover, Sabrah and Sherif (1988) reported that, the partial resistance of Pak. and the susceptibility of Ab. faba bean cultivars could be affected or related to peroxidase isozyme.

The aim of this work was the identification of any peroxidase isozyme(s) as a biochemical genetic marker of *Vicia faba* cultivars for resistance or susceptibility to *Botrytis fabae* for early and effective selection of resistant cultivars.

MATERIALS AND METHODS

I. Materials:

I.a. Plant cultivars:

Selfed seeds of two cultivars of *Vicia faba*; Giza 461 a highly resistant and Giza 402 a highly susceptible were used as host plants for *Botrytis fabae* isolates. Dr. M. El-Hady; Field Crops Research Institute Agricultural Research Center, Giza kindly provided the seeds of these two cultivars.

I.b. Fungal isolates:

Two fungal isolates of *Botrytis fabae* were used in this study for the artificial infection; the first isolate was *B. fabae* No. 10 (virulent) and the second was *B. fabae* No. 40 (avirulent). Department of Leguminous Plant Pathology A.R.C. Giza kindly provided these isolates.

I.c. Media:

I.c.1. Potato dextrose agar (PDA) medium (Difco 1977) was used for *B. fabae* cultivation and maintenance.

I.c.2. Bean leaf extract dextrose agar (BLEDA) medium (Leash and Moore, 1966) was used for spore production.

I.d. Isozyme buffers and reagents:

Electrophoretic buffers, gel preparation, running conditions and peroxidase isozyme developing were carried out according to Stegemann *et al.* (1985) and Scandalios (1964).

II. Methods:

II.a. Field procedures:

Forward and reciprocal Crosses were made between the two parental cultivars to produce the F_1 seeds in 1997. In 1998 the F_1 plants of both crosses were grown to produce the F_2 seed by self-pollination. In 1999, an experiment was conducted in the greenhouse at Gimmiza Agric. Res. Station in a completely randomized design. Therefore, the greenhouse experiment contained the two parental cultivars, the F_1 (reciprocals) and the F_2 generations. Fifteen replications were used and each plot contained 5 plants.

II.b. *Botrytis fabae* spore production:

The maximum number of spores was collected on BLEDA medium at 20 ± 2°C for 12 days with 24 hr. fluorescent daylight according to Last and Hamley (1956), Last (1960) and Leash and Moore (1966). The final concentration of spores after dilution (25 x 10^4 spore/ml) was used

immediately for plant infection (Abou-Zeid and Le-Normand, 1979 and Abou-Zeid *et al.*, 1985).

II.c. Artificial infection:

Plants (65 days old) were artificially infected with a spore suspension for each isolate under study, using a fine mist hand sprayer. Inoculated plants were then covered with polyethylene sheets supported with metal frames to maintain a high relative humidity. Plants were uncovered and remoisted twice at 24 hr. intervals, then covered again. After 120 hr. of inoculation the infection grades were recorded and the disease severity were recorded according to the scale reported by Gondran (1975) and Abou-Zeid (1985)

II.d. Isozymes electrophoresis:

Leaves of the same position were detached from infected and non-infected parents, F_1s and F_2s plants to extract the peroxidase isozyme. One gram from each sample was used for extraction and ths isozymes were separated electrophoretically according to Scandalios (1964), Stegemann *et al.* (1985) and El-Fadly *et al* (1990).

RESULTS AND DISCUSSION

Results presented in Figure (1) and Table (1) showed that although both parents exhibited three bands (2, 3 and 5), as a maximum number of peroxidase isozymes, however, each parent showed completely different intensities. Band No 2 that was identified as faint in Giza 402 cultivar was characterized as dark in Giza 461 cultivar. The other two bands (Nos 3 and 5) which were classified as dark and very dark in Giza 402 appeared as faint and very faint in Giza 461, respectively.

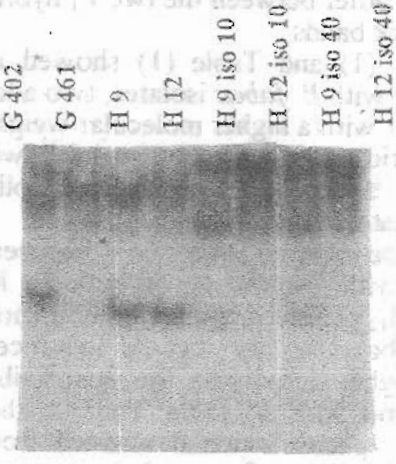

Figure (1) Peroxidase isozyme patterns of Giza 402, Giza 461 cultivars, their uninfected and infected F_1 plants with *B. fabae* 10 and 40 isolates.

* H_9 [G. 402 (♂) x G. 461 (♀)].
* H_{12} [G. 461 (♂) x G. 402 (♀)]

Table (1). Peroxidase isozyme intensity of faba bean noninfected (control) Giza 402, Giza 461 cultivars, their noninfected and infected F_1 plants with *B. fabae* 10 and 40 isolates.

Genotype	G. 402	G 461	402 x 461 (H9)	461 x 402 (H12)	Isolate 10		Isolate 40	
					H9	H12	H9	H12
Susceptibility	Cont.	Cont.	Cont.	Cont.	R	S	R	S
Band No.								
1	0	0	0	0	2	4	3	4
2	2	3	4	4	1	1	0	0
3	3	2	1	2	1	3	1	2
4	0	0	0	0	0	2	0	0
5	4	1	4	4	2	0	2	0

0 = absent 1 = very faint 2 = faint 3 = dark 4 = very dark

These results showed clearly that the activities of peroxidase isozymes in the susceptible cultivar of faba bean (Giza 402) were (in general) higher than that of the resistant parent (Giza 461). The same finding was obtained in a previous study made by Tarrad *et al.* (1993), where they found that the peroxidase activities were increased by infection in contrast to healthy leaf tissues. Also, El-Shimi (1998) observed an increasing activity of peroxidase associated with the susceptibility of *Cucumis melo* to the infection with *Pseudoperonospora cubensis*.

The hybrid F_1 plants via cross of the Giza 402 (♂) x Giza 461 (♀) and its reciprocal exhibited a slight difference at only band 3, since it was very faint when Giza 461 was used as a female parent while it was faint in the reciprocal F_1 hybrid. Moreover, the other two bands (2 and 5) did not differ between the two F_1 hybrids ,since they consistently appeared as very dark bands.

Both Figure (1) and Table (1) showed also that when the hybrid plants were infected with *B. fabae* isolates, two additional bands appeared. A common band No.1 with a higher molecular weight than the original ones of the uninfected hybrid plants was obtained following the infection with both isolates. The other band No.4 was induced following the infection of H_{12} plants with only isolate 10.

Moreover, the isozyme band No. 2 disappeared completely as a result of the infection with isolate 40 of both F_1 hybrids. Also, band No.5 disappeared when H_{12} plants were infected with either 10 or 40 isolates.

From the above results it could be noticed that peroxidase isozyme No. 3 exhibited higher activity in the susceptible faba bean cultivar (Giza 402) to the infection with *B. fabae* than in the resistant one (Giza 461). Moreover, the F_1 plants which descended from Giza 402 as a female exhibited the same activity of peroxidase isozyme 3 either without infection or following the artificial infection with the both *B. fabae* isolates. Therefore, 120 plants from each F_2 (as a segregating generation) were used to study the

association between the resistance or susceptibility to *B. fabae* infection and peroxidase isozyme 3 activity.

Figure (2) and Table (2) show the peroxidase isozyme polymorphic patterns and the degree of activities for each tested F_2 descendant plants from the cross (H9) between Giza 402 as a male and Giza 461 as a female and their response to the infection with *B. fabae* isolate 10.

The data indicated that the majority of the F_2 plants exhibited three peroxidase isozymes. Fifteen plants (No. 46-60) showed an extra peroxidase isozyme. Moreover, the majority of the infected plants, i.e., 98 out of the tested 120 plants with 81.67 percentage were classified as resistant plants to *B. fabae* isolate 10. The remaining 22 plants were susceptible to the same isolate.

To correlate the chocolate spot disease severity and peroxidase isozyme activity in this cross, peroxidase isozyme 3 presented high activity, since it was classified as dark and very dark in 20 susceptible out of 22 plants with 90.91 percent; the remaining two plants (No. 27 and 36) exhibited faint band for the same isozyme. On the contrary, 18 plants (18.37%) out of 98 resistant ones showed the same degree of peroxidase isozyme 3 activity since it appeared as dark or very dark band. The majority of the resistant plants to *B. fabae* isolate 10 showed faint band of the peroxidase isozyme No.3.

Figure (3) and Table (3) show the peroxidase isozyme polymorphic patterns of 120 F_2 plants obtained from the reciprocal cross (H_{12}) between Giza 461 (resistant male) and Giza 402 (susceptible female) of faba bean following the infection with *B. fabae*, virulent isolate 10.

The results revealed that the activity of peroxidase isozymes in the plants of this cross (H_{12}) has increased as a result of isolate 10 infection compared with that obtained in the forward cross (H_9) infected plants with the same isolate. The peroxidase isozyme bands number increased to five (as a maximum number of peroxidase isozyme) in H_{12} plants infected with isolate 10.

Moreover, data given in Table (3) showed that 92 out of the tested 120 plants with 76.67 percentage proved to be susceptible to *B. fabae* isolate 10. Out of these susceptible plants, 82 with 89.13 percent exhibited dark peroxidase isozyme band 3. Meanwhile, 10.87 percentage of the susceptible plants showed faint band of the same band. On contrary, the majority of the resistant plants i.e., 26 out of 28 resistant ones exhibited faint peroxidase isozyme band 3. The remaining two resistant plants showed the same degree of darkness as the majority of the susceptible ones.

Figure (4) and Table (4) illustrate peroxidase isozyme patterns and their intensity as well as the F_2 plants of H_9 cross response to *B. fabae* isolate 40.

The result shows that the majority of the tested plants exhibited three peroxidase isozymes, while 13 plants showed an extra faint band.

Data in Table (4) clearly show that 106 out of the tested 120 plants with percentage of 88.33 proved to be resistant to the chocolate spot disease

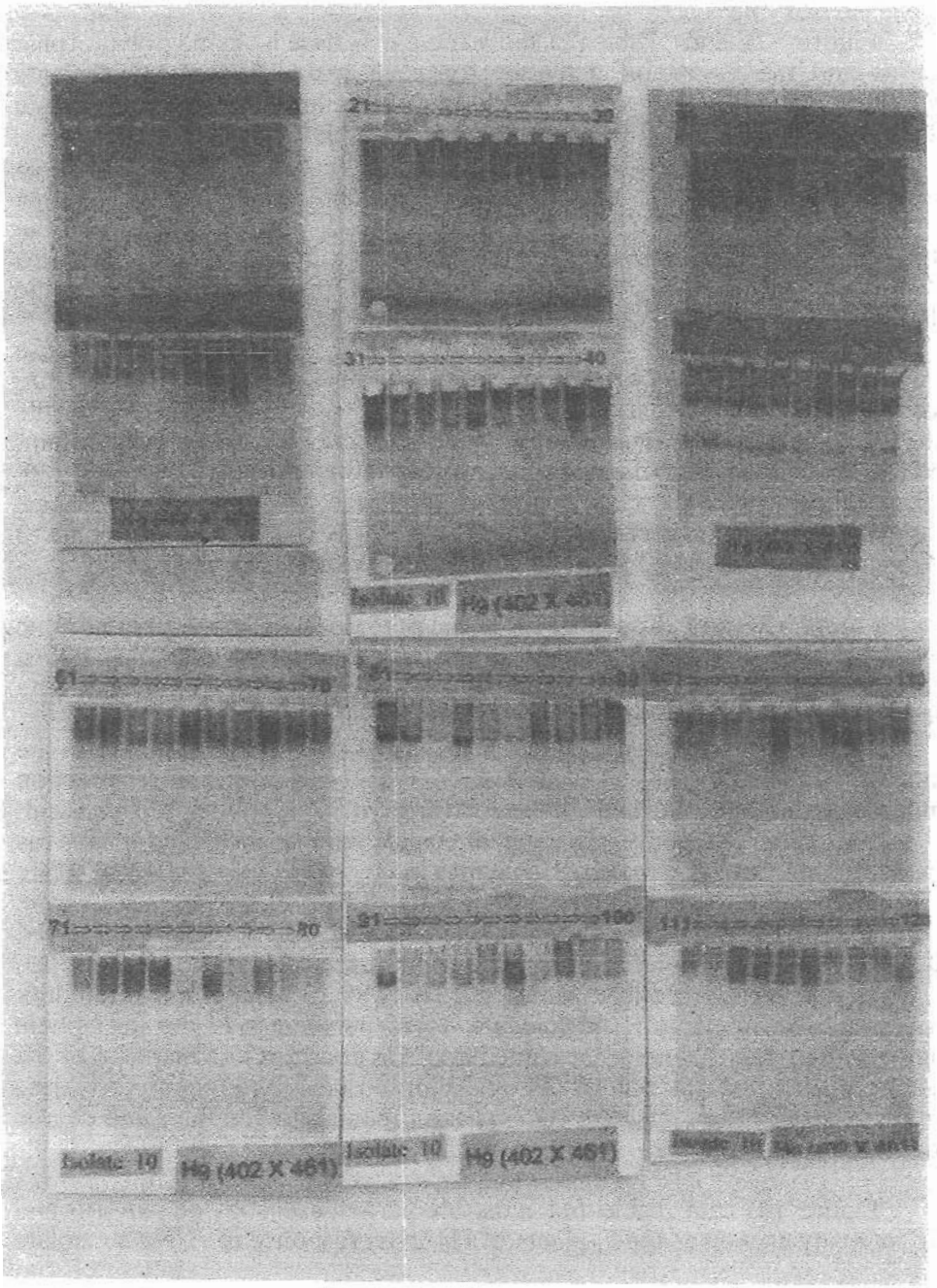

Figure (2). Peroxidase isozyme patterns of isolate 10 infected F_2 plants obtained from Giza 402 (♂) x Giza 461 (♀) cross

Table (2): Response of G 402 (♂) × G 461 (♀) F2 plants infected with *Botrytis fabae* isolate 10 and their peroxidase isozyme intensity.

Plant No.	F2 1	F2 2	F2 3	F2 4	F2 5	F2 6	F2 7	F2 8	F2 9	F2 10	F2 11	F2 12	F2 13	F2 14	F2 15	F2 16	F2 17	F2 18	F2 19	F2 20	F2 21	F2 22	F2 23	F2 24	F2 25	F2 26	F2 27	F2 28	F2 29	F2 30
Suscep-Ptibility	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	S	S	S	R	R	R	R	R	R	R	R	S	R	R	R
Band No.																														
1	2	2	2	2	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
2	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2
3	2	2	2	2	2	2	2	3	2	2	2	3	4	2	2	4	4	4	2	2	2	2	2	2	2	2	2	2	2	2
4																														
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Plant No.	F2 31	F2 32	F2 33	F2 34	F2 35	F2 36	F2 37	F2 38	F2 39	F2 40	F2 41	F2 42	F2 43	F2 44	F2 45	F2 46	F2 47	F2 48	F2 49	F2 50	F2 51	F2 52	F2 53	F2 54	F2 55	F2 56	F2 57	F2 58	F2 59	F2 60
Suscep-Ptibility	R	R	R	R	R	S	R	R	R	R	S	S	S	R	R	R	R	R	R	R	R	R	R	R	S	R	S	S	S	S
Band No.																														
1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	2	2	2	2	3	2	3	3	3	3
2	2	0	2	2	0	2	2	0	0	0	3	0	0	2	2	2	2	0	0	0	0	0	2	2	2	2	0	0	0
3	3	2	2	2	3	2	2	2	4	2	4	4	4	4	3	3	3	2	4	4	2	2	2	2	3	2	4	4	4	4
4																														
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3

Plant No.	F2 61	F2 62	F2 63	F2 64	F2 65	F2 66	F2 67	F2 68	F2 69	F2 70	F2 71	F2 72	F2 73	F2 74	F2 75	F2 76	F2 77	F2 78	F2 79	F2 80	F2 81	F2 82	F2 83	F2 84	F2 85	F2 86	F2 87	F2 88	F2 89	F2 90
Suscep-Ptibility	R	R	R	R	S	R	R	S	S	R	R	S	S	S	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R
Band No.																														
1	2	2	2	2	2	2	2	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0	2	2
2	0	0	0	0	0	0	0	4	2	2	0	3	3	4	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
3	2	2	2	2	3	2	2	4	3	2	2	3	3	3	1	2	2	2	2	2	3	3	2	3	2	2	2	2	2	2
4																														
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Plant No.	F2 91	F2 92	F2 93	F2 94	F2 95	F2 96	F2 97	F2 98	F2 99	F2 100	F2 101	F2 102	F2 103	F2 104	F2 105	F2 106	F2 107	F2 108	F2 109	F2 110	F2 111	F2 112	F2 113	F2 114	F2 115	F2 116	F2 117	F2 118	F2 119	F2 120
Suscep-Ptibility	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R
Band No.																														
1	2	2	2	0	2	2	2	2	2	2	2	2	2	2	2	2	3	2	2	2	2	2	3	2	2	3	2	2	2	0
2	0	0	0	2	0	2	0	0	0	0	0	0	0	0	2	2	2	3	2	2	2	2	3	3	3	4	2	2	0	2
3	3	2	2	2	2	3	2	2	2	2	2	2	2	2	3	0	2	3	2	2	0	0	3	2	2	4	2	2	2	2
4																														
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

0 = absent 1 = very faint 2 = faint 3 = dark 4 = very dark S = Susceptible R= Resistant

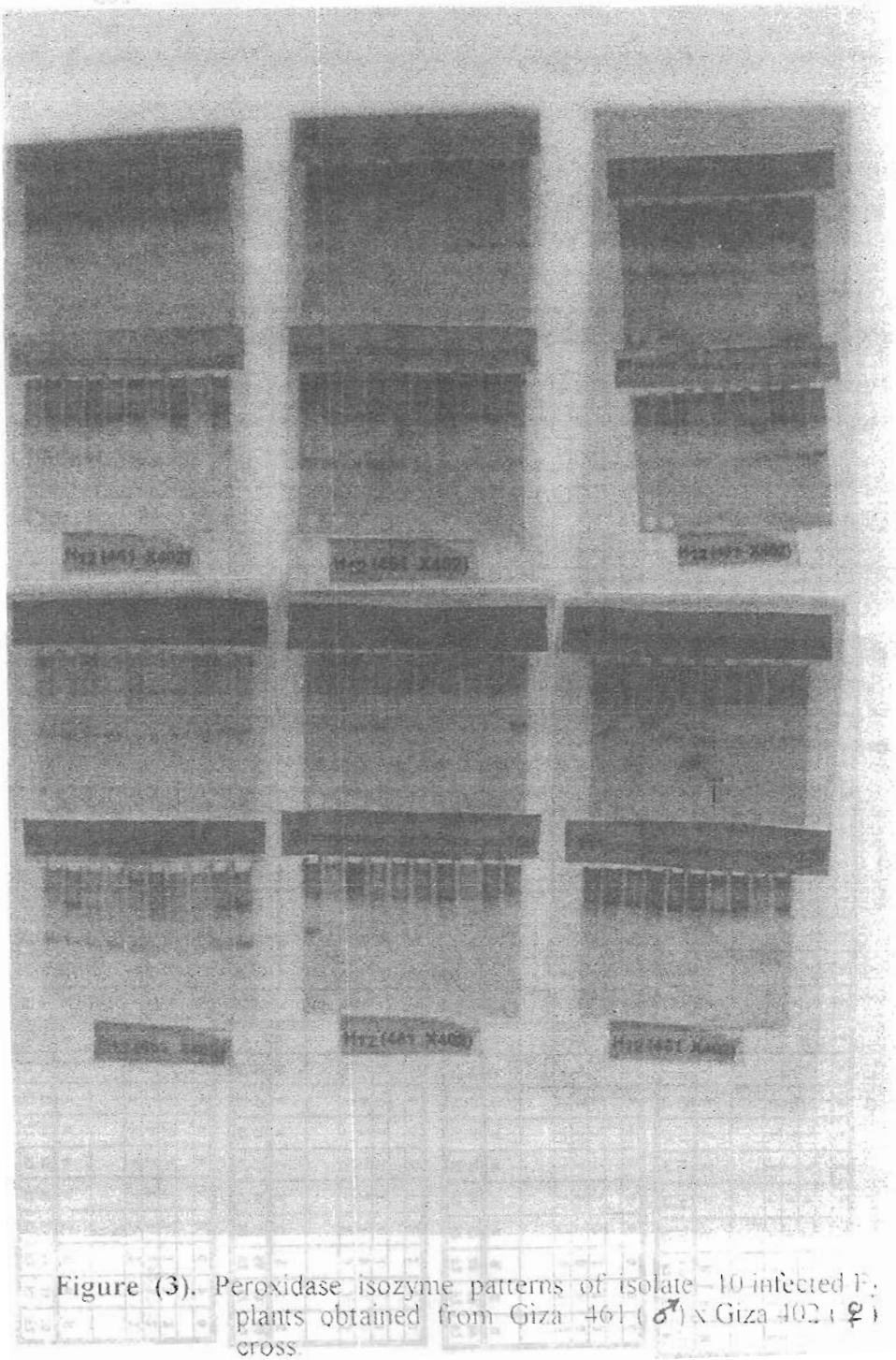

Figure (3). Peroxidase isozyme patterns of isolate 10 infected F$_2$ plants obtained from Giza 461 (♂) x Giza 402 (♀) cross.

Table (3): Response of G461(♂) × G402(♀) F2 plants infected with *Botrytis fabae* isolate 10 and their peroxidase isozyme intensity.

Plant No.	F2 1	F2 2	F2 3	F2 4	F2 5	F2 6	F2 7	F2 8	F2 9	F2 10	F2 11	F2 12	F2 13	F2 14	F2 15	F2 16	F2 17	F2 18	F2 19	F2 20	F2 21	F2 22	F2 23	F2 24	F2 25	F2 26	F2 27	F2 28	F2 29	F2 30
Susceptibility	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S
Band No.																														
1	2	0	2	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3	2	2	2	3	2
2	2	1	1	0	1	1	1	1	1	1	2	2	2	2	2	0	3	2	3	2	3	2	2	0	2	2	2	3	3	1
3	3	4	2	4	2	4	4	4	4	3	3	4	4	4	4	3	3	4	3	4	4	4	4	2	4	4	4	4	4	3
4	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	2	0	3	0	0	0	0	0	0	0	0	0	0
5																														
6																														
7	2	2	2	2	2	2	2	2	2	0	2	0	2	2	2	2	2	2	2	2	2	2	2	0	3	2	2	2	2	3

Plant No.	F2 31	F2 32	F2 33	F2 34	F2 35	F2 36	F2 37	F2 38	F2 39	F2 40	F2 41	F2 42	F2 43	F2 44	F2 45	F2 46	F2 47	F2 48	F2 49	F2 50	F2 51	F2 52	F2 53	F2 54	F2 55	F2 56	F2 57	F2 58	F2 59	F2 60
Susceptibility	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	S	S	S
Band No.																														
1	3	3	2	2	2	2	3	2	2	2	2	3	3	3	3	3	3	2	3	3	2	2	2	2	2	2	2	3	3	2
2	3	2	2	2	2	3	4	2	2	2	0	4	0	2	2	0	0	0	0	0	1	2	2	0	2	2	2	2	2	2
3	4	3	3	3	2	2	3	3	3	2	4	4	4	4	4	4	4	3	3	3	2	2	2	1	2	2	2	4	3	2
4	0	0	0	0	0	0	2	0	0	0	0	1	0	0	0	3	0	1	1	1	0	0	0	0	0	0	0	0	0	0
5																														
6																														
7	2	3	2	3	2	2	2	2	2	3	3	3	3	3	2	3	2	2	3	3	2	2	2	2	2	2	2	2	3	3

Plant No.	F2 61	F2 62	F2 63	F2 64	F2 65	F2 66	F2 67	F2 68	F2 69	F2 70	F2 71	F2 72	F2 73	F2 74	F2 75	F2 76	F2 77	F2 78	F2 79	F2 80	F2 81	F2 82	F2 83	F2 84	F2 85	F2 86	F2 87	F2 88	F2 89	F2 90
Susceptibility	S	R	S	R	R	S	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	R	R	S
Band No.																														
1	2	2	2	2	2	2	2	2	2	2	2	2	3	2	3	2	2	2	0	2	3	2	2	2	2	2	3	2	2	2
2	0	0	0	0	0	0	2	2	0	0	0	0	2	1	1	0	0	0	0	2	0	0	0	0	0	0	0	0	0	2
3	2	2	3	2	3	3	3	3	2	2	3	3	3	3	3	3	2	2	3	3	2	3	3	4	2	2	4	2	2	3
4																														
5																														
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0	0
7	3	2	2	2	2	2	2	2	0	0	3	2	2	3	3	2	2	2	2	3	2	2	3	3	2	2	0	0	0	3

Plant No.	F2 91	F2 92	F2 93	F2 94	F2 95	F2 96	F2 97	F2 98	F2 99	F2 100	F2 101	F2 102	F2 103	F2 104	F2 105	F2 106	F2 107	F2 108	F2 109	F2 110	F2 111	F2 112	F2 113	F2 114	F2 115	F2 116	F2 117	F2 118	F2 119	F2 120
Susceptibility	S	R	S	R	R	R	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S
Band No.																														
1	2	2	3	2	3	3	3	2	3	3	3	3	0	2	2	2	0	3	2	2	3	2	2	2	2	3	2	3	2	4
2	2	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
3	3	1	2	2	2	2	3	2	2	2	4	4	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	2	4
4	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5																														
6																														
7	3	0	0	2	0	0	0	0	0	2	2	0	2	2	0	2	2	0	2	2	0	0	0	0	0	0	0	0	0	0

0 = absent 1 = very faint 2 = faint 3 = dark 4 = very dark S = Susceptible R = Resistant

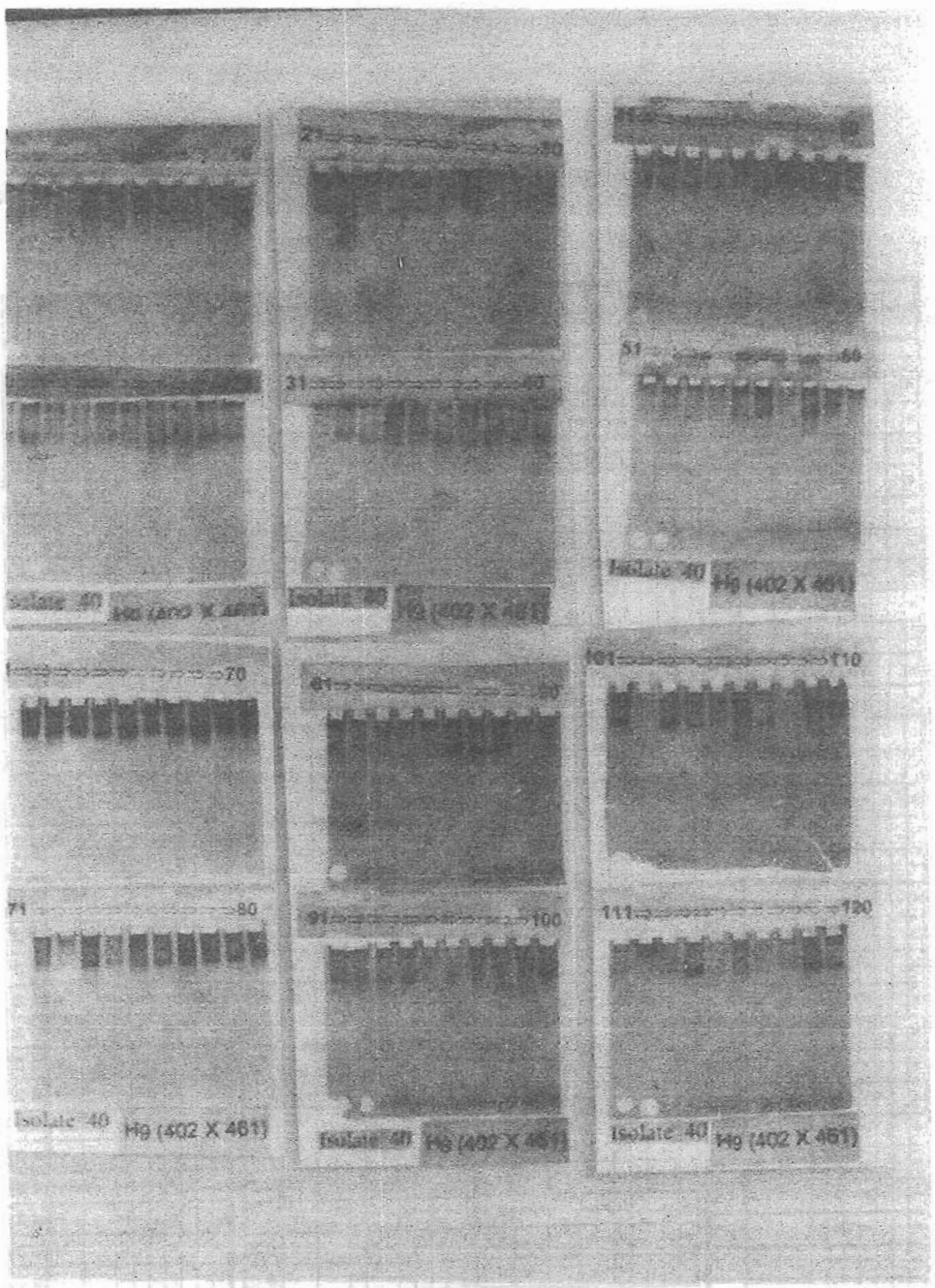

Figure (4). Peroxidase isozyme patterns of isolate 40 infected F1 plants obtained from Giza 402 (♂) x Giza 461 (♀) cross.

Table (4): Response of G402 (♂)× G 461 (♀)- F2 plants infected with *Botrytis fabae* isolate 40 and their peroxidase isozyme intensity.

Plant No.	F2 1	F2 2	F2 3	F2 4	F2 5	F2 6	F2 7	F2 8	F2 9	F2 10	F2 11	F2 12	F2 13	F2 14	F2 15	F2 16	F2 17	F2 18	F2 19	F2 20	F2 21	F2 22	F2 23	F2 24	F2 25	F2 26	F2 27	F2 28	F2 29	F2 30
Suscep-Ptibility	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	S	R	R	R
Band No.																														
1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	2	2	2
2	0	0	0	0	2	2	0	2	0	0	0	0	0	0	0	0	2	2	0	2	2	3	0	0	2	2	0	0	0	0
3	2	2	2	3	2	2	2	2	2	3	2	2	2	2	2	2	2	2	2	2	3	4	2	2	2	2	3	2	2	2
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0
5																														

Plant No.	F2 31	F2 32	F2 33	F2 34	F2 35	F2 36	F2 37	F2 38	F2 39	F2 40	F2 41	F2 42	F2 43	F2 44	F2 45	F2 46	F2 47	F2 48	F2 49	F2 50	F2 51	F2 52	F2 53	F2 54	F2 55	F2 56	F2 57	F2 58	F2 59	F2 60
Suscep-Ptibility	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R
Band No.																														
1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
2	0	0	0	2	0	0	0	0	0	0	0	0	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	1	2	2	2	2	2	2	2	2	2	2	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	2	2
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5																														

Plant No.	F2 61	F2 62	F2 63	F2 64	F2 65	F2 66	F2 67	F2 68	F2 69	F2 70	F2 71	F2 72	F2 73	F2 74	F2 75	F2 76	F2 77	F2 78	F2 79	F2 80	F2 81	F2 82	F2 83	F2 84	F2 85	F2 86	F2 87	F2 88	F2 89	F2 90
Suscep-Ptibility	S	S	R	S	S	R	R	S	R	R	R	R	R	R	R	R	R	S	S	R	R	R	R	R	R	R	R	R	R	R
Band No.																														
1	3	3	3	2	2	3	3	2	3	3	2	2	2	2	2	2	3	2	2	2	2	2	2	2	2	2	2	2	2	2
2	2	2	0	0	3	0	0	2	3	4	2	2	0	0	2	0	2	0	0	2	0	2	2	2	0	2	0	2	2	2
3	3	3	4	3	4	3	3	4	4	4	2	2	2	2	2	3	3	3	4	4	3	2	2	2	2	3	3	3	2	2
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	2
5																														

Plant No.	F2 91	F2 92	F2 93	F2 94	F2 95	F2 96	F2 97	F2 98	F2 99	F2 100	F2 101	F2 102	F2 103	F2 104	F2 105	F2 106	F2 107	F2 108	F2 109	F2 110	F2 111	F2 112	F2 113	F2 114	F2 115	F2 116	F2 117	F2 118	F2 119	F2 120
Suscep-Ptibility	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	R	R	R	R
Band No.																														
1	2	2	2	2	2	2	2	2	2	2	3	2	2	2	2	2	2	0	2	2	0	2	2	2	2	2	2	2	2	2
2	0	0	0	0	0	0	2	2	2	2	0	0	0	2	2	2	2	2	2	2	2	2	2	0	2	2	2	2	2	2
3	3	3	2	3	2	2	2	2	2	4	4	0	3	2	2	2	2		2	2	2		2	3	2	2	2	2	4	3
4	2	2	2	0	0	0	2	2	2	2	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5																														

0 = absent 1 = very faint 2 = faint 3 = dark 4 = very dark S = Susceptible R = Resistant

caused by the infection with *B. fabae* isolate 40. The remaining 14 plants with percentage of 11.67 exhibited susceptible symptoms to the same isolate infection.

Twelve out of the 14 susceptible plants (85.71%) exhibited dark or very dark intensity for peroxidase isozyme band 3. The other two susceptible plants showed faint intensity for the same band. On the other hand, 21 resistant plants with percentage of 19.81 out of the 106 resistant ones showed dark peroxidase isozyme band 3 similar to that of the susceptible plants.

Figure (5) and Table (5) illustrate peroxidase isozyme patterns of the F_2 descendant plants from H_{19} of the cross of Giza 461 as a male and Giza 402 as a female and infected by *B. fabae* isolate 40.

The obtained results indicated that 67 out of the 120 tested plants with 55.8 percent proved to be resistant to chocolate spot disease. The remaining plants (53 plants) with 44.2 percent were susceptible to the same disease. Regarding band 3 activity and plant response to *B. fabae* isolate 40 infection, the results proved that 38 (71.7%) out of the 53 susceptible ones exhibited high activity of the peroxidase isozyme band 3. Meanwhile, 15 plants with percentage 28.3 from the susceptible plants showed either faint or complete absence of band 3. On the contrary, 3 resistant out of the 67 ones with percentage of 4.5 showed higher activity of band 3 than the 64 resistant plants.

Regarding the correlation between the peroxidase isozymes activity and chocolate spot disease resistance or susceptibility of faba bean plants, it could be concluded that, generally the peroxidase activity was higher in the majority of the susceptible plants than the resistant ones. Band 3 of the electrophoretic peroxidase isozyme patterns exhibited higher activity in the majority of the susceptible plants. Therefore, it could be considered as a preliminary marker for the early discrimination of susceptible faba bean cultivars to chocolate spot disease in breeding programs.

These results are in a good harmony with those reported earlier by Urbanek *et al.* (1987), (Sabrah and Sherif, 1988), (Patykowski *et al.*, 1992) and (Tarrad *et al.*, 1993) since they found that the peroxidase activities increased in faba bean leaves as a result of chocolate spot disease infection in contrast to the healthy leaf tissues. Moreover, Valazhahan and Vidhyasekaran (1994) reported that the inoculation with the pathogen resulted in an increase of peroxidase activity in susceptible varieties of groundnut to the rust disease caused by *Puccinia arachidis*.

The results also showed that the infection with the pathogen induced changes in the kinds of peroxidase isozyme. This result is in an excellent harmony with the results obtained by Okiror *et al.* (1982) since they found that the infection with anthracnose induced change in the kinds of peroxidase isozyme in faba bean lines and El-Fadly *et al.* (1990) who also found an increase in the band numbers in faba bean after infection with broad bean stain virus.

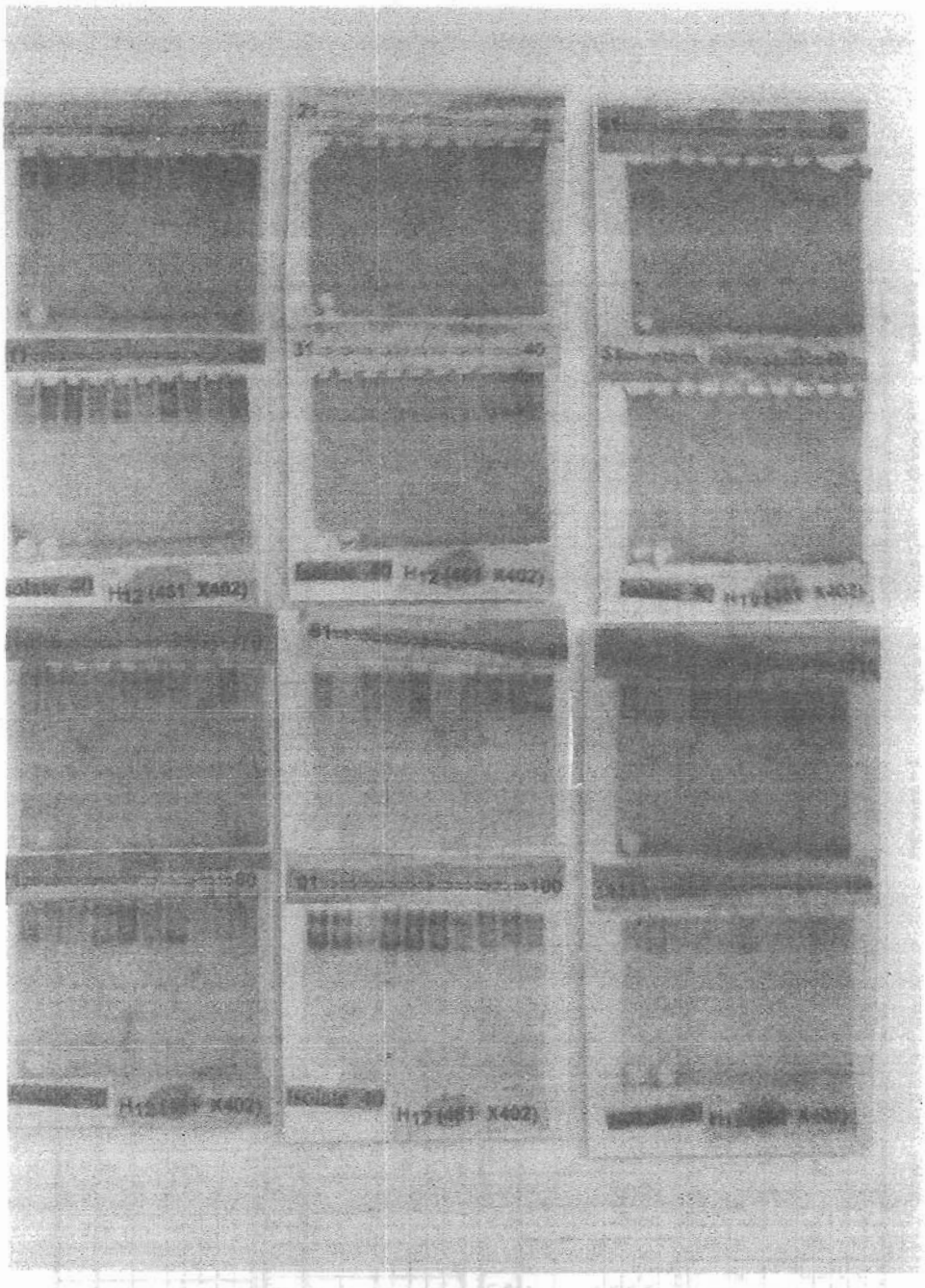

Figure (5). Peroxidase isozyme patterns of isolate 40 infected F1 plants obtained from Giza 401 (♂) x Giza 402 (♀) cross

Table (5): Response of G 461(♂) x G 402 (♀)- F2 plants infected with *Botrytis fabae* isolated 40 and their peroxidase isozyme intensity.

Plant No.	F2 1	F2 2	F2 3	F2 4	F2 5	F2 6	F2 7	F2 8	F2 9	F2 10	F2 11	F2 12	F2 13	F2 14	F2 15	F2 16	F2 17	F2 18	F2 19	F2 20	F2 21	F2 22	F2 23	F2 24	F2 25	F2 26	F2 27	F2 28	F2 29	F2 30
Suscep-Ptibility	R	R	R	R	R	R	R	S	R	S	R	R	S	S	S	R	S	R	R	S	R	R	R	R	R	R	R	S	R	R
Band No.																														
1	3	3	2	2	2	2	2	3	2	2	2	3	2	2	2	2	3	2	2	3	0	0	0	0	0	0	0	0	0	0
2	2	2	2	2	2	2	2	0	2	2	2	2	3	3	2	2	2	2	2	0	0	0	0	0	0	0	0	0	0	0
3	2	3	2	2	2	2	2	4	2	3	2	3	3	2	2	2	2	2	2	4	2	2	2	2	2	2	2	3	2	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5																														

Plant No.	F2 31	F2 32	F2 33	F2 34	F2 35	F2 36	F2 37	F2 38	F2 39	F2 40	F2 41	F2 42	F2 43	F2 44	F2 45	F2 46	F2 47	F2 48	F2 49	F2 50	F2 51	F2 52	F2 53	F2 54	F2 55	F2 56	F2 57	F2 58	F2 59	F2 60
Suscep-Ptibility	R	R	S	R	R	R	R	R	S	R	S	R	R	R	R	R	S	S	R	R	R	R	S	R	R	R	R	R	R	R
Band No.																														
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	2	2	2	3	2	2	2	2	2	3	2	2	2	2	2	0	0	3	3	2	0	0	3	2	2	2	3	2	0	2
4	0	2	3	0	3	3	0	0	4	0	4	0	3	3	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
5																														

Plant No.	F2 61	F2 62	F2 63	F2 64	F2 65	F2 66	F2 67	F2 68	F2 69	F2 70	F2 71	F2 72	F2 73	F2 74	F2 75	F2 76	F2 77	F2 78	F2 79	F2 80	F2 81	F2 82	F2 83	F2 84	F2 85	F2 86	F2 87	F2 88	F2 89	F2 90
Suscep-Ptibility	S	S	R	R	S	S	S	R	S	R	S	S	S	S	S	R	S	R	S	R	S	R	S	S	S	R	R	S	S	S
Band No.																														
1	2	2	3	3	3	3	2	3	2	3	2	3	3	3	4	3	2	2	2	3	2	2	3	3	4	3	3	2	3	3
2	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	2	0	2	2	4	0	0	2	0	0
3	3	3	2	2	2	2	3	2	0	2	3	3	3	4	4	2	4	2	3	2	3	2	3	3	4	2	2	3	4	3
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5																														

Plant No.	F2 91	F2 92	F2 93	F2 94	F2 95	F2 96	F2 97	F2 98	F2 99	F2 100	F2 101	F2 102	F2 103	F2 104	F2 105	F2 106	F2 107	F2 108	F2 109	F2 110	F2 111	F2 112	F2 113	F2 114	F2 115	F2 116	F2 117	F2 118	F2 119	F2 120
Suscep-Ptibility	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S	R	S	S	S	R	S	R	S	R	R	R	R	R	R
Band No.																														
1	3	3	2	2	3	3	3	2	2	2	3	2	1	2	2	2	2	2	2	2	1	2	2	2	0	2	0	2	2	2
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
3	4	3	3	4	4	4	3	2	3	2	3	2	0	2	2	2	0	3	3	2	2	2	1	3	2	2	0	1	1	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5																														

0 = absent 1 = very faint 2 = faint 3 = dark 4 = very dark S = Susceptible R = Resistant

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استخدام المشابهات الإنزيمية للبيروكسيديز كدلائل بيوكيميائية لجينات المقاومة لمرض التبقع الشيكولاتى فى الفول البلدى

الملخص العربى

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أجرى هذا البحث بهدف تحديد كاشفات وراثية بيوكيميائية (البيروكسيديز) ذات علاقة بمقاومة مرض التبقع الشيكولاتى الناتج عن العدوى بفطر بوترايتس فابى للفول البلدى. وترجع أهمية ذلك إلى الأنتخاب السريع والدقيق لأصناف وسلالات الفول البلدى المقاومة لهذا المرض قبل الزراعة. وبذلك يمكن تجنب الخسائر الجسيمة التى تنتج عن الإصابة بهذا المرض الذى كثيرا ما يؤدى إلى فقد نسبة كبيرة من المحصول قد تصل لحد الموت الكامل للنبات الحساس وبالتالى الفقد التام لمحصول البذور من الفول البلدى.

للوصول لهذا الغرض فقد تم استخدام صنفين من الفول البلدى إحدهما حساس للإصابة بالمرض (جيزة ٤٠٢) والآخر مقاوم (جيزة ٤٦١) وتم التهجين وزرعت البذور الهجينية للحصول على نباتات الجيل الثانى التى زرعت عام ١٩٩٩ مع بذور الأباء والجيل الأول للحصول على نباتات الأباء وكل من الجيلين الأول والثانى بتصميم كامل العشوائية.

تمت العدوى الصناعية لكل من نباتات الآباء والجيل الأول والجيل الثانى بالرش بمعلق جرثومى من سلالتين مختلفتين من فطر بوترايتس فابى ، إحداهما ذات مقدرة مرضية عالية (عزلة ١٠) والأخرى ذات مقدرة مرضية منخفضة (عزلة ٤٠).

وقد أوضحت نتائج التفريد الكهربى لمشابهات أنزيم البيروكسيديز الآتى:

١- إختلافات فى كثافة الحزم بين كل من الصنفين ونباتات الجيل الأول لكل من الهجين والهجين العكسى. بينما أدت العدوى بأى من عزلتى الفطر إلى ظهور حزمة إضافية ذات وزن جزيئ أعلى من الحزم الخاصة بالنباتات غير المعداه.

٢- من نباتات الجيل الثانى فقد تم الأستخلاص والتفريد الكهربى لمشابهات إنزيم البيروكسيديز من مائة وعشرين نباتاً مختلفاً من كلا الهجين والهجين العكسى بعد رش تلك النباتات بمعلق جرثومى من كلا العزلتين.

٣- أظهر المشابه الأنزيمى رقم (٣) للبيروكسيديز نشاطا أعلى فى معظم نباتات الجيل الثانى الحساسة لمرض التبقع الشيكولاتى بالإضافة إلى نباتات الصنف الحساس جيزة (٤٠٢) ونباتات الجيل الأول من الهجين رقم (١٢).

٤- كما أظهر عدد قليل من النباتات المقاومة للمرض نشاطا عاليا أيضا لنفس المشابه الأنزيمى رقم (٣) على الرغم من أن الغالبية العظمى من النباتات المقاومة كانت ذات نشاط ضعيف لهذا المشابه الأنزيمى لذلك يمكن اعتبار هذا المشابه الأنزيمى ككاشف بيوكيميائى أولى لجينات المقاومة لمرض التبقع الشيكولاتى فى برامج التربية للفول البلدى.