THE ACTIVITY OF PEROXIDASE AND ESTERASE ISOZYMES IN BROADBEANS LEAVES INFECTED WITH Botrytis fabae.

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نشاط مشابهات انزيمات البيروكسيديز والأستيريز فى أوراق الفول البلدى المصابة بال Botrytis fabae

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ملخص البحث

حددت الاختلافات فى مشابهات انزيمات البيروكسيديز والأستيريز فى أربعة سلالات احداهم محلية والثلاثة الأخريات مستوردة وهى G-1 (محلية) و C.R. و Ab. و Pak. (مستوردة) وكذلك فى الهجين بين السلالة المحلية والسلالات الثلاثة المستوردة وهى G-1 x C.R. و G-1 x Ab. و G-1 x Pak. · وقد أختبرت هذه التراكيب الوراثية بالنسبة للمقاومة والحساسية لمرض تبقع الأوراق الشيكولاتى المتسبب عن الـ Botrytis fabae·

وجد أن السلالة Pak. كانت أكثر مقاومة بينما السلالة Ab. كانت حساسة بينما أظهرت السلالتين G-1 ، C.R. درجات متوسطة من المقاومة وذلك حسب الـ Disease index المحسوب لمرض التبقع الورقى · ووجد أيضا أن التهجين المشتمل على السلالة Pak. كان أكثر مقاومة عن التهجينين الأخريين ·

بالنسبة لنشاط مشابهات انزيم البيروكسيديز ، وجد أن المشابهات المتجهة للمهبط فى الأوراق المصابة من السلالة الباكستانية كانت أكثر نشاطا من تلك الخاصة بالسلالات الأخرى المصابة فقد كانت الحزم فى الأوراق المصابة فى السلالة الباكستانية ١٠ أضعاف والسلالة ٣,٦ ضعفا وذلك قياسا بالأفراد غير المصابة · وقد أرجعت هذه الزيادة فى النشاط الى المقاومة لهذا المرض ·

أيضا وجد أن التهجين المشتمل على السلالة الباكستانية كان أعلى فى نشاطه لعدة أضعاف وذلك فى الحزم المتجهة الى المهبط من مشابهات البيروكسيديز. بالنسبة لنشاط مشابهات انزيم الاستيريز وجد أن جميع التراكيب الوراثية كانت تعطى حزما تتجه الى المصعد بنفس درجة النشاط تقريبا فى كل من الأوراق المصابة وغير المصابة .

ABSTRACT

Electrophoretic variation of peroxidase and esterase isozymes were determined for four broadbean cultivars, G-1, C.R., Ab., and Pak. and their crosses G-1 x C.R., G-1 x Ab. and G-1 x Pak. These genotypes were tested for resistance or susceptibility to chocolate leaf spot disease caused by Botrytis fabae. The Pak. cultivar was more resistant while the Ab. was susceptible and the others, G-1 and C.R., showed moderate degrees of resistance, according to the calculated disease index of leaf spot disease. The cross that involved Pak. cultivar was more resistant than the other two crosses. The activity of cathodal isoperoxidases in infected leaves of the Pakistanian cultivar was higher than those of the other infected cultivars. The activity of cathodal C_2 and C_6 bands in infected Pak. leaves were increased by 10 and 3.6 folds, respectively, more than the non-infected control. These increase of activity could be related to the resistance to this disease. The cross involved Pakistanian, was also higher by several folds in activity of cathodal isoperoxidases. The activity of anodal esterase isozymes in all genotypes were almost the same in infected and non-infected leaves.

INTRODUCTION

The broadbean (Vicia faba, L.) is one of the most important crops in Egypt. There are several diseases which cause serious damage to this crop and cause heavy losses in both yield and quality of seeds. These losses in yield, in Egypt, was estimated in the farms at Sakha, Kafr El-Sheikh Governorate, to be more than 55% (Mohamed, 1982). The brown or checolate spot disease caused by Botrytis fabae reduced the leaf area and decreased the total yield by 27% (Williams, 1975) or by 38.5% (Hanounik, 1981).

Variable results were reported concerning the electrophoretic patterns of some enzymes of resistant and susceptible plants. The leaves of broadbean infected with Botrytis fabae showed changes in the activity of esterase enzymes (Harrison, 1981) and increases in the activity of acid phosphatase enzyme (Griffith and Amin, 1978).

In other crops, the isoenzymes activities of some enzymes were increased at the site of infection with different pathogens. In potato plants, Fehrmann and Diamond (1967) and Borchert (1978), found high correlation between peroxidase activity and resistance to Phytophthora infestans or after wounding respectively. In sweet potato, Weber et al. (1967) reported that the peroxidase, polyphenol oxidase and acid phosphatase activities increase after infection with Ceratocystis fimbriata, but the esterase and alkaline phosphatase activities decreased or did not change.

Finally, in bean plants, Okiror et al. (1982) found that the infection with anthracnose induced changes in the activity and kinds of peroxidase isozymes in both resistant and susceptible lines. Surli and Rivera (1983) showed an increase in peroxidase activity in a resistant line as compared to the susceptible ones after infection with beans common mosaic. Wasfy et al. (1984) noticed that the peroxidase activity increases in hypocotyl of beans infected with Rhizoctonia solani.

MATERIALS AND METHODS

Four cultivars of broadbean (Vicia faba, L.), One local, Giza-1 (G-1) and three introduced cultivars, Cyperian Romi (C.R.), Abyssinian (Ab.) and Pakistanian (Pak.) were used in this study. These cultivars and their F_1's (G-1 x C.R.), (G-1 x Ab.) and (G-1 x Pak.) were planted in the Exp. Station of the Faculty of Agriculture,

Alexandria University, through 1984, 1985, 1986. Disease indices were calculated by using the following equation. (Horsfull and Heuberger, 1942)

$$\text{D. index} = \frac{\text{Sum of (disease class x number of plants in that class x 100}}{\text{Total number of plants x 4}}$$

to determine the effect of Botrytis fabae infection on the leaves (using 100 plants for each genotype).

The naturally infected and healthy leaves (control) of the same plant were used to measure the activity of peroxidase and esterase isozymes.

The extracts of diseased or healthy leaves were absorbed on strips of filter paper and plated on the origin line of the agar-starch-P.V.P.gel plates (Sabrah and El-Metainy, 1985). After one hour, the filter papers were removed and the electrophoretic run began for 1.5 or 2.5 hrs for peroxidase and esterase isozymes, respectively. After electrophoresis, the plates of peroxidase were stained with H_2O_2-benzidine solution in 0.01 M sodium acetate acetic acid buffer, pH 5.0; while the plates of esterase were stained with Fast blue RR, α-and β-naphthyle acetate in 0.01 M tris-Hcl buffer, pH 7.0. The optical density of the isoperoxidases and isoesterases were measured at 400 and 575 nm, respectively, by a denistometer (CS-910 Shinadzu).

RESULTS AND DISCUSSION

Peroxidase isozymes were studied in the leaves of four broad-bean cultivars (G-1, C.R., Ab. and Pak.) and in three F_1 hybrids (G-1 x C.R.), (G-1 x Ab.) and (G-1 x Pak.). The electrophoretic patterns of either infected or non-infected (control) leaves showed six bands migrating towards the cathode and were designated as C_1, C_2, C_3, C_4, C_5 and C_6. The seven genotypes were found to be varying

in band numbers and in their density. Moreover, this variation was found also in infected and non-infected leaves. C_1 band was absent in G-1 only, while the C_5 band was absent in the other three cultivars; C.R., Ab. and Pak. (Fig. 1). The activity of the isoperoxidases in diseased leaves was higher than that of the non-infected leaves (Table 1). The infected leaves showed much higher activity of peroxidase isozymes than the non-infected ones, especially for C_2, C_3, C_4 and C_6 bands (Table 2). The Pakistanian (Pak.) cultivar was characterized by high activity of peroxidases bands C_2 and C_6; since C_2 band increased by 10 folds, while C_6 band increased by 3.6 folds.

The cross between G-1 and Pak. cultivars showed the highest activity of peroxidases when compared with the other two crosses (G-1 x C.R.) and (G-1 x Ab.) according to (Table 2).

The disease indeces (D.I.) of chocolate leaf spots in all genotypes were calculated and recorded (Table 3). Disease index was high (72%) in Abyssinian (Ab.) cultivar, but was low (45%) in the Pakistanian (Pak.) cultivar. The other two cultivars G-1 and C.R. were intermediate in this respect. In the cross (G-1 x Ab.) disease index was high (69%), while in the other two crosses (G-1 x C.R.) and (G-1 x Pak.) was low (50%) and (51%), respectively. Accordingly, it could be concluded that the Pakistanian is partially resistant, while the Abyssinian cultivar is susceptible for chocolate leaf spot disease. This conclusion can be also related to the activity of peroxidase isozymes. The latter was increased in diseased Pakistanian leaves more than the other cultivars. Moreover, the hybrid with the Pak. cultivar showed an increase in activity of peroxidase isozymes. Therefore, the partial resistance of Pak. and the susceptibility of Ab. could be affected or related to peroxidase isozymes activity.

Figure 1 : Diagram of Peroxidase isozyme patterns in leaves of four cultivars and three crosses of broadbean non-infected (C) and infected (I) by Botrytis Fabae.

Table 1 : Densitometric scan arbitrary units at 400 nm of the cathodal isoparoxidase of non-infected (C) and infected (I) with Botrytis fabae in four cultivars of broadbean and their F_1's

Genotypes		O.D. of cathodal isoperoxidase at 400 nm.					
		C_1	C_2	C_3	C_4	C_5	C_6
G-1	-C	--	10	9	10	6	9
	-I	--	20	22	20	14	12
C.R.	-C	13	9	8	9	--	11
	-I	20	30	24	20	--	26
Ab.	-C	11	14	8	13	--	20
	-I	36	30	18	32	--	32
Pak.	-C	8	2	6	10	--	6
	-I	28	20	16	18	--	22
G-1xC.R.	-C	--	7	4	8	4	3
	-I	--	28	24	20	18	12
G-1x Ab.	-C	7	6	2	3	--	5
	-I	15	16	14	12	--	28
G-1x Pak.	-C	--	4	2	6	--	4
	-I	--	18	16	32	--	36

Table 2 : Relative increase in Optical density of Cathodal isoperoxidase bands C_2, C_3, C_4 and C_6 after infection with Botrytis fabae .

Genotypes	C_2	C_3	C_4	C_6
G-1	2.0	2.5	2.o	1.3
C.R.	3.3	3.0	2.2	2.4
Ab.	2.2	2.3	2.7	1.6
Pak.	10.0	2.7	1.8	3.6
G-1x C.R.	4.0	6.0	2.5	4.0
G-1x Ab.	2.7	7.0	4.0	5.6
G-1x Pak.	4.5	8.0	5.3	9.0

Table 3 : Disease indeces (D.I.) of chocolate spots caused by Botrytis fabae in leaves of four broadbean cultivars and their F_1 crosses.

Genotypes	D.I.
Giza-1	54 %
Cyperian-Romi	62 %
Abyssinian	72 %
Pakistanian	45 %
G -1 x C.R.	50 %
G -1 x Ab.	69 %
G -1 x Pak.	51 %

These results are in agreement with those of Okiror et al., 1982; Surli and Rivera, 1983; Wasfy et al., 1984 on common bean. They reported that peroxidase isozymes activity increase due to infection with anthracnose, mosaic virus and Rhizoctonia solani, respectively. Griffiths and Amin (1978) noticed that relative amount of free and bound acid phosphatase enzyme was related to the susceptibility of Vicia faba leaves to B. fabae.

Furthermore esterase isozymes were studied in the leaves of the aforementioned cultivars and crosses. The electrophoretic bands migrating towards the anode were found to be four bands, A_1, A_2. A_3 and A_4 in all cultivars except G-1 cultivar. (Fig. 2). The increase of esterase isozymes activity was found in infected leaves for some bands, when compared with the none infected ones (Table 4). However the increases in optical density of esterase isoenzymes were

Figure 2 : Diagram of esterase isozyme patterns in leaves of four cultivars and three crosses of broadbean, non-infected (C) and infected (I), by Botrytis fabae .

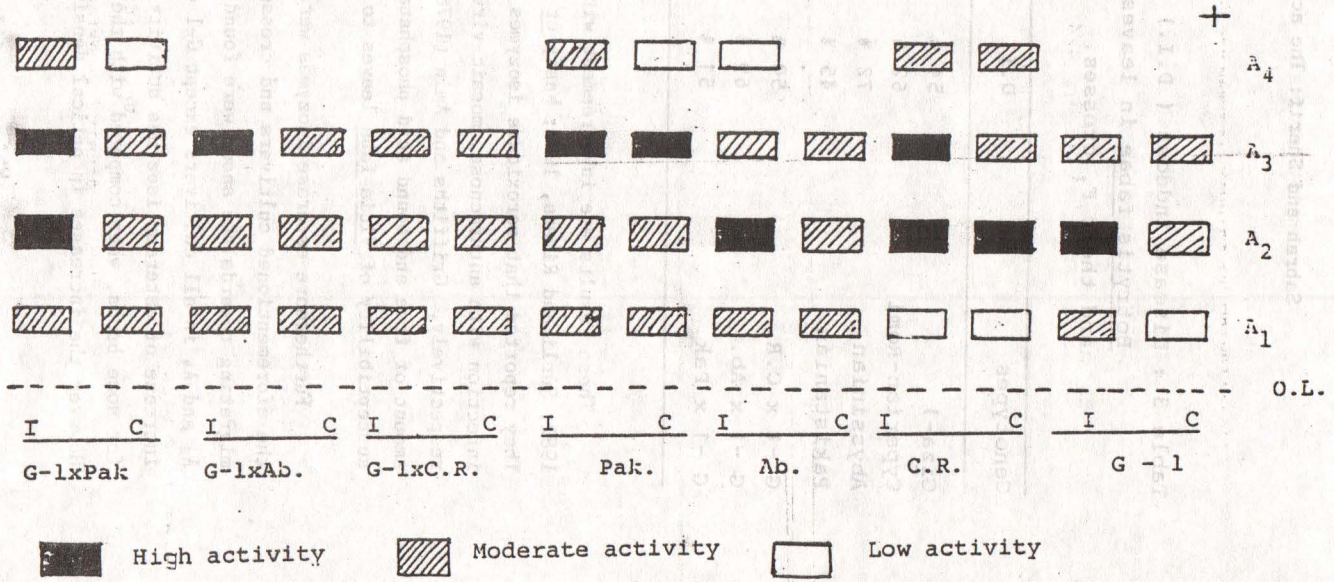

Table 4 : Densitometric scan arbitrary units at 575 nm for anodal esterase isozymes of non-infected (C) and infected (I) with Botrytis fabae in four broadbean cultivars and their F_1's .

Genotypes		O.D. of anodal esterase isozymes at 575 nm			
		A_1	A_2	A_3	A_3
G-1	-C	6	16	10	--
	-I	8	20	16	--
C.R.	-C	4	20	18	14
	-I	6	22	20	16
Ab.	-C	8	16	14	--
	-I	8	20	16	5
Pak.	-C	12	16	20	4
	-I	18	18	20	8
G-1 x C.R.	-C	10	15	14	--
	-I	10	16	15	--
G-1 x Ab.	-C	12	14	18	--
	-I	14	16	20	--
G-1 x Pak.	-C	12	18	18	4
	-I	16	22	20	10

Table 5 : Relative increase in optical density of anodal esterase isozyme bands A_1, A_2 and A_3 after infection by Botrytis fabae in four broadbean cultivars and their F_1's .

Genotypes	A_1	A_2	A_3
G-1	1.3	1.3	1.6
C.R.	1.5	1.1	1.1
Ab.	1.0	1.3	1.1
Pak.	1.5	1.1	1.0
G-1 x C.R.	1.0	1.1	1.1
G-1 x Ab.	1.2	1.1	1.1
G-1 x Pak.	1.3	1.2	1.1

low in diseased leaves and no differences were found (Table 5). The results of activity of isoesterases were in agreement with those of Okiror et al., 1982 in case of anthracnose disease of common bean, and with Weber et al., 1967 on sweet potato after infection with Ceratocystis fimbriata.

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