PEROXIDASE AND ESTERASE ISOZYMES ACTIVITY OF BROADBEAN LEAVES INFECTED WITH UROMYCES FABAE

Sabrah, N.S. and M.K. Youssef
Department of Genetics, Faculty of Agriculture,
Alexandria University, Alexandria, Egypt.

نشاط المشابهات الانزيمية للبيروكسيديز والاستيريز فى أوراق الفول البلدى المصابة بفطر Uromyces fabae.

نوح سليمان صبره ـ محمد خليل يوسف
قسم الوراثة ـ كلية الزراعة ـ جامعة الاسكندرية

ملخص البحث

درست مشابهات انزيمى البيروكسيديز والاستريز بواسطة الفصل الكهربى فى طرز الفول : جيزة ١ ، قبرصى وحبشى وباكستانى وكذلك الهجن بينهم. لاختبار مقاومتهم أو عدم مقاومتهم لمرض صدأ الأوراق والذى يسببه فطر Uromyces fabae حسب معامل الاصابة disease index وأيضا القيمة الوسطية mean value من خلال عدد البقع فى الورقة لكل الطرز والهجن .

الطراز الحبشى كان أكثرهم مقاومة بينما الباكستانى كان أكثرهم اصابة . وجيزة ١ والقبرصى كانا متوسطا الاصابة أما الهجين الذى يشترك فيه الحبشى كان أكثر مقاومة عن الهجن الأخرى .

المشابهات الانزيمية لكل من البيروكسيديز المستقطب للقطب السالب والاستيريز المستقطب للقطب الموجب أظهرت اختلافات من حيث العدد والنشاط بين الطرز المختلفة . كما أظهرت الأوراق المصابة نشاطا أكبر لكلا الانزيمين بالمقارنة بالأوراق الغير مصابة .

وقد أبدى الطراز المقاوم (الحبشى) نشاطا أكبر بالمقارنة بالطراز القابل للاصابة (الباكستانى) غير أن الفروق بينهما فى معدل زيادة النشاط لم تكن واضحة .

ABSTRACT

Electrophoretic patterns of peroxidase and esterase isozymes were examined for seven broadbean genotypes; four cultivars: Giza-1 (G-1) Cyperian-Romé (C.R.) Abyssinian (Ab.) and Pakistanian (Pak.); and their F_1 hybrids; G-1 x C.R., G-1 x Ab. and G-1 x Pak.

To test their resistance or susceptibility to leaf rust disease caused by Uromyces fabae, disease index and mean value of leaf rust spots were calculated for all genotypes. The Abyssinian cultivar was more resistant, while the Pakistanian were more susceptible and the others G-1 and C.R. showed moderate resistance. The cross involving the Abyssinian, i.e., G-1 x Ab. was more resistant than the two other crosses.

The cathodal peroxidase and anodal eterase isozyme bands showed differences in number and activity from the one cultivar to the other. The infected leaves showed more activity in the two examined isozymes than the uninfected leaves. The resistant cultivar (Abyssinian) was higher than the susceptible cultivar (Pakistanian) but no differences appeared between them in the amount of increase in the peroxidase and the esterase isozymes activities.

INTRODUCTION

Boradbean (Vicia faba L.) is considered one of the important legume crops in developing countries especially Egypt. Many diseases caused by fungi, bacteria, virus and pathogenic plants, affect this crop and consequently decrease its seed yield (total seeds) and seed quality. Mohamed (1982) reported that, due to several diseases, the yield decreased 55% in the susceptible plants when compared with the healthy ones.

Many results have been reported dealing with comparisons between susceptible and disease-resistant plants based on their electrophoretic patterns. These results varied not only for different crops, but also from one enzyme system to the other.

Rust disease caused by Uromyces fabae which damages the leaves of broadbean, changed the activity of polyphenol oxidase enzyme (Montalbini et al., 1981). In wheat plants, the rust disease caused by Puccinia graminis tritici increased the activity of peroxidase enzyme (Daly et al., 1970; Seevers and Daly, 1970 and Daly et al., 1971), and also Puccinia recondita f. sp tritici increases the activity of these enzymes in wheat plants, (Wasfy et al., 1983).

Accordingly, this study aimed to assess changes in peroxidase and esterase activity in broadbean cultivars and their crosses due to infection with Uromyces fabae.

MATERIALS AND METHODS

One local cultivar of broadbean; Giza-1 (G-1) and three exotic cultivars; Cyperian-Romé (C.R.), Abyssinian (Ab.) and Pakistanian (Pak.) were used in this study. The four cultivars and their F_1's (G-1 x Pak.), (Ab. x G-1) and (C.R. x G-1) were grown during 1984, 1985 and 1986 at the experimental station of the Faculty of Alexandria Agriculture; University of Alexandria. To determine the effect of Uromyces fabae, disease indices were scored according to the equation proposed by Horsfull and Henberger, 1942 as follows:

$$\text{Disease index} = \frac{\text{Sum of (disease class X No. of plants in that class X 100}}{\text{Total number of plants x 4}}$$

Healthy and naturally infected leaves, were collected for scoring the disease class from three parts of 100 plants for each genotype i.e. lower, medium and upper parts of the plant.

Leaves of each genotype either damaged by natural infection (rust disease) or noninfected (control) in the same plant were used to study the activity of esterase and peroxidase isozymes. Extracts of damaged and non-damaged leaves were absorbed on filter paper

strips and placed 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 electrophoresis started for 1.5 hours for the peroxidase isozymes assay and 2.5 hours for the esterase isozymes assay. Peroxidase plates were then stained with H_2O_2-benzidine solution in 0.01 M sodium acetate-acetic acid buffer, pH 5.0 while the esterase plates were stained with fast blue RR, and B naphthyl acetate in 0.01 M tris H.C.L. buffer, pH 7.0. The optical density of the isozyme patterns was measured at 400 nm for peroxidaze and 575 nm for esterase, using a TLC scanner (CS-910 Shimadzo).

RESULTS AND DISCUSSION

The electrophoretic patterns of peroxidase and esterase isozymes were studied in broadbean leaves of four cultivars; Giza-1, C.R., Ab. and Pak. and also in their F_1 hybrids G-1 x C.R.; G-1 x Ab. and G-1 x Pak. during the flowering period after natural infection by Uromyces fabae which cause's rust spot disease.

Peroxidase isozymes which migrated towards the cathode ranged from 5 to 6 bands in all genotypes. Three cultivars C.R., Ab. and Pak. showed six bands, while G-1 showed five bands only (Figure 1). The hybrids G-1 x C.R. and G-1 x Pak. showed five bands as the G-1 parent; while the third hybrid (G-1 x Ab.) showed six bands (isozymes) like the Abyssinian parent. According to the densitometric scanning at 400 nm. (Table 1), the peroxidase isozymes in the infected leaves were, in general, more active than in uninfected ones for all genotypes studied. Amount of increase in peroxidase activities are shown in Table 2. The relative amounts of activity were almost similar in all genotypes except in cross G-1 x Ab. where C_2 and C_6 bands revealed an increase in relative optical densities of 2.4 and 2.2 folds, respectively.

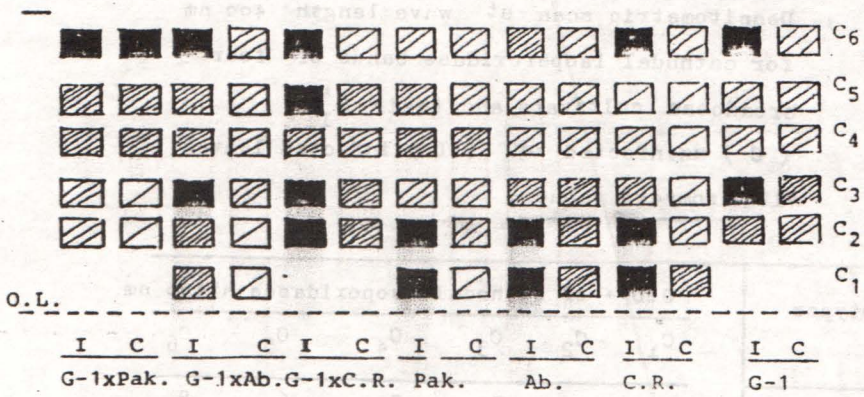

Figure 1: Schematic diagram of cathodal peroxidase isozyme patterns of seven genotypes of broadbean infected (I) or uninfected (C) by Uromyces fabae.

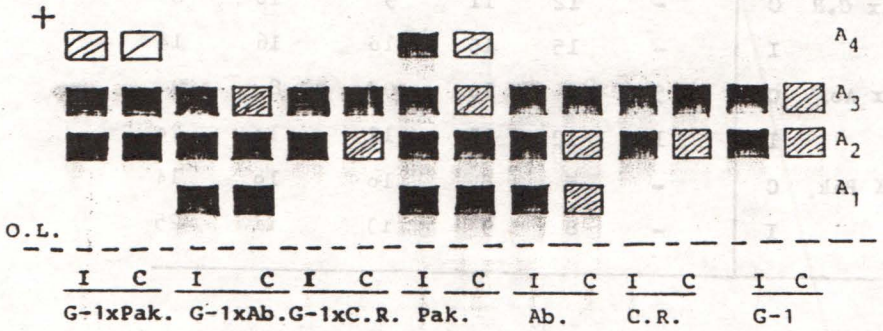

Figure 2: Schematic diagram of anodal esterase isozyme patterns of seven genotypes of broadbean infected (I) or uninfected (C) by Uromyces fabae.

Table 1 : Densitometric scan at wave length 4oo nm for cathodal isoperoxidase bands of four broadbean cultivars and their F_1's . (C) uninfected or (I) infected leaves with Uromyces fabae .

Genotypes		O:D of cathodal isopoxidases at4oo nm					
		C_1	C_2	C_3	C_4	C_5	C_6
G-1	C	-	9	12	7	6	8
	I	-	12	14	8	8	15
C.R	C	12	9	6	5	4	9
	I	18	15	12	8	5	19
Ab.	C	11	1o	11	7	8	9
	I	16	18	13	8	9	12
Pak.	C	6	7	8	1o	9	7
	I	15	14	9	12	1o	9
G-1x C.R	C	-	12	11	9	1o	8
	I	-	15	15	1o	16	14
x Ab.	C	5	5	1o	9	8	9
	I	1o	12	19	12	1o	2o
X Pak.	C	-	5	5	1o	1o	14
	I	-	8	9	13	11	25

Table 2 : Folds for optical density increases of cathodal isoperoxidase bands C_2 - C_6 after infection by Uromyces fabae .

Genotypes	C_1	C_2	C_3	C_4	C_5	C_6
G - 1	-	1.3	1.2	1.1	1.3	1.9
C.R.	1.5	1.7	2.0	1.6	1.2	2.1
Ab .	1.5	1.8	1.2	1.1	1.1	1.3
Pak .	2.5	2.0	1.1	1.2	1.1	1.3
G-1 x C.R	-	1.2	1.4	1.1	1.6	1.7
xAb .	2.0	2.4	1.9	1.3	1.2	2.2
xPak .	-	1.6	1.8	1.3	1.1	1.8

The disease index and spot mean values were calculated for all genotypes. These parameters were estimated in 300 samples (Leaves) for each genotype. Table (3) shows the disease indices and mean values of all genotypes. Disease indices and mean values were low in the Abyssinian cultivar, but were high in the Pakistanian cultivar, and were medium in other cultivars. The disease index and mean value were low in cross G-1 x Ab., but high in cross G-1 x Pak. and the third cross G-1 x C.R. showed intermediate spots of rust caused by Uromyces fabae. These data indicated that the Abyssinian cultivar was more resistant and that the Pakistanian cultivar was more susceptible than the other two cultivars. This result was confirmed with the hybrid results, especially where the Abyssinian cultivar is involved in the cross. Peroxidase isozyme activity was related to some extent with the resistance or susceptibility for rust disease. But the units of densitometric scanning were high in the resistant cultivar (Abyssinian) than the susceptible cultivar (Pakistanian). The final result agreed with the data of Montalbini et al. (1981) on Vicia faba infected with Uromyces fabae. They noticed that the activity of polyphenol oxidase enzyme was reduced in infected plants more than in uninfected ones. This decrease in activity was due to the rust infection which caused a reduction in the rate of photo-oxidation of dihydroxyphenyl alanine. Again, the data on wheat plants infected with Puccinia graminis tritici of the stem rust disease by Daly et al. (1970); Seevers and Daly (1970) and Daly et al. (1971) supported the present results. They reported that peroxidase enzyme activity increased in the infected leaves of wheat plants of either resistant or susceptible lines which carried the Sr-6 or Sr-11 alleles. They concluded that the total peroxidase activity was not related to resistance.

Furthermore, esterase isozyme patterns were examined in all the present genotypes after infection with Uromyces fabae. Figure (2)

Table 3 : Disease indices (D . I .) and mean value ($\bar{x}$) of rust spots caused by Uromyces fabae in leaves of four broadbean cultivars and three crosses.

Genotypes	D.I %	$\bar{X}$
G - 1	5o . 66	21. 41
C . R	48 . oo	2o . 7o
Ab .	36 . oo	6 . 4o
Pak .	61 . 33	33 . 15
G- 1 xC.R	45 . 2	2o . 11
G-1 xAb.	41 . 7	15 . 21
G-1 xPak.	59 . 1	3o . 27

shows the anodal bands of esterase isozymes, which were different in their number and activity between the four cultivar and also between their crosses. The number of bands ranged from 2 to 4 in these genotypes. Table (4) shows the densitometric scaning at a wave length of 575 nm for anodal esterase isozymes. Table (5) shows the amount of density increase of these isozymes after infection. The esterase activity tended to increase in the infected than in the uninfected leaves. The amount of total isozyme activity was almost the same in the resistant or susceptible cultivar except for the A1 band in the Abyssinian which was increased by 2.1 folds.

From these results, it could be concluded that esterase isozyme activity was not related to the resistance of the Abyssinian cultivar which showed low disease index and low mean value for rust spots as well (Table 3). The Pakistanian cultivar (susceptible) had a higher disease index and a higher mean value for rust spots compared to the other cultivars (Table 3). Therefore, the amount of increase in esterase isozyme activity in the resistant and susceptible cultivars were almost the same for all the bands except the A_1 band. A_1 band in the Abyssinian was increased by 2.1 folds, while in the Pakistanian it was increased by only 1.3 fold.

Finally, the three F_1 hybrids were also similar in their esterase isozymes activities irrespective of the resistance or susceptibility to rust spots caused by Uromyces fabae.

These results agree with those of Weber et al. (1967). They noticed that the esterase enzyme activity was decreased or did not change in sweet potato (Ipomoca batatas) varieties after infection with Ceratocystis fimbriata. Okiror et al. (1982) noticed that the biochemical variation based on electrophoresis, among bean lines resistant and susceptible to anthracnose were found with peroxidase and esterase isozymes. The resistant and susceptible plants were

Table 4 : Densitometric scan at wave length 575 nm for anodal esterase bands of four cultivars and their crosses of broadbean , (C) uninfected and (I) infected leaves with Uromyces Tabae .

Genotypes		O.D.of anodal esterase bands at 575 nm.			
		C_1	C_2	C_3	C_4
G- 1	C	—	9	8	—
	I	—	14	13	—
C.R.	C	—	9	13	—
	I	—	14	15	—
Ab.	C	11	12	13	—
	I	23	16	11	—
Pak	C	14	13	1o	8
	I	18	17	13	15
G-1x C.R	C	—	12	15	—
	I	—	19	18	—
x Ab .	C	15	13	11	—
	I	17	16	17	—
x Pak.	C	—	14	13	3
	I	—	15	16	5

Table 5 : Relative amount of optical density increases of anodal esterase bands after infection with Uromyces fabae .

Genotypes	A_1	A_2	A_3	A_4
G - 1	—	1.6	1.6	—
C.R	—	1.6	1.7	—
Ab	2.1	1.3	1.3	—
Pak	1.3	1.3	1.3	1.9
G-1xC.R	—	1.9	1.2	—
xAb.	1.3	1.2	1.5	—
xpak .	—	1.1	1.2	1.7

different in their banding patterns and intensities at certain stages of development. In the 40 day-old plants, the esterase bands were different in intensities, especially in the two bands A_1 and A_2. These two bands showed greater activity in susceptible lines than in the resistant ones. Other anodic bands are not important since they did not show any consistent differences between the resistant and susceptible lines.

REFERENCES

Daly, J.M.; P.M. Seevers and P. Ludden (1970). Studies on wheat stem rust resistance controlled at the Sr_6 locus. III. Ethylene and disease reaction. Phytopathology, 60(11): 1648-1652.

Daly, J.M.; P. Ludden and P. Seevers (1971). Biochemical comparisons of resistance to wheat stem rust disease controlled by the Sr_6 or Sr_{11} alleles. Physiol. Plant path. 1: 397-407.

Horsfull, J.G, and J.W. Henberger (1942). Measuring magnitude system for measuring plant disease. Phytopathology 32: 226-232.

Mohamed, H.Ab. R. (1982). Major disease problems of fababeans in Egypt. Faba Bean Improvement, Page 213.

Montalbini, P.; B.B. Buchanan, and S.W. Hutcheson (1981). Effect of rust infection on rates of photochemical polyphenol oxidation and latent polyphenol oxidase activity of Vicia faba chloroplast membranes. Physiol. Plant. Pathol. 18(1): 51-57.

Okiror, M.A.; U.K. Gupta and W.M. Van Breukelen (1982). Genetic and physiological variation among bean lines resistant and susceptible to bean anthracnose. Theor. Appl. Genet., 62(4): 355-359.

Sabrah, N.S. and A.Y. El-Metainy (1985). Genetic distances between local and exotic cultivars of Vicia faba L. based on esterase isozyme variation. Egypt. J. Genet. Cytol. 14: 301-307.

Seevers, P.M. and J.M. Daly (1970). Studies on wheat stem rust resistance controlled at the Sr_6 locus. II. Peroxidase activities. Phytophathology 60(11): 1642-1647.

Wasfy, E.M.; M.M. Fahim; M.M. Ragab; A.R. El-Mahdy; A.Y. El-Meteny; T. El-Sharkawy, and M.A. Nagieb (1983). Effect of Race 57 of leaf rust fungus on phenolic compounds and peroxidase isoenzymes of resistant and susceptible wheat cultivars 3rd Egyptian-Hungarian Conference on Plant Protection Budapest, May, 30 June, 2, 1983. P. 95.

Weber, D.J.; B. Clare and M.A. Stahmann (1967). Enzymic changes associated with induced and natural resistance of sweet potato to Ceratocystis fimbriata. Phytopathology 57(4): 421-424.