J. Agric. Sci. Mansoura Univ., 30 (6): 3425 - 3438, 2005

USE OF ISOZYME COMPOSITION OF SEED TO QUANTIFY RESISTACE OF FLAX CULTIVARS TO POWDERY MILDEW DISEASE

Aly,A.A.[1];E.M. Hussein[1]; M.T.M. Mansour[1] and M.A.Tag El-Din[2]

1- Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

2- Department of Agricultural Biochemistry, Faculty of Agriculture, Ain Shams Univ., Shoubra El-Kheima, Cairo, Egypt.

ABSTRACT

A field trial was conducted in 2002/2003 and 2003/2004 growing seasons at Giza Agricultural Research Station to evaluate the reactions of ten flax cultivars to powdery mildew (PM) disease. In general, the tested cultivars could be divided into four distinct groups, i.e., highly resistant (Ottowa 770 B, Dakota, and Bombay), resistant (Cass, Wilden, and Clay), susceptible (Koto and Marshall), and highly susceptible (Cortland and C.I. 2008). The cultivars showed considerable variation in disease severity (DS) ranged from 3.69 on Bombay to 100% on C.I. 2008. Isozymes of malate dehydrogenase (MDH), peroxidase (PRX) and esterase (EST) of cultivar seed were separated by polyacrylamide gel electrophoresis (PAGE), and the obtained banding patterns were visualized by using three staining systems. Data for PM severity (dependant variable), and amounts of isozymes (independent variables or predictors) were entered into a computerized stepwise multiple regression analysis. Using the predictors supplied by stepwise regression, three models were constructed to quantify PM severity. Coefficient of determination (R^2) values of the models were 98.67, 83.46, and 83.46% for MDH, PRX and EST, respectively. These results indicate that PAGE of isozymes may provide a supplementary assay to greenhouse and field tests to distinguish quantitatively between PM resistant or susceptible cultivars.

INTRODUCTION

Flax (*Linum usitatissimum* L.) is considered the most important bast fiber crop, it ranks second after cotton (Seedy fiber) regarding economic importance and production. Powdery mildew (PM), caused by *Oidium lini* Škoric, is currently considered the most common, conspicuous, widespread, and easily recognized foliar disease of flax in Egypt. Over the last decade, the importance of this disease has increased probably due to the appearance and rapid distribution of new races capable of attacking the previously resistant cultivars (Aly *et al.*, 1994). In India, Pandey and Misra (1993) reported that as the disease increased, yield losses increased ranging from 11.8 to 38.9%, yield losses were greater when the disease appears earlier in the season. Accurate assessment of losses due to the disease in Egypt has not been reported. However, Aly *et al.* (1994) found significant negative correlations between disease intensity ratings and agronomic traits (yield and yield components).

Currently, resistance is not available in commercially grown flax cultivars in Egypt. Therefore, in years when environmental conditions favor the development of the disease, foliar application of fungicides has become the only commercially available management practice for the disease

control (Aly *et al.*, 1994). However, complete dependence on fungicides for the disease control carries risks for the producers, in that accurate coverage and distribution of fungicides may not be achieved and there are potential problems with correct timing of applications. Furthermore, increasing concern for the environment will likely mean greater regulation of fungicide usage (Pearce *et al.*, 1996).

Use of cultivars with PM resistance can resolve all these problems. However, successful screening for PM resistance in flax requires the development of a reliable method for the quantification of resistance.

Isozymes (Isoenzymes) are defined as genetically determined multiple molecular forms of an enzyme. There are three main causes of formation of multiple molecular forms of enzymes. These are (1) the presence of more than one gene locus coding for the enzyme, (2) the presence of more than one allele at a single gene locus coding for the enzyme, and (3) the post translation modifications of the formed enzymatic polypeptides resulting in formation of nongenetic or so-called "secondary" isozymes. The term isozymes is usually used to denote multiple molecular forms deriving from different genetic loci, where the term "allozymes" is used to denote multiple molecular forms deriving from different alleles of the same genetic locus. The term "allelic isozymes" is also used by some isozymologists (Manchenko, 1994).

The relationship between isozyme composition of host plant and its resistance or susceptibility to PM diseases has been studied in some pathosystems. For example, in a comparison of wheat mutants induced by gamma radiation, it was shown that the PM-susceptible material contained an extra peroxidase isozyme band, 3A. Comparisons of the activity of the band in the 2 susceptible parents showed that activity increased relative to the increase in infection and that the absorption peak was greater in the more susceptible variety (Xia *et al.*, 1986).

Chitinase (EC 3.2.1.14) and beta-1, 3 glucanases (EC 3.2.1.39) have been known to play a vital role in the defense of plants against fungal pathogens. The pattern of induction of these two enzymes subsequent to infection by PM was studied in 10 pairs of near-isogenic lines of barley (*Hordeum vulgare* L.), which possess PM resistance genes. These isogenic lines have been grouped according to their reaction to the fungus. The induction varied between the resistant and the susceptible cultivars within each group and between different groups. More isozymes were induced in susceptible varieties of highly resistant groups and overall levels and the number of isozymes of chitinases and beta-1, 3 glucanases were lower in groups with low resistance (Ignatius *et al.* 1994).

The isoelectric focusing (IEF) gel electrophoretic approach was used to compare the peroxidase isoenzyme bands of 50 wheat varieties resistant or susceptible to PM (*Erysiphe graminis*). Wide differences were observed in the expression level of band pI6.1 in leaves of resistant and susceptible varieties from the jointing stage to the occurrence of the disease. The genes for band pI6.1 in resistant varieties showed life-long normal expression. The band had strong activity throughout the growth period. The expression of this band was suppressed in susceptible varieties after the

jointing stage, resulting in ambiguity or even disappearance of the band (Wang *et al.*, 1999).

Isozyme spectra of peroxidases for 11 selected *Cucurbita pepo* cultivars representing different groups of morphotypes with various levels of PM field resistance were analyzed. They could be ranged into three basic groups, which correspond with the cultivar's level of field resistance (Lebeda *et al.*, 1999).

To date, as far as we know, no attempts have been made to evaluate the degree of association between PM intensity ratings and isozymes composition of flaxseed.

Therefore, the objectives the present study were to (1) evaluate the relationship of PM disease on flax to isozyme composition of seed (2) develop a statistical model to predict PM severity by using isozymes of malate dehydrogenase, peroxidase, and esterase in the seed as biochemical predictors. This approach has not been employed previously for the prediction of PM on flax.

MATERIALS AND METHODS

Reactions of flax cultivars to PM:

A field trial was conducted in 2002/2003 and 2003/2004 growing seasons at Giza Agricultural Research Station. The experiment consisted of a randomized complete block design of five replications (blocks). Plots were 2X3 ($6m^2$) and consisted of ten rows spaced 20 cm apart. Seeds of each cultivar were sown by hand at a rate of 70g/plot. Planting date was in the first week of December. Disease severity was rated visually in the last week of April (Nutter *et al.*, 1991).

Extraction of proteins from flax seeds:

Random samples of seeds, taken from the same seeds used in planting the field trial, were used for the extraction of proteins according to Hussein (1992) in the following way:

Seeds of healthy plants of flax cultivars Ottowa 770B, Dakota, Bombay, Cass, Koto, Clay, Wilden, Marshall, Cortland and C.I. 2008, were slightly ground and defatted by diethyle ether or chloroform for 4 to 5 days. After drying at room temperature, ground seeds were suspended in a solution (1-3ml/g seeds) consisting of 12.5g glucose and 1g ascorbic acid dissolved in 100ml phosphate buffer 8.3 and ground in liquid nitrogen to a fine powder. After thawing, the powder suspended in buffer was centrifuged at 19,000 rpm for 30 minutes at 0°C. The protein content in the supernatant was adjusted to a concentration of 3 to 4 mg/ml according to Bradford (1976) spectrophotometric method by using bovine serum albumin as a standard protein.

Polyacrylamide gel electrophoresis (PAGE) of native protein:

Thawed protein-extract supernatant was mixed with an equal volume of a solution containing 20% glycerol (vol/vol) and 0.1% bromphenol blue (vol/vol) in 0.15 M Tris-HCL, pH 6.8. Twenty microliters of the resulting suspension (40 to 60 µg of protein) was subjected to electrophoresis in 25 mM Tris buffer containing 192 mM glycin at pH 8.3. Electophoresis was

conducted at room temperature (approximately 20 to 25°C) for 9 hr on an 15% polyacrylamide gel with a 6% stacking gel, at 20 and 10 mA, respectively, until the dye reached the bottom of the separating gel (Laemmli, 1970 and Latorre *et al.*, 1995). Electrophoresis was performed in a vertical slab mold (16.5 x 14.5 x 0.1 cm). Gels wre stained according to Manchenko (1994) for the detection of isozymes of malate dehydrogenase (EC 1.1.1.37),peroxidase (EC1.11.1.7) and esterase (EC 3.1.1.1)(Table 1).

Statistical analysis:

Gels were scanned for band R_f (position) and amount (%) by the documentation system AAB (Advanced American Biotechnology 1166). Linear correlation coefficient was calculated to evaluate the degree of association between PM severity and the amount of each isozyme. Stepwise regression technique with the greatest increase in R^2 as the decision criterion was used to describe the effects of isozymes on PM severity. Correlation and regression analyses were performed with a computerized program.

Table 1. Staining solutions used for the detection of isozymes of malate dehydrogenase, peroxidase, and esterase.

Isozymes of	Staining solution	
Malate dehydrogenase	0.1 M tris-HCl buffer, pH 8.0	100 ml
	L-Malic acid (disodium salt)	250 mg
	NAD (Nicotinamide-adenine dinucleotide)*	30 mg
	NBT (Nitro blue tetrazolium)	25 mg
	PMS (Phenazine methosulfate)	2 mg
Peroxidase	50 mM Sodium acetate buffer, pH 5.0	100 ml
	Benzidine dihydrochloride	50 mg
	3% H_2O_2 (freshly prepared)	0.75 ml
Esterase	0.05 M Phosphate buffer, pH 7.2	100 ml
	α- Naphthyl acetate (dissolved in 1 ml of acetone)	10 mg
	Fast Blue RR salt	50 mg

* oxidized.

RESULTS

Environmental conditions in 2002/2003 and 2003/2004 growing seasons were favorable for epiphytotic spread of the disease. This was apparent as these environmental conditions resulted in 100% disease severity (DS) on cultivar C.I. 2008 (Table 2), which is known as highly susceptible (A.A. Aly, *personal observations*). In general, the tested cultivars could be divided into four distinct groups, i.e., highly resistant (Ottowa 770B, Dakota, and Bombay), resistant (Cass, Wilden, and Clay), susceptible (Koto and Marshall), and highly susceptible (Cortland and C.I. 2008). The cultivars showed considerable variation in DS ranged from 3.69 on Bombay to 100% on C.I. 2008 (Table 2).

Malate dehydrogenase (MDH) isozymes:

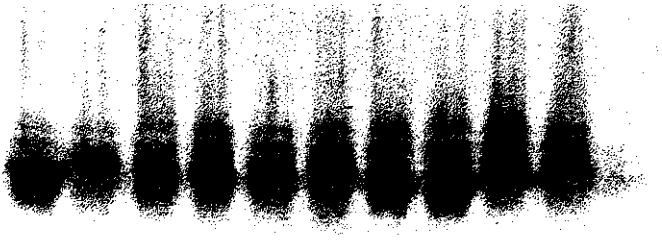
A total of 25 MDH isozymes were identified among the cultivars that were analyzed (Fig.1 and Table 3). No single cultivar was stained for all the 25 isozymes. Ottowa 770B showed the largest number of isozymes (5 isozymes), while Cass showed only one isozyme. The other cultivars showed a number of isozymes ranged from 2 to 4. Each cultivar was characterized by a unique set of isozymes. For example, isozymes nos. 1, 5, 13, 14, and 22 were unique to Ottowa 770B. Dakota was characterized by the unique isozymes 7 and 17.

Table 2. Powdery mildew severity ratings on ten flax cultivars and their disease reactions under field conditions in Giza in 2002/2003 and 2003/2004 growing seasons.

Cultivar	Disease severity [a]	Disease reaction [b]
Ottowa 770 B	9.20	HR
Dakota	3.98	HR
Bombay	3.69	HR
Cass	14.39	R
Koto	28.86	S
Clay	16.14	R
Wilden	16.01	R
Marshall	56.49	S
Cortland	99.76	HS
C.I. 2008	100.00	HS

[a] Disease severity is the percentage of infected leaves/plant in a random sample of 10 plants/plot. Each value is the mean of two growing seasons.

[b] Disease reactions are highly resistant (HR), resistant (R), susceptible (S), and highly susceptible (HS).



1 2 3 4 5 6 7 8 9 10

Fig. 1. Malate dehydrogenase isozyme patterns obtained by polyacrylamide gel electrophoresis (PAGE) from flax seeds. Flax cultivars in lanes 1 through 10 were Ottowa 770 B (1), Dakota (2), Bombay (3), Cass (4), Koto (5), Clay (6), Wilden (7), Marshall (8), Cortland (9), and C.I. 2008 (10).

Table 3. Malate dehydrogenase isozyme patterns for ten flax cultivars obtained by polyacrylamide gel electrophoresis (PAGE).

Isozyme		Flax cultivar									
No.	Position	*Ottowa 770 B*	Dakota	Bombay	Cass	Koto	Clay	*Wilden*	*Marshall*	*Cortland*	C.I. 2008
1	7	18.78[a]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	8	0.00	0.00	0.00	0.00	0.00	16.06	0.00	0.00	0.00	0.00
3	26	0.00	0.00	0.00	0.00	8.00	0.00	0.00	0.00	0.00	0.00
4	51	0.00	0.00	0.00	0.00	0.00	4.27	0.00	0.00	0.00	0.00
5	78	15.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	83	0.00	0.00	39.92	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7	84	0.00	38.73	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8	86	0.00	0.00	0.00	0.00	0.00	20.02	0.00	0.00	0.00	0.00
9	88	0.00	0.00	0.00	0.00	0.00	0.00	37.79	0.00	0.00	0.00
10	89	0.00	0.00	0.00	0.00	0.00	0.00	0.00	38.91	0.00	0.00
11	90	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	45.36
12	91	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	47.46	0.00
13	98	6.62	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14	100	4.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15	111	0.00	0.00	0.00	0.00	0.00	0.00	13.36	0.00	0.00	0.00
16	116	0.00	0.00	0.00	0.00	0.00	59.64	0.00	0.00	0.00	0.00
17	120	0.00	61.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
18	121	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	52.54	0.00
19	122	**0.00**	0.00	0.00	100.0	0.00	0.00	0.00	0.00	0.00	0.00
20	125	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	54.64
21	**130**	0.00	0.00	0.00	0.00	92.00	0.00	0.00	0.00	0.00	0.00
22	132	**54.85**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
23	134	0.00	0.00	60.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	139	0.00	0.00	0.00	0.00	0.00	0.00	48.85	0.00	0.00	0.00
25	**147**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	61.09	0.00	0.00

[a] Amount (%) of the designated isozyme.

Pearson correlation coefficient was calculated to measure the degree of association between PM severity and the amount (%) of each separated isozyme (Table 4). Isozymes nos. 11, 12, 18, and 20 were weakly correlated with PM severity, while none of the other isozymes was satisfactory correlated with PM severity.

Data for PM severity and amounts of isozymes were entered into a computerized stepwise multiple regression analysis. The analysis

constructed a predictive model by adding predictors, in this case, amounts of isozymes, to the model in order of their contribution to R^2. The analysis was effective in eliminating those variables with little or no predictive value by incorporating into the model only those variables that made a satisfactory significant contribution to the R^2 value of the model (Podleckis *et al.*, 1984).

Table 4. Relationship of powdery mildew severity[a] on ten flax cultivars and malate dehydrogenase isozyme composition (%) of seeds from these cultivars.

Isozyme no.	r[b]	Isozyme no.	r
1	- 0.240	14	- 0.240
2	- 0.175	15	- 0.177
3	- 0.056	16	- 0.175
4	- 0.175	17	- 0.289
5	- 0.240	18	0.608 x
6	- 0.292	19	- 0.192
7	- 0.289	20	0.610 x
8	- 0.175	21	- 0.056
9	- 0.177	22	- 0.240
10	0.203	23	- 0.292
11	0.610 x[c]	24	- 0.177
12	0.608 x	25	0.203
13	- 0.240		

[a] Percentage of infected leaves/plant in a random sample of 10 plants/plot.
[b] Pearson correlation coefficient, which measures the degree of association between powdery mildew severity and the designated isozyme.
[c] Significant at $p < 0.10$.

Using the predictors supplied by stepwise regression, a four-factor model was constructed to predict PM severity (Table 5). This model showed that PM severity differences were due to largely to the isozymes nos. 11, 12, 10 and 3, which accounted for 93.08% of the total variation in PM severity ratings.

Table 5. Regression equation that describes the effects of some malate dehydrogenase isozymes (X_s) on severity of flax powdery mildew (Y).

Stepwise regression model	R^2 [a]	F. value [b]
$3.27 + 1.97\ X_{11} + 1.86\ X_{12} + 1.18\ X_{10} + 2.29\ X_3$	98.67%	93.08***

[a] Coefficient of determination. Relative contributions of the predictors X_{11}, X_{12}, X_{10}, and X_3 to R^2 are 37.23, 46.23, 12.96, and 2.26 %, respectively.
[b] F. value is significant at $p < 0.005$ (***).

Peroxidase (PRX) isozymes:

A total of 19 PRX isozymes were identified among the 10 cultivars that were analyzed (Fig. 2 and Table 6). No single cultivar was stained for all the 19 isozymes. C.I. 2008 showed the largest number of isozymes (5 isozymes), while the other cultivars showed a number of isozymes ranged from 2 to 4. Most cultivars were characterized by unique isozyme (s).

Table 6:Peroxidase isozyme patterns for ten flax cultivars obtained by polyacrylamide gel electrophoresis (PAGE).

Isozyme		Flax cultivar									
No.	Position	*Ottowa 770B*	Dakota	Bombay	Cass	Koto	Clay	*Wilden*	*Marshall*	*Cortland*	C.I. 2008
1	0		0.00	7.26	0.00	5.62	0.00	0.00	0.00	0.00	0.00
2	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	31.15
3	2	11.39	16.69	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	18.68	0.00
5	35	37.59	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	**37**	0.00	0.00	50.55	0.00	48.93	0.00	0.00	0.00	0.00	12.46
7	38	0.00	51.37	0.00	59.72	0.00	41.84	52.45	0.00	28.83	0.00
8	39	0.00	0.00	0.00	0.00	0.00	0.00	0.00	52.50	0.00	0.00
9	55	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.27
10	64	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	52.49	0.00
11	**65**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.58
12	**80**	48.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
13	**82**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	35.55
14	84	0.00	0.00	0.00	0.00	45.45	0.00	0.00	0.00	0.00	0.00
15	**85**	0.00	0.00	42.19	40.28	0.00	0.00	0.00	47.50	0.00	0.00
16	**86**	0.00	31.94	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
17	**88**	0.00	0.00	0.00	0.00	0.00	0.00	47.55	0.00	0.00	0.00
18	**89**	0.00	0.00	0.00	0.00	0.00	58.16	0.00	0.00	0.00	0.00
19	**100**	2.97	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

[a] Amount (%) of the designated isozyme.

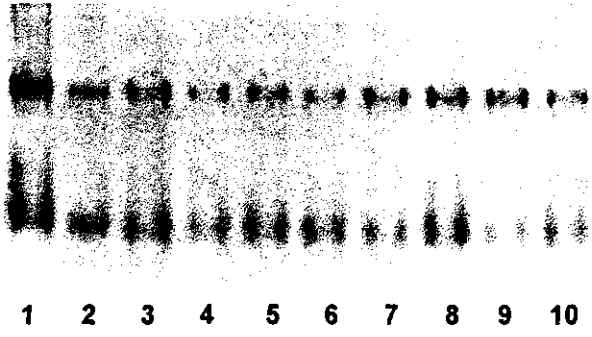


Fig. 2. Peroxidase isozyme patterns obtained by polyacrylamide gel electrophoresis (PAGE) from flax seeds. Flax cultivars in lanes 1 through 10 were Ottowa 770 B (1), Dakota (2), Bombay (3), Cass (4), Koto (5), Clay (6), Wilden (7), Marshall (8), Cortland (9), and C.I. 2008 (10).

Isozymes nos. 2, 4, 9, 10, 11, and 13 were weakly correlated with PM severity, while none of the other isozymes was significantly correlated with PM severity (Table 7).

Using the predictors supplied by stepwise regression, a two-factor model was constructed to quantify PM severity (Table 8). This model showed that PM severity differences were due largely to the isozymes nos. 2 and 4, which accounted for 83.46% of the total variation in severity ratings.

Table 7. Relationship of powdery mildew severity[a] on ten flax cultivars and peroxidase isozyme composition (%) of seeds from these cultivars.

Isozyme no.	r[b]	Isozyme no.	r
1	- 0.281	11	0.610 x
2	0.610 x [c]	12	- 0.240
3	- 0.395	13	0.610 x
4	0.608 x	14	- 0.056
5	- 0.240	15	- 0.157
6	- 0.151	16	- 0.289
7	- 0.309	17	- 0.177
8	0.203	18	- 0.175
9	0.610 x	19	- 0.240
10	0.608 x		

[a] Percentage of infected leaves/plant in a random sample of 10 plants/plot.
[b] Pearson correlation coefficient, which measures the degree of association between powdery mildew severity and the designated isozyme.
[c] Significant at $p < 0.10$.

Table(8):Regression equation that describes the effects of some peroxidase isozymes (X_s) on severity of flax powdery mildew (Y).

Stepwise regression model	R^2 [a]	F. value [b]
$Y = 11.64 + 2.61\ X_2 + 4.35\ X_4$	83.46%	17.65***

[a] Coefficient of determination. Relative contributions of the predictors X_2 and X_4 to R^2 are 37.23 and 46.23 %, respectively.
[b] F. value is significant at $p < 0.005$ (***).

Esterase (EST) isozymes:

A total of 30 EST isozymes were identified among the 10 cultivars that were analyzed (Fig. 3 and Table 9). No single cultivar was stained for all the 30 isozymes. Each of Ottowa 770B and C.I. 2008 showed the largest number of isozymes (7 isozymes), while Cass showed only one isozyme. The other cultivars showed a number of isozymes ranged from 2 to 4. Each cultivar was characterized by unique isozyme(s).

Isozymes nos. 5, 6, 10, 11, 12, 13, 23, 27, and 29 were weakly correlated with PM severity, while none of the other isozymes was satisfactory correlated with PM severity (Table 10).

Using the predictors supplied by stepwise regression, a two-factor model was constructed to predict PM severity (Table 11). This model showed that PM severity differences were due largely to the isozymes nos. 5 and 10, which accounted for 83.46% of the total variation in PM severity ratings.

Table 9: Esterase isozyme patterns for ten flax cultivars obtained by polyacrylamide gel electrophoresis (PAGE).

Isozyme		Flax cultivar									
No.	Position	*Ottowa 770 B*	Dakota	Bombay	Cass	Koto	Clay	*Wilden*	*Marshall*	*Cortland*	C.I. 2008
1	1	0.00[a]	0.00	0.00	0.00	0.00	0.00	31.84	0.00	0.00	0.00
2	2	0.00	12.42	8.29	0.00	0.00	30.79	0.00	35.95	4.57	0.00
3	3	0.00	0.00	0.00	0.00	10.30	0.00	0.00	0.00	0.00	0.00
4	4	19.69	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.03
6	**11**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.57
7	26	19.76	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8	28	24.74	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
9	38	0.00	0.00	0.00	0.00	29.52	0.00	0.00	0.00	0.00	0.00
10	43	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	39.10	0.00
11	**47**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	26.01
12	**63**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.93
13	**73**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.21
14	74	26.66	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15	**75**	0.00	33.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	86	5.39	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
17	88	0.00	0.00	0.00	0.00	0.00	39.56	0.00	0.00	0.00	0.00
18	89	0.00	0.00	0.00	0.00	33.79	0.00	0.00	0.00	0.00	0.00
19	90	0.00	29.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20	91	0.00	0.00	0.00	0.00	0.00	0.00	68.16	0.00	0.00	0.00
21	**92**	8.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
22	94	0.00	0.00	91.71	0.00	0.00	0.00	0.00	0.00	0.00	0.00
23	94	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	38.02
24	98	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00
25	99	18.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
26	**100**	0.00	0.00	0.00	0.00	26.40	29.47	0.00	0.00	0.00	0.00
27	**101**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	56.33	0.00
28	**105**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	64.05	0.00	0.00
29	119	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.23
30	**120**	1.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

[a] Amount (%) of the designated isozyme.

Table 10: Relationship of powdery mildew severity[a] on ten flax cultivars and esterase isozyme composition (%) of seeds from these cultivars.

Isozyme no.	r[b]	Isozyme no.	r
1	- 0.177	16	- 0.240
2	- 0.032	17	- 0.175
3	- 0.056	18	- 0.056
4	- 0.240	19	- 0.289
5	0.610 x [c]	20	- 0.177
6	0.610 x	21	- 0.240
7	- 0.240	22	- 0.292
8	- 0.289	23	0.610 x
9	- 0.056	24	- 0.192
10	0.608 x	25	- 0.240
11	0.610 x	26	- 0.178
12	0.610 x	27	0.608 x
13	0.610 x	28	0.203
14	- 0.240	29	0.610 x
15	- 0.289	30	- 0.240

[a] Percentage of infected leaves/plant in a random sample of 10 plants/plot.
[b] Pearson correlation coefficient, which measures the degree of association between powdery mildew severity and the designated isozyme.
[c] Significant at $p < 0.10$.

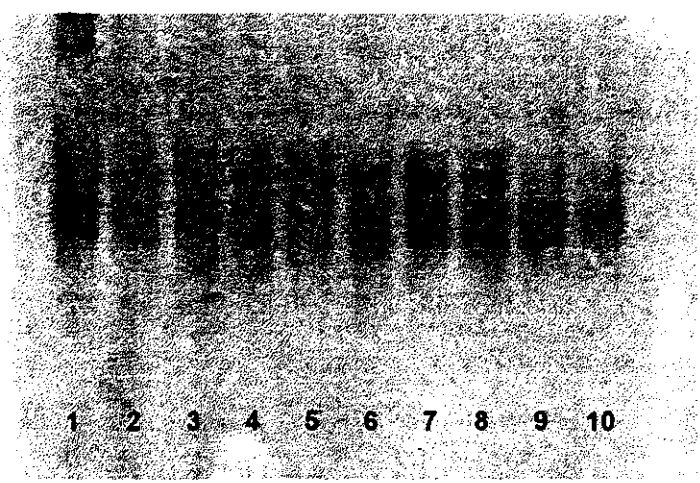


Fig. 3. Esterase isozyme patterns obtained by polyacrylamide gel electrophoresis (PAGE) from flax seeds. Flax cultivars in lanes 1 through 10 were Ottowa 770 B (1), Dakota (2), Bombay (3), Cass (4), Koto (5), Clay (6), Wilden (7), Marshall (8), Cortland (9), and C.I. 2008 (10).

Table (11): Regression equation that describes the effects of some esterase isozymes (X_s) on severity of flax powdery mildew (Y).

Stepwise regression model	R^2 [a]	F. value [b]
$Y = 5.37 + 11.15 X_5 + 2.08 X_{10}$	83.46%	17.65***

[a] Coefficient of determination. Relative contributions of the predictors X_5 and X_{10} to R^2 are 37.23 and 46.23 %, respectively.
[b] F. value is significant at $p < 0.005$ (***).

DISCUSSION

The conventional methods for evaluating flax cultivars for PM resistance are to evaluate them under field and greenhouse conditions. Experience with flax PM showed that each method has its potential limitations. Under field conditions, susceptibility of cultivars to PM may be obscured by the nonhomogenous distribution of the nature inoculum. In some years, susceptible cultivars may escape from infection due to the lack of inoculum or the prevailing of unfavorable environmental conditions. In addition, field tests are expensive and time-consuming. Admittedly, screening of cultivars under greenhouse conditions may overcome these difficulties and improve the efficiency of screening process; however, the greenhouse should be equipped with efficient expensive air-conditioning system to maintain greenhouse temperature at about 25°C. Thus, a new method should be developed to evaluate resistance of flax cultivars to PM. This method should meet two requirements. It should be independent of the pathogen, and should reflect the genetic differences among cultivars. Isozymes reasonably meet these requirements for several reasons. Amino acid sequence of polypeptides (components of isozymes) are dependent on nucleotide sequence of their coding genes, therefore, an analysis of isozymic variation among flax cultivars by electrophoresis, approximates an analysis of their genetic variation (Markert and Faulhaber, 1965). Electrophoretic patterns of isozymes can be obtained rapidly and with small amounts of tissue. Therefore, large number of single plant selections can be tested without sacrificing the plants (Wheeler *et al.*, 1971). The growing conditions have no influence on isozyme patterns (Kobrehel and Gautier, 1974).

The utility of the electrophoretic data depends on the method for statistical analysis. Multiple regression was a logical choice for construction of a predictive model, but the complex nature of banding patterns warranted a method to eliminate bands with no predictive value. Stepwise regression is the best variable selection procedure because it eliminates from the model any variable whose contribution to predictive ability is statistically insignificant (Draper and Smith, 1981 and Podleckis *et al.*, 1984).

In the present study, satisfactory visualizations of isozyme banding patterns were obtained by using three staining systems, and the stepwise regression models they generated proved effective in predicting PM

severity from banding patterns. Coefficient of determination (R^2) values of MDH, PRX and EST models were 98.67, 83.46 and 83.46%, respectively.

The most common technique for selection of PM-resistant flax cultivars has been thoroughly ratings of visible foliar symptoms. The time and effort involved in these selection tests have limited plant breeders in selecting PM-resistant cultivars. PAGE of isozymes, such as that described herein, may provide a supplementary assay to greenhouse and field tests to distinguish quantitatively between PM resistant or susceptible cultivars.

Acknowledgement

This research was supported by the Research Project No. EU. 13.74.96 (Integrated Control of Principal Flax Diseases in Egypt).

REFERENCES

Aly, A.A., A.Z.A. Ashour, E.A.F. El-Kady and M.A. Mostafa. 1994. Effectiveness of fungicides for control of powdery mildew of flax and effect of the disease on yield and yield components. J. Agric. Sci. Mansoura Univ. 19: 4383-4393.

Bradford, M.M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 72: 248-254.

Draper, N.R. and H. Smith. 1981. "Applied Regression Analysis", 2nd Ed. John Wiley, New York, 709p.

Hussein, E.M. 1992. Biochemical and serological studies for determining susceptibility of cotton cultivars to *Fusarium oxysporum* f.sp. *vasinfectum*. (In Russian). Ph.D.Thesis, All-Union Institute of Plant Protection, Leningrad, USSR.

Ignatius, S.M.J., R.K. Chopra, and S. Muthukrishnan. 1994. Effect of fungal infection and wounding on the expression of chitinases and beta-1,3 glucanases in near-isogenic lines of barley. Physiol. Plant 90:584-592.

Koberhel, A.S. and A.H. Gautier. 1974. Peroxidase isozymes from horseradish roots. J. Biol. Chem. 242: 2470-2473.

Laemmli, U.K. 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature 227: 680-685.

Latorre, B.A., G.F. Perez, W.F. Wilcox, and R. Torres. 1995. Comparative protein electrophoretic and isozymic patterns of *Phytophthora cryptogea* isolated from Chilean Kiwifruit and North American deciduous fruits. Plant Dis. 79: 703-708.

Lebeda, A., E. Kristkova, and K. Dolezal. 1999. Peroxidase isozyme polymorphism in *Cucurbita pepo* cultivars with various morphotypes and different levels of field resistance to powdery mildew. Scientia Horticulturae 81:103-112.

Manchenko, G.P. 1994. "Handbook of Detection of Enzymes on Electrophoretic Gels". CRC Press, Inc., Boca Raton, Florida. 341p.

Markert, C.L. and I. Faulhaber. 1965. Lactate dehydrogenase isozyme patterns of fish. J. Exp. Zool. 159: 319-332.
Nutter, F.W., Jr. P.S. Teng, and F.M. Shoks. 1991. Disease assessment terms and concept. Plant Dis. 75: 1187-1188.
Pandey, R.N. and D.P. Misra. 1993. Assessment of yield loss due to powdery mildew of linseed. Indian Botanical Reports 11:62-64.
Pearce, W.L., D.H. Van Sanford, and D.E. Hershman. 1996. Partial resistance to powdery mildew in soft red winter wheat. Plant Dis. 80: 1359-1362.
Podleckis, E.V., C.R. Crutis, and H.E. Heggestad. 1984. Peroxidase enzyme markers for ozone sensitivity in sweet corn. Phytpathology 74: 572-577.
Wang, L.X., Q.Su, T.T. Kang, M.X. Xu, and Z. Chen. 1999. A biochemical marker for resistance to powdery mildew in wheat-peroxidase isozyme band pl6-1. Acta Agriculturae Boreali Sinica 14: 93-97.
Wheeler, H.A., A. Navacky, and H.H. Luke. 1971. Isozymes of Victoria blight-resistant oat lines selected from susceptible cultivars. Phytopathology 61:1147-1148.
Xia, W., Z. Li, and K. Wang. 1986. Studies on the isoenzymes of powdery mildew-resistant wheat mutants induced by gamma radiation. Scientia Agriculture Sinica 1:93.

إستعمال محتوى البذرة من المشابهات الإنزيمية للتعبير الكمى عن مقاومة أصناف الكتان لمرض البياض الدقيقى

على عبد الـهادى على[1] ، عـزت محـمد حسـين[1] ، محمود توفـيق محـمود منـصـور[1] و ممدوح أبو مسلم تاج الدين[2]
[1] معهد بحوث أمراض النباتات – مركز البحوث الزراعية – الجيزة – مصر.
[2] قسم الكيمياء الحيوية الزراعية– كلية الزراعة – جامعة عين شمس – شبرا الخيمة – القاهرة.

أجريت تجربة حقلية بمحطة البحوث الزراعية بالجيزة خلال موسمى ٢٠٠٢/٢٠٠٣ و ٢٠٠٣/٢٠٠٤ لتقييم عشرة أصناف كتان من حيث المقاومة أو القابلية للإصابة بمرض البياض الدقيقى. إنقسمت الأصناف إلى أربع مجموعات محددة هى على النحو التالى: عالية المقاومة (اوتوا ٧٧٠ب وداكوتا وبومباى)، مقاومة (كاس وويلدين وكلاى)، قابلة للإصابة (كوتو ومارشال)، شديدة القابلية للإصابة (كورتلاند والصنف ٢٠٠٨). أظهرت الأصناف تباين واضح فى شدة الإصابة التى تراوحت ما بين ٣,٦٩ على الصنف بومباى إلى ١٠٠% على الصنف ٢٠٠٨. استعملت تقنية التفريد الكهربى لفصل المشابهات الإنزيمية لكل من الماليت ديهيدروجينيز والبيروأوكسيديز والإستريز من بذور الأصناف. استعملت ثلاث نظم صبغ لإظهار أنماط المشابهات الإنزيمية المتحصل عليها. أمكن- باستخدام أسلوب الانحدار المتعدد المرحلى- التوصل إلى ثلاث نماذج رياضية لوصف العلاقة بين شدة الإصابة بالبياض الدقيقى (متغير تابع) و النسب المئوية للمشابهات الإنزيمية المفصولة (متغيرات مستقلة) كانت قيم معامل التحديد للنماذج الثلاثة هى ٩٨,٦٧ و ٨٣,٤١ و ٨٣,٤٦% لانزيمات الماليت ديهيدروجينيز والبيرأوكسيديز والإستريز، على التوالى. تدل نتائج الدراسة الحالية على أنه من الممكن إستخدام تقنية التفريد الكهربى للمشابهات الإنزيمية كوسيلة مكملة لاختبارات الصوبة والحقل للتفرقة الكمية بين أصناف الكتان المقاومة أو القابلة للإصابة بالبياض الدقيقى.