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USE OF AMINO ACID COMPOSITION OF SEED TO QUANTIFY RESISTANCE OF FLAX CULTIVARS TO POWDERY MILDEW DISEASE

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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. GLC analysis of amino acid composition of cultivar seeds revealed the presence of 18 amino acids. Regarding mean percentages of the separated amino acids, glutamic acid showed the highest value (20.29%), while tryptophan showed the lowest value (0.45%). The other amino acids showed intermediate values between these two extremes. DS was positively correlated with serine ($r = 0.858$, $P < 0.01$). On the other hand, none of the other amino acids was significantly correlated with DS. Data for DS ratings and amounts of amino acids were entered into a computerized stepwise multiple regression analysis. Using the predictors supplied by stepwise regression, a six-factor model was constructed to predict PM severity. This model showed that PM severity differences were due largely to the amino acids serine, threonine, isoleucine, leucine, alanine and valine, which accounted for 99.83% of the total variation in DS. These results indicate that amino acid composition of linseed may provide a supplementary assay to greenhouse and field tests to distinguish quantitatively between. PM resistant and susceptible genotypes.

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 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 grater 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.

A considerable body of literature has shown a strong relationship between amino acids and powdery mildew diseases. For example, Ali (1984) studied the relationship between cucumber susceptibility to PM and amino acid content of leaf exudates. He found that in infected plants, total free amino acid content of leaf exudates from the highly susceptible variety Beit alpha was higher than that of the highly resistant variety Yomaki. In general, infection increased total amino acids compared with control in Beit alpha, whereas, in case of Yomaki, the content was lower in infected plants than in healthy ones.

Hwang (1985) reported that ethanol-soluble amino acids in healthy and powdery mildew (*Erysiphe graminis*) infected 1st leaves of the susceptible spring barley cv. Peruvian and the adult plant resistant Asse one day after inoculation were similar to those of comparable healthy controls. During sporulation, increases in amino acids were more pronounces in Peruvian than those in Asse. The changes in amino acid content in the 1st and 5th leaves were closely related to the number of colonies/leaf. Peruvian showed higher amounts of amino acids in these leaves at all infection intensities than did Asse.

El-Desouky (1988) found that PM-highly susceptible cucumber variety Beit alpha contained higher amounts of total free amino acids in healthy leaves than the highly resistant one Medina hybrid.

El-Hafez *et al.* (1990) reported that PM-susceptible cucumber plants contained low total amino acids, while the reverse trend was observed in resistant plants.

Kaushal *et al.* (1992) mentioned that PM-infected leaves of wheat contained histidine, arginine, asparagine and glutamine, whereas healthy ones contained the same 4 amino acids plus threonine.

Through correlations and path coefficient analysis of a field trial with peas cv. Rachana (resistant) and T163 (susceptible), Bhattacharya and Shukla (1996) concluded that severity of powdery mildew (*Erysiphe polygoni*) on field pea is substantially increased by the accumulation of free amino acids.

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

Therefore, the objectives of the present study were to (1) evaluate the relationship of PM disease on flax to amino acid composition of seed (2) develop a statistical model to predict PM severity by using amino acids in the seed as biochemical predictors.

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 total amino acids:

Random samples of seeds, taken from the same seeds used in planting the field trial, were used for the extraction of total amino acids by the following method: Half gram of defatted ground flaxseed was hydrolyzed with HCl 6N at 105°C for 16 hr (AOAC, 1990).

Derivatization of amino acids:

Trifluoroacetyl amino acid butyl esters were prepared by the method of Lamkin and Gehrke (1966).

Separation of total amino acid derivatives by using GLC:

Separation of total amino acid derivatives was executed on a glass column (2m), packed with 3% OV_{225}, coated on 80/100 mesh, chromosorb W.HP. Varian's G.C. (model 3700). Injector and flame ionization detector (FID) temperatures were set at 200°C and 240°C, respectively. Flow rate of the carrier gas N_2 was 20ml/min. Oven temperature was initially started at 80°C for 4 min, then programmed to rise 4°C/min. to 200°C, at that point elution was continued isothermally for 10 min. Amino acid peaks were identified from calibration runs with known mixtures of amino acids (Mahmoud, 2000).

Statistical analysis of the data:

Linear correlation coefficient was calculated to evaluate the degree of association between PM severity and the percentage of each fatty acid. Stepwise regression technique with the greatest increase in R^2 as the decision criterion was used to describe the effects of amino acids on PM severity. Correlation and regression analyses were performed with a computerized program.

RESULTS AND DISCUSSION

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 1), 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 1).

Table 1. 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 770B	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).

The GLC analysis of amino acid composition of seeds (Table 2) revealed the presence of 18 amino acids. Regarding mean percentages of the separated amino acids, glutamic acid showed the highest value (20.29%), while tryptophan showed the lowest value (0.45%). The other amino acids showed intermediate values between these two extremes.

It is well known that the type and degree of association between characters may facilitate or complicate the selection process in breeding programs. Selection for a character may result in an impovement or deterioration in other characters according to the type and degree of correlation. Hence, it was desirable to assess the type and degree of association between DS and amino acids in linseed.

Pearson correlation coefficient was calculated to measure the degree of association between DS and the amount (%) of each amino acid (Table 3). DS was positively correlated with serine ($r= 0.858$, $P<0.01$). On the other hand, none of the other amino acids was significantly correlated with DS (Table 3).

Table 2. Amino acid composition (%) of flax seed.

Amino acid	Cultivar										
	Ottowa770 B	Dakota	Bombay	Cass	Koto	Clay	*Wilden*	*Marshall*	*Cortland*	C.I. 2008	***Mean***
Alanine	3.0	3.4	2.8	5.9	3.7	6.7	3.9	2.9	6.1	5.9	4.43
Valine	11.0	8.6	11.7	7.5	3.9	7.0	4.9	3.9	6.7	7.1	7.23
Threonine	7.4	5.2	6.9	3.0	2.7	5.3	4.3	3.8	4.8	3.0	4.64
Glycine	3.2	8.8	12.7	7.5	8.8	7.0	7.2	5.4	9.2	7.3	7.71
Isoleucine	4.2	5.1	9.8	5.8	3.5	4.2	4.2	5.6	7.5	5.7	5.56
Leucine	1.4	3.0	4.0	2.7	6.8	2.2	1.5	11.6	1.9	2.5	3.76
Serine	2.7	1.6	1.2	1.1	1.6	1.5	1.8	1.9	4.0	4.1	2.15
Proline	8.1	5.2	20.5	11.4	16.2	8.7	4.6	2.9	9.6	9.4	9.66
Cysteine	3.5	0.6	4.5	3.2	4.8	1.5	1.5	1.3	3.6	2.0	2.65
Y-Aminobutyric	2.2	1.6	2.1	2.7	4.8	2.5	2.7	0.9	2.1	2.3	2.39
Metheonine	5.2	2.1	1.5	2.7	3.8	3.8	4.3	2.0	2.9	1.9	3.02
Aspartic	7.6	13.0	2.0	6.3	12.8	14.4	13.4	14.9	8.2	10.0	10.26
Phenylalanine	10.0	11.6	11.2	16.2	5.8	13.3	15.0	12.7	11.1	12.0	11.89
Histidine	2.4	0.6	2.1	1.7	2.7	0.7	1.8	1.9	0.4	1.3	1.56
Glutamic	25.0	24.9	5.6	20.2	10.5	20.3	28.1	25.1	20.7	22.5	20.29
Tyrosine	1.4	2.0	0.5	0.3	2.0	0.4	0.1	0.6	0.6	0.3	0.82
Lysine	1.2	2.0	0.7	1.4	4.5	0.4	0.3	1.9	0.4	1.9	1.47
Tryptophan	0.5	0.6	0.2	0.4	1.1	0.1	0.4	0.6	0.2	0.4	0.45

Table 3. Relationship of powdery mildew severity[a] on ten flax cultivars and amino acid composition (%) of seeds from these cultivars.

Amino acid	r[b]	Amino acid	r
Alanine	0.446	Y-Aminobutyric	- 0.139
Valine	- 0.373	Metheonine	- 0.299
Threonine	- 0.442	Aspartic	0.095
Glycine	- 0.049	Phenylalanine	- 0.086
Isoleucine	0.164	Histidine	- 0.332
Leucine	0.122	Glutamic	0.165
Serine	0.858**[c]	Tyrosine	- 0.291
Proline	- 0.164	Lysine	0.032
Cysteine	- 0.025	Tryptophan	- 0.091

[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 amino acid.
[c] Significant at $p < 0.01$ (**).

The conventional methods for evaluating flax genotypes 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 genotypes to PM may be obscured by the nonhomogeneous distribution of the natural inoculum. In some years, susceptible genotypes may escape from infection due to the lack of natural inoculum or the prevailing of unfavorable environmental conditions. In addition, field tests are expensive and time-consuming. Admittedly, screening of genotypes under greenhouse conditions may overcome these difficulties and improve the efficiency of screening process; however, the greenhouse should be equipped with efficient and expensive air-conditioning system to maintain greenhouse temperature at about 25°C. Thus, a new method should be developed to evaluate resistance of flax genotypes to PM. This method should meet two requirements. It should be independent of the pathogen, and should reflect the genetic differences among genotypes. Amino acid composition of linseed may meet these requirements for several reasons. Amino acids may have a direct effect on pathogenic fungi, they act as fungicides of fungistatics. Amino acids may disturb the normal nitrogen metabolism of the plant resulting in fungicidal of fungistatic substances, they may also result in a change in the nutritional conditions for the fungus (Van Andel, 1966). Amino acid profiles can be obtained rapidly and with small amounts of seed by using GLC. Therefore, large number of genotypes can be tested without sacrificing the seeds.

Data for PM severity and amounts of amino acids were entered into a computerized stepwise multiple regression analysis. The analysis constructed a predictive model by adding predictors, in this case, amounts of amino acids, 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 statistically significant contribution to the R^2 value of the model (Podleckis *et al.*, 1984). Using the predictors supplied by stepwise regression, a six-factor model was constructed to predict PM severity (Table 4). This model showed that PM severity differences were due to largely to serine, threonine, isoleucine,

leucine, alanine and valine, which accounted for 99.83% of the total variation in DS.

Table 4. Regression equation that describes the effects of some amino acids (X_s) on severity of flax powdery mildew (Y).

Stepwise regression model	$R^{2\ a}$	F. value
$Y = -52.11 + 27.34 X_7 - 3.44 X_3 + 4.68 X_5 + 3.23 X_6 + 5.02 X_1 - 2.31 X_2$	99.83%	287.76***

[a] Coefficient of determination. Relative contribution of the predictors X_7 (Serine), X_3 (Threonine), X_5 (Isoleucine), X_6 (Leucine), X_1 (Alanine), and X_2 (Valine) to R^2 were 73.55, 14.47, 5.29, 3.47,2.45, and 0.59%, respectively. F. value was significant at $p < 0.005$ (***).

The most common technique for selection of PM-resistant flax genotypes has been through ratings of visible foliar symptoms. The time and effort involved in these selection tests have limited flax breeders in selecting PM-resistant genotypes. Therefore, regression models, which include amino acids as predictors, as that describe herein, may provide a supplementary method to greenhouse and field tests to distinguish between PM resistant or susceptible genotypes quantitatively.

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استعمال محتوى البذرة من الأحماض الأمينية للتعبير الكمى عن مقاومة أصناف الكتان لمرض البياض الدقيقى

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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 ويلدين 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. أظهر التحليل الكروماتوجرافى (GLC) أن بذور الأصناف احتوت على ١٨ حمض أميني. كان الجلوتامين هو أكثر الأحماض تواجدا فى الأصناف المختلفة إذا وصل متوسط نسبة تواجده إلى ٢٠,٢٩%، فى حين كان التربتوفان هو أقل الأحماض تواجدا فى الأصناف المختلفة إذ انخفض متوسط نسبة تواجده إلى ٠,٤٥%، أما الأحماض الأخرى فإن وجدت بنسب مختلفة محصورة بين هاتين النسبتين. أظهرت الدراسة وجود ارتباط موجب عالى المعنوية بين شدة المرض والحمض سيرين، فى حين لم ترتبط شدة المرض مع أى من الأحماض الأمينية الأخرى. أمكن – باستخدام أسلوب الانحدار المتعدد المرحلى– التوصل إلى نموذج رياضى لوصف العلاقة بين شدة المرض (متغير تابع) و الأحماض الأمينية المفصولة (متغيرات مستقلة) أظهر هذا النموذج أن ٩٩,٨٣% من إجمالى التباين فى شدة المرض من الممكن أن تعزى إلى تأثيرات الأحماض الأمينية سيرين وثريونين وأيسوليوسين وليوسين وألانين وفالين. تدل نتائج الدراسة الحالية على أنه من الممكن إستخدام محتوى بذرة الكتان من الأحماض الأمينية كوسيلة مكملة لاختبارات الصوبة والحقل للتفرقة الكمية بين تراكيب الكتان الوراثية المقاومة أو القابلة للإصابة بالبياض الدقيقى.