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# USE OF FATTY 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 770B, Dakota and Bombay), resistant (Cass, Wilden, and Clay), susceptible (Koto and Manshall), and highly susceptible (Cortland and C.I.2008). The cultivars showed considerable variation in disease severity (DS) ratings ranged from 3.69 on Bombay to 100% on C.I.2008. GLC analysis of fatty acid composition of cultivar seeds revealed the presence of the following fatty acids: Myristic, palmitic, palmitoleic, stearic, oleic, linoleic, linolenic and arachidic. However, the unsaturated fatty acids oleic, linoleic and linolenic were predominant in linseed oil. The total percentage of the three fatty acids ranged from 88 in Cortland to 92.9% in Ottowa 770B. PM severity was positively correlated with each of palmitoleic ($r=0.776$, $p<0.01$) and stearic ($r=0.704$, $p<0.05$). On the other hand, none of the other fatty acids was significantly correlated with PM severity. Data for PM ratings and amounts of fatty acids were entered into computerized stepwise multiple regression analysis. Using the predictors supplied by stepwise regression, a two-factor model was constructed to predict PM severity. This model showed that PM severity differences were due to largely to the fatty acids palmitoleic and myristic, which accounted for 80.16% of the total variation in PM severity. These results indicate that fatty acid composition of linseed oil may provide a supplementary assay of greenhouse and field tests to distinguish quantitatively between PM resistant and susceptible genotypes.

## INTRODUCTION

Powdery mildew (PM) of flax (*Linum usitatissimum* L.) is caused by the obligate parasite *Oidium lini* Škoric. This fungus is found on flax in Egypt only in its imperfect (conidial) stage. The pathogen infects all aboveground flax organs including stems, leaves, flowers, and capsules. PM occurs annually in all flax production areas in Egypt. Physiological races of the pathogen have not been identified because no differential host lines are available (A.A. Aly, *personal observation*).

In flaxseed, both endosperm and embryo are oil storing tissues. The embryo occupies the central cavity of the seed and is the major tissue both in size and oil content. Oil content of the embryo may be as high as 60 to 70% of the tissue dry weight. Linolenic acid comprises 40 to 60% of the total fatty acids of the two tissues. Others include palmitic and stearic acids (less than 10% each), oleic acid (15 to 30%), and linoleic acid (10 to 20%) (Dybing and Lay, 1981).

Oleic, linoleic and linolenic are considered to be unsaturated since they have double bonds between adjoing carbons. These double bonds are the active sites in the molecule where other elements or compounds may combine or react. Oleic has one double bond, linoleic two double bonds and linolenic three double bonds. Quality of linseed oil for industrial uses is determined by the percentage of these three unsaturated fatty acids, particularly linolenic acid. Oil quality is commonly measured as iodine value (Kenaschuk, 1975).

Currently, all commercially grown flax cultivars in Egypt are susceptible to PM. 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).

To date, as far as we know, no attempts have been made to evaluate the degree of association between PM intensity ratings and fatty acid composition of flaxseed. Therefore, the objectives of the present study were to (1) evaluate the relationship of PM disease on flax to fatty acid composition of seed and (2) develop a model to predict PM severity by using fatty 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 crude fat:**

Random samples of seeds, taken from the same seeds used in planting the field trial, were used for extraction of crude fat by using dimethyl ether according to AOAC (1990).

**Identification of fatty acids by using GLC:**

The fatty acids of the oil were converted to methyl esters using sodium methoxide according to Chman *et al.* (1973). Methyl esters fatty acids were identified by using Perkin-Elmer 8310 GC, with column 200 cm stainless steel 10% silar 10C on 100/120 Gas Chrom.Q. Both of injector and flame ionization detector (FID) temperature were 150°C and 250°C, respectively. Carrier gas was nitrogen with 20ml/min. flow rate. The program began with 100°C for 2 min., then increased to 200 °C with rate of 10/min. and isothermally at 200 °C for 25 min.

**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 effect of fatty 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 resistance (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 ratings 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 fatty acid composition of seeds (Table 2) revealed that the unsaturated fatty acids oleic, linoleic and linolenic were predominant in linseed oil. The total percentage of the three fatty acids ranged from 88 in Cortland to 92.9% in Ottowa 770B. The predominance of the three fatty acids in linseed oil, as we have demonstrated herein, is in agreement with the reports of Kenashuk (1975) and Dybing and Lay (1981).

It is well known that the type and degree of correlation between characters may facilitate or complicate the selection process in breeding programs. Selection for a character may result in an improvement 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 fatty acids in linseed oil.

**Table 2. Fatty acid composition (%) of flax seed.**

| Fatty acid | Cultivar | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Ottowa770 B* | Dakota | Bombay | Cass | Koto | Clay | *Wilden* | *Marshall* | *Cortland* | C.I. 2008 |
| Myristic acid (C14) | 0.2 | 0.1 | 0.1 | 0.0 | 0.1 | 0.7 | 0.1 | 0.1 | 0.1 | 0.1 |
| Palmitic acid (C16) | 5.8 | 5.9 | 5.8 | 5.7 | 5.8 | 5.6 | 5.8 | 5.8 | 6.6 | 5.5 |
| Palmitoleic acid (C16:1) | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.0 | 0.1 | 0.1 | 0.2 | 0.2 |
| Stearic acid (C18) | 0.0 | 3.4 | 3.1 | 2.5 | 2.9 | 3.0 | 3.2 | 3.0 | 4.6 | 5.6 |
| Oleic acid (C18:1) | 30.2 | 28.0 | 24.3 | 25.5 | 25.2 | 25.9 | 27.6 | 26.1 | 23.7 | 30.4 |
| Linoleic acid (C18:2) | 13.9 | 15.9 | 14.3 | 14.0 | 14.5 | 14.6 | 14.2 | 14.0 | 14.6 | 13.5 |
| Linolenic acid (C18:3) | 48.8 | 46.3 | 51.7 | 51.2 | 50.9 | 49.6 | 48.8 | 49.9 | 49.7 | 43.6 |
| Arachidic acid (C20) | 0.7 | 0.1 | 0.5 | 0.6 | 0.2 | 0.3 | 0.1 | 0.7 | 0.2 | 0.5 |

Pearson correlation coefficient was calculated to measure the degree of association between PM severity and the amount (%) of each fatty acid (Table 3). PM severity was positively correlated with each of palmitoleic ($r=0.776$, $p<0.01$) and stearic ($r=0.704$, $p<0.05$). In spite of the significance of these correlations, it seems reasonable to conclude that they are not practically important due to the very low percentages of the two fatty acids in linseed oil (Table 2). On the other hand, none of the other fatty acids was significantly correlated with PM severity (Table 3).

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

| Fatty acid | r [b] |
|---|---|
| Myristic acid (C14) | - 0.178 |
| Palmitic acid (C16) | 0.353 |
| Palmitoleic acid (C16:1) | 0.776**[c] |
| Stearic acid (C18) | 0.704* |
| Oleic acid (C18:1) | 0.013 |
| Linoleic acid (C18:2) | - 0.354 |
| Linolenic acid (C18:3) | - 0.419 |
| Arachidic acid (C20) | 0.039 |

[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 fatty acid.
[c] Significant at $p < 0.01$ (**) or $p < 0.05$ (*).

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 cultivars to PM may be obscured by the nonhomogeneous distribution of the natural inoculum. In some years, susceptible cultivars 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. Fatty acid composition of linseed oil may meet these requirements for several reasons. Fatty acids are the major components of flaxseed (Dybing and Lay, 1981). Amounts of fatty acids are highly heritable characters (Kenaschuk, 1975). Some fatty acids are potent inhibitors of germination and inducers of cell death of PM spores (Wang *et al.*, 2002). Currently, fatty acids or their derivatives are widely used as natural fungicides for controlling PM on many plants (Pasini *et al.*, 1997; Giovannantonio *et al.*, 1997; Santomauro *et al.*, 1997; Brunelli *et al.*, 1998; Egger *et al.*, 1998; McGrath and Shishkoff, 1999). Fatty acid profiles can be obtained rapidly and with small amounts of seeds by using GLC. Therefore, large number of genotypes can be tested without sacrificing the seeds.

Data for PM severity and amounts of fatty acids were entered into a computerized stepwise multiple regression analysis. The analysis constructed a predictive model by adding predictors, in this case amounts of fatty 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 two-factor model was constructed to predict PM severity (Table 4). This model showed that PM severity differences were due to largely to palmitoleic acid and myristic acid, which accounted for 80.16% of the variation in PM severity.

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

| Stepwise regression model | $R^2$ [a] | F. value |
|---|---|---|
| $Y = -68.40 + 772.66\,X_3 + 114.10\,X_1$ | 80.16% | 14.14*** |

[a] Coefficient of determination. Relative contribution of the predictors $X_3$ (Palmitoleic acid) and $X_1$ (Myristic acid) to $R^2$ were 60.25 and 19.92 %, 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 fatty 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 الدهنية للتعبير الكمى عن مقاومة أصـــناف الكتان لمرض البياض الدقيقى

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أجريت تجربة حقلية بمحطـة البحـوث الزراعيـة بالجيـزة خلال موسمى ٢٠٠٢/٢٠٠٣ و٢٠٠٣/٢٠٠٤ لتقييم عشرة أصناف كتان من حيث المقاومة أى القابلية للإصابة بمرض البياض الدقيقى. انقسمت الأصناف إلى أربع مجموعات محددة هى على النحو التالى: عاليـة المقاومـة (أوتوا ٧٧٠ب وداكوتا وبومباى)، مقاومة (كاس و 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 رقم ٢٠٠٨. أظهر التحليل الكروماتوجرافى (GLC) أن بذور الأصناف تحتوى على الأحماض الدهنية الآتية: ميريستيك وبالمتيك وبالميتوأوليك وستياريك وأوليك ولينوليك ولينولنيك وأراكيديك. الأحماض الدهنية الغير مشبعة أوليك ولينوليك ولينولنيك كانت هى السائدة فى زيت بذرة الكتان إذ تراوحت النسبة المئوية الكلية لهذه الأحماض من ٨٨ فى الصنف كورتلاند إلى ٩٢,٩% فى الصنف أوتوا ٧٧٠ب. أظهرت الدراسة وجود ارتباط موجب بين شدة الإصابة بالمرض والنسبة المئوية لكل من بالميتوليك وستياريك، فى حين لم ترتبط شدة المرض مع أى من الأحماض الدهنية الأخرى. أمكن– باستخدام أسلوب الانحدار المتعدد المرحلى– التوصل إلى نموذج رياضى لوصف العلاقة بين شدة المرض (متغير تابع) والأحماض الدهنية المفصولة (متغير مستقل). أظهر هذا النموذج أن ٨٠,١٦% من التباين فى شدة المرض من الممكن أن تعزى إلى تأثير الحمضين بالميتوليك وميريستيك. تدل نتائج الدراسة الحالية على أنه من الممكن استخدام محتوى بذرة الكتان من الأحماض الدهنية كوسيلة مكملة لاختبارات الصوبة والحقل للتفرقة الكمية بين تراكيب الكتان الوراثية المقاومة أو القابلة للإصابة بالبياض الدقيقى.