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USE OF SEROLOGICAL INTERACTIONS TO QUANTIFY RESISTANCE OF FLAX CULTIVARS TO POWDERY MILDEW DISEASE

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ABSTRACT

Eight flax cultivars were evaluated for powdery mildew resistance under field conditions in 2001/2002 and 2002/2003 growing seasons. The tested cultivars could be divided into two distinct groups. The first group included the resistant cultivars Dakota, Wilden, and Williston Brown, while the second one included the susceptible cultivars Cortland, Linore, C.I. 2008, Giza 7, and Giza 8. The difference in disease severity was highly significant ($P \leq 0.01$) between any cultivar belonged to the first group and any cultivar belonged to the second group. The antigenic relationship between *Oidium lini* and flax cultivars was studied by two methods. In the first method, the antiserum of the infected whole plants of cultivar Giza 8 interacted against antigens of seeds from the healthy flax cultivars. In the second method, antiserum of *O. lini* alone interacted against the same antigens after the elimination of Giza 8 antibodies from the antiserum by the reciprocal preceptitation. In the first method, regression analysis indicated that the common antigens between the infected Giza 8 and the healthy cultivars accounted for 86% of the explained (model) variation in severity of powdery mildew on these cultivars. In the second method, regression analysis indicated that the common antigens between *O. lini* alone and the healthy cultivars accounted for 76% of the explained (model) variation. The first method was superior to the second method for two reasons. First, it did not require the application of the reciprocal precipitation, which greatly simplified the test. Second, this method gave higer coefficient of determination (R^2) value, which indicated that it was more reliable for predicting resistance to powdery mildew.

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 the aboveground flax organs including stems, leaves, flowers and capsules. PM occurs annually in all flax production areas in Egypt (Mansour, 1998).

Significant negative correlations were found between disease intensity ratings and agronomic traits (Aly *et al.*, 1994). Currently, all commercially grown flax cultivars are susceptible to the disease, although field observations indicated that some experimental lines were more susceptible than others (Aly *et al.*, 2001).

Fungicides are currently the only commercially available management practices for controlling the disease and minimizing associated losses in seed and straw yield (Aly *et al.*, 1994 and Mansour, 1998). 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 application. Furthermore,

increasing cocern for the environment will likely mean greater regulation of pesticide usage (Pearce *et al.*, 1996).

Use of cultivars with PM resistance can resolve all these problems. Currently, field evaluation is the only reliable method to distinguish flax genotypes with PM resistance. Howevere, the precision of field evaluation of genetic resistance is adversely affected by environmental variation and heterogeneous levels of natural inoculum. In addition, field evaluation is expensive and time-consuming.

Therefore, another reliable method, either alternative or complementary to field evaluation, is required for the identification of flax genotypes with PM resistance.

In several instances, it has been found that plant hosts have antigenic substances in common with parasitic microorganisms. These substances have been termed "Common antigens" (Charudattan and DeVay, 1972). One of the theories that has been proposed to explain the primary factor in the plant-parasite interaction, which would lead either to susceptibility or resistance of the plant is the degree of antigenic parity between the plant and the pathogen. According to this concept the greater the antigenic parity between the plant and the pathogen, the greater will be susceptibility of the plant to the pathogen (Wimalajeewa and DeVay, 1971). It seems that the presence of common antigens may be an important factor that prevents triggering of the plant defense mechanism, thus allowing the pathogen to parasitize the plant (Charudattan and DeVay, 1972).

Charudattan and Devay (1972) demonstrated a common antigen relationship among *Fusarium* spp. and wilt-susceptible and wilt-tolerant varieties of cotton. When the common antigenic substance was isolated and purified, it was found to be polysaccharide-protein complex. It was postulated that the common antigen may be involved in the establishment and survival of *Fusarium* isolates in host tissue.

When the seed globulins of cotton cultivars susceptible to Fusarium wilt reacted with antiserum of *F. oxysporum* f.sp. *vasinfectum*, more precipitin lines were formed than with the resistant cultivars. On the other hand, no obvious reaction was detected in case of antiserum of *F. moniliforme*, which was nonpathogenic on cotton (Abd-El-Rehim *et al.*, 1988).

Proteins of *Rhizoctonia solani* were compared with those of host and nonhost plants by double diffusion. Flax and Kenaf were used as host plants, while wheat, barley and sorghum were the nonhost plants. In the reactions of antiserum of *R. solani* with homologous antigen and plant antigens, among the four bands formed in the homologous reaction, two were common with the antigens of host plants. No common antigens were shared between *R. solani* and any of the nonhost plants. This result, supports the concept that the greater the antigenic (protein) parity between the host and the pathogen, the greater will be the susceptiblity of the host to the pathogen (Hussein *et al.*, 1997). Shady *et al.* (2000) reported that common antigens were detected only between *Fusarium oxysporum* f.sp. *vasinfectum* and the susceptible cultivars Giza 71 and Giza 74, while no common bands were detected between this pathogen and the resistant cultivars Dandara, Giza 70, Giza 75, Giza 77 and Giza 85.

The present study was initiated to determine whether the PM resistance of fax cultivars can be quantified by using the double diffusion technique.

MATERIALS AND METHODS

Evaluation of flax genotypes for PM resistance

Experiments were conducted in 2001/2002 and 2002/2003 growing season. Experiment consisted of a randomized complete block design of 5 replicates (blocks). Plots were 2 x 3 m (6 m^2) and consisted of ten rows spaced 20 cm apart. Seeds of each genotype were sown by hand at a rate of 70 g/plot. Planting dates were 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:

Protein extract was prepared according to Hussein (1992) in the following way: Seeds of healthy plants of flax cultivars Giza 7, Giza 8, C.I. 2008, Linore, Cortland, Williston Brown, Wilden and Dakota were slightly ground and defatted by diethyl either or chloroform for 4 to 5 days. After drying at room temperature, ground seeds were suspended in a solution (1-3 ml/g seeds) consisting of 12.5 g glucose and 1 g ascorbic acid dissolved in 100 ml phosphate buffer 8.3 and ground in liquid nitrogen to a fine powder.After thawing, the powder suspended in buffer was centrifugated at 19,000 rpm for 30 minurtes at 0°C. The protein content in the supernatant was adjusted to a concentration of 3 to 4 mg/ml according to Bradford spectrophotometric method (1976) by using bovine serum albumin as a standard protein.

Extraction of protein from healthy and powdery mildew infected-cultivars

Healthy and infected fresh whole plants of flax Giza 7 was ground in liquid nitrogen to a fine powder. The grounded plants were suspended in a solution (1-3 ml/g plants) consisting of 12.5% glucose and 1 g ascorpic acid dissolved in 100 ml phosphate buffer (pH 8.3) and contrifuged at 19000 rpm for 30 minutes at 10°C (Hussein, 1992).

The protein content in supernatant was adjusted according to Bradford (1976) as previously mentioned.

Immunization and preparation of antisera

New Zealand rabbits, 3-4 kg weight, were immunized by flax antigens (infected whole plants of Giza 8). The first injection was given intra-cutaneously in the back between ears. This injection consisted of 0.5 mg protein suspended in 1 ml phosphate buffer and mixed in 1 ml Freund's incomplete adjuvant (Difco). After one week, each animal was received 4 mg protein administered intramuscularly every third day in the thigh in a series of twelve injections. One week after the last injection, the animal was bled in the marginal ear vein. Collected blood was kept at room temperature for 1 to 2 hr. Clots were then gently lossened and stored over night at 4°C. Antisera were then decanted and clarified by centrifugation at 10000 rpm for 30 minutes,

subdivided into small portions developed by reciprocal precipitation in serum vials, and stored frozen until the time of use (Hussein, 1992). Antiserum of *Oidium lini* was obtained by the elimination of Giza 8 antibodies from the antiserum of the infected whole plant. The obtained antisera were assayed by double diffusion technique (Ouchterlony and Nilsson, 1978).

Double diffusion (DD) technique

The technique was carried out according to Ouchterlony and Nilsson (1978). Molten 2% ionagar (Sigma), in saline (8.5 g NaCl to I Liter D.W) and supplemented with merthiolate (1:10.000, 37°C), was poured into 9 cm diameter petri dishes to obtained a layer of agar 1-2 mm thick. The diameter of the central and the peripheral wells was 10 and 5 mm, respectively. The distance between the central well and the peripheral ones was 15 mm. The central well was filled with infected whole plants antiserum or the pathogen (*O. lini*) antiserum, and the peripheral wells with antigens of healthy flax (Giza 8, CI 2008, Linore, Cortland, Williston Brown, Wilden, Giza 7 and Dakota). Plates were kept under humid conditions at room temperature (18-24°C) in the dark for 48-72 hours. Agar was stained with Commassie Brilliant Blue R-250 (Weeke, 1973). The developing precipitin lines were examined and recorded by hand drawing.

Statistical analysis of the data

Field trials

The experimental design of the field trials was a randomized complete block with five replications (Blocks). Analysis of variance (ANOVA) of the data was performed with the MSTAT-C Statistical Package (A Microcomputer Program for the Design, Management and Analysis of Agronomic Research Experiments, Michigan State Univ., USA). Least significant differences (LSD) test was used to compare cultivar means.

b. Serology test

Regression analysis was used to measure the amount of variation in disease severity (dependent variable), which was explained by the number of common antigens (independent variable) shared between the infected Giza 8 and seeds from the healthy cultivars in the first method or shared between *O. lini* alone and the seeds in the second method.

RESULTS AND DISCUSSION

Evaluation of flax cultivars to PM (Table 1) revealed that the tested cultivars could be divided into two distinct groups. The first group included the resistant cultivars Dakota, Wilden, and Williston Brown, while the second group included the susceptible cultivars Cortland, Linore, C.I. 2008, Giza 7 and Giza 8. The difference in disease severity was highly significant between any cultivar belonged to the first group and any cultivar belonged to the second group. Within the resistant group, the differences were nonsignificant. Giza 7 was less susceptible than Cortland or C.I. 2008 in the susceptible group.

There were two sources for the protein fractions shown in Table 2. Some of these fractions were the common antigens shared by *O. lini* and flax cultivars, while the remaining fractions could be attributed to the interaction between the antibodies of Giza 8 and the antigens of the other flax cultivars. Consequently, the elimination of Giza 8 antibodies from the antiserum of infected Giza 8 by reciprocal precepitation would decrease the number of the observed protein fractions. In this case, the remaining fractions would represent the common antigens shared by *O. lini* and the tested flax cultivars. The greater the percentage of the remaining bands, the more susceptible the cultivar was. The percentage of the remaining bands was 0% for any resistant cultivar, while it ranged from 20 for Giza 8 to 40% for Cortland or Linore in the susceptible group.

Table (1): Reaction of eight flax cultivars to powdery mildew under field conditions in El-Giza in 2001/2002 and 2002/2003 growing seasons.

Cultivar	Disease severity [a] %
Dakota	19.67
Wilden	25.11
Williston Brown	27.58
Cortland	99.48
Linore	95.26
C.I. 2008	100.00
Giza 7	87.26
Giza 8	93.46

LSD = 8.06 ($P \leq 0.05$)
LSD = 11.19 ($P \leq 0.01$)

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

Fig (1). Diagram showing the double-difussion reaction of antiserum (S_2) of the infected whole plant of Giza 8 (1 and 2) and the antiserum (S_1) of *Oidium lini* (3, 4) against antigens of seeds from eight healthy cultivars.
Cultivars were: Cortland (22), Linore (27), C.I. 2008 (29), Dakota (2), Wilden (14), Williston Brown (15), Giza 7 and Giza 8.

Table (2): Number and distribution of protein fractions obtained by double diffusions reaction of infected hole plant antiserum of Giza 8 against antigens of seeds from eight hralthy flax cultivars.

Protein fraction no.	Antiserum of infected Giza 8 x antigens of seeds from flax cultivars							
	Giza 7	Giza 8	Dakota	Wilden	Williston Brown	Cortland	Linore	CI 2008
1	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+
3	-	+	-	-	+	-	-	-
4	+	+	+	+	-	+	+	+
5	-	+	-	-	-	-	-	+
6	-	-	-	-	-	+	+	+
7	+	-	-	-	-	+	+	+

(+) = Protein fraction was present.
(-) = Protein fraction was absent.

Fig. 2: Regression equation that describes the relationship between number of common antigens (x) found in *Oidium lini*-infected whole plants of flax cultivar Giza 8 and in seeds from healthy cultivars and susceptibility (Y) of these cultivar to *O. lini*.

Some views (Doubly *et al*., 1960 and DeVay *et al*., 1967) on the occurrence of common antigens between host and pathogen emphasized a quantitative relationship in regard to their effect on disease susceptibility. This quantitative relationship has been confirmed by the application of regression analaysis, which indicated that the common antigens between infected Giza 8 and the healthy cultivars accounted for 86% of the explained (model) variation in severity of PM on the cultivars (Fig. 2). When the antiserum of *O. lini* alone interacted against the antigens of the healthy cultivars, regression analysis revealed that the common antigens between *O. lini* and the cultivars

accounted for 76% of the explianed (model) variation in severity of PM on the cultivars (Table 3). These results led us to conclude that the common antigenic determinants shared by flax cultivars and *O. lini* were related to the severity of PM.

Table (3):Number and distribution of protein fractions obtained by double diffusion reaction of *Odiium lini* antiserum against antigens of seeds from eight healthy flax cultivars.

Protein fraction no.	Antiserum of *O. lini* x antigens of seeds from flax cultivars							
	Giza 7	Giza 8	Dakota	Wilden	Williston Brown	Cortland	Linore	CI 2008
1	-	-	-	-	-	-	-	+
2	-	-	-	-	-	+	+	+
3	+	+	-	-	-	+	+	+

(+) = Protein fraction was present.
(-) = Protein fraction was absent.

Fig. 3: Regression equation that describes the relationship between number of common antigens (X) found in *Oidium lini* and in seeds from healthy cultivars and susceptibility (Y) of these cultivar to *O. lini*.

At this point, the question that may arise is which method is better for doing the DD test? The first method or the second method. One should keep in mind that the numbers of common antigens obtained by the two methods were highly correlated (Fig. 4). However, the first method was better because this method did not require the application of the reciprocal precepitation, which greatly simplified the test. In addition, this method gave higher coefficient of determination (R^2) value, which indicated that it was more reliable for predicting resistance to PM.

Fig. 4: Correlation between two methods used to study the antigenic relationship between *Oidium lini* and flax cultivars. In the first method, antiserum of whole flax plants of cultivar Giza 8 infected with *O. lini* interacted against antigens of seeds from healthy flax cultivars. In the second method, antiserum of *O. lini* interacted against antigens of seeds from healthy flax cultivars after elimination of Giza 8 antibodies from the antiserum of infected whole plants by reciprocal precepitation.

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إستعمال التفاعلات السيرولوجية للتعبير الكمى عن مقاومة أصناف الكتان لمرض البياض الدقيقى

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قيمت ثمانية أصناف من الكتان وذلك من حيث المقاومة لمرض البياض الدقيقى تحت ظروف الحقل خلال موسمى ٢٠٠٢/٢٠٠١ و ٢٠٠٣/٢٠٠٢. انقسمت الأصناف إلى مجموعتين محددتين. المجموعة الأولى اشتملت على الأصناف المقاومة داكوتا وويلدن وويلستون براون، فى حين اشتملت المجموعة الثانية على الأصناف القابلة للإصابة كورتلاند ولينور والصنف ٢٠٠٨ وجيزة ٧ وجيزة ٨. كان الفرق فى شدة الإصابة عالى المعنوية بين أى صنف يقع فى المجموعة الأولى وأى صنف يقع فى المجموعة الثانية. درست العلاقة الأنتيجينية بين فطر أويديم لينای وأصناف الكتان بطريقتين، فى الطريقة الأولى تفاعل المصل المضاد للنباتات الكاملة لصنف جيزة ٨ المصاب بالبياض الدقيقى مع أنتيجينات البذور المأخوذة من الأصناف السليمة، أما فى الطريقة الثانية فقد تفاعل المصل المضاد لفطر اويديم لينای فقط مع نفس الأنتيجينات وذلك بعد التخلص من الأجسام المضادة للصنف جيزة ٨ الموجود بالمصل المضاد باستعمال تقنية الترسيب العكسى. أظهر تحليل البيانات باستخدام اسلوب الانحدار الخطى أن الأنتيجينات المشتركة بين جيزة ٨ المصاب والأصناف السليمة تفسر ٨٦% من التباين فى شدة المرض على هذه الأصناف وذلك فى الطريقة الأولى، أما فى الطريقة الثانية، فقد أظهر تحليل الانحدار أن الأنتيجينات المشتركة بين فطر أويديم لينای منفرداً والأصناف السليمة تفسر ٧٦% من

التباين فى شدة المرض على هذه الأصناف. من الواضح أن الطريقة الأولى تتفوق على الثانية وذلك لسببين. الأول هو بساطتها إذ أنها لاتحتاج إلى إجراء تقنية الترسيب العكسى. الثانى هو أنها أعطت قيمة أعلى لمعامل التحديد مما يدل على أنها الأكثر مصداقية فى التنبؤ بمقاومة الاصناف للمرض.