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EFFECT OF STAINING METHOD ON THE EFFICIENCY OF PROTEIN ELECTROPHORESIS IN QUANTIFYING RESISTANCE OF FLAX TO POWDERY MILDEW DISEASE

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ABSTRACT

Seed proteins of ten flax genotypes, having different levels of susceptibility to powdery mildew (PM), were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Gels were stained with Coomassie Brilliant Blue R-250 (CBB) or silver nitrate (SN). Data for PM severity and amounts of protein fractions were entered into a computerized stepwise multiple regression. Using the predictors supplied by stepwise regression, a two-factor model was constructed to predict PM severity when the gel was stained with CBB. This model showed that PM severity differences were due largely to the protein fractions nos. 24 and 3, which accounted for 76.18% of the total variation in severity ratings. On staining the gel with SN, a five-factor model was constructed. This model showed that PM severity differences were due to largely to the protein fractions nos. 36, 22, 32, 2, and 21, which accounted for 96.03% of the total variation in PM severity. These results indicate that the SN regression model was superior, compared with that of CBB, in predicting PM severity from banding patterns. This superiority was attributed to the higher R^2 value of the model. Therefore, SDS-PAGE of proteins and staining with SN may provide a supplementary assay to field trials to distinguish between PM resistant or susceptible genotypes quantitatively.

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 falx organs including stems, leaves, flowers, and capsules. PM occurs annually in all flax production areas in Egypt (A.A. Aly, *personal observations*).

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 procedures, in that accurate coverage and distribution of fungicides may not be achieved and there are potential problems with correct timing of application. Furthermore, increasing concern 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. However, 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 identification of flax genotypes with PM resistance.

The use of gel electrophoresis to analyze plant protein and hence distinguish between and identify cultivars of crop species is a firmly established technique (Cook, 1988). Proteins are primary products of gene expression and reflect gene system specificity in the best manner. Therefore, they are used as very effective markers for genotype identification and evaluation of the species and cultivar constitution (Konarev, 1988).

Some attempts were made to differentiate among flax cultivars by using protein electrophoresis. For example, Khalil (1981) found very high degree of similarity among electrophoreteic protein banding patterns of resistant and susceptible flax cultivars to *Melampsora lini*. Following infection, certain changes occurred in the protein patterns of susceptible cultivars, but not in that of the resistant one. The changes were in the form of a shift in the intensity of some bands and the disappearance of some other bands. Such changes were not evident in the resistant cultivar (Bombay), probably due to the very limited activity of the fungus in that cultivar.

Lapina and Rullin (1985) analyzed the protein fractions electrophoretically in the stems of four flax varieties at different phases of growth. They reported that some fractions were present in each variety throughout the growth period, and that the greater number of fractions were found at the phase of rapid growth. They identified each variety with a characteristic protein fraction (or a group of fractions) at each stage of growth.

In a study of protein banding patterns of eight flax varieties differing in resistance to lodging and fungal diseases. Lapina (1989) reported that these patterns contained 15-22 bands, with the fewest being found in the patterns of the varieties susceptible or only moderately resistant to lodging and fungi. There were cultivar specific bands by which the cultivars could be identified.

Lapina and Kel'ner (1990) examined the electrophoretic characteristics of the seed protein of four flax cultivars differing in yield, resistance to lodging and resistance to fungal diseases. They found that there were differences between protein banding patterns of the studied cultivars, and that each pattern had bands in common and cultivar specific bands. There were 45 bands common to all the cultivars and 2-6 associated with the genotype of the particular seeds. They also reported that the cultivar, which had the widest range of economically useful traits had the highest number of bands in its pattern (71 bands).

Abd El-Salam (1998) differentiated by protein electrophoresis among six monogenic flax cultivars carrying the major genes for rust resistance. However, grouping the cultivars by cluster analysis based on their protein banding patterns was not related to their resistance to rust.

Flax cultivar specific protein bands, separated by electrophoresis, may be useful as biochemical markers for seed purity tests (Hussein *et al.,* 2002) or cultivar identification (El-Sweify *et al.,* 2003).

The present investigation was initiated to determine whether reaction of flax genotypes to PM can be quantified by their electrophoretic protein patterns separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) technique and stained with Coomassie Brilliant Blue R-250 or with silver nitrate.

MATERIALS AND METHODS

Evaluation of flax genotypes for PM resistance:

Experiments were conducted over four successive growing seasons, beginning in the fall of 1997. Experiments consisted of a randomized complete block design of 5 replicates (blocks). Plots were 2 x 3 (6 m^2) and consisted of 10 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. Disease severity was measured as the percentage of infected leaves/plant in a random sample of 10 plants/plot (Nutter *et al.*, 1991).

Extraction of flax proteins:

Flax seeds were slightly ground and defatted by diethyl either or chloroform. After drying at room temperature, seeds were suspended in a solution (1-3 ml/g seeds) consisting of 12.5 g glucose and 1g ascorbic acid dissolved in 100ml phosphate buffer 8.3, mixed thoroughly with glass beads, and ground in liquid nitrogen to a fine powder. The powder was centrifugated at 19,000 rpm for 30 minutes at 0^{o}C. The protein content in the supernatant was estimated according to Bradford (1976) by using bovine serum albumin as a standard protein. If protein concentration was low, protein would be precipitated from the clarified supernatant by adding ammonium sulfate at 70% of saturation (60 g / 100 ml) then kept in the refrigerator for 30 hrs. Pellets, collected by centrifugation at 11.000 rpm for 30 minutes, were resuspended in phosphate buffer pH 8.3 and subjected to dialysis for 24 hrs against the buffer and centrifugation at 11,000 for 30 minutes.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE):

Each supernatant was mixed with an equal volume of a solution consisting of (by volume) 64% buffer (0.15 M Tris-HCl, pH 6.8); 20% glycerol; 6% sodium dodecyl sulfate (SDS); 10% 2-6 mercaptoethanol, and 0.1% bromophenol blue, before boiling in a water bath for 3 minutes. Twenty-microliter samples (40 µg of protein) were subjected to electrophoresis in 15% polyacrylamide prepared in 0.1% SDS. Electrophoresis was conducted at room temperature (approximately 20 to 25^{o}C), for 9 hrs on 15% polyacrylamide gel with 6% stacking gel, at 20 and 10 mA, respectively, until the dye band reached the bottom of the separating gel. Electrophoresis was preformed in a vertical slab mold (16.5 x 14.5 x 0.1 cm). Gels were stained with Coomassie Brilliant Blue R-250 (Laemmli, 1970 and Latorre *et al.*, 1995) or with silver nitrate (Sammons *et al.*, 1981).

Statistical analysis of the data:

The experimental design of the field trials was a randomized complete block with five replications. 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). Duncan's multiple range test was used to compare genotypes means. Percentage data were transformed into arc sine angles before carrying out ANOVA to produce approximately constant variance.

Gel was scanned for band molecular weight and amount (%) by the gel documentation system AAB (Advanced American Biotechnology 1166). Stepwise regression technique with greatest increase in R^2 as the decision criterion was used to describe the effects of proteins (predictors or independent variables) on PM severity (dependent variable). Correlation and regression analyses were preformed with a computerized program.

RESULTS AND DISCUSSION

Environmental conditions in the four years of the present study were favorable for epiphytotic spread of the disease. This was apparent as these environmental conditions resulted in high levels of disease severity (DS) on all the tested genotypes. These high levels of DS indicate that the genotypes were screened for PM resistance under high disease pressure. This high disease pressure is considered a prerequisite condition for any meaningful field evaluation for disease resistance. Cultivar Sakha 1 showed the highest DS (86.8%), while line 366/2/1/2 showed the lowest DS (63.5%). The other genotypes showed intermediate levels of DS between these two extremes (Table 1).

Table 1. Susceptibility of ten flax genotypes to powdery mildew under field conditions in El-Gemmeiza.

Genotypes	Disease severity (%) [a]
Cultivar Giza 7	67.0 [b] BC
Line 1	81.2 ABC
Line 3	85.0 AB
Line 366/2/1/2	63.5 C
Line 422/10/1/2	80.9 ABC
Cultivar Giza 8	75.8 ABC
Cultivar Sakha 1	86.8 A
Cultivar Sakha 2	77.9 ABC
Line 5	77.6 ABC
Line 402/20/18/3	87.3 AB

[a] Disease severity was the percentage of infected leaves/plant in a random sample of 10 plants/plot.

[b] Means of 4 years. Percentage data were transformed into arc sine angles before carrying out the analysis of variance to produce approximately constant variance. Means followed by the same letter(s) were not significantly different (P < 0.05) according to Duncan's multiple range test.

In the present study, SDS dissociated each oligomeric protein into its subunits (Bohinski, 1983). When protein preparation was treated with mercaptoethanol and SDS, the mercaptoethanol disrupted (reduced) all disulfide (-S-S-) bonds present at proteins, whereas the detergent SDS bound to all regions of protein and unravelled all intramolecular protein associations. This resulted in total disruption of associated subunits

organization and then yielded SDS-carrying highly-anionic polypeptide chains (Clark and Switzer, 1977).

Electrophoretic banding patterns of Coomassie Brilliant Blue R-250 (CBB) are shown in Fig. 1 and Table 2, while those stained with silver nitrate (SN) are shown in Fig. 2 and Table 4. Twenty five bands were detected by CBB. Of these bands, one (4.0%) was common to all genotypes. The number of detected bands increased to 39 with SN. Of these bands, five (12.8%) were common to all the genotypes. All the genotypes showed variable increases in the number of bands when SN was used to visualize the protein patterns. These increases ranged from 50.0% (cultivar Sakha 2) to 166.7% (Line 402/20/18/3). The increases in the number of bands detected with SN indicate that SN was a highly sensitive visualization technique for detection of the small amounts of proteins, which cannot be seen with CBB. Generally, SN is 100-fold more sensitive than CBB (Anonymous, 2001).

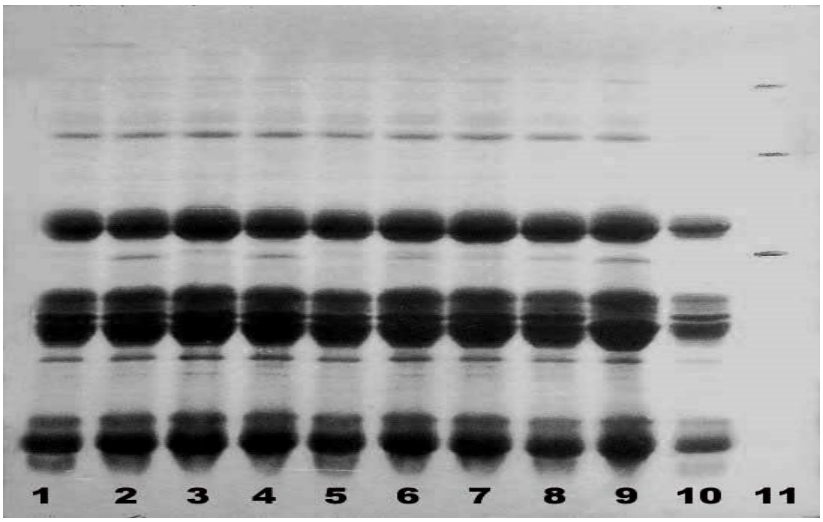

Fig. 1: Protein banding patterns for flax genotypes obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and stained with Coomassie Brilliant Blue R-250. Lanes 1 through 11 were line 1, cultivar Sakha 2, cultivar Sakha 1, line 366/2/1/2, cultivar Giza 8, line 422/10/1/3, cultivar Giza 7, line 402/20/18/3, line 5, line 3, and a marker, respectively.

Pearson correlation coefficient was calculated to measure the degree of association between PM severity and the amounts of the separated protein fractions (Tables 3 and 5). When the gel was stained with CBB, few proteins were satisfactory correlated with PM severity. Thus, of the 25 correlation coefficients shown in Table 3, only two (8.0%) were significant ($p<0.10$ or $p<0.05$). On staining the gel with SN, none of the separated proteins was significantly correlated with PM severity (Table 5).

Table 2. Protein banding patterns of flax genotypes obtained by sodium dodecyl sulfate-polyacrylamide gel electrophorasis (SDS-PAGE) and stained with Coomassie Brilliant Blue R-250.

Band		Genotype [c]									
No	MW [a]	1	2	3	4	5	6	7	8	9	10
1	72	0.00 [b]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.00
2	71	0.00	0.00	0.00	0.00	0.00	2.25	2.31	2.10	2.24	0.00
3	70	0.00	0.00	0.00	2.42	0.00	0.00	0.00	0.00	0.00	0.00
4	69	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	63	0.00	1.83	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.19	1.90	0.00
7	57	0.00	0.00	0.00	0.00	2.49	0.00	0.00	0.00	0.00	0.00
8	54	0.00	0.00	0.00	0.00	0.00	0.00	5.03	3.21	2.50	4.36
9	53	0.00	0.00	0.00	0.00	2.76	4.79	0.00	0.00	0.00	0.00
10	52	9.16	5.41	10.07	5.25	0.00	0.00	0.00	0.00	0.00	0.00
11	43	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.26
12	34	0.00	0.00	0.00	0.00	0.00	0.00	21.20	0.00	21.54	21.37
13	33	25.42	21.87	18.38	25.16	22.74	25.11	0.00	22.14	0.00	0.00
14	30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.01	0.00	0.00
15	29	0.00	0.00	2.15	0.00	4.24	0.00	3.87	0.00	4.33	0.00
16	28	0.00	4.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
17	24	0.00	0.00	0.00	0.00	0.00	13.22	12.26	13.34	11.35	13.57
18	23	12.22	12.82	0.00	0.00	11.86	0.00	0.00	0.00	0.00	0.00
19	21	5.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20	20	14.29	16.65	32.99	32.18	19.36	19.57	19.66	19.13	19.38	21.07
21	18	0.00	0.00	0.00	0.00	7.70	7.69	7.85	7.69	7.54	7.23
22	17	6.55	9.21	9.48	8.31	0.00	0.00	0.00	0.00	0.00	0.00
23	13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.69	8.28
24	12	0.00	0.00	0.00	0.00	0.00	0.00	27.81	0.00	0.00	0.00
25	11	27.34	27.20	26.94	26.68	28.84	27.36	0.00	28.18	20.53	17.86

[a] Molecular weight in kilodalton.

[b] Amount (%) of the designated protein fraction.

[c] The genotypes were line 3 (1), line 5 (2), line 402/20/18/13 (3), cultivar Giza 7 (4), line 422/10/1/3 (5), cultivar Giza 8 (6), line 366/2/1/2 (7), cultivar Sakha 1 (8), cultivar Sakha 2 (9), and line 1 (10).

Data for PM severity and amounts of protein fractions were entered into a computerized stepwise multiple regression analysis. The analysis constructed predictive models by adding predictors, in this case, amounts of protein fractions, to the models 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 models only those variables that made a satisfactory 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 when the gel was stained with CBB (Table 6). This model showed that PM severity differences were due largely to the protein fractions no_s. 24 and 3, which accounted for 76.18% of the total variation in severity ratings. On staining the gel with SN, a five-factor model was constructed. This model showed that PM severity differences were due largely to the protein fractions no_s. 36, 22, 32, 2, and 21, which accounted for 96.03% of the total variation in PM severity.

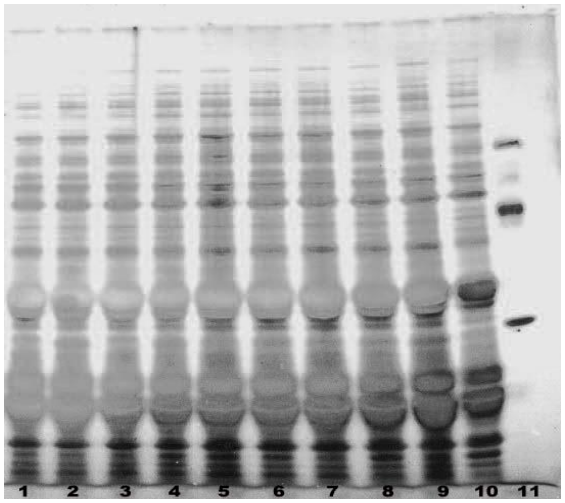

Fig. 2. Protein banding patterns for flax genotypes obtained by sodium dodecyl sulfate- polyacrylamide gel electrophoresis (SDS-PAGE) and stained with silver nitrate. Lanes 1 through 11 were line 1, cultivar Sakha 2, cultivar Sakha 1, line 366/2/1/2, cultivar Giza 8, line 422/10/1/3, cultivar Giza 7, line 402/20/18/3, line 5, line 3, and a marker, respectively.

Table 3. Relationship between PM severity [a] on 10 flax genotypes and protein content [b] of seeds from these genotypes. Protein banding patterns were stained with Coomassie Brilliant Blue R-250.

No. [c]	r [d]	No.	r
1	0.128	14	0.376
2	- 0.278	15	- 0.203
3	- 0.500	16	- 0.031
4	- 0.031	17	- 0.138
5	- 0.031	18	0.244
6	0.289	19	0.297
7	0.115	20	- 0.127
8	- 0.242	21	- 0.114
9	- 0.040	22	0.092
10	0.302	23	0.080
11	0.128	24	- 0.655 *
12	- 0.353	25	0.624 x
13	0.274		

[a] Percentage of infected leaves/plant in a random sample of 10 plants/plot.
[b] Amount of protein (%).
[c] No. of protein fraction.
[d] Pearson correlation coefficient, which measured the degree of association between PM severity and the designated protein fraction, was significant at p< 0.10 (x) or p < 0.05 (*).

The utility of the electrophoretic data depends on the method of statistical analysis. Multiple regression was a logical choice for construction of predictive models, 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).

Table 4. Protein banding patterns for flax genotypes obtained by sodium dodecyl sulfate-polyacrylamide gel electrophorasis (SDS-PAGE) and stained with silver nitrate.

Band		Genotype [c]									
No	MW [a]	1	2	3	4	5	6	7	8	9	10
1	97	0.00 [b]	1.32	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	96	0.00	0.00	0.00	0.00	1.53	0.00	1.82	0.00	0.00	0.00
3	86	0.00	0.00	0.00	0.00	2.50	3.97	2.70	0.00	0.00	0.00
4	83	5.39	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	82	0.00	6.02	7.48	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	81	0.00	0.00	0.00	5.90	3.95	1.81	2.03	8.79	8.83	8.88
7	79	1.74	0.00	0.00	2.01	0.00	0.00	0.00	0.00	0.00	0.00
8	78	0.00	0.00	0.00	2.01	0.00	0.00	0.00	0.00	0.00	0.00
9	77	0.00	0.00	0.00	0.00	0.00	1.89	2.04	0.00	0.00	0.00
10	71	3.51	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11	70	0.00	3.29	3.76	4.75	4.75	4.66	4.30	4.65	4.20	4.40
12	65	3.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
13	64	0.00	3.37	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14	63	0.00	0.00	3.39	4.32	3.82	5.13	4.35	3.78	4.33	3.80
15	59	1.98	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16	58	0.00	0.00	2.23	0.00	2.69	3.09	2.67	2.71	2.72	2.71
17	57	3.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
18	56	0.00	5.07	3.07	5.94	3.56	3.71	3.35	3.57	3.30	3.35
19	52	4.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20	51	0.00	4.96	5.30	0.00	5.65	5.86	5.39	5.57	5.83	4.87
21	50	0.00	0.00	0.00	5.46	0.00	0.00	0.00	0.00	0.00	0.00
22	46	3.58	4.10	3.75	4.41	3.79	4.34	3.92	3.54	3.64	4.01
23	41	8.42	9.07	14.34	14.53	13.56	14.19	13.34	13.58	13.08	13.75
24	35	0.00	4.36	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
25	32	17.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
26	31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.72	0.00
27	30	0.00	9.37	9.54	0.00	8.79	7.74	7.86	7.27	4.98	8.56
28	29	0.00	0.00	0.00	9.05	0.00	0.00	0.00	0.00	0.00	0.00
29	27	0.00	4.77	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
30	25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.00	12.69	12.28
31	24	0.00	0.00	0.00	13.43	13.56	12.17	12.17	0.00	0.00	0.00
32	23	16.06	10.62	13.88	0.00	0.00	0.00	0.00	0.00	0.00	0.00
33	21	4.54	5.30	6.83	5.77	6.48	5.36	0.00	5.63	4.94	5.18
34	19	9.83	0.00	0.00	0.00	5.33	5.20	14.38	0.00	0.00	0.00
35	18	0.00	11.21	8.56	7.95	0.00	0.00	0.00	0.00	0.00	0.00
36	17	6.24	5.54	6.86	6.34	8.26	8.11	6.08	14.45	13.45	14.04
37	16	4.02	3.72	3.49	2.85	3.43	3.62	4.10	3.73	3.11	3.96
38	15	2.50	7.92	3.16	7.56	2.54	2.78	2.89	10.74	10.19	10.22
39	14	3.98	0.00	4.35	0.00	5.82	6.37	6.59	0.00	0.00	0.00

[a] Molecular weight in kilodalton.

[b] Amount (%) of the designated protein fraction.

[c] The genotypes were line 3 (1), line 5 (2), line 402/20/18/13 (3), cultivar Giza 7 (4), line 422/10/1/3 (5), cultivar Giza 8 (6), line 366/2/1/2 (7), cultivar Sakha 1 (8), cultivar Sakha 2 (9), and line 1 (10).

Table 5. Relationship between PM severity [a] on 10 flax genotypes and protein content [b] of seeds from these genotypes. Protein banding patterns were stained with silver nitrate.

No. [c]	r [d]	No.	r	No.	r
1	0.112	14	- 0.182	27	- 0.341
2	0.251	15	0.212	28	0.156
3	0.108	16	- 0.319	29	0.112
4	0.212	17	0.212	30	- 0.444
5	0.045	18	- 0.021	31	0.244
6	- 0.374	19	0.212	32	0.195
7	0.212	20	- 0.199	33	- 0.203
8	0.156	21	0.156	34	0.339
9	0.124	22	- 0.277	35	0.153
10	0.212	23	- 0.265	36	- 0.483
11	- 0.220	24	0.112	37	- 0.251
12	0.212	25	0.212	38	- 0.333
13	0.112	26	0.160	39	0.237

[a] **Percentage of infected leaves/plant in a random sample of 10 plants/plot.**

[b] **Amount of protein (%).**

[c] **No. of protein fraction.**

[d] **Pearson correlation coefficient, which measured the degree of association between PM severity and the designated protein fraction, was nonsignificant.**

Table 6. Regression equations that describe the effect of some protein fractions (X_s) on severity [a] of flax powdery mildew.

Staining method	Stepwise regression model	R^2	F. value
Coomassie Brilliant Blue			
R-250	$Y = 81.56 - 0.65\ X_{24} - 6.02\ X_3$	76.18 [b]	11.19 [c] **
Silver nitrate	$Y = 1000.13 - 21.24\ X_{36} - 174.61\ X_{22} -$		
	$9.43\ X_{32} - 55.69\ X_2 - 11.18\ X_{21}$	96.03 [d]	19.36 **

[a] **PM severity was measured as the percentage of infected leaves/plant in a random sample of 10 plants/plot.**

[b] **Relative contributions of the predictors X_{24} and X_3 to R^2 (coefficient of determination) were 42.93 and 33.25%, respectively.**

[c] **F. value is significant at $p < 0.01$ (**).**

[d] **Relative contribution of the predictors X_{36} , X_{22} , X_{32} , X_2 , and X_{21} to R^2 were 23.35, 23.21, 15.53, 16.51, and 17.43%, respectively.**

In the present study, satisfactory visualization of banding patterns were obtained by using the SN staining system for general proteins, and the stepwise regression model they generated proved superior, compared with that of CBB, in predicting PM severity from banding patterns. The superiority of SN model was attributed to its high R^2 value. Therefore, SDS-PAGE of proteins and staining with SN, such as that described herein, may provide a supplementary assay to field trials to distinguish between PM resistant or susceptible genotypes quantitatively.

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تأثير طريقة الصبغ على كفاءة التفريد الكهربى للبروتينات فى التعبير الكمى عن مقاومة الكتان لمرض البياض الدقيقى

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أجريت دراسة مقارنة لأنواع البروتينات المستخلصة من بذور عشرة تراكيب وراثية لنبات الكتان – تختلف فيما بينها من حيث درجة القابلية للإصابة بمرض البياض الدقيقى - وذلك باستعمال تقنية التفريد الكهربى للبروتينات بعد تفكيكها باستعمال مادة صوديوم دوديسيل سلفيت. إستعملت مادة كوماسى بريليانت بلو أو ملح نترات الفضة لصبغ أنماط البروتينات المتحصل عليها ، أمكن – باستخدام أسلوب الإنحدار المتعدد المرحلى – التوصل إلى نموذج رياضى لوصف العلاقة بين شدة المرض (متغير تابع) وكميات البروتينات المفصولة (متغير مستقل). أظهر هذا النموذج – عند إستعمال الكوماسى بريليانت بلو لصبغ أنماط البروتين المتحصل عليها - أن ٧٦,١٨% من التباين الكلى فى شدة المرض من الممكن أن تعزى إلى تأثير البروتين رقم ٢٤ والبروتين رقم ٣. عند إستعمال نترات الفضة لصبغ أنماط البروتين ، أظهر النموذج أن ٩٦,٠٣% من التباين الكلى فى شدة المرض من الممكن أن تعزى إلى تأثير البروتينات أرقام ٣٦ ، ٢٢ ، ٣٢ ، ٢ و ٢١. تدل هذه النتائج على أن النموذج المتحصل عليه عند إستخدام نترات الفضة فى صبغ أنماط البروتين هو الأكثر تفوقاً فى التنبؤ بشدة المرض – مقارنة بنموذج الكوماسى بريليانت بلو – ويعزى هذا التفوق إلى إرتفاع قيمة معامل التحديد لهذا النموذج ، كما تدل هذه النتائج على أنه من الممكن إستخدام تقنية التفريد الكهربى للبروتينات المفككة مع الصبغ بملح نترات الفضة – كوسيلة مكملة لاختبارات الحقل – للتفرقة الكمية بين تراكيب الكتان الوراثية المقاومة أو القابلة للإصابة بمرض البياض الدقيقى.