

USE OF PROTEIN ELECTROPHORESIS TO QUANTIFY SOME AGRONOMIC TRAITS OF FLAX

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ABSTRACT

Ten flax genotypes were evaluated for 11 agronomic traits under field conditions in 2002/2003 and 2003/2004 growing seasons. The tested agronomic traits were technical length, stem diameter, straw yield per plant, straw yield per fed., apical branching zone length, no. of capsules per plant, no. of seeds per plant, seed yield per plant, seed yield per fed., fiber yield per plant, and fiber yield per fed. Seed proteins of the genotypes were separated by SDS-PAGE, and the obtained banding patterns were visualized by using silver nitrate staining system. Data for agronomic traits (dependent variables) and amounts of protein fractions (independent variables or predictors) were entered into a computerized stepwise multiple regression analysis. Using the predictors supplied by stepwise regression, 11 regression models were constructed to predict agronomic traits. Coefficient of determination (R^2) values of the models ranged from 37.90 to 100 %. It is noteworthy that the three one-variable models of technical length, stem diameter, and straw yield per fed. showed the lowest R^2 values, which may indicate that seed proteins are of limited value as biochemical markers to predict these agronomic traits. The results of the present study suggest that SDS-PAGE of seed proteins may provide a supplementary assay to field trials to quantify agronomic traits of flax genotypes.

INTRODUCTION

Common flax (*Linum usitatissimum* L.) is the only member of a genus containing 200 or more species that is cultivated commercially as a field crop. There are approximately 3000 entries from this species (Dybing and Lay, 1981).

Economically, important tissues of flax include vascular bundles of the stem and oil-containing tissues of the seeds. Fibers obtained from stems for use in linen cloth and paper consist of strands of elongated, thick-walled sclerenchyma cells from the phloem regions of vascular bundles. Such fiber strands attain length over 90 cm. Individual fiber cells grow in length at both apices and increase in wall thickness by deposition of secondary wall layers. They extend from the base of the stem into panicle branches, but the latter do not contribute fiber of commercial value. In the seed, 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).

Flax production in Egypt is mainly concentrated in the northern and middle governorates in the Nile Delta. The Egyptian cultivars are dual purpose produce both fibers and seeds.

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 protein electrophoresis. For example, 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, resistant 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).

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

In the present study, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of proteins was employed to develop regression models to quantify flax agronomic traits. To the best of our knowledge, this approach has not been employed previously in the quantification of flax agronomic traits.

MATERIALS AND METHODS

Field evaluation of agronomic traits of ten flax genotypes:

Experiments were conducted over two successive growing seasons on a heavy clay soil at Sakha Agricultural Research Station, beginning in the fall of 2002. Experiments consisted of a randomized complete block design of five replications (blocks). Plots were 4.5 m² (1.5 x 3.0 m) and consisted of 21 rows spaced 7 cm apart. Plots were mechanically planted with flax genotypes at a rate of 50 kg/feddan on 15 November 2002 and 20 November 2003. At harvest, 11 agronomic traits (Table 1) were recorded for each genotype.

Table 1. Agronomic traits of ten flax genotypes grown under field conditions in Sakha in 2002/2003 and 2003/2004.

Genotype	Y ₁ Technical length (cm)	Y ₂ Stem diameter (mm)	Y ₃ Straw yield per plant (g)	Y ₄ Straw yield per fed (ton)	Y ₅ Apical branching zone length(cm)	Y ₆ No. of capsules per plant
Giza 7	78.84 ^a	1.58	3.42	3.64	17.03	14.52
420/140	70.59	2.47	3.08	3.03	10.33	8.28
420/4	73.24	2.50	3.17	3.17	12.74	10.34
420/153	74.88	2.13	3.21	3.32	13.45	11.02
421/3	72.15	2.67	3.16	3.15	11.76	9.57
421/43	76.75	1.99	3.35	3.58	16.09	12.83
421/60	75.47	2.28	3.29	2.87	15.43	12.37
110/3	80.18	1.12	3.46	3.60	17.92	15.67
282/37	74.73	1.72	3.43	3.51	17.64	16.20
282/98	69.01	2.64	3.09	3.09	14.22	12.23

Table 1. cont.

Genotype	Y ₇ No. of seeds per plant	Y ₈ Seed yield per plant (g)	Y ₉ Seed yield per fed. (kg)	Y ₁₀ Fiber yield per plant (g)	Y ₁₁ Fiber yield per fed. (kg)
Giza 7	116.62	0.96	561.65	0.69	727.63
420/140	81.08	0.46	479.13	0.62	606.00
420/4	86.21	0.59	492.47	0.63	634.75
420/153	92.31	0.61	508.92	0.64	629.00
421/3	79.51	0.50	483.60	0.64	629.00
421/43	107.55	0.71	554.30	0.67	710.38
421/60	104.23	0.80	527.40	0.66	681.00
110/3	123.50	0.98	572.91	0.77	718.00
282/37	123.99	1.01	586.17	0.72	701.25
282/98	96.45	0.77	510.18	0.62	618.25

^a Mean of two years.

Extraction of proteins from flax seeds:

Protein extract was prepared according to Hussein (1992) in the following way: Seeds of genotypes were slightly ground and defatted by diethyl ether or chloroform for 4 to 5 days. After drying at room temperature, ground seeds were suspended in a solution (1-3 ml/o seeds) consisting of 12.5 g glucose and 1g ascorbic acid dissolved in 100ml phosphate buffer, pH 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.

Electrophoresis of dissociated protein (SDS-PAGE):

For electrophoresis of dissociated protein, 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% 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 5-20% gradient polyacrylamide prepared in 0.1% SDS (Laemmli, 1970). Electrophoresis was conducted at 10°C, for 4hr on 5-20% gradient polyacrylamide gel with 3.5% stacking gel, at 30 and 15 mA, respectively, until the dye reached the bottom of the separating gel (Laemmli, 1970). Electrophoresis was performed in a vertical slab mold (16 x 18 x 0.15 cm). Gel was stained with silver nitrate for the detection of protein bands (Sammons *et al.*, 1981).

Statistical analysis:

Gel was scanned for band R_r (position) 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 agronomic traits (dependent variables). Correlation and regression analyses were performed with a computerized program.

RESULTS AND DISCUSSION

The tested genotypes showed considerable variation in their agronomic traits (Table 1).

Amino acid sequences of polypeptides (components of proteins) are dependent on nucleotide sequences of their coding genes; therefore, an analysis of protein variations among flax genotypes by SDS-PAGE approximates an analysis of their genetic variation (Aly *et al.*, 2003). Electrophoretic patterns can also be obtained rapidly and with small amounts of tissues. Therefore, large number of single plant selections can be tested without scarifying the plants (Wheeler *et al.*, 1971).

In the present study, a total of 60 protein bands were identified among the 10 genotypes that were analyzed (Fig. 1 and Table 2). This large number of bands was due to the effect of SDS, which 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 in 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 yield SDS-carrying, highly-anionic polypeptide chains (Clark and Switzer, 1977). No single genotype was stained for all the 60 bands. Similarly, no single band was common to all the genotypes. Line 420/140 showed the least number of bands (8 bands), while the other genotypes showed a number of bands ranged from 10 to 15. Each genotype was characterized by unique bands. For example, bands no. 2, 20, 24, 29, 40, 44, 46, 50, and 60 were unique to Giza 7.

Table 2: Protein banding patterns for ten flax genotypes obtained by SDS-PAGE (gradient gel) and stained with silver nitrate.

Band No.	Position	Genotype									
		Giza 7	420/140	420/4	420/153	421/3	421/43	421/60	110/3	282/37	282/98
1	0	2.20	0.00	0.00	2.63	0.00	0.00	3.53	1.73	1.44	2.54
2	23	9.61	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	25	0.00	9.93	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	26	0.00	0.00	9.97	14.23	10.94	0.00	8.05	9.63	10.49	0.00
5	27	0.00	0.00	0.00	0.00	0.00	10.86	0.00	0.00	0.00	10.07
6	32	3.24	3.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7	33	0.00	0.00	7.91	0.00	0.00	0.00	0.00	3.06	3.28	3.21
8	34	0.00	0.00	0.00	0.00	3.62	3.76	2.90	0.00	0.00	0.00
9	41	2.79	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.04
10	42	0.00	0.00	0.00	0.00	0.00	2.84	0.00	0.00	2.70	0.00
11	47	4.03	5.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.52
12	48	0.00	0.00	4.25	7.05	7.24	0.00	0.00	5.79	2.77	0.00
13	49	0.00	0.00	0.00	0.00	0.00	0.00	5.99	0.00	0.00	0.00
14	55	0.00	0.00	0.00	0.00	0.00	0.00	2.86	0.00	0.00	0.00
15	56	0.00	0.00	3.40	7.52	3.51	0.00	0.00	5.62	3.35	0.00
16	61	0.00	7.70	2.95	0.00	3.56	0.00	0.00	0.00	0.00	0.00
17	62	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.42	0.00
18	72	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.59
19	74	0.00	0.00	0.00	0.00	0.00	19.43	13.35	0.00	0.00	0.00
20	75	16.54	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
21	77	0.00	22.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
22	78	0.00	0.00	18.23	9.37	0.00	0.00	0.00	10.48	0.00	0.00
23	79	0.00	0.00	0.00	0.00	10.38	0.00	0.00	0.00	8.58	0.00
24	85	9.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
25	88	0.00	0.00	0.00	0.00	7.97	7.87	0.00	0.00	0.00	0.00
26	89	0.00	0.00	0.00	7.29	0.00	0.00	0.00	0.00	0.00	8.52
27	90	0.00	0.00	0.00	0.00	0.00	0.00	3.85	7.37	8.01	0.00
28	96	0.00	0.00	0.00	0.00	0.00	0.00	4.59	0.00	0.00	0.00
29	106	3.61	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
30	107	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.96
31	108	0.00	0.00	0.00	4.49	0.00	0.00	0.00	0.00	0.00	0.00
32	109	0.00	0.00	0.00	0.00	0.00	5.73	4.09	0.00	0.00	0.00
33	110	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.02	5.01	0.00
34	116	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.28
35	117	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.51	0.00	0.00
36	118	7.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.04	0.00
37	119	0.00	11.48	18.65	15.62	12.63	0.00	0.00	0.00	0.00	0.00
38	121	0.00	0.00	0.00	0.00	0.00	7.21	0.00	0.00	0.00	0.00
39	123	0.00	0.00	0.00	0.00	0.00	0.00	7.81	0.00	0.00	4.18
40	130	2.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 2. Cont.

Band	No.	Position	Genotype									
			Giza 7	420/140	420/4	420/153	421/3	421/43	421/60	110/3	282/37	282/98
41	131	0.00	0.00	0.00	0.00	6.90	0.00	0.00	0.00	0.00	0.00	3.01
42	132	0.00	0.00	0.00	0.00	0.00	2.98	3.03	2.80	3.03	0.00	0.00
43	138	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.14
44	142	3.44	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
45	143	0.00	0.00	0.00	0.00	0.00	5.95	5.63	5.80	5.25	0.00	0.00
46	164	8.44	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
47	165	0.00	0.00	0.00	0.00	0.00	0.00	8.52	0.00	0.00	0.00	0.00
48	166	0.00	11.94	8.94	0.00	0.00	0.00	0.00	7.73	7.98	7.44	0.00
49	167	0.00	0.00	0.00	7.29	8.72	0.00	0.00	0.00	0.00	0.00	0.00
50	1 4	3.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
51	175	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.25
52	176	0.00	0.00	0.00	0.00	0.00	14.64	0.00	0.00	0.00	0.00	0.00
53	181	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.52	0.00	0.00
54	183	0.00	0.00	13.11	11.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00
55	185	0.00	0.00	0.00	0.00	11.58	0.00	0.00	0.00	0.00	0.00	0.00
56	186	7.92	0.00	0.00	0.00	0.00	0.00	10.32	0.00	0.00	0.00	0.00
57	187	0.00	12.48	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
58	189	0.00	0.00	0.00	0.00	0.00	3.86	0.00	11.09	0.00	0.00	0.00
59	207	0.00	0.00	14.61	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9.58
80	209	16.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

^a Amount (%) of the designated protein fractions.

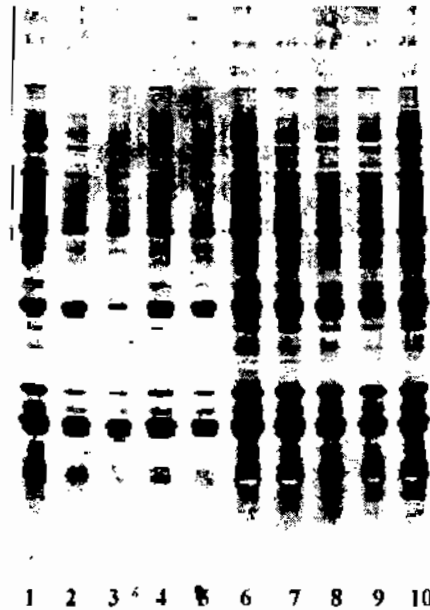


Fig. 1. Protein patterns obtained by SDS-PAGE (gradient gel) from seeds of ten flax genotypes. Genotypes in lanes 1 through 10 were (1) Giza 7, (2) 420/140, (3) 420/4, (4)420/153, (5) 421/3, (6) 421/43, (7) 421/60, (8) 110/3, (9) 282/37 and (10) 282/98.

Pearson correlation coefficient was calculated to measure the degree of association between agronomic traits and the amounts of the separated protein fractions (Table 3). However, few proteins were satisfactory correlated with agronomic traits. Thus, of the 660 correlation coefficients shown in Table 3, only 49 (7.42 %) were significant ($p < 0.05$) or highly significant ($p < 0.01$).

Table 3. Correlation between agronomic traits (Y_a) of ten flax genotypes and protein content of seeds from these genotypes.

No.	Y_1^0	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7	Y_8	Y_9	Y_{10}	Y_{11}
1	0.215 ^c	-0.236	0.234	-0.163	0.437	0.417	0.453	0.521	0.307	0.179	0.209
2	0.428	-0.366	0.377	0.429	0.322	0.230	0.304	0.388	0.308	0.174	0.469
3	-0.402	0.248	-0.456	-0.312	-0.588	-0.544	-0.663*	-0.490	-0.440	-0.333	-0.450
4	0.150	-0.076	0.119	-0.063	0.005	0.046	0.092	-0.028	-0.027	0.205	-0.092
5	-0.561	0.366	-0.431	-0.239	-0.060	-0.010	-0.047	0.055	-0.159	-0.333	-0.358
6	0.029	-0.095	-0.050	0.095	-0.190	-0.174	-0.259	-0.067	-0.091	-0.114	0.024
7	-0.132	0.040	-0.057	0.019	0.051	0.119	0.094	0.115	-0.005	0.055	-0.107
8	0.036	0.271	0.001	-0.199	-0.075	-0.198	-0.082	-0.265	-0.103	-0.132	0.119
9	-0.131	0.024	-0.066	0.120	0.184	0.207	0.181	0.321	0.097	-0.136	0.057
10	0.173	-0.266	0.457	0.469	0.451	0.452	0.436	0.323	0.581	0.318	0.455
11	-0.529	0.302	-0.465	-0.198	-0.230	-0.172	-0.265	-0.049	-0.244	-0.398	-0.358
12	0.118	-0.067	0.002	0.144	-0.145	-0.094	-0.063	-0.200	-0.162	0.166	-0.189
13	0.089	0.117	0.059	-0.616	0.104	0.009	0.089	0.107	-0.003	-0.043	0.116
14	0.089	0.117	0.059	-0.616	0.104	0.009	0.089	0.107	-0.003	-0.043	0.116
15	0.243	-0.271	0.146	0.261	0.039	0.094	0.111	-0.011	0.036	0.295	-0.080
16	-0.533	0.498	-0.626	-0.417	-0.817**	-0.766**	-0.852**	-0.737*	-0.702*	-0.486	-0.624
17	0.015	-0.269	0.402	0.271	0.405	0.527	0.432	0.476	0.530	0.391	0.269
18	-0.561	0.366	-0.431	-0.239	-0.060	-0.010	-0.047	0.055	-0.159	-0.333	-0.358
19	0.243	-0.002	0.214	-0.059	0.231	0.067	0.180	0.020	0.209	-0.001	0.364
20	0.428	-0.366	0.377	0.429	0.322	0.300	0.304	0.388	0.308	0.174	0.469
21	-0.402	0.248	-0.456	-0.312	-0.588	-0.544	-0.663*	-0.490	-0.440	-0.333	-0.450
22	0.180	-0.103	-0.029	0.086	-0.080	-0.082	-0.038	-0.119	-0.151	0.067	-0.130
23	-0.190	0.134	0.059	0.047	-0.049	0.054	0.013	-0.022	0.032	0.110	-0.040
24	0.428	-0.368	0.377	0.429	0.322	0.300	0.304	0.368	0.308	0.174	0.489
25	-0.022	0.230	-0.043	0.140	-0.153	-0.226	-0.149	-0.355	-0.122	-0.120	0.047
26	-0.431	0.304	-0.442	-0.165	-0.162	-0.127	-0.120	-0.112	-0.245	-0.398	-0.478
27	0.446	-0.852*	0.672*	0.251	0.667*	0.735*	0.670*	0.710*	0.702*	0.822*	0.534
28	0.089	0.117	0.059	-0.616	0.104	0.009	0.089	0.107	-0.003	-0.043	0.116
29	0.428	-0.368	0.377	0.429	0.322	0.300	0.304	0.388	0.308	0.174	0.469
30	-0.561	0.386	-0.431	-0.239	-0.060	-0.010	-0.047	0.055	-0.159	-0.333	-0.358
31	0.030	0.014	-0.137	0.040	-0.164	-0.173	-0.119	-0.227	-0.170	-0.188	-0.276
32	0.242	0.001	0.213	-0.072	0.231	0.067	0.180	0.022	0.206	-0.002	0.363
33	0.434	-0.715*	0.658*	0.488	0.635*	0.738*	0.642*	0.675*	0.705*	0.857*	0.499
34	-0.561	0.366	-0.431	-0.239	-0.060	-0.010	-0.047	0.055	-0.159	-0.333	-0.358
35	0.583	-0.683*	0.475	0.380	0.443	0.455	0.424	0.424	0.410	0.752*	0.396
36	0.335	-0.477	0.584	0.526	0.544	0.618	0.552	0.648*	0.627	0.422	0.555
37	-0.405	0.530	-0.622	-0.317	-0.795*	-0.789*	-0.748*	-0.604*	-0.770*	-0.574	-0.716*
38	0.218	-0.083	0.206	0.356	0.194	0.071	0.146	-0.051	0.241	0.029	0.338
39	0.090	0.117	0.059	-0.616	0.104	0.009	0.089	0.107	-0.003	-0.043	0.118
40	0.428	-0.366	0.377	0.429	0.322	0.300	0.304	0.388	0.308	0.174	0.469

Table 3. Cont.

No. ^a	Y ₁ ^b	Y ₂	Y ₃	Y ₄	Y ₅	Y ₆	Y ₇	Y ₈	Y ₉	Y ₁₀	Y ₁₁
41	-0.468	0.522	-0.428	-0.259	-0.402	-0.357	-0.346	-0.379	-0.448	-0.319	-0.409
42	0.525	-0.542	0.691*	0.223	0.694*	0.643*	0.662*	0.579	0.716*	0.671*	0.679*
43	-0.561	0.368	-0.431	-0.239	-0.060	-0.010	-0.047	0.055	-0.159	-0.333	-0.358
44	0.428	-0.366	0.377	0.429	0.322	0.300	0.304	0.388	0.308	0.174	0.469
45	0.556	-0.563	0.694*	0.246	0.695*	0.635*	0.659*	0.568	0.711*	0.685*	0.689*
46	0.428	0.366	0.377	0.429	0.322	0.300	0.304	0.388	0.308	0.174	0.469
47	0.089	0.117	0.059	-0.816	0.104	0.009	0.089	0.107	-0.003	-0.043	0.116
48	-0.376	0.044	-0.255	-0.111	-0.208	-0.080	-0.234	-0.047	-0.124	0.010	-0.323
49	-0.179	0.324	-0.304	-0.108	-0.432	-0.418	-0.359	-0.496	-0.440	-0.281	-0.409
50	0.428	-0.366	0.377	0.429	0.322	0.300	0.304	0.388	0.308	0.174	0.469
51	-0.561	0.366	-0.431	-0.239	-0.060	-0.010	-0.047	0.055	-0.159	-0.333	-0.358
52	0.218	-0.083	0.206	0.356	0.194	0.071	0.146	-0.051	0.241	0.029	0.338
53	0.015	-0.269	0.402	0.271	0.405	0.527	0.432	0.476	0.530	0.391	0.269
54	0.089	0.227	-0.284	-0.088	-0.324	-0.333	-0.284	-0.367	-0.375	-0.339	-0.377
55	0.245	0.387	-0.260	-0.166	-0.394	-0.369	-0.342	-0.420	-0.399	-0.188	-0.272
56	0.351	-0.137	0.293	-0.241	0.295	0.201	0.270	0.340	0.198	0.075	0.400
57	-0.402	0.248	-0.456	-0.312	-0.588	-0.544	-0.663*	-0.490	-0.440	-0.333	-0.450
58	0.625	-0.697*	0.535	0.493	0.499	0.470	0.465	0.397	0.484	0.746*	0.503
59	-0.444	0.449	-0.457	-0.264	-0.265	-0.240	-0.226	-0.200	-0.373	-0.422	-0.412
60	0.428	-0.366	0.377	0.429	0.322	0.300	0.304	0.388	0.308	0.174	0.469

^a Number of protein fraction.

^b Identification of the agronomic traits is shown in Table 1.

^c Pearson correlation coefficient (r), which measures the degree of association between agronomic traits and amount of the designated protein fractions. Value of r is significant at $p < 0.01$ (**) or $p < 0.05$ (*).

Data for agronomic traits 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 significant contribution to the R^2 value of the model (Podleckis *et al.*, 1984). Using the predictors supplied by stepwise regression, 11 models were constructed to predict agronomic traits (Tables 4 and 5). R^2 values of the models ranged from 37.90 to 100.00 %. It is noteworthy that the three one-variable models of technical length, stem diameter, and straw yield per fed. showed the lowest R^2 values, which may indicate that seed proteins are of limited value as biochemical markers to predict these agronomic traits.

The utility of the electrophoretic data depends on the method of statistical analysis. Multiple regressions 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).

In the present study, satisfactory visualization of banding patterns were obtained by using the silver nitrate staining system for general proteins, and the stepwise regression models they generated proved effective in predicting most of the agronomic traits from banding patterns. Therefore, SDS-PAGE of proteins, such as that described herein, may provide a supplementary assay to field traits to quantify agronomic traits of flax genotypes.

Table 4. Stepwise regression models that describe the relationship between agronomic traits (Y_s) of ten flax genotypes and protein content (X_s) of seeds from these genotypes.

Agronomic trait	Stepwise linear regression model	Coefficient of determination (R ²) ^a (%)	F-value ^b
Technical length	$Y_1 = 73.67 + 0.61X_{58}$	39.10	5.14 x
Stem diameter	$Y_2 = 2.28 - 0.17X_{33}$	51.06	8.35*
Straw yield per plant	$Y_3 = 3.14 + 0.04X_{45} + 0.08X_{50}$	87.74	16.78***
Straw yield per fed.	$Y_4 = 3.34 - 0.09X_{13}$	37.90	4.88 x
Apical branching length	$Y_5 = 15.99 - 0.87X_{16} + 0.52X_{33} + 0.32X_{6} - 0.21X_{26} - 0.07X_{4} - 0.04X_{23} + 0.04X_{10} - 0.008X_{31}$	100.00	426749.06***
No. of capsules per plant	$Y_6 = 12.12 - 3.4X_{37} + 0.39X_{15} + 0.33X_{36} + 0.13X_{22} + 0.15X_{10} + 0.09X_{8} + 0.01X_{59} + 0.001X_{48}$	100.00	1632112.25***
No. of seeds per plant	$Y_7 = 106.11 - 7.17X_{16} + 3.99X_{33} - 1.42X_{26} + 3.24X_{6} - 0.24X_{4} + 0.25X_{59} + 0.19X_{25}$	99.99	3749.69***
Seed yield per plant	$Y_8 = 0.78 - 0.03X_{37} + 0.02X_{15} + 0.02X_{36} + 0.01X_{22} - 0.005X_{52}$	99.71	279.74***
Seed yield per fed.	$Y_9 = 521.91 - 4.08X_{37} + 7.55X_{15} + 5.54X_{36} + 2.21X_{52}$	95.31	25.39***
Fiber yield per plant	$Y_{10} = 0.67 + 0.03X_{33} - 0.004X_{48} - 0.003X_{23} - 0.004X_{26} + 0.007X_{9} - 0.002X_{53} - 0.002X_{28}$	99.96	738.29***
Fiber yield per fed.	$Y_{11} = 705.21 - 5.49X_{37} - 8.21X_{18}$	83.72	17.99***

^a Relative contributions of the predictors to R² are shown in Table 5.

^b F. value is significant at P < 0.10 (x), P < 0.05 (*), or P < 0.005 (***).

Table 5. Identification of the predictors included in stepwise regression models in Table 4 and their relative contributions to R².

Agronomic trait	Predictor	Relative contribution to R ² %
Technical length	X58	39.10
Stem diameter	X33	51.06
Straw yield per plant	X45	48.16
	X50	34.58
Straw yield per fed.	X13	37.90
Apical branching zone length	X16	66.81
	X33	17.38
	X6	8.48
	X26	5.31
	X4	1.84
	X23	0.18
	X10	0.01
	X31	0.02
No. of capsules per plant	X37	59.12
	X15	26.58
	X36	9.10
	X22	4.53
	X10	0.53
	X8	0.12
	X59	0.02
No. of seeds per plant	X48	0.0002
	X16	72.55
	X33	17.12
	X26	6.15
	X6	3.47
	X4	0.49
	X59	0.18
Seed yield per plant	X25	0.03
	X37	64.56
	X15	17.26
	X36	10.67
	X22	6.31
Seed yield per fed.	X52	0.92
	X37	59.36
	X15	20.34
	X36	9.96
Fiber yield per plant	X52	5.64
	X33	73.50
	X48	11.56
	X23	6.42
	X26	4.79
	X9	2.21
	X53	1.10
Fiber yield per fed.	X28	0.38
	X37	51.28
	X18	32.44

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إستعمال التفريد الكهربى للبروتينات للتعبير الكمي عن بعض الصفات المحصولية لنبات الكتان

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قيمت ١١ صفة محصولية لعشيرة تراكيب وراثية من الكتان تحت ظروف الحقل خلال موسمي ٢٠٠٢/٢٠٠٣ و ٢٠٠٣/٢٠٠٤. الصفات المحصولية موضوع الدراسة كانت على النحو التالي: الطول الفعال وقطر الساق ومحصول القش للنبات الواحد ومحصول القش للقدان وطول منطقة لتفرع القمي وعدد كبسولات النبات الواحد وعدد بذور النبات الواحد ومحصول البذرة للنبات الواحد ومحصول البذرة للقدان ومحصول الألياف للنبات الواحد ومحصول الألياف للقدان. إستعملت تقنية التفريد الكهربى لفصل بروتينات بذرة التراكيب الوراثية، وذلك بعد تفكيك هذه البروتينات بإستعمال مادة صوديوم ثوديوسيل سلفيت. إستعملت مادة نترات الفضة لصبغ أنماط البروتين المتحصل عليها. أمكن - بإستخدام أسلوب الإتحدار المتعدد المرحلي - التوصل إلى ١١ نموذج إتحدار لوصف العلاقة بين الصفات المحصولية (متغير تابع) وكميات البروتينات المفصولة (متغير مستقل). تراوحت قيم معامل التحديد لنماذج الإتحدار المتحصل عليها من ٣٧,٩٠ إلى ١٠٠ % . الجدير بالذكر أن نماذج الإتحدار الخاصة بالطول الفعال وقطر الساق ومحصول القش للقدان أظهرت أقل قيم لمعامل التحديد ، مما يدل على أن بروتينات البذرة ذات قيمة محدودة عند استعمالها كمعاملات بيوكيميائية للتنبؤ بهذه الصفات. تكل نتائج الدراسة الحالية على أنه من الممكن استخدام تقنية التفريد الكهربى لبروتينات البذرة كوسيلة مكملة للتجارب الحقلية للتعبير الكمي عن الخواص المحصولية للتراكيب الوراثية لنبات الكتان.