Early bolting prediction in sugar beet plants using isoperoxidase activity

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ABSTRACT

The present study was carried out at El-Sabahia Agricultural Research Station, Alexandria, Egypt. The main objective of this study was to determine biochemical marker in sugar beet bolting plant at early stages of plant development. Field experiment was carried out in the experimental farm of El-Sabahia station. The plant samples of bolting and non-bolting plants were collected at different period of plant ages and samples were analyzed employing the well known electrophoresis technique. Cluster analysis was done to recognize relation between bolting and non-bolting plants. The cluster differentiates between bolting and non-bolting plants in different clusters and this may mean that technique would be effective in selection against bolting at early stages of plant development.

INTRODUCTION

Sugar beet (*Beta vulgaris* L.) belongs to the family *Chenopodiaceae*. It is a diploid species with 18 chromosomes (x=n=9) and a nuclear DNA content of 758 Mbp per haploid genome (Arumuganathan and Earle, 1991).

Sugar beet as a biennial plant needs two seasons to complete its life cycle, in the first season (vegetative season) it produce leaves and the succulent roots while in the second one (reproductive season) it produces the seed stalk and flowers Bosemark (1993). Some plants produce the seed stalk in the first growing season (bolting). This undesirable character reduces sugar yield and percentage about half, (Poehlman, 1979). O'Connor, (1970) reported that while the inheritance of bolting is not well understood, selection has been found to be effective in developing varieties which are highly bolting resistance. Lysgaard (1978) reported that genetic effect of bolting can be controlled by rigorous selection, and bolting susceptibility can be reduced by 88-98% in beet populations after three selection cycles. (Zinecker and Rinzier, 1979) suggested that selection was more effective within populations than between populations to produce sugar beet bolting resistance materials.

Isozymes had been defined by (Shaw, 1969), as multiple forms of enzymes in the same organism and having similar or identical catalytic

activity. Weising *et al.* (1995) reported that isozymes are enzymes that convert the same chemical substrate, but are not necessarily products of the same gene. Isozymes may be active at different life stages or in different cell component.

Regarding the different isozymes of peroxidase, the present investigation was designed to study their activity in bolting as well as in non-bolting plants to determine any possible biochemical marker. Several attempts have been carried out by many investigators to detect and/or estimate any biochemical genetic marker. Bolelova and Red'ko (1985) showed that the biennial sugar beet forms were proven to contain a higher amount of protein activity of sucrose synthetase. Alimgazinova (1986) found relationship between peroxidase isozyme activity and the sucrose content of the sugar beet roots. Yu *et al.* (2001) discovered an isozyme marker associated with a gene for resistance to root-knot nematode in *Beta*. Peroxidase of the sugar beet are located on chromosome 6 (Van Geyt *et al.*, 1988) and the gene products are active as monomers. In the present work biochemical technique was used to detected biochemical bolting marker in the early stages of plant life to reduce the selection period. Because every selection cycle needed one year to know the bolting plant and the resistance ones and second year to seed production of resistance ones.

MATERIALS AND METHODS

The sugar beet materials used throughout the present study, were obtained from the Sugar Crops Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt. Selection against the bolting phenomenon was carried out six open pollinated populations of sugar beet (*Beta vulgaris* L.) were employed in this work and classified into three categories. These categories are: two original populations (before selection) [C_{11}-R_{540} (Eg.1) and EL-Kasr (Eg.8)]; two populations after one selection cycle (Eg.3 and Eg.10); and two populations after two selection cycles (Eg.5 and Eg.11).

Seeds of the six open–pollinated populations were grown in October 2001 and October 2002 as well. Seeds were planted in rows (50 cm spaced) and on a distance of 20 cm from plant to plant. The plants received the normal agricultural treatments throughout the vegetative seasons. Plants in rows were numbered and the observations and leaf samples were taken during the season. Leaf samples were kept in the deep freezer until the bolting and non-bolting plants had observed.

Biochemical assay (peroxidase isozymes study):

To detect biochemical marker for bolting trait, investigation of isozymes was done on leaf samples of bolting and non-bolting plants.

Electrophoresis was carried out to obtain the peroxidase isozyme patterns in the leaves of the sugar beet samples. The following are the buffers, gel media, staining solution and the used electrophoretic procedure (Sabrah and El-Metainy, 1985):

Buffers

- 0.23 M Tris - Citric acid buffer, pH 8.0 (Sabrah, 1980): 27.7 gm of Tris dissolved in 200 ml distilled water and 11.0 gm Citric acid were added and completed to 1000 ml.
- 0.01 M Sodium Acetate Acetic acid buffer, pH 5.0 (Sabrah, 1980): 29.6 ml. of 0.01 M Acetic acid were added to 70.4 ml. of 0.01 M Sodium Acetate to prepare one liter of this buffer.

Gel media

Agar-Starch-PVP (polyvinyl pyrrolidine) gel: 1gm Agar, 0.5 gm PVP and 0.4 gm of hydrolyzed starch were added to 100 ml of 0.023 M Tris-Citric acid buffer (pH 8.0) and the mixture was cooked in a boiling water bath until the solution became transparent. Gel plates were prepared by pouring the solution on a glass plates and keeping them in refrigerator at 4°C until utilization.

Staining solution

100 ml of 0.01 M Sodium Acetate-Acetic acid buffer (pH 5.0), containing 0.1 gm Benzidine and 1.5 ml Hydrogen peroxide (H_2O_2), which is added immediately before staining, was used to stain the peroxidase isozyme patterns.

Procedure

Leaf samples were taken during five plant ages of bolting and non-bolting plants (60, 90, 120, 150 and 180 days). Samples were homogenized in a cool mortar, and the homogenate was absorbed on 1 X 0.2 cm strips of filter paper. These strips were placed on the origin line of agar gel plate for about one hour, and then the filter paper strips were removed. After that, a constant current of 13-14 V/cm, electrophoretic started for 2 hours at 4°C, using 0.23 M Tris-Citric acid buffer, pH 8.0, as

electrode buffer. After separating the peroxidase isozymes, the gel plates were incubated at 38°C for 5 min. and were stained with peroxidase staining solution.

Isozymes analysis

The isozyme data were scored by software TOTALLAB V. 1.11. Data was analyzed with computer program NTSYS-pc ver 2.1 (Rohlf, 2000). to develops the cluster analysis.

RESULTS AND DISCUSSION

Isozymes

The obtained data showed differences in band numbers, band volume, peak height and R.f. parameter in the investigated materials whereas:

Band volume: it indicates the value resulting from the interaction between band area and band density. It refers to the amount of isozyme, which was expressed from a given gene.

Peak height: it refers to the density of the band and this indicates to the activity of the isozyme.

R.f. (Retardation factor): it refers to the position of band from the original line to its position as relative number–typically between 0 and 1.

Bolting and non-bolting plants of original populations:

Leaf samples of young plants were collected from the two original populations Eg.1 and Eg.8. Samples were classified into three categories as follows: 1- (r) resistance to bolting; 2- (BB) easy bolting; and 3- (B) late bolting. Samples were subjected to electrophoresis. The obtained banding patterns of electrophoresis analysis are illustrated in figure (1). Table (1) shows bands of the two original populations observed from leaf samples of bolting and non-bolting plants. The data indicated that there were six bands migrated towards the cathode; while there were three bands migrated towards the anode. Band existence, band volume, peak height and R.f. parameter were found to be different from bolting and non-bolting plants as well as from one population to another. For example band No.1 in the cathode side and band No.3 at the anode were absent in resistant plants in population Eg.1; while they were found in population Eg.8. Band No.3 in the cathodal side was absent in the resistance sample plants; while it was present in easy bolting and late bolting plants in two original populations. Band No.1 in the anodal side was found in almost all studied materials.

Figure (2) shows dendrogram of cluster analysis based on (0 and 1) data whereas: (o) refer to band absences while (1) refer to presence band. The figure shows that there were three clusters in the dendrogram tree. The analysis was capable to classify the studied twenty two plant leaf samples into three big clusters, cluster No.1 contained eight plant samples (1r, 4r, 2r, 3r, 12r, 15r, 13r and 14r) these samples belong to the bolting resistant plants in two original studied populations (Eg.1 and Eg.8). Cluster No.2 contained late bolting plant samples in the two original populations (8B, 9B, 10B, 19B, 20B, 11B, 21B and 22B). Easy bolting plants presents in the third cluster (5BB, 7BB, 6BB, 17BB, 16BB and 18BB). Alimgazinova (1986) found relationship between peroxidase isozyme activity and the sucrose content of the sugar beet roots.

Bolting and flowering plants of original populations

Bolting is considered to be flowering without vernalization and in this part of the present investigation the comparison between easy bolting, non-bolting and after vernalization flowering plants were studied in two original populations to examine the effect of vernalization on peroxidase isozyme activity. Figure (3) and Table (2) illustrates the isozyme patterns for the two original populations. From the analysis of these data, it was proven that (band existence, band volume, peak height and R.f. parameter) were found to be different from one population to another, and from bolting resistant, flowering and bolting plants. Bands No.3 & 4 in cathodal side and band No.2 in anodal were present in easy bolting plant samples only in the two examined populations. While band No.2 in the cathodal side was present in bolting resistant samples plant in two studied original populations. On the other hand, band No.1 in the anodal side was found in all plant samples. Analysis of electrophoretic anodal bands revealed that there was a specific anodal band detected in bolting plants while in non-bolting was not. This conclusion is in agreement with that reported by (Saleh, 1999), who found the same band in bolting sugar beet plants.

Figure (4) presents dendrogram of cluster analysis based on (0 and 1). The analysis was proven to be capable to separate the thirty studied material samples into three main clusters, cluster No.1 contained ten bolting resistant sugar beet samples (1r, 2r, 3r, 4r, 5r, 19r, 20r, 16r, 17r and 18r); and the ten easy bolting samples were found in cluster No.3 (9BB, 6BB, 7BB, 8BB, 25BB, 10BB, 21BB, 22BB, 23BB and 24BB); while cluster No.2 contained the ten after vernalization samples (26F, 28F, 29F, 30F, 11F, 12F, 13F, 14F, 15F and 27F).

Bolting and non-bolting plants of original and selected populations

Figure (5) shows five gel electrophoretic isozyme patterns of the two original populations and their selected progenies. Table (3) shows the TOTALLAB software scoring data resulting from the analysis of the electrophoretic patterns of the isozyme for the two original sugar beet populations and their selected progenies. The data showed that bands No.3 & 4 were absent in the resistant bolting samples; while they exist in the easy bolting samples, except in the leaf bolting samples of the second selected population Eg.11. Band No.2 in the anodal side was not found in the bolting resistant samples plants, while they exist in almost all the easy bolting samples. On the other hand, band No.2 in the cathodal side was present in bolting resistant samples plant. The bands was absent in all bolting studied plants. Band No.1 in the anodal side was detected in all plants.

Figure (6) illustrates the dendrogram cluster analysis based on (0 and 1) this figure shows that there were five main different clusters that classify the 46 samples. Cluster No.1 contains twelve samples, the eight bolting resistant samples were found in this cluster (1r, 3r, 4r, 2r, 5r, 19r, 41r and 42r). In the small branch cluster that belongs to the same cluster there were four easy bolting plant samples (43BB, 46BB, 44BB and 45BB). These samples belong to population Eg.11 (such population was subjected to two selection cycles). Easy bolting sugar beet plant samples were found in cluster No.2 & No.3. Cluster No.2 contained eight easy bolting plant samples (6BB, 7BB, 10BB, 34BB, 35BB, 36BB, 37BB and 38BB), and cluster No.3 contained eight easy bolting sugar beet leaf plant samples (8BB, 9BB, 24BB, 25BB, 26BB, 28BB, 27BB and 15BB).

Cluster No.4 contained three easy bolting plant samples in a small branch (16BB, 17BB and 18BB). These plant materials belong to population Eg.3 (a population that exposed to one selection cycles), the cluster contained also seven plant samples resistant to bolting (20r, 22r, 23r, 32r, 21r, 31r and 33r), last cluster contained eight plant samples resistant to bolting (11r, 13r, 14r, 12r, 29r, 30r, 40r and 39r).Finally it can be concluded that isoperoxidase may differentiate between bolting and non-bolting sugar beet plant at early stages of plant development.

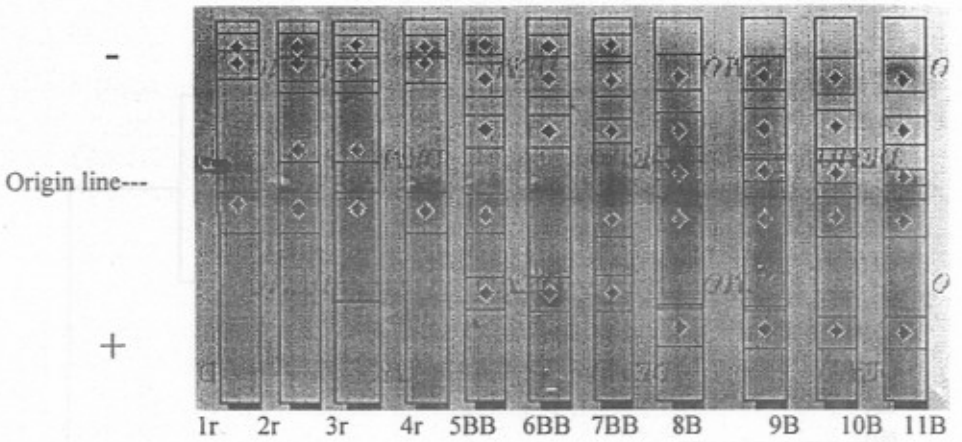

Figure (1a): Peroxidase gel electrophoresis analysis for the original populations Eg.1 of easy bolting (BB), late bolting (B) and resistance bolting plants (r).

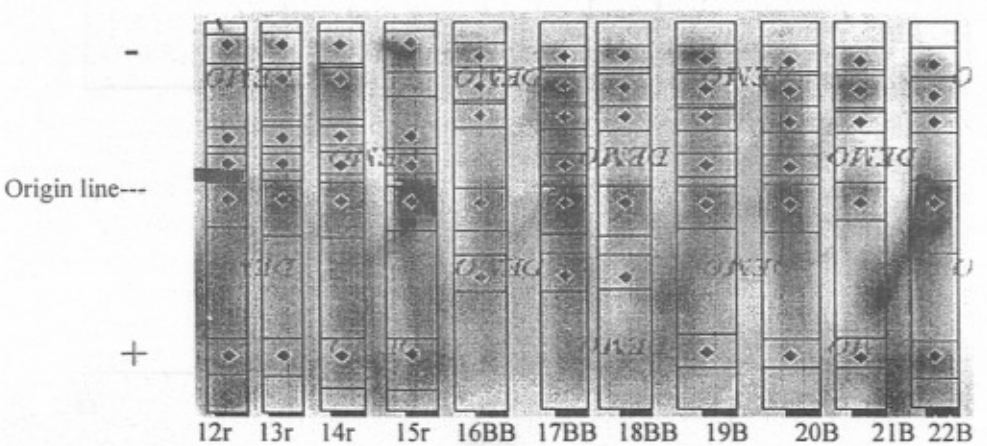

Figure (1b): Peroxidase gel electrophoresis analysis for the original populations Eg.8 of easy bolting (BB), late bolting (B) and resistance bolting plants (r).

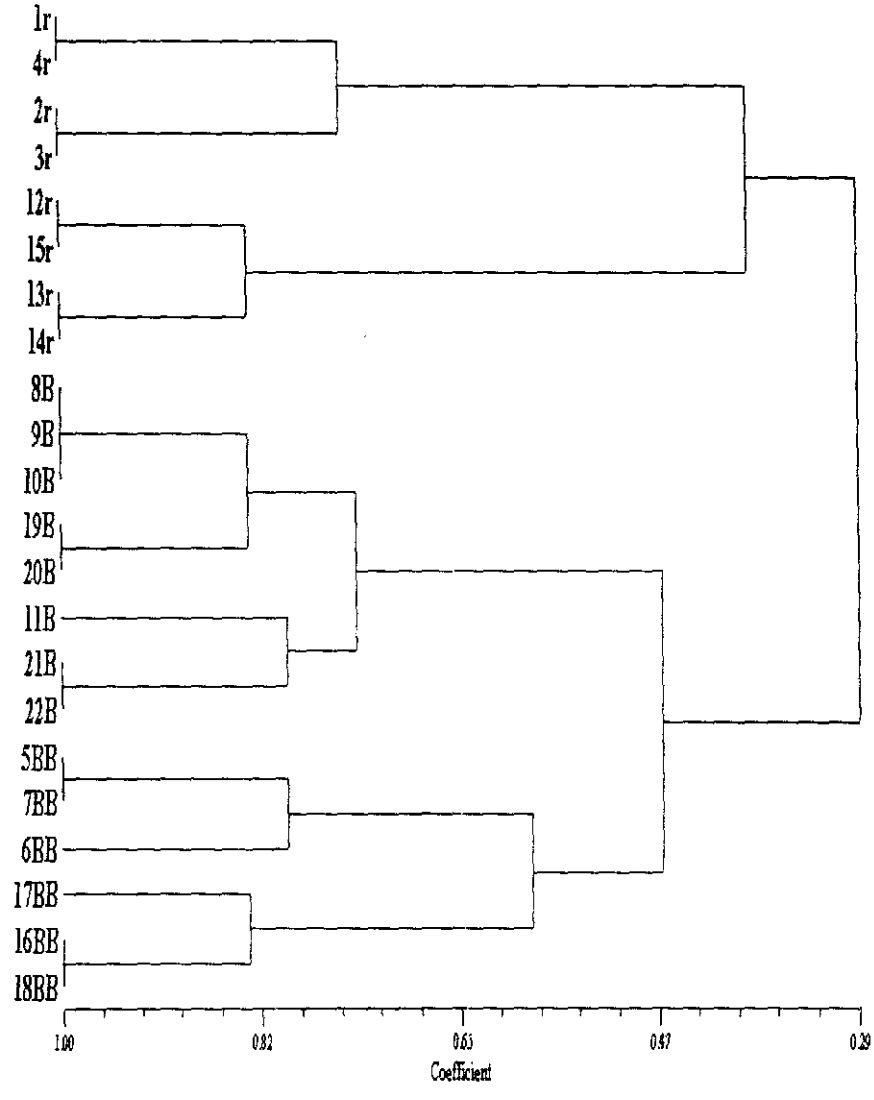

Figure (2): Dendrogram of cluster analysis of population Eg.1 and Eg.8 in the bolting and non-bolting plants based on (0 and 1) data. Whereas: (r): Resistance bolting – (BB): Easy bolting – (B): Late bolting plants.

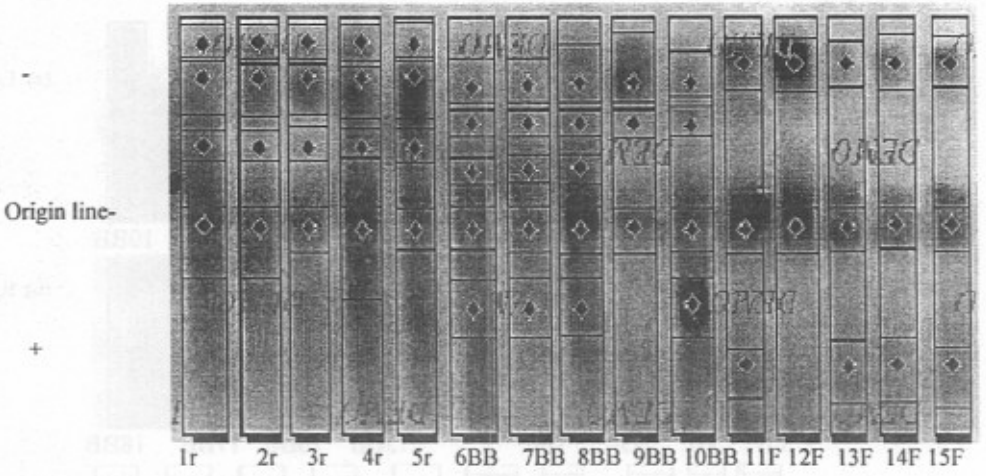

Figure (3a): Peroxidase gel electrophoresis analysis for the original populations Eg.1 of easy bolting (BB), resistance bolting plants (r) and after vernalization plant (F).

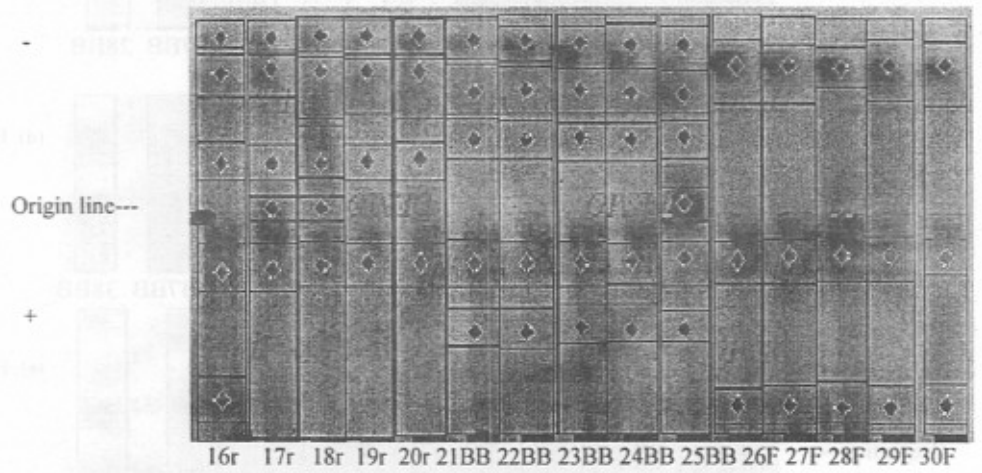

Figure (3b): Peroxidase gel electrophoresis analysis for the original populations Eg.8 of easy bolting (BB), resistance bolting plants (r) and after vernalization plant (F).

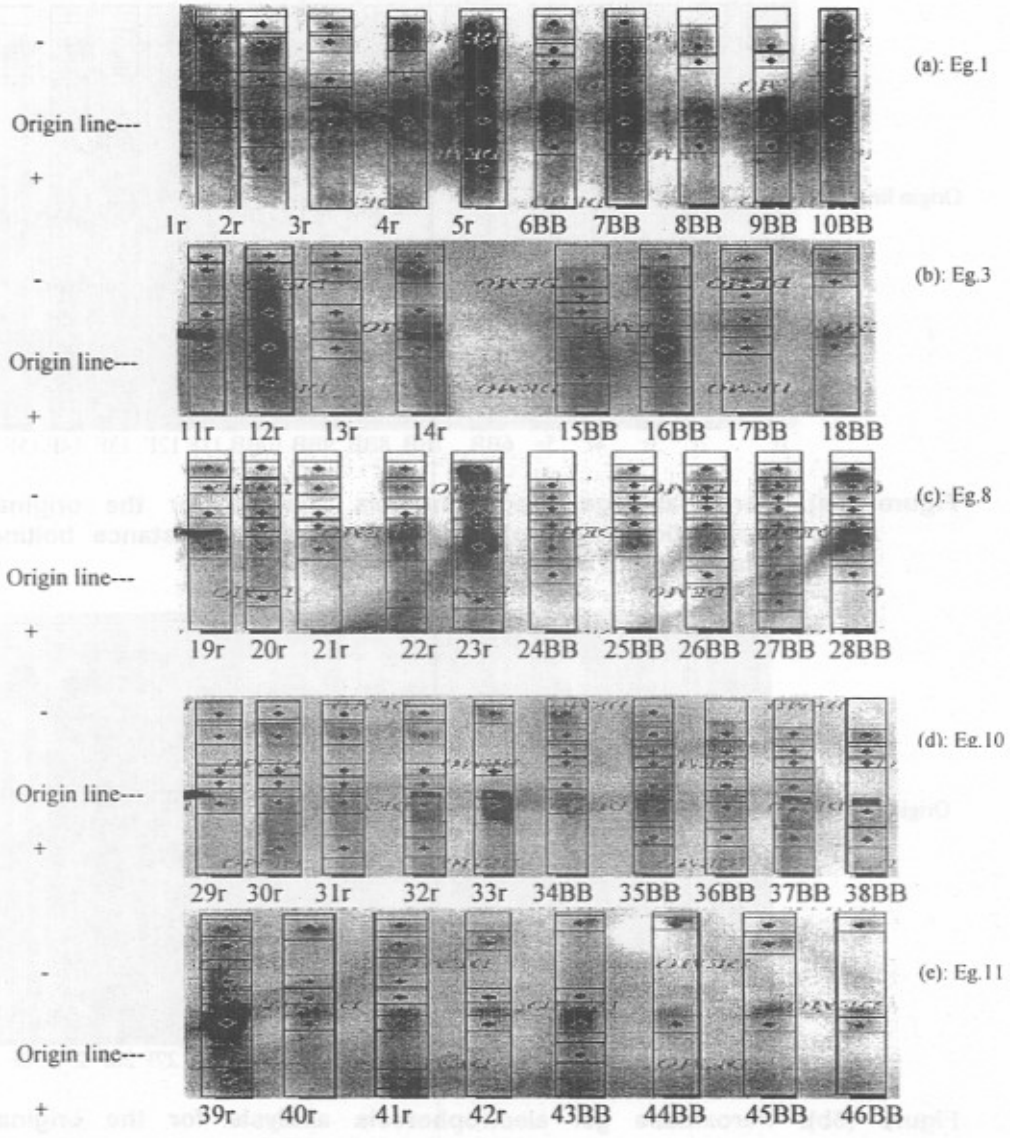

Figure (5) Five gel electrophoretic patterns of peroxidase isozymes for the two original populations and their selected progenies. Whereas: (r): resistance bolting – (BB): bolting.

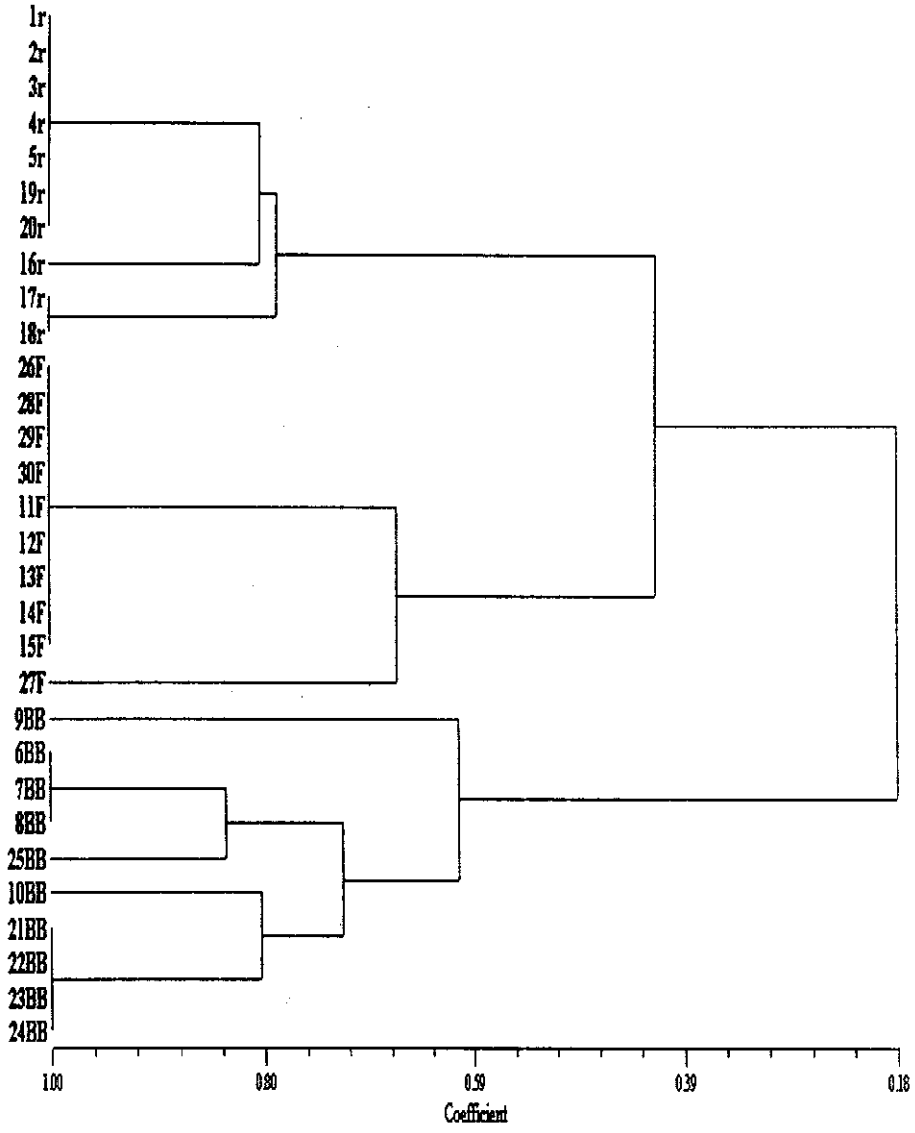

Figure (4): Dendrogram of cluster analysis of population Eg.1 and Eg.8 in the bolting, non-bolting and after vernalization plants based on (0 and 1) data. Whereas: (r): Resistance bolting - (BB): Easy bolting – (F): After vernalization plants.

Table (1): Analysis of electrophoretic data obtained from Eg.1 and Eg.8 populations of the bolting and non-bolting plants

Population	Plant number	Band 1 (-)			Band 2 (-)			Band 3 (-)			Band 4 (-)			Band 5 (-)			Band 6 (-)			Band 1 (+)			Band 2 (+)			Band 3 (+)		
		Vol.	Peak Heig.	R.f.	Vol.	Peak Heig.	R.f.	Vol.	Peak Heig.	R.f.	Vol.	Peak Heig.	R.f.	Vol.	Peak Heig.	R.f.	Vol.	Peak Heig.	R.f.	Vol.	Peak Heig.	R.f.	Vol.	Peak Heig.	R.f.	Vol.	Peak Heig.	R.f.
	1r	-	-	-	-	-	-	-	-	-	-	-	-	871.73	0.81	0.832	624.24	0.43	0.912	1.641.3	0.56	0.100	-	-	-	-	-	-
	2r	-	-	-	4.595.4	0.50	0.298	-	-	-	-	-	-	810.25	0.92	0.831	843.41	0.78	0.911	1.541.2	0.52	0.111	-	-	-	-	-	-
	3r	-	-	-	4.603.1	0.44	0.300	-	-	-	-	-	-	993.11	0.72	0.832	738.46	0.51	0.916	4.527.1	0.78	0.117	-	-	-	-	-	-
	4r	-	-	-	-	-	-	-	-	-	-	-	-	837.91	0.94	0.834	782.34	0.66	0.919	1.612.7	0.53	0.123	-	-	-	-	-	-
	5BB	-	-	-	-	-	-	1.468.1	0.13	0.494	1.439.4	0.52	0.631	-	-	-	852.31	0.86	0.929	1.531.7	0.56	0.128	951.32	0.36	0.44	-	-	-
Eg.1	6BB	-	-	-	-	-	-	1.580.3	0.25	0.478	1.865.3	0.47	0.632	-	-	-	828.14	0.41	0.921	-	-	-	914.36	0.89	0.44	-	-	-
	7BB	-	-	-	-	-	-	919.62	0.68	0.475	1.425.9	0.38	0.633	-	-	-	672.31	0.51	0.924	1.429.7	0.75	0.126	851.36	0.31	0.43	-	-	-
	8B	670.95	1.81	0.113	-	-	-	1.517.7	0.72	0.478	1.576.1	0.75	0.641	-	-	-	-	-	-	4.534.1	1.11	0.125	-	-	-	1.932.2	0.41	0.85
	9B	852.50	0.29	0.138	-	-	-	2.580.4	0.62	0.488	1.461.2	0.78	0.642	-	-	-	-	-	-	4.866.7	0.96	0.120	-	-	-	1.142.3	0.37	0.85
	10B	745.20	0.25	0.137	-	-	-	1.474.6	0.14	0.491	1.498.6	0.66	0.643	-	-	-	-	-	-	1.582.6	0.35	0.125	-	-	-	961.66	0.42	0.86
	11B	399.25	0.23	0.112	-	-	-	969.03	0.11	0.474	1.448.0	0.88	0.640	-	-	-	-	-	-	1.528.9	0.52	0.129	-	-	-	954.12	0.51	0.86
	12r	963.33	0.12	0.130	29.22	0.03	0.261	-	-	-	-	-	-	-	-	-	593.93	0.98	0.901	1.491.3	0.14	0.112	-	-	-	1.171.3	0.44	0.87
	13r	81.00	0.13	0.130	30.53	0.02	0.273	-	-	-	401.32	1.35	0.652	-	-	-	548.23	1.04	0.901	2.510.3	0.95	0.119	-	-	-	1.139.5	0.20	0.87
	14r	210.59	0.49	0.130	136.93	0.06	0.280	-	-	-	380.56	1.82	0.652	-	-	-	759.34	0.86	0.907	2.881.5	0.67	0.114	-	-	-	1.911.5	0.31	0.87
	15r	359.39	0.89	0.130	457.54	0.02	0.273	-	-	-	-	-	-	-	-	-	3.720.1	1.13	0.919	2.358.7	1.15	0.117	-	-	-	2.128.6	0.33	0.86
	16BB	-	-	-	-	-	-	73.61	0.06	0.451	335.15	1.69	0.654	1.280	1.41	0.833	-	-	-	2.354.9	0.45	0.107	1.219.2	0.35	0.48	-	-	-
Eg.8	17BB	119.37	0.05	0.118	-	-	-	1.075.3	1.22	0.447	327.09	0.67	0.658	326.39	0.53	0.845	-	-	-	2.431.4	0.83	0.100	1.014.4	0.41	0.46	-	-	-
	18BB	-	-	-	-	-	-	293.42	0.37	0.453	449.82	1.41	0.658	970.35	1.36	0.845	-	-	-	2.979.4	0.66	0.107	1.216.4	0.31	0.45	-	-	-
	19B	44.13	0.04	0.112	-	-	-	419.86	0.41	0.453	370.23	1.62	0.640	1.970.	2.29	0.832	-	-	-	2.418.3	0.79	0.106	-	-	-	2.013.3	0.29	0.84
	20B	44.36	0.02	0.136	-	-	-	470.33	0.46	0.457	125.30	0.23	0.654	139.84	0.16	0.833	-	-	-	2.422.1	0.96	0.102	-	-	-	1.142.5	0.21	0.85
	21B	-	-	-	-	-	-	854.11	0.94	0.469	280.31	1.31	0.654	1.075	0.67	0.827	-	-	-	1.322.4	0.29	0.109	-	-	-	1.390.2	0.25	0.85
	22B	-	-	-	-	-	-	388.19	0.34	0.469	214.32	2.16	0.656	888.54	0.29	0.831	-	-	-	3.325.4	1.29	0.106	-	-	-	1.122.3	0.51	0.85

Whereas (r): Resistance bolting – (BB): Easy bolting – (B): Late bolting plants.

Table (2): Analysis of electrophoretic data obtained from Eg.1 and Eg.8 populations of the bolting, non-bolting and after vernalization plants

Population	Plant number	Band 1 (-)			Band 2 (-)			Band 3 (-)			Band 4 (-)			Band 5 (-)			Band 6 (-)			Band 1 (+)			Band 2 (+)			Band 3 (+)		
		Vol.	Peak Heig.	R.f.	Vol.	Peak Heig.	R.f.	Vol.	Peak Heig.	R.f.	Vol.	Peak Heig.	R.f.	Vol.	Peak Heig.	R.f.	Vol.	Peak Heig.	R.f.	Vol.	Peak Heig	R.f.	Vol.	Peak Heig	R.f.	Vol.	Peak Heig	R.f.
	1r	-	-	-	1.032.2	0.6	0.311	-	-	-	-	-	-	2.915.8	0.53	0.846	1.258.21	0.33	0.932	3.307.3	1.09	0.091	-	-	-	-	-	-
	2r	-	-	-	928.42	0.25	0.318	-	-	-	-	-	-	2.369.1	0.52	0.848	1.284.31	0.42	0.931	3.546.0	0.75	0.091	-	-	-	-	-	-
	3r	-	-	-	1.075.9	0.29	0.310	-	-	-	-	-	-	2.362.1	0.67	0.843	1.193.98	0.49	0.936	1.382.9	0.75	0.091	-	-	-	-	-	-
	4r	-	-	-	998.84	0.75	0.312	-	-	-	-	-	-	2.314.8	0.77	0.844	1.274.36	0.68	0.939	4.429.6	0.85	0.093	-	-	-	-	-	-
	5r	-	-	-	999.36	0.78	0.313	-	-	-	-	-	-	2.555.2	0.88	0.844	1.194.16	0.46	0.933	1.384.4	0.53	0.101	-	-	-	-	-	-
	6BB	976.45	0.57	0.133	-	-	-	1.052.9	0.45	0.488	2.258.3	0.43	0.651	-	-	-	-	-	-	1.697.6	0.54	0.106	2.345.7	0.41	0.43	-	-	-
	7BB	936.87	0.55	0.131	-	-	-	1.081.2	0.48	0.485	2.336.5	0.35	0.652	-	-	-	-	-	-	1.326.3	0.52	0.104	2.225.3	0.40	0.44	-	-	-
Eg.1	8BB	937.61	0.63	0.143	-	-	-	1.020.4	0.58	0.488	3.214.1	0.54	0.655	-	-	-	-	-	-	1.479.2	0.95	0.112	2.231.1	0.60	0.44	-	-	-
	9BB	-	-	-	-	-	-	1.014.4	0.26	0.488	4.333.5	0.87	0.652	-	-	-	-	-	-	1.556.5	0.17	0.128	-	-	-	-	-	-
	10BB	-	-	-	-	-	-	1.012.5	0.29	0.481	2.396.9	0.68	0.653	-	-	-	-	-	-	1.491.4	0.64	0.127	2.247.12	1.52	0.42	-	-	-
	11F	-	-	-	-	-	-	-	-	-	-	-	-	2.447.5	0.74	0.871	-	-	-	1.499.3	0.96	0.112	-	-	-	1.936	0.35	0.822
	12F	-	-	-	-	-	-	-	-	-	-	-	-	2.543.7	0.92	0.876	-	-	-	1.495.3	0.93	0.111	-	-	-	-	-	-
	13F	-	-	-	-	-	-	-	-	-	-	-	-	2.443.8	0.29	0.873	-	-	-	1.393.8	0.83	0.112	-	-	-	2.382	0.35	0.826
	14F	-	-	-	-	-	-	-	-	-	-	-	-	2.582.6	0.45	0.874	-	-	-	1.300.6	0.67	0.114	-	-	-	2.257	0.34	0.821
	15F	-	-	-	-	-	-	-	-	-	-	-	-	2.477.6	0.69	0.871	-	-	-	1.329.2	0.81	0.101	-	-	-	2.336	0.41	0.824
	16r	-	-	-	1.468.1	0.38	0.354	-	-	-	-	-	-	1.915.9	0.64	0.851	1.354.96	0.64	0.955	1.927.2	1.01	0.106	-	-	-	2.054	0.55	0.873
	17r	727.28	0.51	0.115	1.484.3	0.29	0.351	-	-	-	-	-	-	1.945.8	0.74	0.851	1.268.31	0.74	0.956	1.890.2	0.61	0.084	-	-	-	-	-	-
	18r	799.38	0.58	0.115	2.571.6	0.88	0.350	-	-	-	-	-	-	3.314.4	0.44	0.853	1.333.54	0.67	0.968	1.676.5	0.79	0.097	-	-	-	-	-	-
	19r	-	-	-	1.448.1	0.14	0.352	-	-	-	-	-	-	3.023.3	0.51	0.854	1.387.36	0.46	0.969	1.948.2	0.12	0.103	-	-	-	-	-	-
	20r	-	-	-	1.784.8	0.23	0.361	-	-	-	-	-	-	3.622.1	0.65	0.857	1.687.36	0.78	0.963	1.872.5	0.72	0.079	-	-	-	-	-	-
	21BB	-	-	-	-	-	-	1.953.4	0.52	0.497	2.337.2	0.69	0.663	-	-	-	1.952.25	0.66	0.956	1.890.7	0.72	0.095	1.814.25	0.32	0.46	-	-	-
	22BB	-	-	-	-	-	-	1.746.8	0.58	0.493	2.985.7	0.61	0.677	-	-	-	1.954.13	1.05	0.955	1.814.2	0.74	0.111	1.847.14	0.37	0.46	-	-	-
Eg.8	23BB	-	-	-	-	-	-	1.505.2	0.32	0.508	2.052.1	0.48	0.674	-	-	-	1.654.36	1.06	0.949	1.725.1	0.73	0.123	1.654.31	0.32	0.45	-	-	-
	24BB	-	-	-	-	-	-	1.478.5	0.32	0.509	2.384.8	0.53	0.664	-	-	-	1.965.28	0.98	0.948	1.744.8	0.82	0.111	1.718.24	0.33	0.45	-	-	-
	25BB	1.092.7	0.98	0.138	-	-	-	1.465.2	0.58	0.503	2.228.6	0.85	0.664	-	-	-	1.753.33	0.72	0.944	1.876.5	0.27	0.145	1.455.36	0.25	0.45	-	-	-
	26F	-	-	-	-	-	-	-	-	-	-	-	-	3.247.1	1.04	0.871	-	-	-	1.687.3	0.94	0.148	-	-	-	1.323	0.43	0.882
	27F	-	-	-	-	-	-	-	-	-	-	-	-	3.328.4	0.94	0.874	-	-	-	1.751.6	0.85	0.135	-	-	-	1.474	0.46	0.885
	28F	-	-	-	-	-	-	-	-	-	-	-	-	1.745.6	0.94	0.871	-	-	-	1.858.1	1.29	0.121	-	-	-	1.783	0.44	0.889
	29F	-	-	-	-	-	-	-	-	-	-	-	-	2.952.4	1.01	0.884	-	-	-	1.625.4	0.67	0.114	-	-	-	1.356	0.22	0.889
	30F	-	-	-	-	-	-	-	-	-	-	-	-	2.297.5	0.85	0.887	-	-	-	1.336.2	0.69	0.135	-	-	-	1.300	0.23	0.887

Whereas: (r): Resistance bolting – (BB): Easy bolting – (F): After vernalization plants.

Table (3): Analysis of electrophoretic data obtained from two original sugar beet (Eg.1 & Eg.3) populations and their three selected progenies (Eg.8, Eg.10 and Eg.11) of the bolting and non-bolting plants

Population	Plant number	Band 1 (-)			Band 2 (-)			Band 3 (-)			Band 4 (-)			Band 5 (-)			Band 6 (-)			Band 1 (+)			Band 2 (+)			Band 3 (+)		
		Vol.	Peak Heig	R.f	Vol.	Peak Heig	R.f.	Vol.	Peak Heig.	R.f.	Vol.	Peak Heig.	R.f.	Vol.	Peak Heig.	R.f.	Vol.	Peak Heig	R.f.	Vol.	Peak Heig	R.f.	Vol.	Peak Heig	R.f.	Vol.	Peak Heig.	R.f.
Eg.1	1r	-	-	-	815.6	0.88	0.304	-	-	-	-	-	-	2.369.23	0.84	0.859	1.247.2	0.68	0.941	1.277.21	0.88	0.124	-	-	-	-	-	-
	2r	-	-	-	917.6	0.65	0.301	-	-	-	-	-	-	2.587.18	0.98	0.859	1.364.1	0.45	0.949	1.328.23	0.88	0.135	-	-	-	1.284.3	0.58	0.881
	3r	-	-	-	898.3	0.42	0.304	-	-	-	-	-	-	1.001.35	0.49	0.851	1.271.4	0.68	0.933	1.412.36	0.76	0.108	-	-	-	-	-	-
	4r	-	-	-	719.2	0.48	0.311	-	-	-	-	-	-	858.24	0.99	0.854	625.1.5	0.88	0.938	1.028.84	0.77	0.115	-	-	-	-	-	-
	5r	1.952.2	1.43	0.115	-	-	-	-	-	-	-	-	-	2.347.98	1.26	0.859	1.428.1	0.99	0.939	1.417.36	0.99	0.134	-	-	-	1.248.6	0.83	0.877
	6BB	-	-	-	-	-	-	732.12	0.22	0.502	787.25	0.25	0.648	-	-	-	2.171.6	0.45	0.932	1.362.81	0.81	0.127	1.242.33	0.34	0.466	-	-	-
	7BB	2.025.6	1.36	0.119	-	-	-	855.31	1.01	0.503	678.25	1.02	0.643	-	-	-	2.138.4	0.50	0.939	1.287.39	0.97	0.109	1.211.13	0.85	0.471	-	-	-
	8BB	-	-	-	-	-	-	763.05	0.23	0.504	1.782.31	0.69	0.658	-	-	-	-	-	-	1.369.25	0.64	0.118	1.285.55	0.58	0.468	-	-	-
	9BB	-	-	-	-	-	-	772.55	0.21	0.501	1.565.21	0.69	0.651	-	-	-	-	-	-	1.258.36	0.74	0.135	1.074.67	0.67	0.459	-	-	-
	10BB	1.936.1	1.06	0.121	-	-	-	851.54	1.07	0.502	658.25	1.08	0.658	-	-	-	2.587.3	0.35	0.956	1.283.54	0.81	0.122	1.001.25	0.72	0.466	-	-	-
Eg.3	11r	-	-	-	607.6	0.21	0.281	-	-	-	-	-	-	1.482.36	0.48	0.842	671.39	0.37	0.971	1.625.36	0.48	0.105	-	-	-	-	-	-
	12r	-	-	-	995.2	0.96	0.294	-	-	-	-	-	-	1.658.14	0.67	0.845	1.184.2	0.30	0.977	1.958.36	0.91	0.121	-	-	-	1.341.8	0.87	0.861
	13r	-	-	-	999.3	0.28	0.295	-	-	-	-	-	-	1.847.39	0.49	0.859	1.658.5	0.36	0.979	1.958.13	0.28	0.114	-	-	-	-	-	-
	14r	-	-	-	993.2	0.61	0.304	-	-	-	-	-	-	1.628.73	0.71	0.854	1.228.1	0.54	0.978	1.565.29	0.31	0.119	-	-	-	-	-	-
	15BB	-	-	-	991.2	0.38	0.303	1.141.5	0.32	0.531	1.458.36	0.54	0.658	-	-	-	-	-	-	2.251.98	0.37	0.109	1.289.35	0.46	0.431	-	-	-
	16BB	967.56	0.74	0.135	-	-	-	1.098.5	0.61	0.538	1.411.39	0.64	0.661	-	-	-	1.337.5	0.44	0.961	2.237.15	0.91	0.091	1.214.44	0.57	0.438	-	-	-
	17BB	947.74	0.34	0.139	-	-	-	999.36	0.27	0.537	1.498.32	0.28	0.647	-	-	-	1.479.1	0.44	0.963	1.331.22	0.25	0.089	-	-	-	-	-	-
	18BB	-	-	-	-	-	-	-	-	-	717.35	0.36	0.653	-	-	-	1.381.4	0.54	0.965	1.221.39	0.29	0.099	-	-	-	-	-	-

Whereas: (r): Resistance bolting – (BB): Easy bolting plants.

Cont. Table (3): Analysis of electrophoretic data obtained from two original sugar beet (Eg.1 & Eg.3) populations and their three selected progenies (Eg.8, Eg.10 and Eg.11) of the bolting and non-bolting plants

Population	Plant number	Band 1 (-)			Band 2 (-)			Band 3 (-)			Band 4 (-)			Band 5 (-)			Band 6 (-)			Band 1 (+)			Band 2 (+)			Band 3 (+)		
		Vol.	Peak Heig.	R.f	Vol.	Peak Heig.	R.f.	Vol.	Peak Heig.	R.f.	Vol.	Peak Heig.	R.f.	Vol.	Peak Heig.	R.f.	Vol.	Peak Heig	R.f.	Vol.	Peak Heig.	R.f.	Vol.	Peak Heig.	R.f.	Vol.	Peak Heig	R.f.
Eg.8	19r	-	-	-	274.28	0.35	0.281	-	-	-	-	-	-	578.36	0.23	0.843	803.69	0.89	0.943	1.847.36	0.87	0.126	-	-	-	-	-	-
	20r	359.21	0.75	0.126	314.58	0.73	0.304	-	-	-	-	-	-	597.58	0.70	0.838	701.31	0.40	0.954	1.699.85	0.76	0.121	-	-	-	486.36	0.69	0.873
	21r	268.35	0.42	0.132	428.36	0.19	0.279	-	-	-	-	-	-	719.25	0.51	0.842	735.61	0.32	0.939	1.958.36	0.69	0.124	-	-	-	-	-	-
	22r	301.58	0.96	0.129	309.36	0.22	0.273	-	-	-	-	-	-	620.36	0.83	0.861	710.36	0.82	0.932	1.843.69	1.14	0.129	-	-	-	500.36	1.06	0.881
	23r	369.25	0.99	0.103	835.64	1.06	0.295	-	-	-	-	-	-	840.36	1.16	0.865	1.310.3	1.69	0.934	2.458.33	1.74	0.118	-	-	-	1.045.2	0.72	0.887
	24BB	394.25	0.67	0.109	-	-	-	284.65	0.59	0.456	298.35	0.62	0.657	470.25	0.49	0.872	-	-	-	1.589.32	0.69	0.116	1.093.1	0.71	0.421	-	-	-
	25BB	299.58	0.54	0.096	-	-	-	257.35	0.44	0.461	256.35	0.45	0.649	420.14	0.30	0.879	787.25	0.42	0.926	1.891.36	1.20	0.114	-	-	-	-	-	-
	26BB	276.35	0.63	0.109	-	-	-	237.25	0.39	0.453	239.54	0.47	0.645	416.52	0.50	0.821	736.25	0.43	0.929	1.901.36	1.02	0.120	851.37	0.53	0.415	-	-	-
	27BB	310.36	0.59	0.111	-	-	-	219.25	0.35	0.437	369.28	0.62	0.643	468.25	0.94	0.851	716.25	0.43	0.920	1.941.36	0.99	0.123	902.36	1.06	0.409	889.28	0.99	0.888
	28BB	284.25	0.42	0.121	-	-	-	224.35	0.26	0.426	229.25	0.54	0.631	470.36	0.91	0.853	499.25	1.24	0.919	1.836.36	1.31	0.126	709.21	0.55	0.417	-	-	-
Eg.10	29r	430.25	0.23	0.149	421.25	0.25	0.298	-	-	-	-	-	-	996.25	0.52	0.875	985.21	0.57	0.946	851.23	0.53	0.125	-	-	-	-	-	-
	30r	442.36	0.26	0.142	430.25	0.26	0.293	-	-	-	-	-	-	1.098.3	0.74	0.879	916.25	0.31	0.951	836.25	0.69	0.126	-	-	-	1.101.3	0.89	0.888
	31r	410.21	0.28	0.137	430.24	0.22	0.302	-	-	-	-	-	-	1.006.7	0.69	0.875	901.25	0.28	0.947	826.36	0.63	0.129	-	-	-	997.36	0.61	0.876
	32r	426.32	0.30	0.133	428.32	0.19	0.309	-	-	-	-	-	-	998.25	0.67	0.871	899.25	0.29	0.949	1.003.8	0.87	0.135	-	-	-	910.25	0.60	0.874
	33r	-	-	-	410.25	0.20	0.305	-	-	-	-	-	-	-	-	-	941.25	0.79	0.941	1.001.2	1.59	0.138	-	-	-	-	-	-
	34BB	399.21	0.19	0.132	-	-	-	286.21	0.26	0.462	403.25	0.25	0.635	409.25	0.62	0.870	990.36	0.88	0.947	540.23	0.63	0.140	-	-	-	-	-	-
	35BB	451.36	0.76	0.129	-	-	-	310.25	0.52	0.456	398.25	0.56	0.632	412.36	0.59	0.876	970.25	0.32	0.959	940.25	0.85	0.140	680.25	0.65	0.421	-	-	-
	36BB	462.32	0.22	0.131	-	-	-	395.25	0.59	0.453	402.36	0.63	0.629	701.25	0.61	0.875	-	-	-	1.010.3	0.59	0.145	740.36	0.58	0.429	-	-	-
	37BB	443.25	0.41	0.133	-	-	-	249.32	0.30	0.458	400.25	0.32	0.629	430.25	0.51	0.871	-	-	-	1.090.3	0.63	0.147	731.25	0.64	0.432	-	-	-
	38BB	-	-	-	-	-	-	268.21	0.29	0.451	405.36	0.33	0.630	506.36	0.81	0.866	-	-	-	1.070.6	0.59	0.149	736.36	0.59	0.434	-	-	-
Eg.11	39r	569.23	0.95	0.149	985.25	0.89	0.347	-	-	-	-	-	-	974.25	0.61	0.873	903.25	0.72	0.961	548.25	1.95	0.149	-	-	-	709.25	0.97	0.803
	40r	571.36	0.49	0.147	564.21	0.41	0.339	-	-	-	-	-	-	-	-	-	989.36	0.76	0.969	543.25	0.59	0.152	-	-	-	-	-	-
	41r	581.36	0.28	0.143	576.25	0.29	0.340	-	-	-	-	-	-	985.23	0.59	0.866	504.36	0.43	0.975	564.36	0.78	0.154	-	-	-	712.25	0.99	0.806
	42r	499.35	0.27	0.146	-	-	-	-	-	-	-	-	-	723.58	0.56	0.878	-	-	-	510.36	0.29	0.152	-	-	-	-	-	-
	43BB	522.36	0.29	0.147	-	-	-	-	-	-	-	-	-	-	-	-	688.25	0.56	0.981	567.95	1.81	0.159	577.25	0.39	0.459	-	-	-
	44BB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	510.25	0.66	0.971	571.52	0.36	0.151	-	-	-	-	-	-
	45BB	-	-	-	-	-	-	-	-	-	-	-	-	820.36	0.58	0.876	701.25	0.24	0.970	576.25	0.34	0.154	-	-	-	-	-	-
	46BB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	640.25	0.54	0.969	521.36	0.42	0.151	-	-	-	-	-	-

Whereas: (r): Resistance bolting – (BB): Easy bolting plants.

REFERENCES

Alimgazinova, B.Sh. (1986). Using the isoperoxidases to identify inbred sugar beet lines in breeding work. Biokhimicheskie pokazateli v selektsii zernovykh kul'tur. Alma-Ata, Kazakh SSR. 32-39. (Pl. Breed. Abs. 58 (1): 631).

Arumuganathan K. and E.D. Earle (1991). Nuclear DNA content of some important plant species. Plant Mol Biol. Rep 9: 208-218.

Bosemark, N.O. (1993). Genetics and breeding. In: The sugar beet crop (ed. Cooke D.A. and R.K. Scott) Chapman and Hall. New York. PP. 67-119.

Lysgaard, C.P. (1978). Selection for reduced bolting susceptibility in beet and swedes, and the influence of environmental factors on bolting. Kgl. Vet-og Landbohojsk. Arsskr. 138-158.

O'Connor, L.J. (1970). Environmental influence during beet seed production on bolting and quality characteristics of the subsequent root crop. I.I.R.B. 4 (4): 207-216.

Poehlman, J.M. (1979). Breeding sugar beets. In: breeding field crops. 2nd ed. Avi. Pub. Co. Westport. Conn. U.S.A.

Rohlf, F.J. (2000). NTSYS-pc numerical taxonomy and multivariate system, version 2.1. Applied Biostatics Inc., New York.

Sabrah, N.S. (1980). Genetical and cytological studies on Maize. Ph. D. Thesis, Faculty of Agriculture, University of Alexandria, Egypt.

Sabrah, N.S. and A.Y. El-Metainy (1985). Genetic distances between local and exotic cultivares of *Vicia fabae* L. based on esterase isozyme variation. Egypt. J. Genet. Cytol. 14: 301-307.

Saleh, M.S. (1999). Genetical Studies on some sugar crops Ph.D. Thesis, Faculty of Agriculture University of Alexandria Egypt.

Shaw, C.R. (1969). Isozymes classification, frequency and significance. International Review of cytology. 25: 297-332.

Van Geyt, J.P.C.; M. Oleo and TH.S.M. De Bock (1988). Monosomic addition in beet (Beta vulgaris) carrying extra chromosomes of Beta procumbens. I. Identification of the alien chromosomes with the help of isozyme markers. Theor. Appl. Genet. 76: 577-586.

Weising, K.; H. Nybom; K. Wolff and W. Meyer (1995). DNA Fingerprinting in Plants and Fungi. (CRC Press, Boca Raton, FL).

Yu, M.H.; L.M. Pakish and H. Zhou (2001). An isozyme marker for resistance to root-knot nematode in sugarbeet. Crop Sci. 41: 1051– 1053.

Zinecker, M. and D. Rinzier (1979). Results of breeding for resistance to bolting in monocarpic sugar beet. Archiv fur Zuchtungsforschung. 9: 299-310. (Pl. Breed. Abs. 50 (3): 2220).

الملخص العربى

التنبؤ المبكر للتزهير الكاذب في بنجر السكر بأستخدام المشابهات الأنزيمية لإنزيم البيروكسيديز

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**كلية الزراعة –قسم الوارثة _جامعة الإسكندرية –فرع سابا باشا.

اجريت هذه الدراسه في محطة البحوث الزراعية بالصبحية في الأسكندرية بغرض الحصول علي واسمات كيموحيوية قي بنجر السكر بغرض الكشف المبكر عن ظاهرة التزهير الكاذب وهي صفة غير مرغوب فيها قي بنجر السكر لأنها تؤدي الى نقص المحصول ونقص نسبة السكر وبالتالى نقص محصول السكر.

أجريت هذه التجربة الحقلية في المزرعة البحثية لمحطة البحوث الزراعية بالصبحية. وقد تم أخذ العينات من نباتات بنجر السكر التي ظهر بها تزهير كاذب والتي لم يظهر بها هذه الصفة وذلك في مراحل مختلفة من عمر النبات وهذا قبل ان تظهر هذه الصفة على النباتات وذلك بترقيم النباتات في الحقل وعند ظهور الصفة على النباتات تم معرفة النباتات التى أظهرت الصفة من تلك التي لم تظهر بها الصفة. وقد تم تحليل العينات الورقية المتحصل عليها في اعمار مختلفة من عمر النباتات بطريقة الفصل الكهربي لإنزيم البيروكسيديز.

تم عمل تحليل الشجرة وقد أدى هذا التحليل الى وضع النباتات التي اظهرت صفة التزهير الكاذب وتلك التي لم تظهر بها الصفة في مجاميع مختلفة مما يعني انة يمكن استخدام مثل هذه الطريقة في الأنتخاب ضد صفة التزهير الكاذب في مراحل مبكرة من عمر النبات.