

Isozymes Variability in Sugar Beet Genotypes Resistant to Root-Knot Nematode, (*Meloidogyne javanica*)

Saleh, M.S.,¹A.K. EL-Sayed²; M. A. Ghonema¹and Nancy A. Abo Ollo²

ABSTRACT

In Egypt, sugar beet is cultivated in 257667 faddans with an average production of about 18.593 tons per faddan 2007- 2008. Recently, reclaimed desert irrigated lands at West Nubaryia and El-Bostan regions has shown that sugar beet can be successfully grown under sandy soil area condition and is considered as an extended area for sugar beet production in Egypt. The most serious problem against sugar beet extension in new lands is root-knot nematode, *Meloidogyne javanica* which were reported as major nematode pests of sugar beet in Egypt. Importance of employed resistance nematode sugar beet genotypes (cultivars/hybrids) in infested areas has a great concern. The present study was carried out during the growing season 2007 - 2008 in Sabahia Agricultural Research Station, Alexandria, Egypt to study the activity of three different isozymes (esterase, amylase and peroxidase) in twenty seven sugar beet genotypes had different background in immune behavior (susceptible or resistance) to nematode *Meloidogyne javanica* to recognized biochemical marker for this trait. To facilitate the choice of resistance ones for planting in nematode contaminated areas and can be used in evaluative purposes breeding programs to identify resistant breeding materials. From the three studied isozymes only peroxidase may differentiate between susceptible or resistant sugar beet genotypes to root-knot nematode.

INTRODUCTION

Plant parasitic nematodes, especially root-knot nematodes are known to be among the most serious pests of sugar beet in many countries. Of some 50 described species of *Meloidogyne*, only few parasitized sugar beet, viz, *M. arenaria*, *M. incognita*, *M. javanica*, *M. hapla* and *M. naasi* are economically important to sugar beet production. *M. arenaria*, *M. incognita* and *M. javanica* essentially are hot-weather organisms and most important where beets are grown in regions with long, hot summers and short, mild winter (Arnold, 1984). In Egypt, the most serious problem against sugar

beet extension in new lands is root-knot nematode. *Meloidogyne incognita* and *M. javanica*, (Ibrahim, 1982; Oteifa and El-Gindi, 1982; Abd El-Massih, 1985; Maareg *et al.*, 1988 and Ismail *et al.*, 1996).

Isozyme term was first introduced by Markert and Moller (1959), to refer to multiple molecular form of an enzyme with similar or identical substrate specificity occurring with the same organism. Gaspar and Bouchet (1973) studied peroxidase as biochemical measure of fresh weight and sugar yield in sugar beet. They found that peroxidase activity was always much higher in the roots of sugar beet populations characterized by low fresh weight and high content of sugar. This correlation could be detected in seedlings only a few days old.

Liu *et al.* (1992) studied esterase, peroxidase and polyphenol oxidase isozymes in 5 wild species belonging to the 3 sections of the genus *Beta* and in 2 sugar beet cultivars using PAGE. The results indicated that there is a distant phylogenetic relationship between *B. patellaris* and *B. vulgaris*, with *B. corolliflora* intermediate to these 2 species. *B. patellaris* had a more distant relationship with the 2 sugar beet cultivars than did *B. maritima*. 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. Abe (1998) reported that isozymes have been used as useful markers in genetic studies of many plant species. Up to date, approximately thirty isozyme loci were identified in sugar beet. Some of the loci, however, may be of use in genetic studies of agronomically important traits. El-Kholi *et al.* (2005) examined enzymatic activity of Chitinase β -1,3 glucanase, poly-phenol oxidase, peroxidase and invertase in sugar beet roots infected by *Rhizoctonia solani*. They found that isozymes activity was significantly increased in infected sugar beet roots

¹Sugar Crops Research Institute, Department of Genetics and breeding.

²Department of Genetics, Faculty of Agriculture, University Of Alexandria-(Saba-Bacha),

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than in healthy roots. The levels of the tested enzymes were varied significantly between the tested sugar beet varieties. The qualitative and quantitative analysis of the tested isozymes (malat-dehydrogenase, esterase, indo-phenol oxidase and β -1, 3 glucanase) in healthy and infected sugar beet varieties showed different enzyme patterns between the tested varieties as well as between the healthy and infected ones. The resulted number of bands and the banding intensity score of tested isozymes increased in infected sugar beet varieties than in healthy one. Srivastava *et. al.* (2007) examined four diploid populations of sugar beet using isozymes and molecular markers. Seventy-one bands consisting of 28 isomorphs of 6 isozyme systems *viz.* superoxide dismutase, guaiacol peroxidase, malate dehydrogenase, amylase, esterase and aspartate amino transferase were resolved.

The main objective of this study was to study the activity of three different isozymes (esterase, amylase and peroxidase) to achieve such a purpose twenty seven

sugar beet genotypes that displayed differential immune behavior (susceptible or resistance) to root knot nematode *Meloidogyne javanica* to recognized biochemical marker for this trait. To facilitate the choice of resistant ones for planting in nematode contaminated areas and can be used in breeding programs to identify resistant breeding materials.

MATERIALS AND METHODS

1. Sugar beet genotypes:

The different genotypes used in this study were classified into four categories one genotype was highly susceptible (HS), nine were susceptible (S), thirteen moderate resistant (MR) and four genotypes were resistant (R). Table (1) presents the twenty seven sugar beet genotypes and its description (Saleh *et. al.*, 2009). The examined sugar beet genotypes used in this study were introduced from Sugar Crops Research Institute, Agriculture Research Center, Egypt.

Table 1. Twenty seven sugar beet investigated genotypes and its description, (HS) highly susceptible, (S) susceptible, (MR) moderate resistant and (R) resistant to root knot nematode

Genotypes reaction	Sugar beet genotypes	Genotypes handling category	Seed type	Code
HS	FD 9902	Commercial var,	Poly	A13
	Glorius	Commercial var,	Poly	A1
S	DS 9004	Commercial var,	Poly	A3
	Rosanna	Commercial var,	Mono	A5
	02-99	Commercial var,	Mono	A6
	Rhist	Commercial var,	Mono	A9
	Toro	Commercial var,	Poly	A12
	Type	Commercial var,	Poly	A25
	Eg.6	Breeding material	Poly	A26
	Armure	Commercial var,	Mono	A27
	Helwes	Commercial var,	Poly	A2
	Francesca	Commercial var,	Mono	A4
MR	LP-10	Commercial var,	Mono	A7
	LP-13	Commercial var,	Mono	A8
	05-99	Commercial var,	Mono	A10
	01-99	Commercial var,	Mono	A11
	Despreze(2003)	Commercial var,	Poly	A14
	Baraca	Commercial var,	Poly	A15
	Eg-2701	Breeding material	Poly	A18
	SP-270	Breeding material	Poly	A19
	C.39	Breeding material	Poly	A20
	Asthos poly	Commercial var,	Poly	A21
	Eg.26	Breeding material	Poly	A24
	R	Sultan	Commercial var,	Poly
Amile		Commercial var,	Mono	A17
Eg.27		Breeding material	Poly	A22
Monte Bianco		Commercial var,	Mono	A23

2. METHODS:

Agar- Starch- Polyvinyl pyrrolidine (PVP) gel was applied to study different isozymes variations. Esterase, Amylase and Peroxidase were examined to detect biochemical marker for (susceptible or resistance) sugar beet plants to root knot nematode (*Meloidogyne javanica*). Electrophoresis was carried out to obtain the isozyme patterns in the leaves of the sugar beet samples. The following are the buffers, gel media, staining solution and electrophoretic procedure:

2.1- Isozyme buffers:

2.1.1. Esterase and amylase isozymes:

0.23 M Tris- Boric acid buffer, pH 8.6 (Sabrah, 1980) 9.1 gm of Tris dissolved in 200 ml distilled water and 18.55 gm Boric acid were added to 2.5 g of NaOH and completed to 1000 ml volume.

2.1.2. Peroxidase isozyme:

This buffer was prepared by dissolving 27.7 gm of Tris in 200 ml distilled water 11.0 gm citric acid were added and completed to 1000 ml volume, then pH was adjusted to 8.0 (Sabrah, 1980).

2. 2 - Gel media:

2. 2. 1. Esterase and amylase isozymes:

Agar- Starch- Polyvinyl pyrrolidine (PVP) gel were added to 100 ml of 0.23 M Tris- Boric acid buffer, pH 8.6. The mixture was cooked in boiling water bath until the solution became transparent.

2. 2. 2. Peroxidase isozyme:

Agar- Starch- Polyvinyl pyrrolidine (PVP) gel (1 gm Agar; 0.5g PVP and 0.3gm of hydrolyzed starch) were added to 100 ml of 0.023M Tris- Citric acid buffer (pH 0.8). The mixture was cooked in 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 (Sabrah and El- Metainy, 1985).

2. 3 - Procedure:

The plant samples was homogenized in cold mortar containing 0.1 ml of 0.23M Tris-Acetate buffer (pH 8.0). The homogenate was absorbed into stripes of filter paper (0.5 X 0.2cm). Filters were placed on the agar gel plates about 30 min, at 4 °C. The, filter papers were removed and a constant current of 13-14 v/ cm was applied for 90 min, at 4°C using 0.23M Tris- Acetate buffer (pH 8.0) as electrode buffer. The plates were stained with staining solution.

2. 4 - staining solution:

2. 4. 1. Esterase:

Gel plate was rinsed in 10 ml of 0.01 M Tris HCl pH 7.0, 3.0 ml of substrate (40 mg of α Naphthyl acetate, 40 β Naphthyl acetate , 4 ml of acetone and H₂O(1:1), 60 ml of fast blue RR and 100 ml of phosphate buffer pH 6.0) and 87 ml of distilled water.

2. 4. 2. Amylase:

Gel plate was raised in 10 ml of 0.2 M Tris- acetate pH 5.0, and then incubates in 1 % of starch buffer for 2-2.5. incubation in 0.1% of iodine + 0.5 % KI + 0.5 ml Glacial acetic acid.

2. 4. 3. Peroxidase:

100 ml of 0.01M sodium acetate – acetic acid buffer (pH 5.0) containing 0.1g benzidine and 0.5 % hydrogen peroxide (H₂O₂) were used as staining solution of peroxidase isozymes.

2.5. Isozymes analysis:

Measurement of bands was carried out using the computer program software TOTALLAB 100. Data was analyzed with computer program NTSYS-pc ver 2.1 (Rohlf, 2000), to develop the cluster analysis. The following parameters were estimated during the electrophoretic analysis 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 migration distance between original line and band position as relative number.

RESULTS AND DISCUSSION

1. Isozyme pattern in the twenty seven genotypes:

Twenty seven sugar beet genotypes were employed to study (esterase, amylase and peroxidase) isozymes for (susceptible or resistant) plants. The data were found to be varied between the three employed isozymes.

1.1. Esterase isozyme:

Figure (1a&b) illustrates electrophoretic patterns of esterase isozyme for the twenty seven sugar beet genotypes of 90 days old plants. Table (2a) presents data for cathode migration of the studied genotypes. The data indicated that there were seven bands migrated towards the cathode. Band existence, band volume, peak height and R.f. parameters could not differentiated between susceptible or resistant plants as well as from one genotype to another. Same directional was found in the anode migration of esterase isozyme presented in

Table (2b). There were five bands in anode migration of esterase isozyme in sugar beet studied genotypes.

Figure (2) presents dendrogram of cluster analysis for esterase isozyme based on (0 and 1) data employing the NTSYS-pc ver. 2.1 software. The data indicated that cluster analysis differentiate the twenty seven sugar beet

genotypes in three clusters, cluster number one contain five genotypes two susceptible and three moderate resistant and cluster number three contain seven genotypes (five susceptible, one highly susceptible, and one moderate resistant).

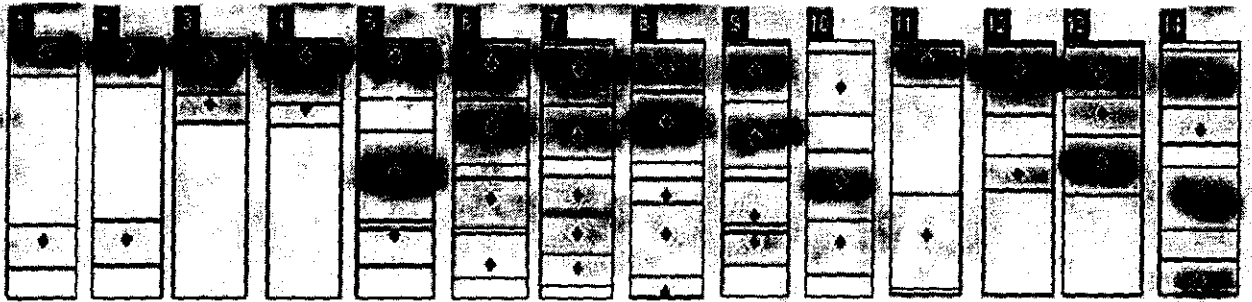
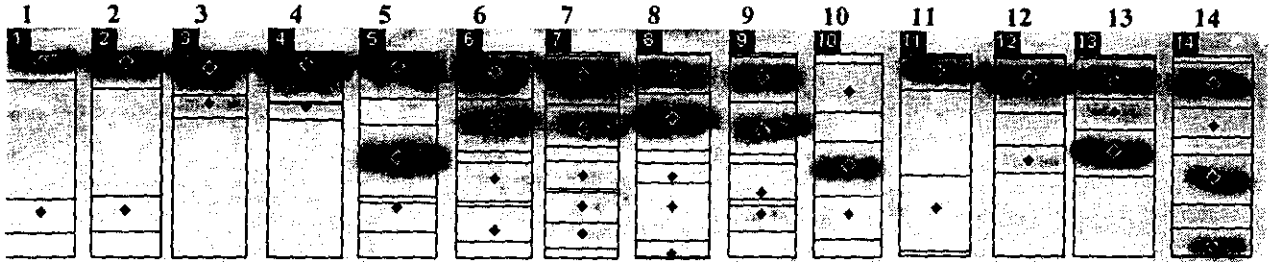


Figure 1 a. Esterase pattern for control sugar beet genotypes from (1 to 14) after 90 days from planting

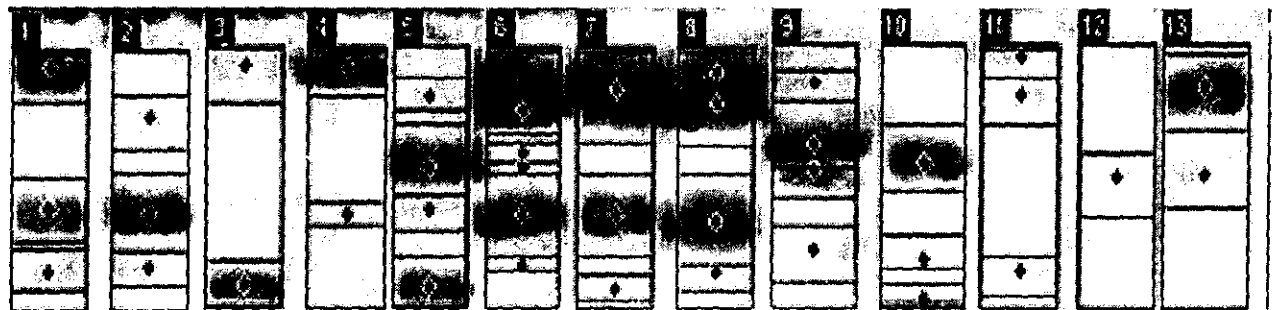
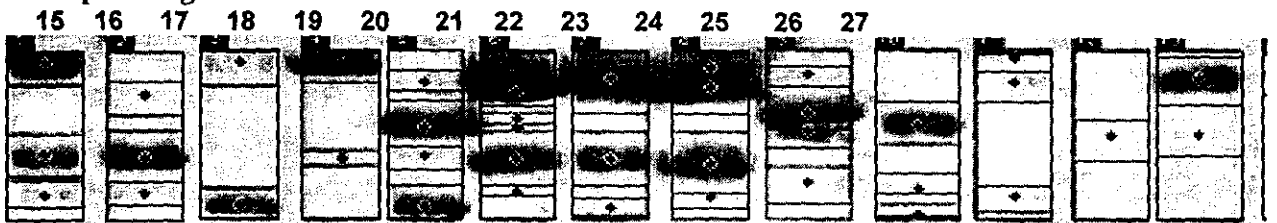


Figure 1 b. Esterase pattern for control sugar beet genotypes from (15 to 27) after 90 days from planting

Table 2a. Analysis of esterase isozyme data obtained from the twenty seven sugar beet genotypes in cathode side

Plant number	Band 1 (-)			Band 2 (-)			Band 3 (-)			Band 4 (-)			Band 5 (-)			Band 6 (-)k height			Band 7 (-)		
	Vol	Peak height	R.F	Vol	Peak height	R.F	Vol	Peak height	R.F	Vol	Peak height	R.F	Vol	Peak height	R.F	Vol	Pea	R.F	Vol	Peak height	R.F
1(S)	70000.55	134.77	0.071	-	-	-	-	-	-	-	-	-	28012.10	49.73	0.616	2677.00	6.45	0.866	-	-	-
2(MR)	-	-	-	3384.29	5.48	0.259	-	-	-	-	-	-	74.095.40	115.65	0.634	2642.94	6.47	0.848	-	-	-
3(S)	6905.14	10.56	0.071	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	42597.64	86.23	0.920
4(MR)	57178.46	117.73	0.080	-	-	-	-	-	-	-	-	-	1141.19	4.74	0.646	-	-	-	-	-	-
5(S)	-	-	-	2998.61	7.72	0.195	-	-	-	74453.30	121.22	0.460	6475.09	16.46	0.628	-	-	-	55265.81	109.21	0.929
6(S)	-	-	-	151155.42	157.27	0.248	1735.00	8.95	0.407	746.00	4.36	0.460	96769.50	159.11	0.646	686.75	4.66	0.841	-	-	-
7(MR)	-	-	-	173252.36	167.14	0.168	-	-	-	-	-	-	68212.50	100.76	0.646	-	-	-	1079.50	3.69	0.938
8(MR)	68278.66	139.32	0.106	122178.80	170.40	0.221	-	-	-	-	-	-	113001.00	133.97	0.673	2493.00	8.16	0.876	-	-	-
9(S)	-	-	-	1949.00	5.58	0.149	108817.00	153.53	0.377	28165.50	71.06	0.482	-	-	-	5736.00	9.42	0.781	-	-	-
10(MR)	-	-	-	-	-	-	-	-	-	64444.18	94.56	0.447	-	-	-	1700.04	4.23	0.816	1055.48	4.62	0.947
11(MR)	5192.80	12.65	0.044	4146.87	7.56	0.193	-	-	-	-	-	-	-	-	-	4973.96	8.90	0.868	-	-	-
12(S)	-	-	-	-	-	-	-	-	-	4899.03	7.10	0.509	-	-	-	-	-	-	-	-	-
13(HS)	-	-	-	69413.00	93.67	0.167	-	-	-	9309.49	10.20	0.500	-	-	-	-	-	-	-	-	-
14(MR)	69818.20	99.96	0.123	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15(MR)	52800.20	115.35	0.073	2959.04	9.71	0.239	4720.46	10.26	0.330	-	-	-	-	-	-	3236.00	7.80	0.734	5188.51	14.98	0.936
16(R)	3837.36	6.27	0.064	11861.50	17.60	0.275	-	-	-	-	-	-	61018.53	126.24	0.560	-	-	-	-	-	-
17(R)	7109.97	16.70	0.101	-	-	-	50813.50	89.80	0.330	-	-	-	-	-	-	10660.12	16.67	0.688	2077.83	6.42	0.881
18(MR)	30215.54	40.33	0.092	-	-	-	-	-	-	2683.50	3.94	0.477	837.48	4.22	0.596	5900.47	18.43	0.807	16023.24	25.76	0.908
19(MR)	51069.50	112.69	0.156	-	-	-	640.89	3.78	0.394	-	-	-	58228.61	103.65	0.560	-	-	-	6969.75	13.83	0.936
20(MR)	18077.73	24.55	0.083	-	-	-	7317.99	11.71	0.376	-	-	-	8757.00	12.70	0.606	3589.75	11.14	0.835	-	-	-
21(MR)	48201.43	111.84	0.110	-	-	-	13862.12	18.38	0.413	-	-	-	8056.44	17.86	0.560	997.00	5.87	0.706	44061.91	106.61	0.945
22(R)	58214.86	128.31	0.073	4957.92	11.41	0.284	-	-	-	-	-	-	433.23	8.43	0.578	-	-	-	8317.50	15.96	0.881
23(R)	54199.62	104.23	0.101	-	-	-	-	-	-	-	-	-	34201.31	81.49	0.541	4358.39	8.13	0.734	-	-	-
24(MR)	65110.27	105.48	0.147	-	-	-	1813.59	5.11	0.303	-	-	-	6012.11	13.18	0.532	27932.89	34.49	0.725	-	-	-
25(S)	63821.69	126.51	0.101	450.12	2.67	0.257	-	-	-	4470.34	5.19	0.477	-	-	-	-	-	-	3042.61	10.84	0.972
26(S)	78675.53	128.04	0.092	-	-	-	43364.67	88.63	0.376	-	-	-	6988.33	11.34	0.532	3809.00	7.16	0.853	-	-	-
27(S)	27174.02	107.25	0.110	-	-	-	2695.50	10.53	0.320	37590.25	114.52	0.486	-	-	-	-	-	-	-	-	-

Table 2b. Analysis of esterase isozyme data obtained from the twenty seven sugar beet genotypes in anode side

Plant number	Band 1 (+)			Band 2 (+)			Band 3 (+)			Band 4 (+)			Band 5 (+)		
	Vol	Peak height	R.F	Vol	Peak height	R.F	Vol	Peak height	R.F	Vol	Peak height	R.F	Vol	Peak height	R.F
1(S)	44194.57	93.27	0.054	-	-	-	-	-	-	2342.67	4.17	0.791	-	-	-
2(MR)	72009.13	121.55	0.047	-	-	-	-	-	-	4086.82	6.26	0.783	-	-	-
3(S)	100606.45	131.72	0.078	5175.40	14.20	0.256	-	-	-	-	-	-	-	-	-
4(MR)	89984.51	110.14	0.062	1303.10	4.65	0.271	-	-	-	-	-	-	-	-	-
5(S)	96530.14	117.57	0.070	-	-	-	117041.50	132.52	0.519	2415.04	5.33	0.767	-	-	-
6(S)	94604.52	116.63	0.101	65018.48	84.27	0.341	7447.80	11.32	0.620	-	-	-	3759.15	3.96	0.876
7(MR)	101110.03	118.92	0.116	55468.20	81.82	0.372	8072.92	15.99	0.605	11533.43	16.94	0.760	6216.48	12.73	0.891
8(MR)	82010.44	124.60	0.116	139000.06	166.73	0.318	2344.49	6.02	0.605	11070.51	8.77	0.760	1098.16	4.40	0.984
9(S)	74892.00	127.71	0.152	56127.82	92.82	0.383	4890.00	10.24	0.688	7477.36	15.98	0.797	-	-	-
10(MR)	6063.00	7.58	0.188	-	-	-	69634.00	126.02	0.555	4639.00	7.29	0.797	-	-	-
11(MR)	47272.00	87.69	0.087	-	-	-	-	-	-	10868.00	8.06	0.764	-	-	-
12(S)	88571.50	124.14	0.118	-	-	-	7754.00	23.74	0.528	-	-	-	-	-	-
13(HS)	81506.33	121.28	0.134	15405.67	29.91	0.283	102716.00	147.94	0.480	-	-	-	-	-	-
14(MR)	77400.00	114.15	0.142	3142.75	6.07	0.346	56287.00	83.44	0.606	-	-	-	33508.03	66.85	0.953
15(MR)	-	-	-	-	-	-	32762.00	71.83	0.468	-	-	-	40585.88	79.69	0.894
16(R)	42398.65	83.92	0.096	-	-	-	50821.94	88.18	0.426	-	-	-	27692.19	64.12	0.883
17(R)	66054.95	126.57	0.074	-	-	-	44529.35	74.31	0.453	-	-	-	-	-	-
18(MR)	74927.28	123.71	0.095	-	-	-	77392.00	134.94	0.411	2875.50	7.99	0.642	6384.54	12.18	0.832
19(MR)	56877.00	108.69	0.116	-	-	-	2301.50	5.65	0.432	8771.48	17.82	0.663	61788.09	99.19	0.842
20(MR)	65819087	114.07	0.126	-	-	-	101003.77	144.35	0.421	3912.23	9.59	0.674	2389.40	6.69	0.863
21(MR)	41102.69	87.81	0.036	1690.00	8.10	0.274	6164.47	13.36	0.474	2703.47	7.17	0.726	2423.53	5.98	0.926
22(R)	39720.42	84.57	0.166	-	-	-	66693.33	122.88	0.442	3587.13	13.10	0.632	8211.44	16.26	0.842
23(R)	-	-	-	-	-	-	85917.00	132.07	0.442	-	-	-	54826.65	101.29	0.874
24(MR)	49385.81	88.14	0.074	-	-	-	101495.61	146.47	0.379	1201.39	5.61	0.621	81886.00	134.62	0.853
25(S)	68665.20	115.75	0.126	-	-	-	6343.02	13.60	0.495	-	-	-	-	-	-
26(S)	67915.15	101.43	0.126	-	-	-	69621.45	104.53	0.442	8371.55	12.02	0.726	-	-	-
27(S)	37874.91	119.68	0.112	-	-	-	638.00	4.42	0.429	2813.33	9.56	0.796	6684.19	16.13	0.796

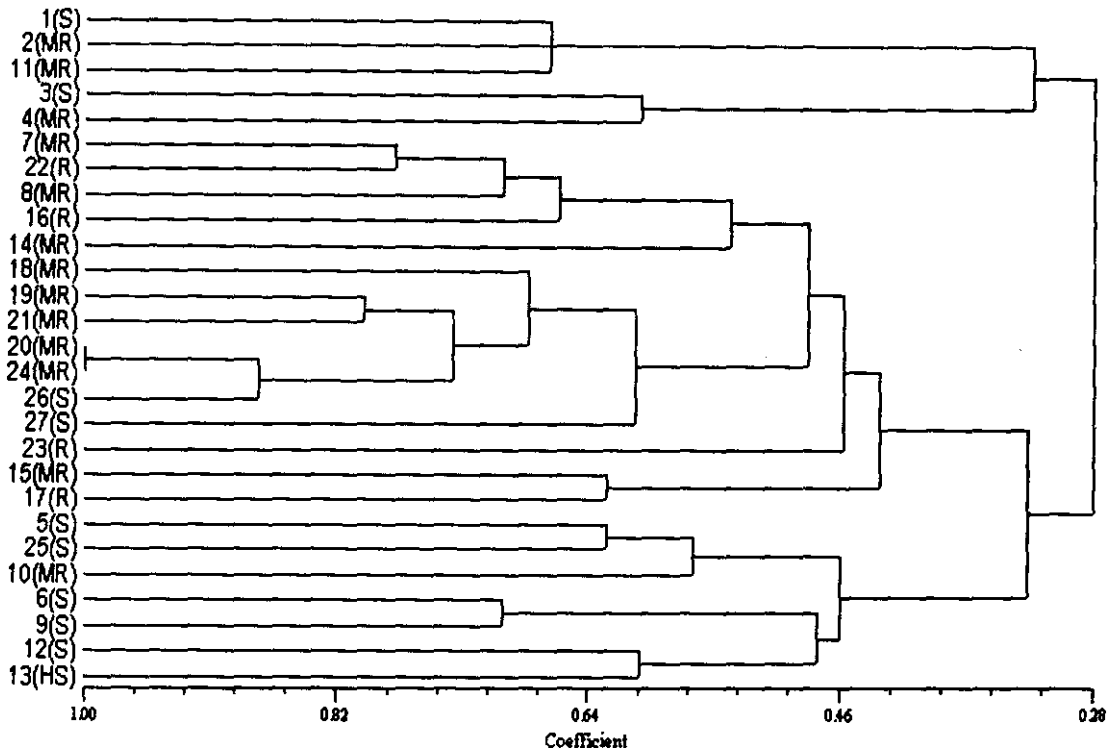


Figure 2. Dendrogram of cluster analysis for esterase of twenty seven sugar beet genotypes at control treatment

1.2. Amylase isozyme:

Figure (3 a&b) shows amylase pattern of twenty seven sugar beet genotypes of 90 days old plants. Cathode migration for amylase isozyme presents in (Table 3a). The data indicated that there were four bands in cathode migration. Table (3b) presents amylase anode migration. The data indicated that there were six bands in anode migration. Band parameters in amylase isozyme cannot differentiate between susceptible or resistant genotypes. Figure (4) shows dendrogram of cluster analysis for amylase isozyme. There were four big clusters in the dendrogram, cluster number one contain 18 genotypes in seven sub-clusters, cluster number two contain seven genotypes in two sub-clusters, while clusters number three and four contain one genotype in each. Clusters number one and two contain mix in genotype behavior (susceptible or resistant), in another word we can say cluster analysis cannot differentiate between the twenty seven sugar beet genotypes according to its resistant behavior.

1.3. Peroxidase isozyme:

Figures (5 a&b) shows isoperoxidase pattern for the twenty seven sugar beet genotypes at 90 days from planting. Table (4) presents the analysis data of

peroxides isozyme. The data indicated that there were six bands migrated towards the cathode; while there were two bands migrated towards the anode. Band existence, band volume, peak height and R.f. parameter were found to be different from susceptible or resistant plants as well as from one genotype to another. For example band No.1 and No.3 in the cathode side was found in ten different genotypes nine of them were susceptible and one of them was highly susceptible genotype, while, band No2 and No4 was found in resistant and moderate resistant genotypes only. In the anode side the two existed bands were found in all studied genotypes (monomorphic).

Figure (6) shows dendrogram of cluster analysis for peroxides of twenty seven sugar beet genotypes. The analysis differentiae the twenty seven sugar beet genotypes in two big clusters one of them contain ten genotypes (susceptible and highly susceptible), and the other contain seventeen genotypes (resistant and moderate resistant) genotypes. The results are in agreement with that described by (El-Kholi *et al.* 2005) they found that the enzymatic activity of Chitinase β -1,3 glucanase, poly-phenol oxidase, peroxidase and invertase were significantly increased in infected sugar beet roots with *Rhizoctonia solani* than in healthy roots.

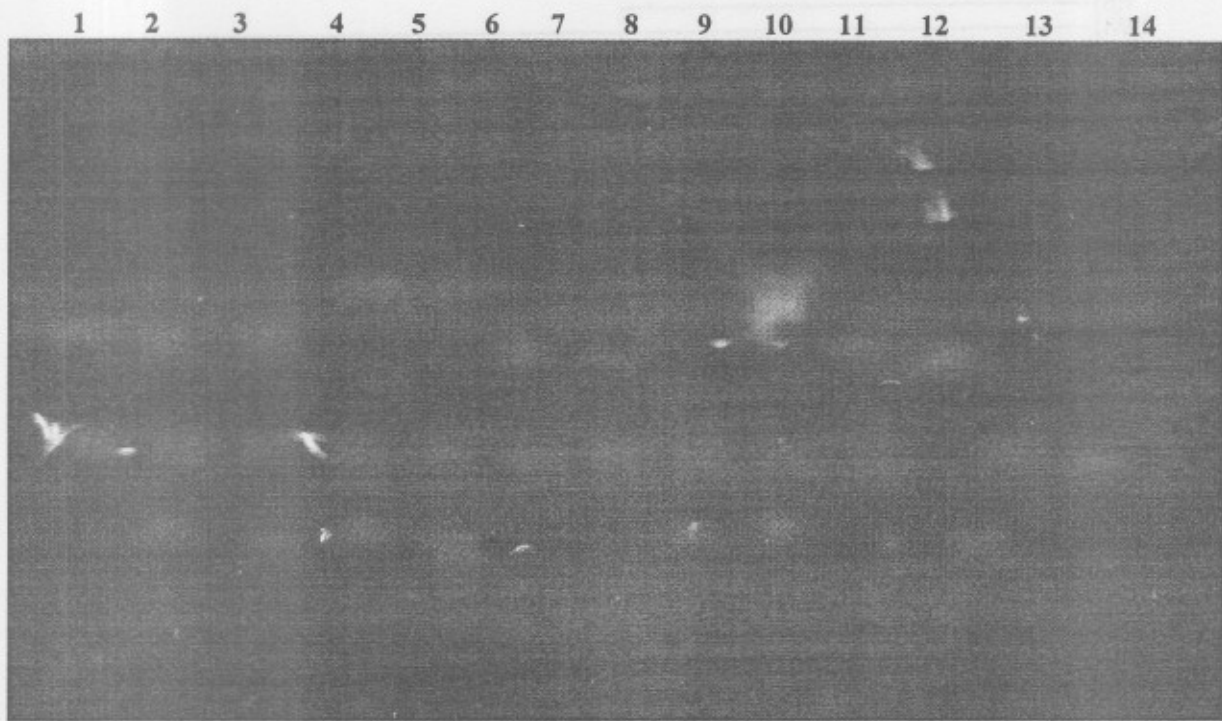


Figure 3 a. Amylase pattern for control sugar beet genotypes from (1 to 14) after 90 days from planting

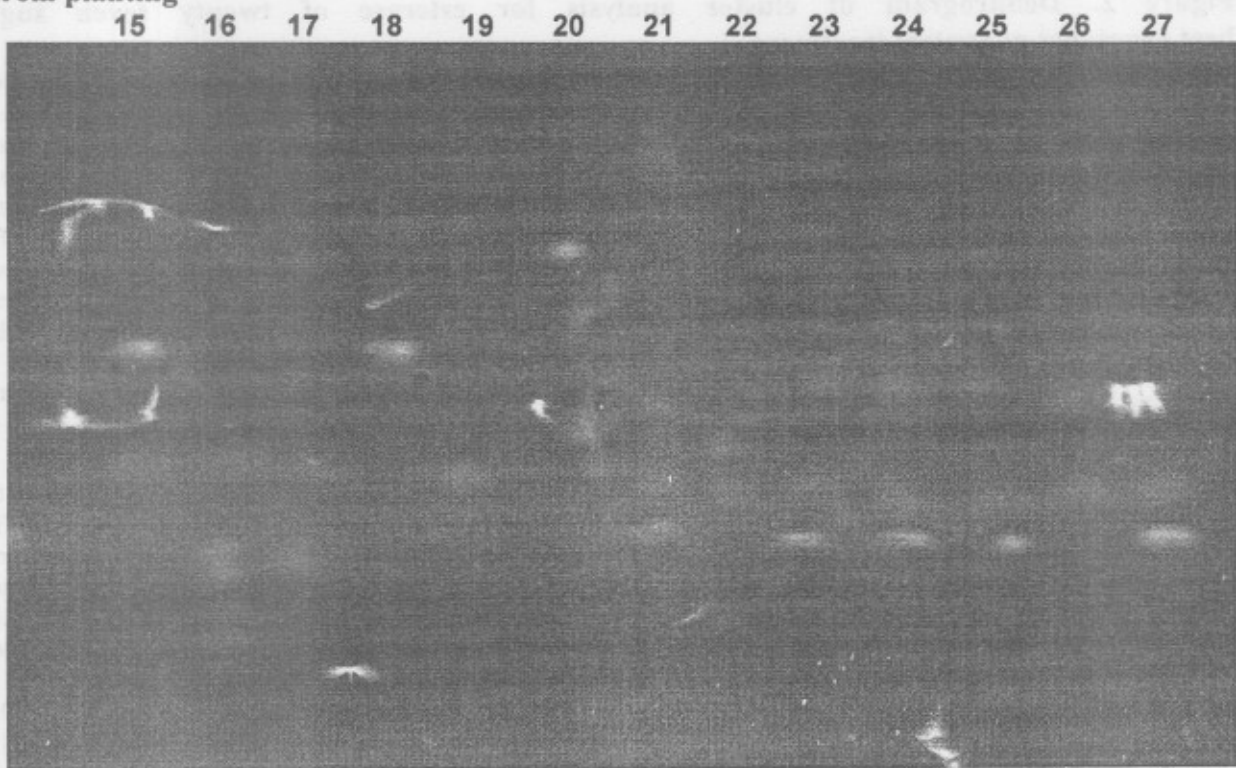


Figure 3 b. Amylase pattern for control sugar beet genotypes from (15 to 27) after 90 days from planting

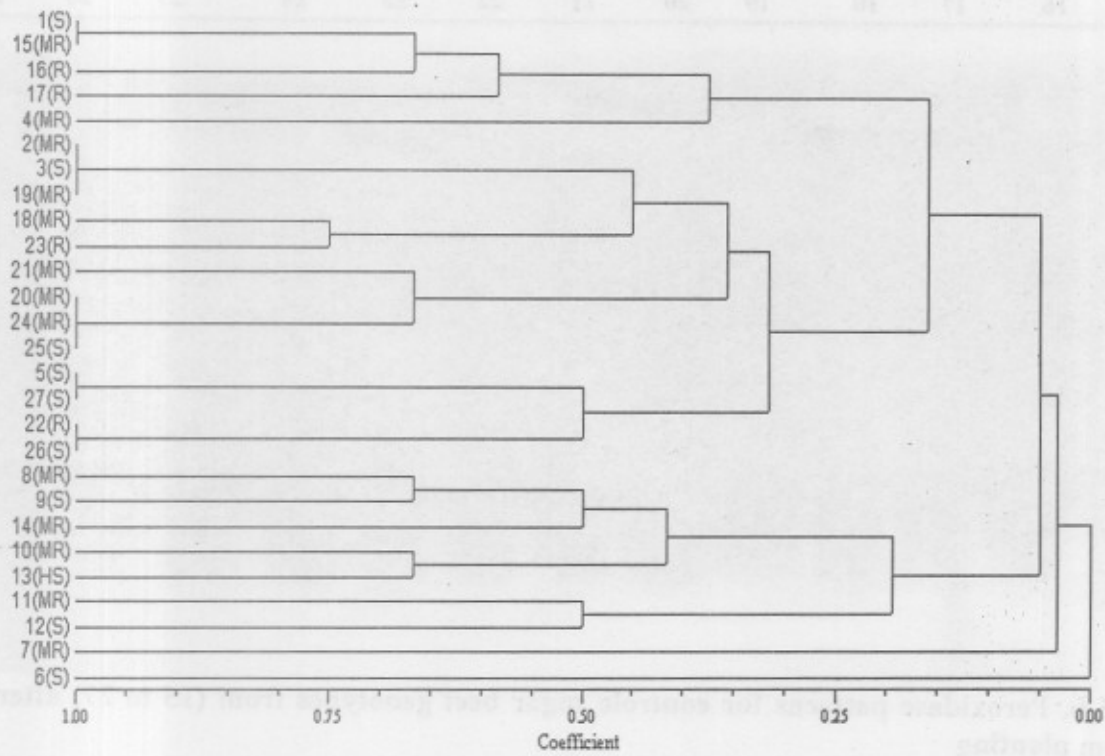


Figure 4. Dendrogram of cluster analysis for amylase of twenty seven sugar beet genotypes

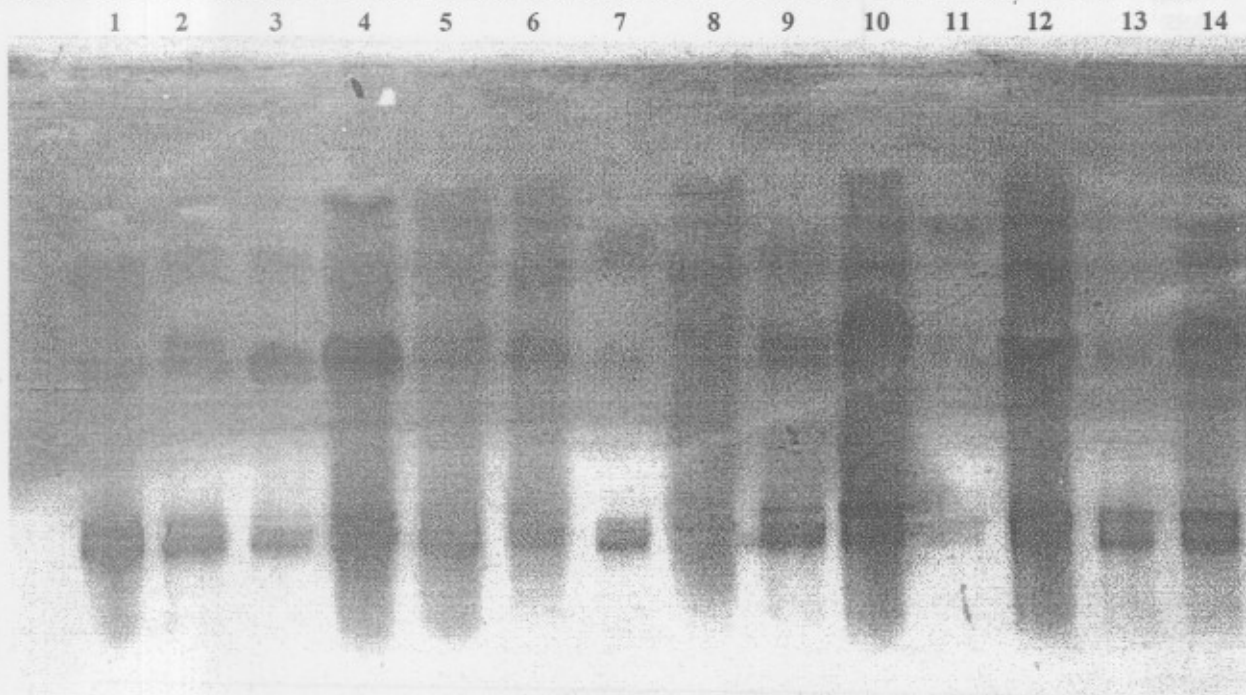


Figure 5 a. Isoperoxidase patterns for control sugar beet genotypes from (1 to 14) after 90 days from planting

15 16 17 18 19 20 21 22 23 24 25 26 27

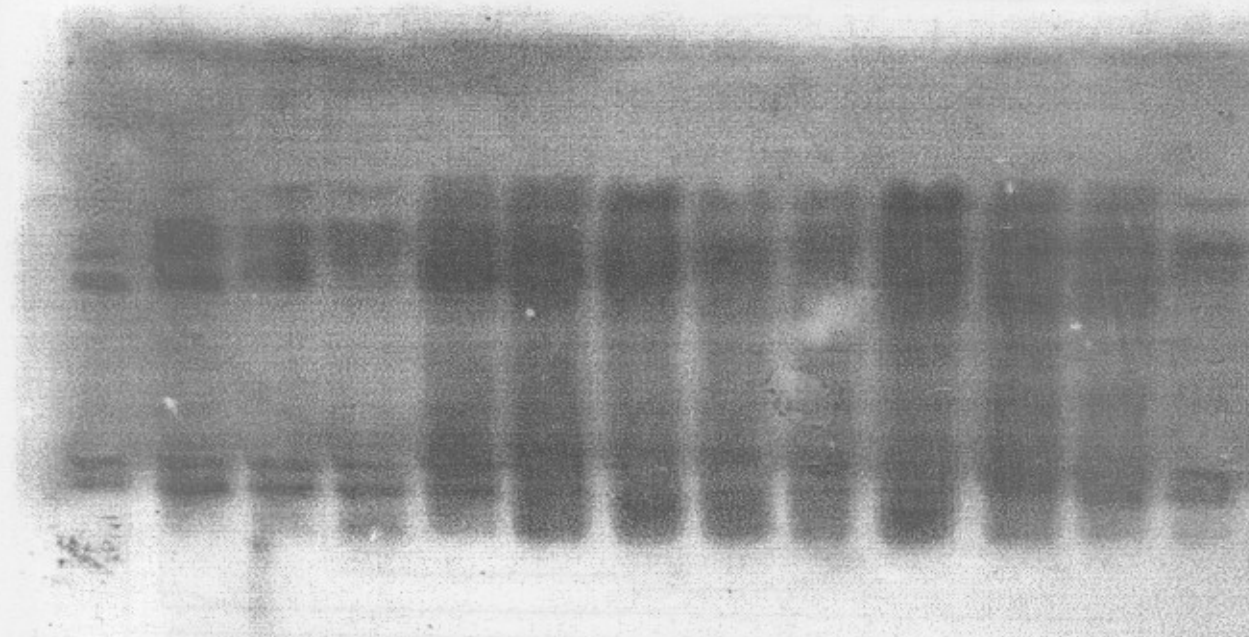


Figure 5 b. Peroxidase patterns for control sugar beet genotypes from (15 to 27) after 90 days from planting

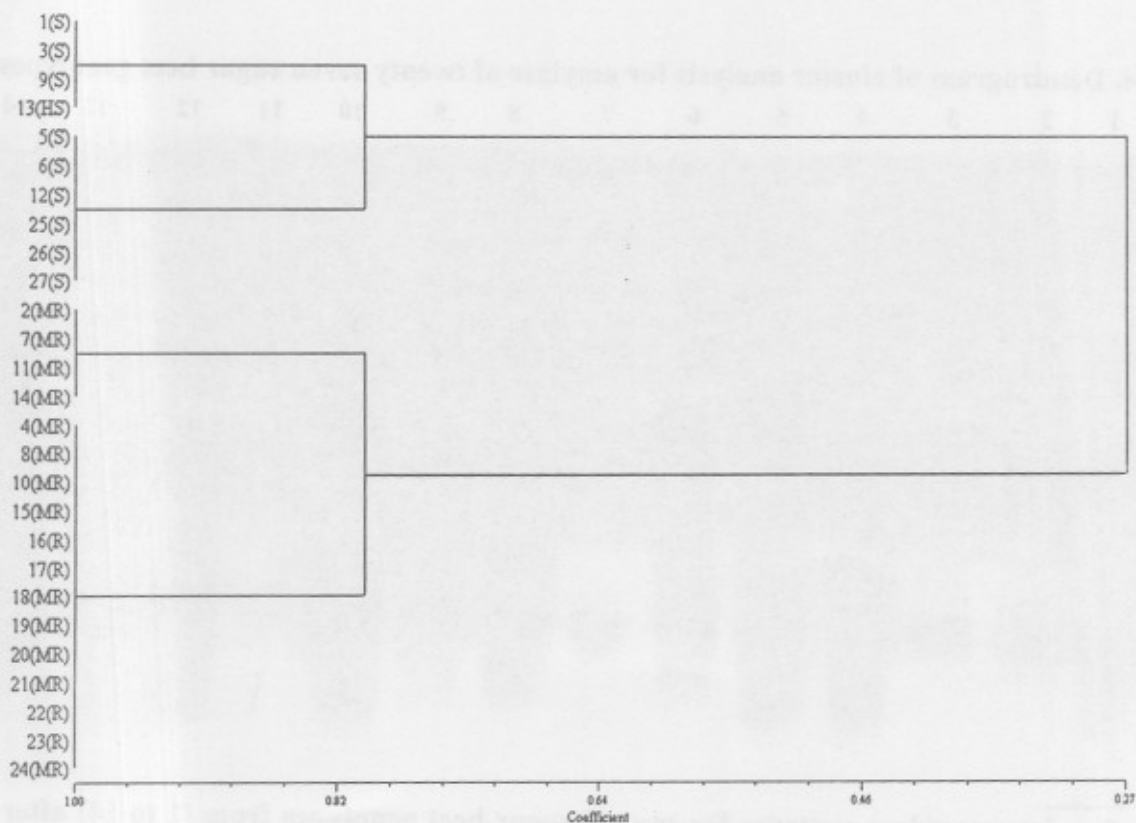


Figure 6. Dendrogram of cluster analysis for peroxidases of twenty seven sugar beet genotypes

Table 3a. Analysis of amylase isozyme data obtained from the twenty seven sugar beet genotypes in cathode side

Plant Number	Band 1 (-)			Band 2 (-)			Band 3 (-)			Band 4 (-)		
	Vol.	Peak height	R.F	Vol	Peak height	R.F	Vol	Peak height	R.F	Vol	Peak height	R.F
1(S)	-	-	-	-	-	-	7222.48	53.07	0.474	-	-	-
2(MR)	-	-	-	-	-	-	-	-	-	2418.32	16.28	0.923
3(S)	-	-	-	-	-	-	-	-	-	2988.74	20.92	0.872
4(MR)	-	-	-	-	-	-	7404.36	59.99	0.462	-	-	-
5(S)	-	-	-	-	-	-	-	-	-	-	-	-
6(S)	-	-	-	-	-	-	-	-	-	-	-	-
7(MR)	3873.44	27.46	0.115	-	-	-	-	-	-	5597.14	50.81	0.910
8(MR)	1299.73	12.31	0.051	-	-	-	-	-	-	-	-	-
9(S)	2420.53	22.14	0.051	-	-	-	-	-	-	-	-	-
10(MR)	-	-	-	3091.00	20.31	0.321	-	-	-	-	-	-
11(MR)	-	-	-	3948.52	22.51	0.295	-	-	-	-	-	-
12(S)	-	-	-	3983.50	21.79	0.295	-	-	-	-	-	-
13(HS)	-	-	-	4165.74	25.84	0.256	-	-	-	-	-	-
14(MR)	-	-	-	-	-	-	-	-	-	-	-	-
15(MR)	-	-	-	-	-	-	7274.42	28.26	0.585	-	-	-
16(R)	-	-	-	-	-	-	7096.57	29.89	0.538	-	-	-
17(R)	-	-	-	-	-	-	7900.30	27.53	0.554	-	-	-
18(MR)	-	-	-	1190.20	5.41	0.004	-	-	-	6462.26	28.20	0.877
19(MR)	-	-	-	-	-	-	-	-	-	2788.54	16.68	0.877
20(MR)	-	-	-	6342.39	30.14	0.477	-	-	-	-	-	-
21(MR)	-	-	-	4125.62	17.78	0.431	-	-	-	-	-	-
22(R)	-	-	-	-	-	-	-	-	-	-	-	-
23(R)	-	-	-	-	-	-	-	-	-	2774.06	20.33	0.923
24(MR)	-	-	-	7065.65	33.56	0.492	-	-	-	-	-	-
25(S)	-	-	-	10721.58	46.64	0.431	-	-	-	-	-	-
26(S)	-	-	-	-	-	-	-	-	-	-	-	-
27(S)	-	-	-	-	-	-	-	-	-	-	-	-

Table 4. Analysis of peroxides data obtained from the twenty seven sugar beet genotypes at 90 days from plant age

Plant number	Band 1 (-)			Band 2 (-)			Band 3 (-)			Band 4 (-)			Band 5 (-)			Band 6 (-)			Band 1 (+)			Band 2 (+)		
	Vol.	Peak height	R.F	Vol	Peak height	R.F	Vol	Peak height	R.F	Vol	Peak height	R.F	Vol	Peak height	R.F	Vol	Peak height	R.F	Vol	Peak height	R.F	Vol	Peak height	R.F
1(S)	6400.42	0.23	0.113	-	-	-	4943.14	1.49	0.541	-	-	-	-	-	-	-	-	-	6832.86	1.89	0.508	9742.47	0.10	0.608
2(MR)	-	-	-	4268.76	1.44	0.188	-	-	-	8030.32	4.20	0.602	-	-	-	-	-	-	4859.92	20.76	0.500	2516.47	0.01	0.575
3(S)	9344.89	11.13	0.120	-	-	-	12727.27	10.36	0.556	-	-	-	-	-	-	-	-	-	9909.59	23.80	0.496	3075.77	7.17	0.588
4(MR)	-	-	-	8852.35	0.01	0.195	-	-	-	22868.41	25.81	0.602	-	-	-	17801.29	15.43	0.827	10344.01	3.56	0.462	22242.92	12.39	0.580
5(S)	7611.22	10.16	0.143	-	-	-	3771.47	0.87	0.564	-	-	-	7116.02	4.21	0.797	-	-	-	12883.83	13.21	0.479	2743.67	0.13	0.580
6(S)	5842.88	10.35	0.150	-	-	-	2750.33	2.11	0.571	-	-	-	6583.78	8.99	0.805	-	-	-	5034.79	3.59	0.437	1202.25	1.97	0.563
7(MR)	-	-	-	9464.43	11.88	0.188	-	-	-	6065.61	0.01	0.624	-	-	-	-	-	-	11080.40	28.69	0.475	4443.40	0.01	0.568
8(MR)	-	-	-	7032.36	9.47	0.188	-	-	-	5927.66	5.97	0.624	-	-	-	5616.55	0.24	0.880	8287.82	5.22	0.466	5949.49	8.75	0.568
9(S)	7327.37	7.25	0.158	-	-	-	10352.22	10.24	0.564	-	-	-	-	-	-	-	-	-	2599.25	10.80	0.432	1940.70	0.45	0.551
10(MR)	-	-	-	10033.88	7.90	0.211	-	-	-	26645.81	32.60	0.632	-	-	-	27806.23	34.06	0.910	12252.72	12.23	0.483	28176.42	15.02	0.559
11(MR)	-	-	-	4821.41	2.81	0.211	-	-	-	7199.04	7.46	0.647	-	-	-	-	-	-	2350.98	7.53	0.471	2825.48	6.40	0.571
12(S)	7955.24	5.89	0.165	-	-	-	18783.13	17.96	0.602	-	-	-	18410.78	22.59	0.827	-	-	-	14269.07	0.17	0.471	11127.38	20.10	0.588
13(HS)	3210.48	3.17	0.165	-	-	-	6183.85	5.91	0.602	-	-	-	-	-	-	-	-	-	2405.78	1.51	0.462	3498.34	1.96	0.588
14(MR)	-	-	-	14694.80	2.96	0.226	-	-	-	41481.10	27.68	0.677	-	-	-	-	-	-	15209.07	1.19	0.471	6249.08	13.54	0.588
15(MR)	-	-	-	3665.30	0.01	0.238	-	-	-	13733.28	29.44	0.583	-	-	-	12662.37	33.56	0.833	1843.48	0.85	0.569	6248.36	23.47	0.688
16(R)	-	-	-	3955.61	0.29	0.250	-	-	-	5375.97	6.20	0.595	-	-	-	22981.60	44.94	0.845	1363.75	0.01	0.569	2868.16	2.71	0.706
17(R)	-	-	-	3853.88	6.63	0.250	-	-	-	7420.41	7.39	0.571	-	-	-	22088.67	31.42	0.821	2704.82	1.50	0.587	3181.55	15.45	0.725
18(MR)	-	-	-	9353.08	24.40	0.262	-	-	-	3789.03	4.52	0.583	-	-	-	22155.10	33.06	0.821	1600.21	0.01	0.587	3076.93	2.35	0.716
19(MR)	-	-	-	8449.03	7.93	0.214	-	-	-	12152.34	21.83	0.607	-	-	-	26737.46	50.09	0.833	3931.11	2.33	0.596	6016.82	25.71	0.743
20(MR)	-	-	-	4069.81	4.69	0.181	-	-	-	12223.80	20.41	0.614	-	-	-	15132.28	33.82	0.843	4037.26	7.37	0.624	23043.13	20.38	0.743
21(MR)	-	-	-	5238.26	6.76	0.181	-	-	-	7610.57	14.36	0.614	-	-	-	7321.77	16.63	0.843	5755.91	11.67	0.624	15671.42	0.85	0.752
22(R)	-	-	-	4703.66	7.27	0.193	-	-	-	6605.06	12.78	0.602	-	-	-	15280.00	34.43	0.843	6766.10	11.06	0.624	14341.00	9.39	0.743
23(R)	-	-	-	2276.30	3.44	0.193	-	-	-	4235.12	6.95	0.602	-	-	-	1169.09	28.13	0.843	11898.37	11.22	0.624	9940.41	30.06	0.743
24(MR)	-	-	-	5351.60	2.82	0.193	-	-	-	4677.05	9.13	0.614	-	-	-	10099.65	13.21	0.831	4594.15	5.24	0.633	18438.53	12.52	0.743
25(S)	3808.95	0.69	0.133	-	-	-	8675.33	9.54	0.530	-	-	-	22998.31	30.91	0.795	-	-	-	6835.08	3.00	0.624	43748.82	30.47	0.725
26(S)	3195.90	4.52	0.133	-	-	-	7944.31	8.55	0.530	-	-	-	16514.63	38.60	0.795	-	-	-	5441.43	0.01	0.615	11206.10	14.35	0.725
27(S)	10693.78	21.48	0.133	-	-	-	5029.04	8.74	0.542	-	-	-	15796.48	36.49	0.795	-	-	-	3195.75	3.86	0.596	9360.11	15.93	0.743

2. Cluster analysis based on the three studied isozymes:

Figure (7) illustrates dendrogram of cluster analysis for the three studied isozymes (Esterase, amylase and peroxidase) in plant age of 90 days. There were two big clusters, cluster number one contain the ten susceptible genotypes (nine susceptible and one highly susceptible) in four sub-clusters. Cluster number two contain the resistant and moderate resistant genotypes in four sub-clusters, sub-clusters one and two contain seven

moderate resistant genotypes. Sub-cluster number three contains five moderate resistant and one resistant genotypes, while, sub-cluster four contain three resistant and one moderate resistant genotypes. Peroxidase isozymes were proven to play the major role in differentiates between susceptible or resistant plants. These results are in agreement with that reported by (Yu *et al.*, 2001) they established isozyme marker for sugar beet plants resistance to root-knot nematode.

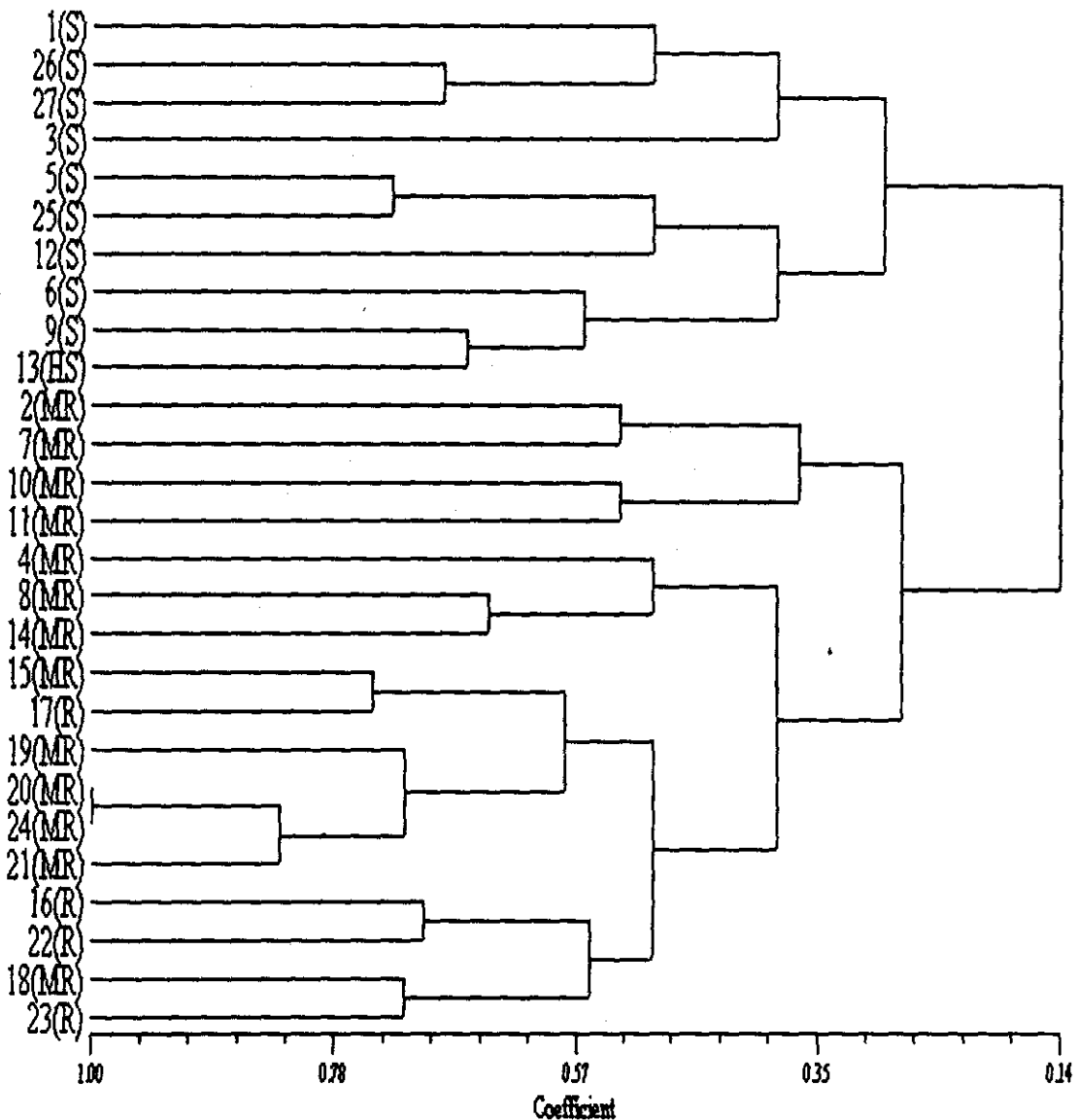


Figure 7. Dendrogram of cluster analysis for (Esterase, amylase and peroxidase) isozymes of twenty seven sugar beet genotypes

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الملخص العربي

الإختلافات في المشاهات الإنزيمية لتراكيب وراثية من بنجر السكر مقاومة لنيما تودا تعقد الجذور

مجدى سعد صالح، أحمد السيد محمد خالد، عماد عبد المنعم غنيمه، نانسي عبد السلام أبوعلو

تم أخذ عينات ورقية سواء من النباتات تحت الدراسة عند ٩٠ يوم من عمر النبات لدراسة المشاهات الإنزيمية لثلاث أنزيمات هي (الاستيريز والأميليز والبيروكسيديز) وذلك بهدف الحصول على واسمات كيمو حيوية يمكن إستخدامها في المستقبل لأمكانية التنبؤ بالأصناف المقاومة أو الحساسة لنيما تودا تعقد الجذور دون الحاجة إلى إجراء العدوى الصناعية سواء في برامج التربية أو عند اختيار الأصناف للزراعة في المناطق الموبوءة بالنيما تودا في المستقبل.

أظهرت نتائج دراسة المشاهات الإنزيمية لثلاث أنزيمات تحت الدراسة (الاستيريز والأميليز والبيروكسيديز) وجود أختلافات ما بين الأصناف في عدد الحزم سواء في الجانب السالب أو الجانب الموجب من الهجرة الكهربية كذلك كان هناك أختلاف بين الأصناف في مواصفات الحزم المنفصلة في كل أنزيم من الأنزيمات الثلاث تحت الدراسة.

وعند عمل تحليل الشجرة للنتائج المتحصل عليها من دراسة المشاهات الإنزيمية الثلاث أنزيمات تحت الدراسة لكل أنزيم على حدى وجد أن أنزيم البيروكسيديز هو الوحيد الذى أستطاع التفرقة ما بين الطرز الوراثية الحساسة للإصابة والأخرى المقاومة حيث أستطاع هذا الأنزيم أن يضع كل مجموعة من المجموع السابق ذكرها في مجموعة منفصلة عن الأخرى الذى عجز عنه الأنزيمان الأخران تحت الدراسة حيث كانت المجموع التي أشار إليها تحليل الشجرة بجميع مختلطة من كل الطرز الوراثية سواء حساسة أو مقاومة.

وعند إستخدام نتائج تحليل الثلاث أنزيمات معا في تحليل الشجرة تم الحصول على نتيجة مثالية من حيث تفريق الطرز الوراثية سواء الحساسة أو المقاومة في جميع مختلفة مما يعطى أنطباع أن أنزيم البيروكسيديز هو الذى له الدور الأكبر في هذه النتيجة.

أجرى هذا البحث في محطة بحوث الصباحية بالإسكندرية حيث تم زراعة ٢٧ طراز وراثي من بنجر السكر معروفة درجة مقاومتهم لنيما تودا تعقد الجذور حيث تم تقسيم السبعة وعشرون طراز وراثي تحت الدراسة إلى ٤ مجاميع من حيث المقاومة إلى:

١- المجموعة الأولى (شديدة الحساسية)

وقد أحتوت هذه المجموعة الأولى على صنف واحد تجارى هو الصنف عديد الأجنة (FD - 9902).

٢- المجموعة الثانية (الحساسة)

وقد أحتوت هذه المجموعة على تسعة أصناف أربعة أصناف منهم وحيدة الأجنة هي كما يلي (ريست وأرمور وروزانا و 02-99) كما أحتوت هذه المجموعة على أربعة أصناف أيضا عديدى الأجنة هم (تيب وتورو وجولوريا و DS- 9004) بالإضافة إلى مادة واحدة من مواد التربية هي (Eg.6).

٣- المجموعة الثالثة (متوسط المقاومة)

وأحتوت هذه المجموعة على النصيب الأكبر من السبع وعشرون طرازا وراثيا حيث أحتوت على ثلاثة عشر طرازا وراثيا خمسة منهم أصناف وحيدة الأجنة هم (فرنسيسكا و 05-99 و 01-99 و LP-10 و LP-13) وأربع أصناف عديدة الأجنة هم (هلويس وبركة وأتوس بولى وديسيريز) بالإضافة إلى أربعة من مواد التربية هم (C.39 و SP-270 و Eg.26 و Eg.2701).

٤- المجموعة الرابعة (المقاومة)

أحتوت هذه المجموعة على أربعة طرز وراثية أثنان منهم عبارة عن أصناف وحيدة الأجنة هما (أميل ومونت بيانكو) وصنف عديد الأجنة هو (سلطان) ومادة واحدة من مواد التربية هي (Eg.27).