ALEXANDRIA

SCIENCE EXCHANGE JOURNAL

VOL. 30 APRIL- JUNE 2009

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

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## 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 phyilogenetic 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 ? -1,3 glucanase, poly-phenol oxodase, peroxidase and invertase in sugar beet roots infected by *Rhizoctonia solani*. They found that isozymes activity was significantly increased in infected sugar beet roots than

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Received April 1, 2009, Accepted April 21, 2009

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 ? -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 |
| | DS 9004 | Commercial var, | Poly | A3 |
| | Rosanna | Commercial var, | Mono | A5 |
| S | 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 |
| | LP-10 | Commercial var, | Mono | A7 |
| | LP-13 | Commercial var, | Mono | A8 |
| MR | 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 |
| | Sultan | Commercial var, | Poly | A16 |
| R | Amile | Commercial var, | Mono | A17 |
| | Eg.27 | Breeding material | Poly | A22 |

| | | | | |
|---|---|---|---|---|
| | Monte Bianco | Commercial var, | Mono | A23 |

**2. METHODS:**

Agar- Starch- Polyvinyl pyrolidine (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.I- 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 pyrolidine (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 pyrolidine (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 rainsed 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).

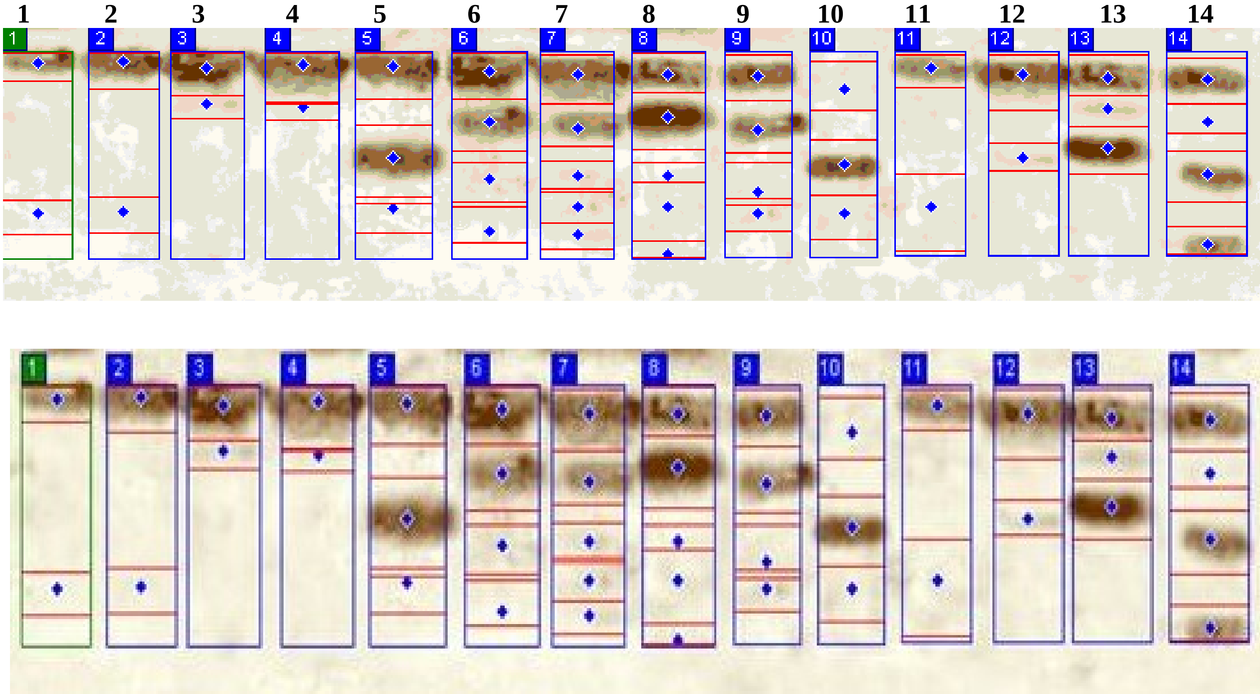


**Figure 1 a. Esterase pattern for control sugar beet genotypes from (1 to14) after 90 days from planting**

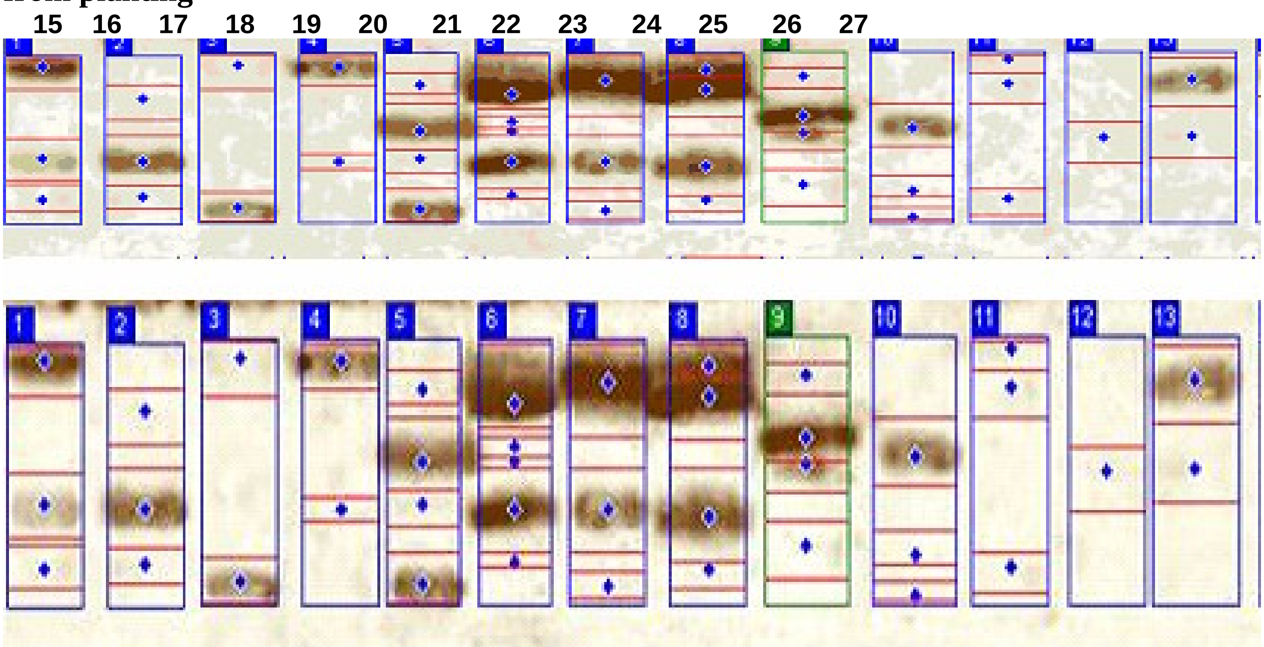


**Figure 1 b. Esterase pattern for control sugar beet genotypes from (1 5 to 27) after 90 days from planting**

**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 |

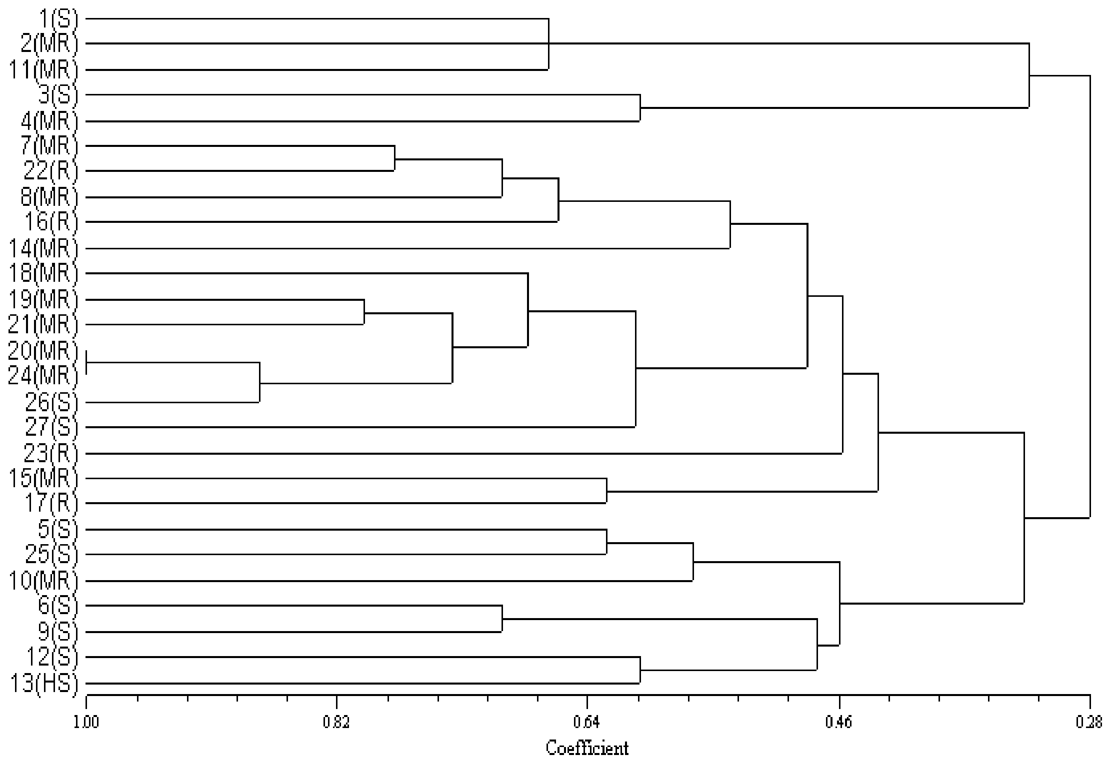


**Figure 2. Dendrogram of cluster analysis for esterase of twenty seven sugar beet genotypes at control treatment**

**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 ? -1,3 glucanase, poly-phenol oxodase, peroxidase and invertase were significantly increased in infected sugar beet roots with *Rhizoctonia solani* than in healthy roots.

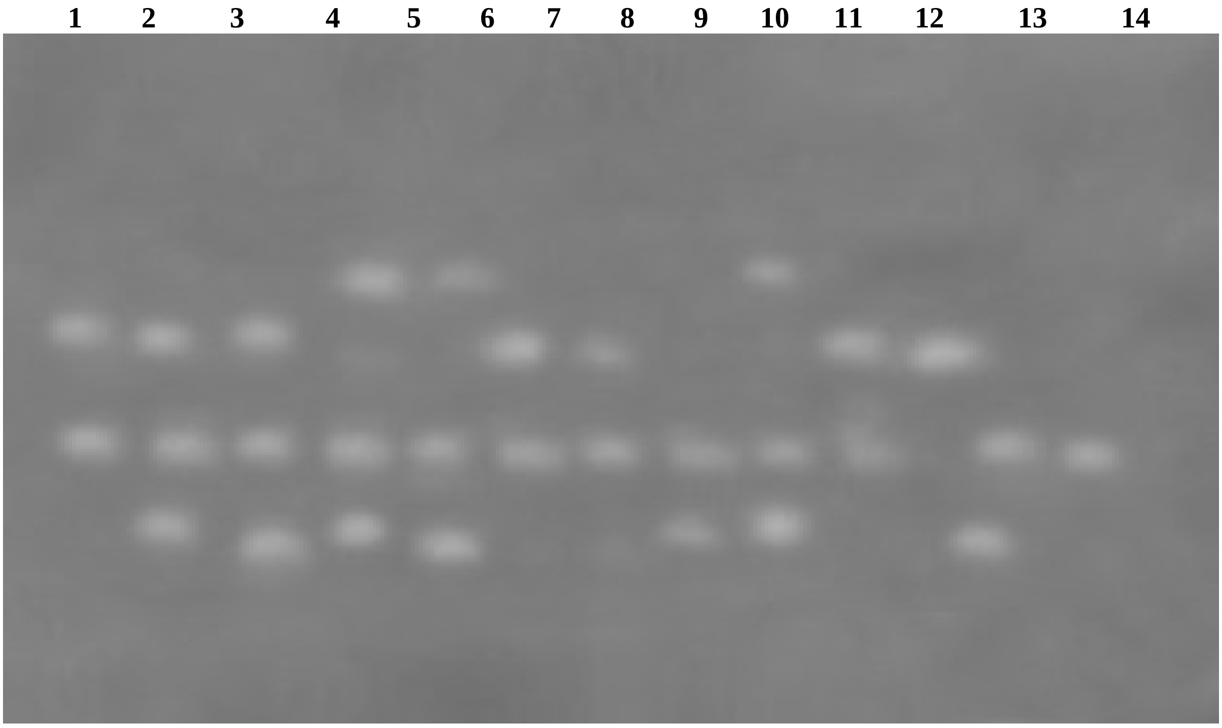


**Figure 3 a. Amylase pattern for control sugar beet genotypes from (1 to 14) after 90 days from planting**

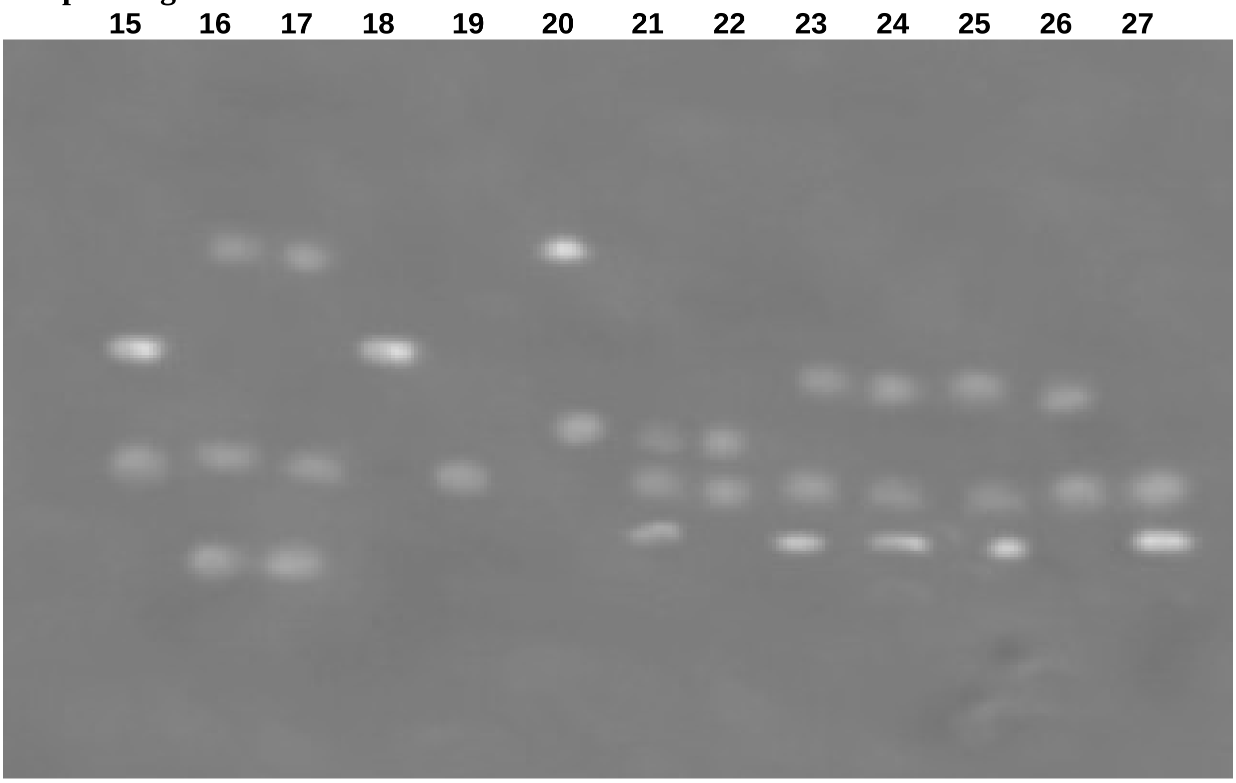


**Figure 3 b. Amylase pattern for control sugar beet genotypes from (15 to 27) after 90 days from planting**

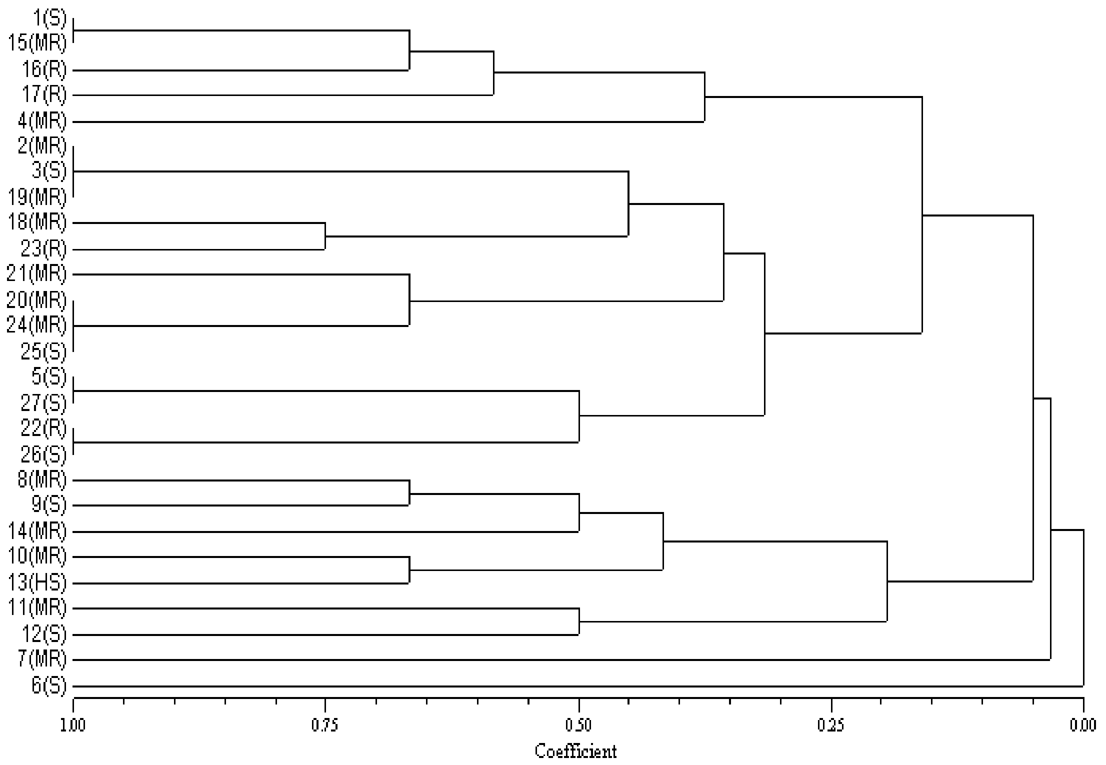


**Figure 4. Dendrogram of cluster analysis for amylase of twenty seven sugar beet genotypes**

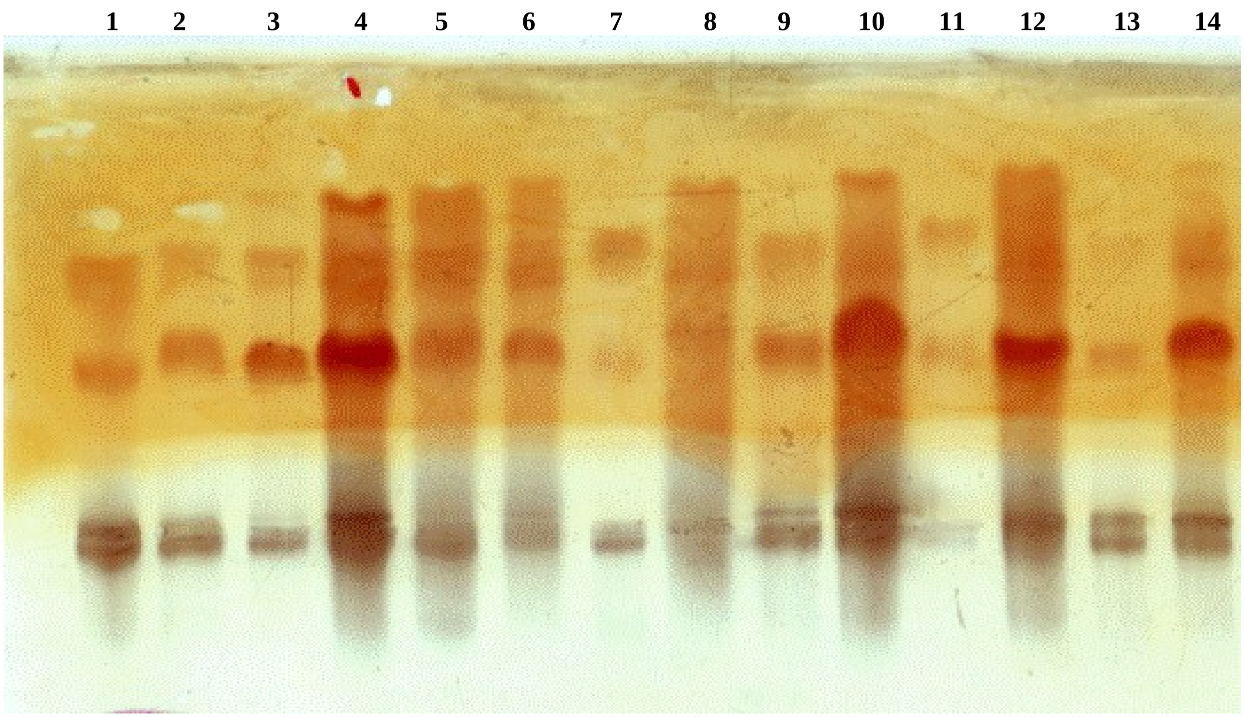


**Figure 5 a. Isoperoxidase patterns for control sugar beet genotypes from (1 to 14) after 90 days from planting**

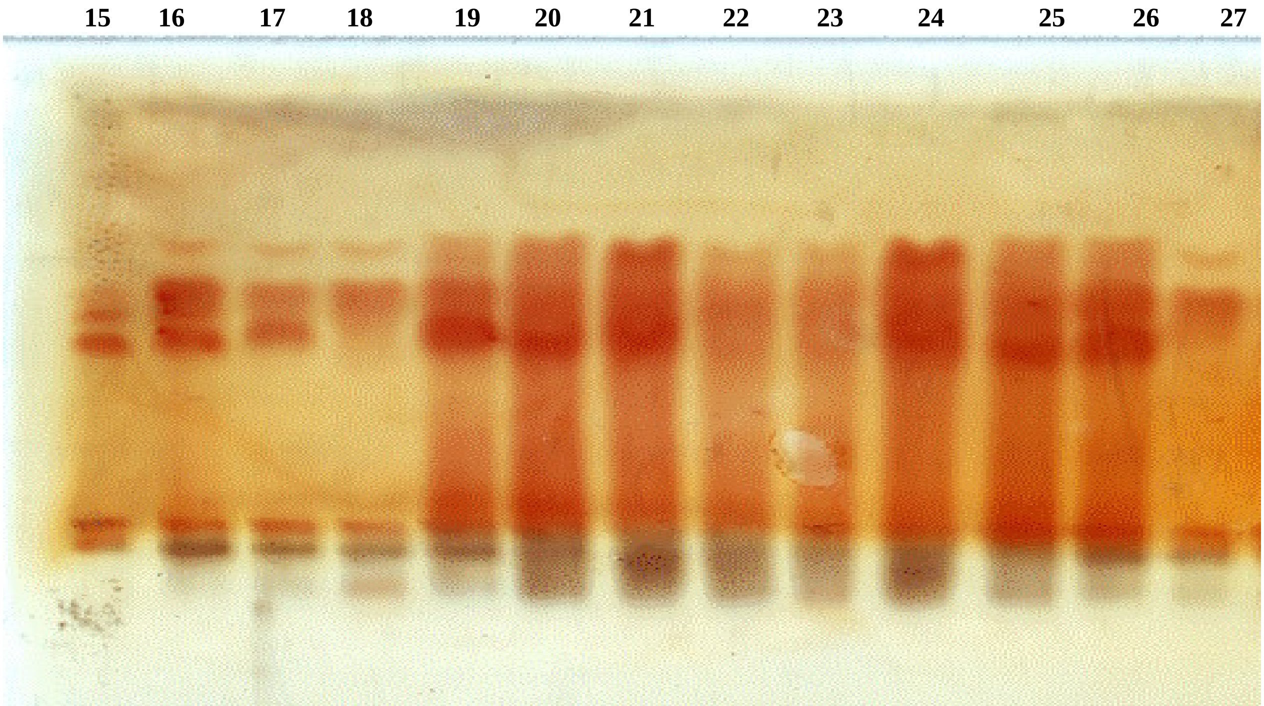


**Figure 5 b. Peroxidase patterns for controle sugar beet genotypes from (15 to 27) after 90 days from planting**

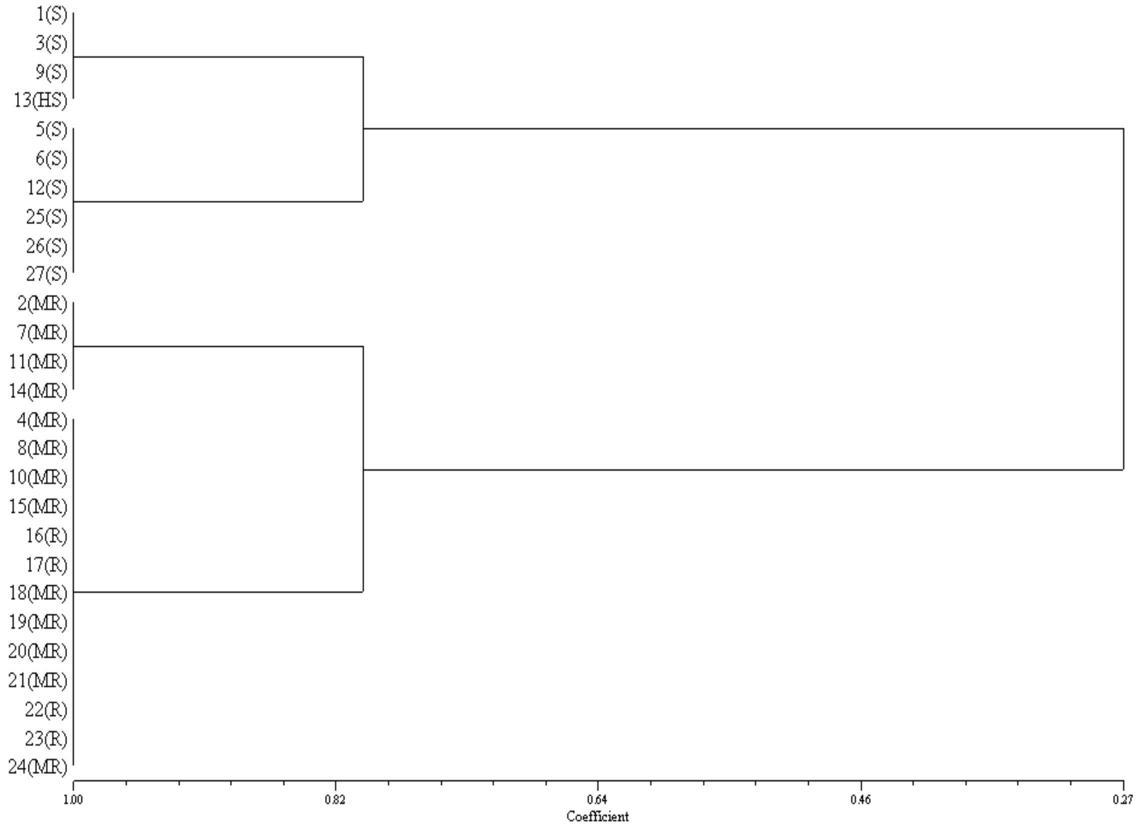


**Figure 6. Dendrogram of cluster analysis for peroxides of twenty seven sugar beet genotypes**

**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 3b. Analysis of amylase 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 (+) | | | Band 6 (+) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 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) | 3492.80 | 24.87 | 0.087 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2(MR) | 2317.24 | 21.20 | 0.087 | - | - | - | - | - | - | - | - | - | - | - | - | 3759.97 | 25.85 | 0.804 |
| 3(S) | 1828.52 | 16.28 | 0.111 | - | - | - | - | - | - | - | - | - | - | - | - | 3720.16 | 27.12 | 0.867 |
| 4(MR) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5(S) | 3478.47 | 30.03 | 0.178 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6(S) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 7(MR) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 8(MR) | - | - | - | 2407.00 | 18.79 | 0.244 | - | - | - | 2771.00 | 31.84 | 0.622 | - | - | - | - | - | - |
| 9(S) | - | - | - | 3429.13 | 22.52 | 0.295 | - | - | - | - | - | - | - | - | - | - | - | - |
| 10(MR) | - | - | - | 3514.00 | 22.04 | 0.250 | - | - | - | 3777.79 | 42.34 | 0.682 | - | - | - | - | - | - |
| 11(MR) | - | - | - | - | - | - | 2893.33 | 16.53 | 0.341 | 4416.67 | 42.60 | 0.682 | - | - | - | - | - | - |
| 12(S) | - | - | - | - | - | -- | 2999.64 | 16.65 | 0.341 | - | - | - | 4390.23 | 36.08 | 0.750 | - | - | - |
| 13(HS) | - | - | - | 4840.63 | 27.59 | 0.273 | - | - | - | - | - | - | - | - | - | - | - | - |
| 14(MR) | - | - | - | 5595.42 | 32.16 | 0.273 | - | - | - | 7288.37 | 71.52 | 0.682 | - | - | - | - | - | - |
| 15(MR) | 5330.36 | 29.62 | 0.088 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 16(R) | 5614.80 | 28.25 | 0.123 | - | - | - | - | - | - | 5961.29 | 24.76 | 0.682 | - | - | - | - | - | - |
| 17(R) | 5105.92 | 26.96 | 0.123 | - | - | - | - | - | - | - | - | - | - | - | - | 7051.55 | 29.45 | 0.807 |
| 18(MR) | 5798.54 | 27.22 | 0.140 | - | - | - | - | - | - | 5831.29 | 28.67 | 0.702 | - | - | - | - | - | - |
| 19(MR) | 6276.02 | 25.26 | 0.140 | - | - | - | - | - | - | - | - | - | - | - | - | 6566.54 | 31.16 | 0.825 |
| 20(MR) | 6393.28 | 27.77 | 0.175 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 21(MR) | 5232.06 | 28.07 | 0.158 | - | - | - | - | - | - | - | - | - | - | - | - | 1149.37 | 6.04 | 0.877 |
| 22(R) | 4470.21 | 23.18 | 0.175 | - | - | - | - | - | - | - | - | - | 3573.14 | 20.02 | 0.737 | - | - | - |
| 23(R) | 4017.16 | 23.07 | 0.175 | - | - | - | - | - | - | 7048.19 | 33.24 | 0.667 | - | - | - | - | - | - |
| 24(MR) | 4054.65 | 19.12 | 0.158 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 25(S) | 1134.98 | 6.25 | 0.211 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 26(S) | 4402.39 | 28.29 | 0.121 | - | - | - | - | - | - | - | - | - | 3038.00 | 18.71 | 0.776 | - | - | - |
| 27(S) | 5395.44 | 28.22 | 0.190 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

**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 | - | - | - | - | - | - | - | - | - | 6852.86 | 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 | 0.5.68 |
| 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 | 11891.37 | 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.

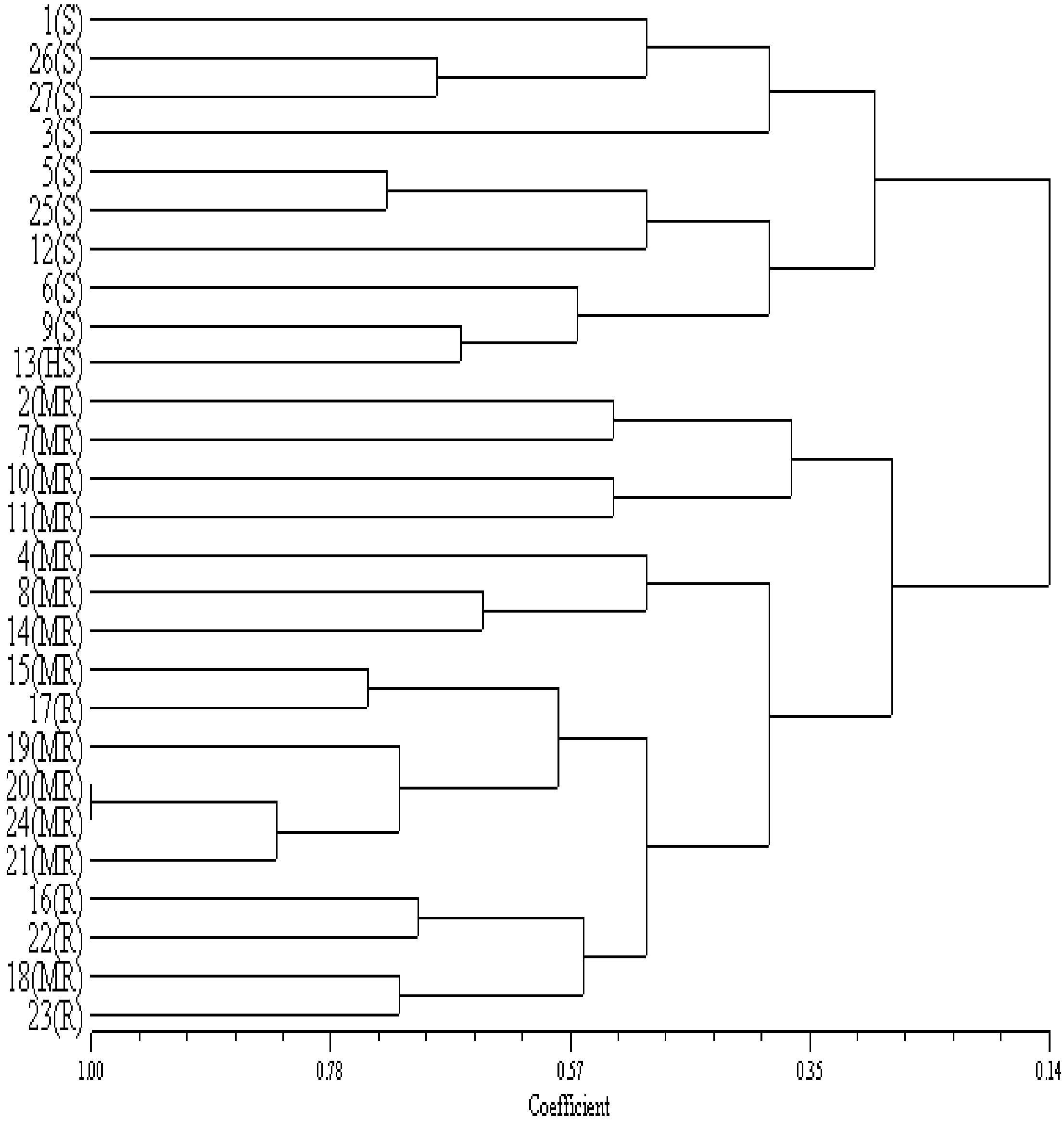


**Figure 7. Dendrogram of cluster analysis for (Esterase, amylase and peroxidase) isozymes of twenty seven sugar beet genotypes**

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## الملخص العربي

# الإختلافات فى المشابهات الإنزيمية لتراكيب وراثية من بنجر السكر مقاومة لنيماتودا تعقد الجذور

مجدى سعد صالح، أحمد السيد محمد خالد، محمد عبد المنعم غنيمة، نانسى عبد السلام أبوعلو

لنيماتودا تعقد الجذور حيث تم تقسيم السبعة وعشرون طراز وراثى تحت الدراسة إلى٤ 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 هى كما يلى (ريست وأرمور وروزانا و 02-99) كما أحتوت هذه المجموعة على أربعة أصناف أيضا عديدى الأجنة هم (تيب وتورو وجلوريا و9004 –DS) بالأضافة إلى مادة واحدة من مواد التربية هى (Eg.6).

**٣- المجموعة الثالثة (متوسط المقاومة)**

وأحتوت هذه المجموعة على النصيب الأكبر من السبع وعشرون طرازا وراثيا حيث أنها أحتوت على ثلاثة عشرا طرازا وراثيا خمسة منهم أصناف وحيدة الأجنة هم (فرنسيسكا و99-05 و99-01 وLP-10 وLP-13) وأربع أصناف عديدة الأجنة هم (هلويس وبركة وأثوس بولى وديسبريز) بالأضافة إلى أربعة من مواد التربية هم (C.39 و SP-270 و Eg.26 وEg.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 هى (Eg.27).

تم أخذ عينات ورقية سواء من النباتات تحت الدراسة عند ٩٠ يوم من عمر النبات لدراسة المشابهات الأنزيمية لثلاث أنزيمات هى (الأستيريز والأميليز والبيروكسيديز) وذلك بهدف الحصول على واسمات كيمو حيوية يمكن إستخدامها فى المستقبل لأمكانية التنبؤ بالأصناف المقاومة أوالحساسة لنيماتودا تعقد الجذور دون الحاجة إلى إجراء العدوى الصناعية سواء فى برامج التربية أوعند أختيار الأصناف للزراعة فى المناطق الموبؤة بالنيماتودا فى المستقبل.

أظهرت نتائج دراسة المشابهات الأنزيمية للثلاث أنزيمات تحت الدراسة (الأستيريز والأميليز والبيروكسيديز) وجود أختلافات ما بين الأصناف فى عدد الحزم سواء فى الجانب السالب أو الجانب الموجب من الهجرة الكهربية كذلك كان هناك أختلاف بين الأصناف فى مواصفات الحزم المنفصلة فى كل أنزيم من الأنزيمات الثلاث تحت الدراسة.

وعند عمل تحليل الشجرة للنتائج المتحصل عليها من دراسة المشابهات الأنزيمية الثلاث أنزيمات تحت الدراسة لكل أنزيم على حدى وجد أن أنزيم البيروكسيديز هو الوحيد الذى أستطاع التفرقة مابين الطرز الوراثية الحساسة للإصابة والأخرى المقاومة حيث أستطاع هذا الأنزيم أن يضع كل مجموعة من المجاميع السابق ذكرها فى مجموعة منفصلة عن الأخرى الذى عجز عنه الأنزيمان الآخران تحت الدراسة حيث كانت المجاميع التى أشار إليها تحليل الشجرة مجاميع مختلطة من كل الطرز الوراثية سواء حساسة أومقاومة.

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

أجرى هذا البحث فى محطة بحوث الصبحية بالإسكندرية حيث تم زراعة ٢٧ طراز وراثى من بنجر السكر معروفة درجة مقاومتهم