Isoperoxidase Activity in Root- Knot Nematode Sugar Beet Resistance Plants

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ABSTRACT

The present study was carried out in a pot experiment at Sabahia Agricultural Research Station, Alexandria, Egypt, during successive season 2007-2008 for evaluating activity of peroxidase isozymes (isoperoxidase), in sugar beet plants (susceptible and/or resistant), to root-knot nematode *Meloidogyne javanica*. Sixteen sugar beet (*Beta vulgaris* L.), genotypes that displayed differential resistance background to root-knot nematode were employed in this work. Computed damage index classified the sixteen genotypes into three categories four genotypes were found to be susceptible (S), eight moderate resistant (MR), and four genotypes resistant (R). Peroxidase isozyme pattern was studied in control and infected plants. Cluster analysis of (susceptible and resistant), genotypes differentiate control and infected plants in two clusters; which mean nematode infection effect on isoperoxidase activity. The cluster analysis differentiated the eight moderate resistant genotypes in three clusters, cluster number one contained five control and one infected plant, cluster number two contained three control and four infected plants, while cluster number three contained three infected plants. It can be concluded that isoperoxidase in moderate resistant genotypes cannot differentiate between infected and control plants.

INTRODUCTION

Peroxidases are ubiquitous in plants. They have become standard isozyme markers due to their easy detection. In addition, the enzymes are extremely stable (Rasmussen and Kerby 1993). Usually, peroxidases are analyzed using starch gel or polyacrylamide gel electrophoresis. Most plants contain peroxidases of migrating towards to anode. Even if less frequently studied, peroxidases of cathodal migration were detected in different, taxonomically very distant groups of plants, e.g. in bryophytes (Krzakowa 1993), grasses (Felder 1976, Krzakowa 1996).

Peroxidases of the sugar beet (*Beta vulgaris* L.) also exhibit cathodal migration. They are located on chromosome 6 (Van Geyt *et al.*, 1988) and the gene products are active as monomers. The peroxidases of sugar beets are also thought to represent useful markers in the detection of interspecific differences (Abe and Tsuda, 1987; Nagamine *et al*., 1989).

Plant peroxidases are contained mainly in cell walls and such localization results in their exhibiting activity already at early stages of defense reactions of the plant to pathogen attacks (Rautela and Payne, 1970; Van Geyt, 1986 and Harrison *et al*., 1995). Besides, peroxidases can produce oxidized phenolics or other substances toxic for invading pathogens (Gaspar *et al*., 1991).

Peroxidase in sugar beet cultivars was not intensively examined in comparison with other enzyme systems (Abe *et al*., 1993 and Szkutnik, 1997). The main objective of this work was to study the activity of peroxidase isozyme (isoperoxidase) in control and infected sugar beet genotypes (susceptible or resistance) to recognize effect of nematode infection on isoperoxidase activity.

MATERIALS AND METHODS

1. Materials:

1.1. Sugar beet genotypes:

Sixteen sugar beet genotypes used in this study were classified into three categories with respect to its root-knot nematode resistance. Four genotypes were susceptible (S), eight were moderate resistant (MR) and four genotypes were resistant (R). Table (1) presents the sixteen sugar beet investigated genotypes and its description (Saleh *et. al.*, 2009). The examined sugar beet genotypes used in this study were kindly obtained from Sugar Crops Research Institute, Agriculture Research Center, Egypt.

1.2. The nematode:

The root-knot nematode, (*Meloidogyne javanica*), was originating from a sugar beet field in Nubariya district. Genera identification was based on the morphology of adult and larval form as described by (Mai and Lyon, 1975). Species of the root-knot nematode were identified on the basis of perineal pattern morphology of the adult females as described by (Eisenback *et al*., 1980, and Eisenback, 1985).

2. METHODS:

Pot experiment was employed in this investigation to study isoperoxidase activity in the infected plants

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Table 1. The sixteen sugar beet investigated genotypes and their description,(S) susceptible, (MR) moderate resistant and (R) resistant to root-knot Nematode

Genotypes reaction	Sugar beet genotypes	Genotypes handling category	Seed type	Code
S	Toro	Commercial var,	Poly germ	1
	Rosanna	Commercial var,	Monogerm	2
	Eg.6	Breeding material	Poly germ	3
	Glorius	Commercial var,	Poly germ	4
MR	C.39	Breeding material	Poly germ	5
	05-99	Commercial var,	Monogerm	6
	01-99	Commercial var,	Monogerm	7
	Baraca	Commercial var,	Poly germ	8
	LP-10	Commercial var,	Monogerm	9
	SP-270	Breeding material	Poly germ	10
	Asthos poly	Commercial var,	Poly germ	11
	LP-13	Commercial var,	Monogerm	12
R	Sultan	Commercial var,	Poly germ	13
	Amile	Commercial var,	Monogerm	14
	Monte Bianco	Commercial var,	Monogerm	15
	Eg.27	Breeding material	Poly germ	16

after inculcation with root-knot nematode (*Meloidogyne javanica*), to detect biochemical marker in susceptible or resistance sugar beet plants to root-knot nematode.

2.1. Inoculum preparation:

The root knot nematode, *Meloidogyne javanica* was cultured alternately on tomato or eggplant (*Solanum melongena* cv. Blackbeauty) and sugar beet (*Beta vulgaris* cv. Chems). Eggs were extracted from tomato or eggplant roots by agitating in 0.05% NaOCl for 2 to 3 min (Hussey and Barker, 1973). The eggs were then collected and rinsed with tap water on nested 150- and 25-µm pore sieves. To collect the second-stage juveniles (J2) for use as inoculum infected tomato or eggplant roots were placed in hatching dishes and incubated in a mist chamber. The J2 were then collected using 150- and 25-µm-pore sieves once a day for 3 to 5 d. During the collection period, J2 were stored in a 1-cm aqueous suspension at 5°C prior to inoculation of sugar beet plants.

2.2. Inoculation procedure:

Beet seeds of each tested genotype were sown in 25 cm diameter pots, filled with steam sterilized sandy clay soil (sand : clay, 2:1) in three replicates. Seedlings were thinned to two per pot at the four to six leaves stage. Each plant was inoculated with about 2000 second-stage juveniles (J2) or/and (eggs + J2s) suspension**.** Two holes about 5-cm deep and 1-cm wide were made in the soil around each four week old sugar beet seedling in 2.5 ml aliquot of inoculum suspension was applied to each hole with a pipette. Unless otherwise noted, the plants were maintained in a greenhouse at 25± 2.5°C. Nutrients were supplied as liquid feed one each week with 5 ml per pot of diluted Vitafeed III® (N : P_2O_5: K_2O, 19: 19 : 19 %) and watered daily as required. Pots were maintained 45 days after inoculation. Sugar beet plants were lifted to complete its growing season after nematode inculcation (180 days), to collect leaf samples from control or infected plants for isoperoxidase analysis. Figure (1), illustrates sugar beet plants after nematode inculcation at 180 days old plants. Figure (2), shows sugar beet (control & infected) roots after nematode inculcation at the experiment end.

2.3. Isozyme analysis:

Sugar beet leaf samples were collected for isozyme analysis. Electrophoresis was carried out to obtain the isoperoxidase patterns in control and infected plants. The following are the buffers, gel media, staining solution and the used electrophoretic procedure (Sabrah and EI-Metainy, 1985): 0.23 M Tris - Citric acid buffer, pH 8.0, in agar-starch-PVP (polyvinyl pyrolidine) gel. 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 (H2O2), which is added immediately before staining, to stain the peroxidase isozyme patterns.

Figure 1. Sugar beet plants after nematode inculcation at 180 days from plant age (control in the right rows and infected plants in the left)

a

b

Figure 2. Sugar beet roots in the end of experiment (a) control roots at harvest (b)infected roots after nematode

RESULTS AND DISCUSSION

1. Peroxidase activity in control and infected plants:

Peroxidase isozyme pattern was studied in control and infected plants to examine effect of root-knot nematode infection on isoperoxidase activity.

1.1. Peroxidase activity in control and infected (susceptible and resistant) plants:

In the presents investigation eight sugar beet genotypes were employed (four susceptible and four resistant genotypes), to recognized variations in isoperoxidase activity in both susceptible or resistant plants. Control and infected plants were pot together in the same plate. Figure (3) shows isoperoxidase pattern for 130 days from plant age. Table (2a) presents TOTAL LAB analysis data for four susceptible and four resistant genotypes in control and infected plants. The data indicated that there were six bands migrating to the cathode. Table (2b) presents anode migration bands indicating that there were three bands migrating toward anode. Figure (4) shows dendrogram of cluster analysis for control and treated plants based on (0 and 1) data. The data indicated that cluster analysis differentiate control and infected plants in two big clusters which mean nematode infection effect on isoperoxidase activity. The results are in agreement with that reported by (Van Geyt, 1986), he studied isozyme system in virus infected sugar beet plants as active defense mechanisms, **(**Yu *et. al.*, 2001) they established isozyme marker for resistance to root-knot nematode and (El-Kholi *et al.*, 2005) reported that the enzymatic activity of Chitinase ?-1, 3 glucanase, poly-phenol oxodase, peroxidase and invertase were significantly increased in infected sugar beet roots than in healthy roots.

1.2. Peroxidase activity in control and infected (moderate resistant) plants:

Eight sugar beet moderate resistant genotypes were used in this experiment to study effect of nematode infection on isoperoxidase activity. Figure (5) shows peroxidase pattern of 130 days control and infected plants. Table (3 a&b), illustrate analysis data for moderate resistant plants. There were six bands in cathode migration and three in the anode. Figure (6) shows dendrogram of cluster analysis for control and infected plants based on (0 and 1) data. The cluster analysis differentiates the eight moderate resistant genotypes in three clusters, cluster number one contain five control and one infected plant, cluster number two contain tree control and four infected, while cluster number three contain three infected plants. It can be concluded that isoperoxidase in moderate resistant plants cannot precisely differentiate between infected and control plants.

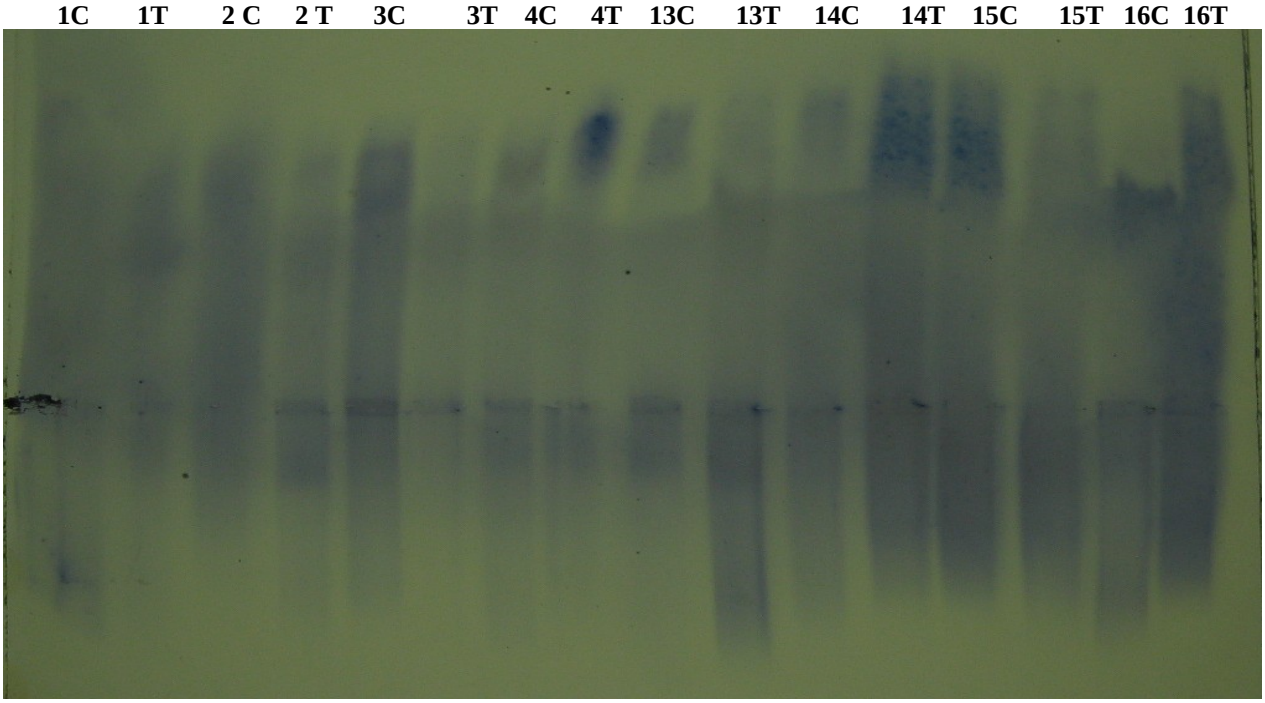

Figure 3. Peroxidase isozyme pattern for(C) control and (T)treated plants (four susceptible and four resistant genotypes)

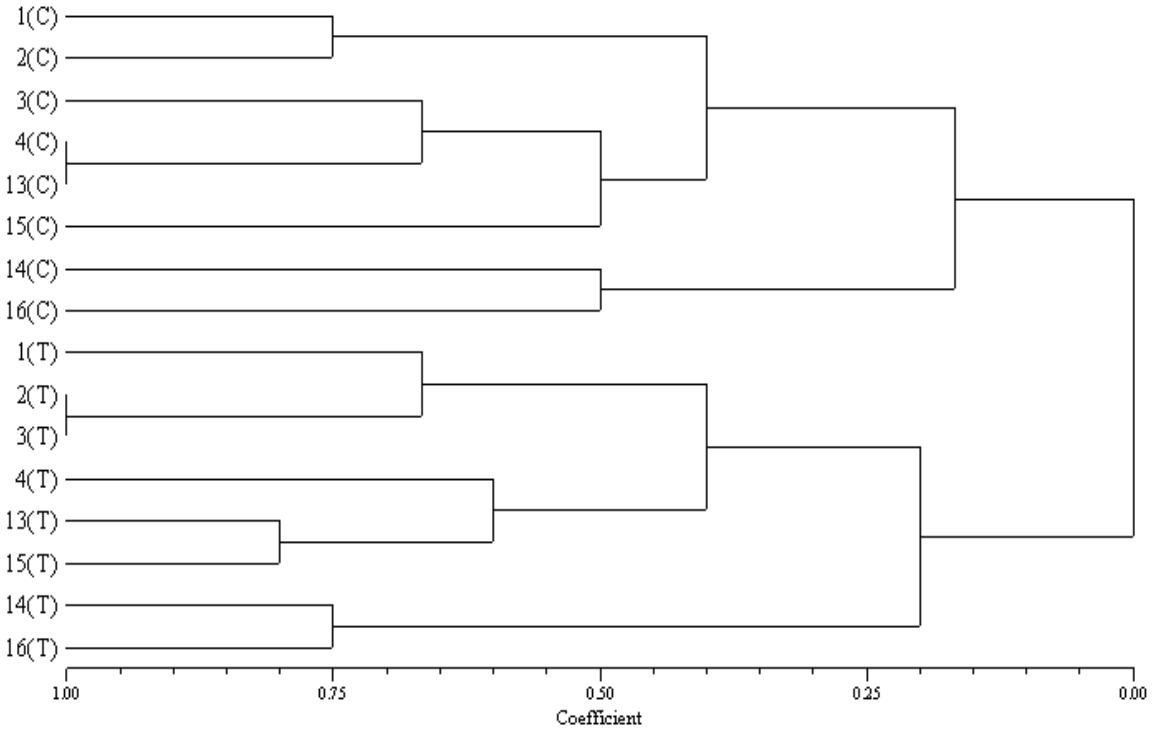

Figure 4. Dendrogram of cluster analysis for peroxides isozyme of (C) control and(T) treated plants (four susceptible and four resistant genotypes)

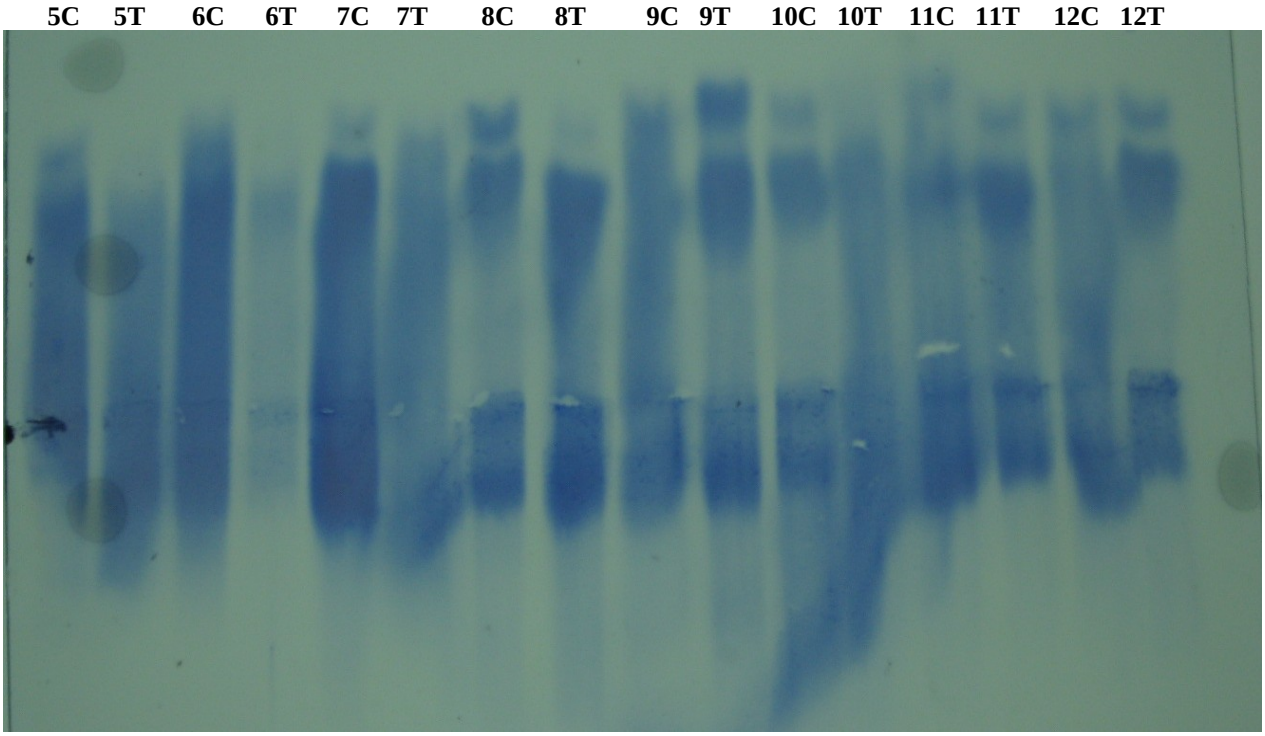

Figure 5. Peroxidase isozyme pattern for (C) control and (T) treated plants of (M R) genotypes

Table 2a. Analysis of peroxidase isozyme for(C) control and (T)treated plants in (susceptible and resistant) plants in cathode 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(C)	2199.95	5.13	0.266	-	-	-	-	-	-	-	-	-	1280.25	3.47	0.758	-	-	-
1(T)	2993.65	14.52	0.065	-	-	-	15530.66	17.59	0.46	-	-	-	-	-	-	-	-	-
2 (C)	-	-	-	15790.66	19.71	0.266	-	-	-	20373.19	18.43	0.734	-	-	-	-	-	-
2 (T)	-	-	-	-	-	-	25190.73	20.36	0.468	-	-	-	-	-	-	-	-	-
3 (C)	-	-	-	-	-	-	-	-	-	-	-	-	28335.5	25.3	0.718	-	-	-
3 (T)	-	-	-	-	-	-	13448.98	17.54	0.508	-	-	-	-	-	-	-	-	-
4 (C)	-	-	-	-	-	-	11368.41	16.9	0.508	-	-	-	6763.33	17.45	0.774	-	-	-
4 (T)	-	-	-	-	-	-	4553.87	8.88	0.524	-	-	-	13863.33	31.18	0.879	-	-	-
13 (C)	-	-	-	-	-	-	6566.15	13.19	0.532	-	-	-	7818.73	16.1	0.823	-	-	-
13 (T)	5358.03	11.78	0.073	-	-	-	10501.36	18.44	0597	-	-	-	1627.42	4.99	0.855	-	-	-
14 (C)	-	-	-	-	-	-	-	-	-	2691.57	9.24	0.653	-	-	-	1591.36	5.49	0.952
14 (T)	5052.45	11.14	0.121	-	-	-	-	-	-	-	-	-	11557.81	24.81	0.806	-	-	-
15 (C)	3115.21	7.6	0.096	-	-	-	-	-	-	-	-	-	12785.62	23.96	0.824	-	-	-
15 (T)	9305.23	15.5	0.104	-	-	-	6394.87	13.65	0.576	-	-	-	3205.76	7.73	0.816	-	-	-
16 (C)	-	-	-	-	-	-	-	-	-	19036.36	33.93	0.648	-	-	-	-	-	-
16 (T)	6699.91	15.89	0.152	4755.24	14.07	0.408	-	-	-	-	-	-	10707.51	12.82	0824	-	-	-

Table 2 b. Analysis of peroxidase isozyme for(C) control and (T)treated plants in (susceptible and resistant) plants in anode side

Plant number	Band 1(+)			Band 2(+)			Band 3(+)		
	Vol	Peak height	R.F	Vol	Peak height	R.F	Vol	Peak height	R.F
1(C)	12578.9	9.11	0.071	-	-	-	36279.21	16.7	0.741
1(T)	-	-	-	.33926.94	9.8	0.214	-	-	-
2 (C)	76765.82	19.76	0.165	-	-	-	-	-	-
2 (T)	-	-	-	57753.55	17.41	0.237	-	-	-
3 (C)	34290.37	12.17	0.17	-	-	-	-	-	-
3 (T)	-	-	-	30967.73	8.48	0.238	-	-	-
4 (C)	54937.86	18.08	0.156	-	-	-	-	-	-
4 (T)	-	-	-	55694.86	14.87	0.237	-	-	-
13 (C)	72161.23	20	0.179	-	-	-	-	-	-
13 (T)	-	-	-	39260.96	11.15	0.268	13114	5.91	0.853
14 (C)	66230.44	16.55	0.165	-	-	-	-	-	-
14 (T)	-	-	-	150520.4	21.57	0.25	-	-	-
15 (C)	162148.8	21.48	0197	-	-	-	-	-	-
15 (T)	-	-	-	130486.6	21.35	0.26	-	-	-
16 (C)	25687.77	9.38	0.192	-	-	-	14468.85	7.24	0.817
16 (T)	-	-	-	61110.19	7.18	0.25	-	-	-

Table 3a. Analysis of peroxidase isozyme for(C) control and(T) treated plants in (moderate resistant) plants in cathode 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
5 (C)	18880.35	39.38	0.128	-	-	-	20811.97	36.17	0.543	-	-	-	4589.76	17.38	0.766	-	-	-
5 (T)	-	-	-	21205.69	40.55	0.436	-	-	-	-	-	-	-	-	-	-	-	-
6 (C)	15743.17	43.84	0.128	-	-	-	31905.38	47.53	0.521	-	-	-	8445.3	26.19	0.798	-	-	-
6 (T)	-	-	-	-	-	-	8201.85	21.48	0.564	-	-	-	-	-	-	-	-	-
7 (C)	14564.93	41.05	0.106	-	-	-	32194.83	41.7	0.5	-	-	-	-	-	-	2585.35	11.36	0.819
7 (T)	-	-	-	-	-	-	36832.24	35.77	0.532	-	-	-	-	-	-	-	-	-
8 (C)	-	-	-	-	-	-	-	-	-	19429.66	39.22	0.67	-	-	-	7629.79	30.95	0.84
8 (T)	-	-	-	-	-	-	-	-	-	29465.93	41.98	0.624	-	-	-	1032.22	6.07	0.828
9 (C)	13050.9	22.58	0.151	-	-	-	17981.61	29.83	0.548	-	-	-	-	-	-	9420.35	29.45	0.839
9 (T)	-	-	-	-	-	-	-	-	-	18918	38.41	0.656	-	-	-	9329.33	38.7	0.892
10 (C)	-	-	-	-	-	-	-	-	-	13442.85	32.09	0.613	-	-	-	3464.85	15.66	0.849
10 (T)	9371.65	22.71	0.161	-	-	-	-	-	-	19670.11	27.23	0.634	-	-	-	-	-	-
11(C)	3468.49	18.42	0.161	-	-	-	-	-	-	1554.48	34.19	0.613	-	-	-	2719.54	10.82	0.882
11(T)	8161.21	19.91	0.172	-	-	-	20161.95	42.32	0.591	-	-	-	-	-	-	3761.32	16.67	0.849
12 (C)	8574.69	25.09	0.191	-	-	-	-	-	-	-	-	-	-	-	-	6016.4	19.76	0.851
12 (T)	-	-	-	-	-	-	-	-	-	16974.69	38.65	0.66	-	-	-	4311.79	23.31	0.84

Table (3 b) Analysis of peroxidase isozyme for(C) control and(T)treated plants in (moderate resistant) plants in anode side

Plant number	Band 1(+)			Band 2(+)			Band 3(+)		
	Vol.	Peak height	R.F	Vol	Peak height	R.F	Vol	Peak height	R.F
5 (C)	10985.15	27.23	0.141	-	-	-	-	-	-
5 (T)	-	-	-	37688.55	43.89	0.25	-	-	-
6 (C)	34712.3	50.68	0.13	-	-	-	-	-	-
6 (T)	-	-	-	10244.07	17.88	0.217	-	-	-
7 (C)	49721.38	55.12	0.161	-	-	-	-	-	-
7 (T)	-	-	-	36654.45	43.02	0.304	-	-	-
8 (C)	23459.89	45.1	0.196	-	-	-	-	-	-
8 (T)	-	-	-	31092.15	49.79	0.293	-	-	-
9 (C)	26698.14	41.52	0.161	-	-	-	-	-	-
9 (T)	-	-	-	23635.93	38.23	0.269	1864.85	5.1	0.71
10 (C)	7968.99	22.87	0.213	-	-	-	4217.33	11.08	0.83
10 (T)	6070.58	32.55	0.096	40010.26	38.51	0.351	-	-	-
11(C)	29716.56	49.14	0.181	-	-	-	-	-	-
11(T)	20554.91	46.18	0.181	-	-	-	-	-	-
12 (C)	2452.26	47.79	0.17	-	-	-	-	-	-
12 (T)	13026.96	36.02	0.202	-	-	-	-	-	-

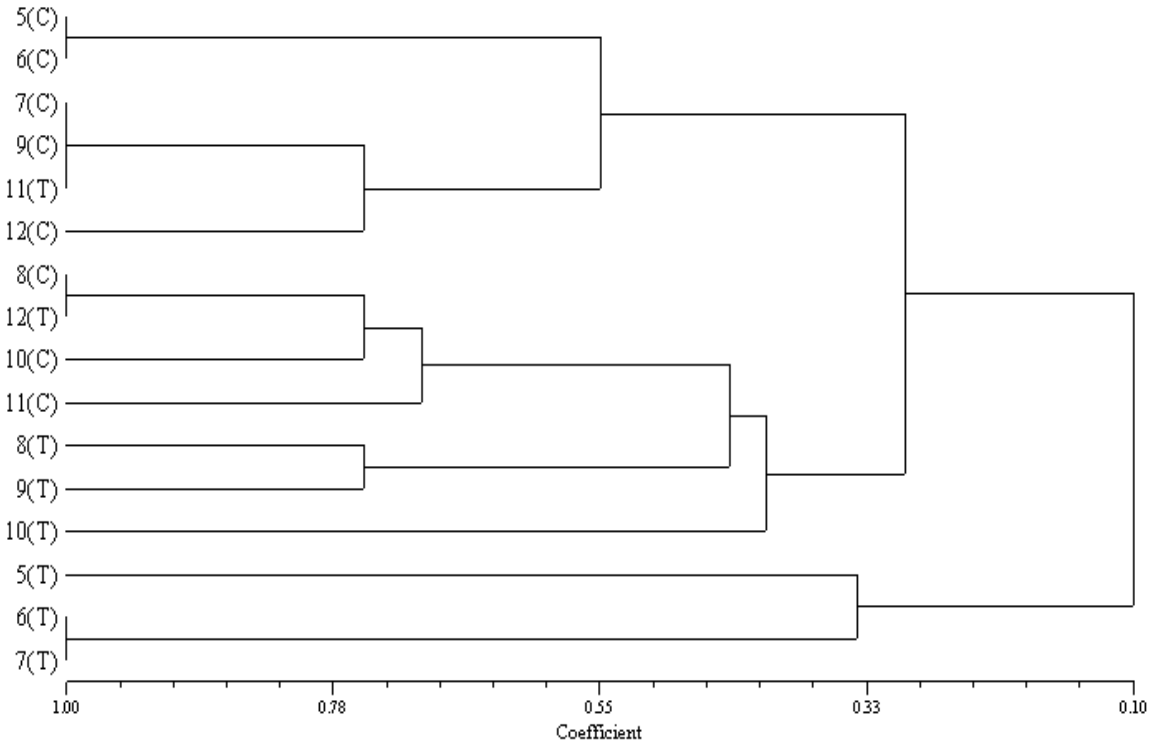

Figure 6. Dendrogram of cluster analysis for peroxides isozyme of (C) control and (T) treated plants of (M R) genotypes

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الملخص العربي

نشاط مشابهات إنزيم البيروكسيديز فى نباتات بنجر السكر المقاومة لنيماتودا تعقد الجذور

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أجرى هذا البحث فى محطة بحوث الصبحية بالإسكندرية فى موسم الزراعة ٢٠٠٧-٢٠٠٨ بغرض دراسة مشابهات إنزيم البيروكسيديز فى نباتات بنجر السكر الحساسة والمقاومة لنيماتودا تعقد الجذور، حيث تم زراعة ستة عشر طراز وراثى من بنجر السكر فى شوالى فخار، تم ملء الشوالى الفخار ذات قطر ٢٥سم بالتربة الخفيفة بعد أجراء تعقيم للتربة لها بالبخار وقد كانت التربة عبارة عن (٢جزء رمل إلى ١جزء طمى).

وقد قسمت هذه التراكيب الوراثية الستة عشر من بنجر السكر إلى ثلاثة مجاميع تبعا لدرجة مقاومتها لنيماتودا تعقد الجذور (المجموعة الأولى "الحساسة" أحتوت على٤طرز وراثية، المجموعة الثانية "متوسطة المقاومة" أحتوت على ٨ طرز وراثية أما المجموعة الثالثة "المقاومة" فقد أحتوت على ٤ طرز وراثية).

تم إجراء العدوى الصناعية بنيماتودا تعقد الجذور (ميلودوجين جفنيكا) بعد إجراء الخف عند عمر شهر تقريبا للبادرات (عند وجود من ٤ إلى ٦ ورقات) وبعد ذلك تم ترك النباتات حتى نهاية موسم الزراعة (١٨٠ يوم) وذلك لأمكانية أخذ عينات أوراق من كل من النباتات المعاملة والنباتات الغير معاملة (الكنترول) فى مواعيد مختلفة من عمر النبات لدراسة مشابهات أنزيم البيروكسيديز.

وقد أظهرت النتائج المتحصل عليها مايلى:

أ- دراسة البيروكسيديز فى نباتات الكنترول والمعاملة (للمقاوم والحساس)

تم أخذ ٤طرز وراثية من الطرز الوراثية للنباتات الحساسة بالأضافة إلى ٤طرز المقاومة ومقارنة النبات المعامل بالنبات الغير معامل (الكنترول)لنفس الصنف وعند عمل تحليل الشجرة وجد أن التحليل أستطاع أن يضع النباتات المعاملة والنباتات الغير معاملة كل فى مجموعة منفصلة بغض النظر عما أذا ما كانت مقاومة أوحساسة للأصابة بالنيماتودا مما يعطى أنطباع أن النشاط الأنزيمى أختلف مابين النباتات نتيجة للأصابة بالنيماتودا.

ب-دراسة إنزيم البيروكسيديز فى نباتات الكنترول والمعاملة (للمتوسطة المقاومة)

تم أخذ ثمانية نباتات متوسطة المقاومة ونم دراسة نشاط البيروكسيديز فى كل من النباتات المعاملة والغير معاملة لنفس الصنف وعند عمل تحليل الشجرة تم الحصول على ثلاث مجموعات الأولى تحتوى على خمسة نباتات عير معاملة بالأضافة إلى نبات معامل والثانية تحتوى على أربع نباتات معاملة وثلاث غير معاملة أما المجموعة الرابعة فتحتوى على ثلاث نباتات معاملة، وهذا يعطى أنطباع أن النباتات متوسطة المقاومة ليس لها سلوك ثابت تجاه الأصابة حيث أن هناك نباتات تستجيب للمعاملة أوالأصابة فيختلف النشاط الأنزيمى تبعا للأصابة أوأن الأصابة لاتؤثر على النشاط الأنزيمى فتسلك سلوك النباتات الغير معاملة.