Evaluation of Twenty Seven Sugar Beet Genotypes for Resistant to Root- Knot Nematode, (*Meloidogyne Javanica*)

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ABSTRACT

Sugar beet has been commercially introduced in Egypt since 1982 with cultivated area about 20,000 feddans and increased gradually to be 25,7667 fed. in 2008. The plan is to increase the sugar beet area and beet sugar factories to fill the gap between sugar consumption and production which reaches about one million tons a broadly imported every year. Recently, reclaimed desert irrigated lands at West Nubariya and El-Bostan regions has shown that sugar beet can be successfully grown under sandy soil area condition and it's considered as the extended area for sugar beet production in Egypt. The root-knot nematode, (*Meloidogyne incognita* and *M.-javanica*), is the most serious problem against sugar beet expansion in the new arable lands which was reported as major nematode pests of sugar beet in Egypt. The present study was carried out during the growing season 2007 - 2008 at pots experiment in Sabahia Agricultural Research Station, Alexandria, Egypt, for evaluating the reaction of twenty seven sugar beet genotypes against the most serious nematode, (*Meloidogyne javanica*). The twenty seven sugar beet genotypes used in this test were 21 commercial varieties and 6 breeding materials.

Computed damage index classified the twenty seven sugar beet genotypes into four categories one commercial variety was highly susceptible (HS), nine genotypes were susceptible (S), thirteen genotypes moderate resistant (MR), and four genotypes resistant (R), three of them are commercial varieties while the best one in computed damage index was proven to be the breeding material (Eg.27).

INTRODUCTION

Sugar beet (*Beta vulgaris*), is an important arable crop, that traditionally used for sugar extraction, and recently, for biofuel production. A wide range of pests, including beet cyst nematode (*Heterodera schachtii*), root-knot nematodes (*Meloidogyne* spp.), green peach aphids (*Myzus persicae*) and beet root maggot (*Tetanops myopaeformis*), infest the roots or leaves of sugar beet, which leads to direct yield loss or through transmission of beet pathogens such as viruses. The average annual loss in yield of sugar beet due to *M. incognita* in different states in the U. S. A. was estimated to be as high as 10-50 % and in Italy as 5-15 % (Altman and Thomson, 1971). Conventional pest control approaches based on chemical application have led to high economic costs. Development of pest-resistant sugar beet varieties could play an important role towards sustainable crop production while minimizing environmental impact. Yu, *et al* (1999) reported that development of commercially available plant resistant varieties to *Meloidogyne* spp. is essential for sugar beet caltivation. Intensive *Beta* germplasm screening has been fruitful, and genetic lines resistant to nematodes, aphids and root maggot have been identified and integrated into sugar beet breeding programmes. The use of transgenic technology is discussed with regard to biodiversity and food safety Zhang *et al*, (2008).

In Egypt, sugar beet is cultivated in 25,7667 feddans with an average production of about 18.593 tons per feddan 2007- 2008 (Annual Report of Sugar Crops Council, 2008). The most serious problem against sugar beet extension in new areas is root-knot nematode. *Meloidogyne incognita* and *M. javanica* were reported as major nematode pests of sugar beet in Egypt, (Ibrahim, 1982; Oteifa and El-Gindi, 1982; Abd El-Massih, 1985; Maareg *et al*. 1988, (a & b), Maareg *et al*. 1998 and Ismail *et al*. 1996). Also, in Egypt, Koura (1983) carried out a survey work in sugar beet producing areas and recorded the presence of seven nematode genera, viz. *Helicotylenchus*, *Hirschmanniella*, *Tylenchorhynchus*, *Hoplolaimus*, *Criconemoides* and *Pratylenchus* in decreasing order. Maareg *et al*. (1988b) reported that the root-knot nematodes, *Meloidogyne javanica* and *M. incognita* are known among the most serious pests of sugar beet crop in Egypt.

Present investigation was carried out to study the reaction of twenty seven sugar beet genotypes (21 commercial varieties and 6 breeding materials) to the root-knot nematode (*M. javanica*), to select resistant ones for planting in nematode contaminated areas and as a prospect to use in the evaluation purposes needed for breeding programs.

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MATERIAL AND METHODS

1. Materials:

1.1. Sugar beet genotypes:

The twenty seven sugar beet genotypes used in this study consisted of twenty one commercial varieties and six breeding materials, sixteen polygerm and eleven monogerm. The tested genotypes were obtained from Sugar Crops Research Institute, Agriculture Research Center, Egypt. Table (1) presents the twenty seven sugar beet genotypes and its description.

1.2. The root-knot nematode:

The root-knot nematode, *Meloidogyne javanica* was originating from a sugar beet field in Nubariya district and its generic 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). Second stage juveniles (J2) of the root-knot nematodes, *M. javanica* were obtained from a pure culture of *M. javanica* that was previously initiated by a single eggmass and propagated on tomato plants, (*Lycopersicon esculentum* Mill. cv. Moneymaker) in the greenhouse

2. METHODS:

2.1. Inoculum preparation:

The root knot nematode, *Meloidogyne javanica* was cultured alternately on tomato or eggplant (*Solanum melongena* cv. Blackbeauty) and then 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.

Table 1. Description of the twenty seven sugar beet genotypes evaluated for their resistance to the root-knot nematode, *Meloidogyne javanica* and its seed types

Code	Sugar beet varieties	Genotypes handling category	Seed type
A1	Glorius	Commercial var.	polygerm
A2	Helwes	Commercial var.	polygerm
A3	Ds 9004	Commercial var.	polygerm
A4	Francesca	Commercial var.	monogerm
A5	Rossanna	Commercial var.	monogerm
A6	02-99	Commercial var.	monogerm
A7	Lp-10	Commercial var.	monogerm
A8	Lp-13	Commercial var.	monogerm
A9	Rhist	Commercial var.	monogerm
A10	05-99	Commercial var.	monogerm
A11	01-99	Commercial var.	monogerm
A12	Toro	Commercial var.	polygerm
A13	FD-9902	Commercial var.	polygerm
A14	Despreze	Commercial var.	polygerm
A15	Baraca	Commercial var.	polygerm
A16	Sultan	Commercial var.	polygerm
A17	Amile	Commercial var.	monogerm
A18	Eg. 2701	Breeding material	polygerm
A19	SP. 270	Breeding material	polygerm
A20	C. 39	Breeding material	polygerm
A21	Athos poly	Commercial var.	polygerm
A22	Eg. 27	Breeding material	polygerm
A23	Monte Bianco	Commercial var.	monogerm
A24	Eg. 26	Breeding material	polygerm
A25	Type	Commercial var.	polygerm
A26	Eg. 6	Breeding material	polygerm

A27	Armure	Commercial var.	monogerm

2.2. Inoculation procedure:

Beet seeds of each tested genotype were sown in 25 cm diameter pots, filled with steam sterilized sandy clay soil (2:1, v/v) and three replicates were used for each genotype. 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. All pots were layout in a complete randomized block design in the greenhouse. 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 irrigated daily as required. Pots were maintained for 45 days after inoculation.

2.3. Resistance assessment:

After 45 days from inoculation, plants were uprooted carefully from pots. Infected roots of each plant were washed with tap water, fixed in 4% formalin for 24 h and stained in 0.01 lactic acid fuchsin (Byrd and Barker, 1983) and then examined for recording number of galls and eggmasses per root system.

The roots were, then graded for gall and egg-mass numbers, gall size (GS), gall index (G.I) and egg-mass index (E.I)., where a 1-9 scale for each of galls and egg-masses numbers was as follows:

-Gall index (G.I): 1 = no galls, 2 = 1-5, 3 = 6-10, 4 = 11-20, 5 = 21-30, 6 = 31-50, 7 = 51-70, 8 = 71-100 and 9 > 100 galls or egg-masses per plant.

-Gall size (G.S): was rated as 1 = no galls, 3 = very small, 5 = small, 7 = medium, and 9 = big galls.

-Galled area (G.A.): = 1 = < 10%, 2 = 10- 20%, 3 = 21- 30%, 4 = 31-40%, 5 =41- 50, 6 = 51 – 60%, 7 = 61 – 70%, 8 = 71 – 80% and 9 = > 80% of surface root area.

The damage index (D.I) was deduced by dividing the sum of G.I, G.S and G.A by 3 (D.I = G.I+G.S+G.A / 3). The different ratings of D.I were moderately resistant (< 5), susceptible (5.1 -7) and highly susceptible (7.1 – 9). Nematode damage index (D.I.) was evaluated according to (Sharma *et al*., 1994).

2.4. Statistical analysis:

The experimental design was randomized completed block design (RCBD) with three replicates, and the data was analyzed according to (Steel and Torrie, 1981).

RESULTS AND DISCUSSION

1. The pots experimental results:

After 45 days from artificial nematode inculcation the plants were carefully uprooted from the soil and the observations were recorded. Figure (1) shows uprooted seedlings after 45 days from pots. Figure (2) illustrates examined seedling for the twenty seven studied genotypes. There were significant differences in all three studied parameters.

1.1. Gall index (GI):

There were highly significant differences between genotypes in this gall index (GI). Table (2) presents mean values for gall index, these values ranged from 7 in commercial polygerm varieties (Helwes and FD-9902), to 2.3 in breeding material Eg.27.

1.2. Gall size (GS):

Significant differences were found between genotypes for galls size. Figure, (3) illustrates different size of galls in seedling roots. Mean values in Table (2) indicates that these values ranged from 7 to 1.6. Highest values were found in ten genotypes, nine of them are commercial ones (Glorius, Ds 9004, Rossanna, 02-99, Rhist, Toro, FD-9902, Type and Armure), while one of them breeding material (Eg.6). Lowest values in this character found in three genotypes, two of them are commercial varieties (Sultan and Amile), while one breeding material (Eg.27).

1.3. Galled area (GA):

The data indicated that there were highly significant differences between genotypes in the values of root galled area. Table (2) shows mean values for gall area character, these values ranged from 7.6 in polygerm commercial variety (FD-9902) to 1.3 in monogerm commercial variety (Monte Bianco).

2. Genotypes reaction against nematode:

Categorization of the tested genotypes according to the damage index (DI) of (Sharma *et. al.*, 1994) is shown in Table (3). Computed damage index classified the twenty seven sugar beet genotypes into four categories highly susceptible (HS), susceptible (S), moderate resistant (MR) and resistant (R).

2.1- Highly susceptible genotypes (HS):

Data indicated that there was one commercial variety as highly susceptible (HS), sugar beet polygerm commercial variety (FD-9902).

2.2- Susceptible genotypes (S):

There were nine genotypes considered as susceptible (S), four of them are monogerm (Rhist, 02-99, Armure and Rosanna), four polygerm (DS 9004,

Table 2. Investigated sugar beet genotypes and their reactions after nematode inoculation

Sugar beet genotypes	Code	Gall index (GI)	Gall size index (GS)	Galled area index (GA)
Glorius	A1	4 cde	7 a	6.8 a
Helwes	A2	7 a	4 ab	3.7 bc
Ds 9004	A3	5 abcd	7 a	6.6a
Francesca	A4	3.3 de	4.6ab	3.4 c
Rossanna	A5	4.6 bcd	7 a	6.9 a
02-99	A6	5.3 abcd	7 a	7.2 a
Lp-10	A7	4.3 bcde	4 ab	3.7 bc
Lp-13	A8	3.3 de	4 ab	3.9 bc
Rhist	A9	5.6 abc	7 a	7.5 a
05-99	A10	5.6 abc	4 ab	4.2 bc
01-99	A11	5.3 abcd	4 ab	3.7 bc
Toro	A12	5.6 abc	7 a	6.9 a
FD-9902	A13	7 a	7a	7.6 a
Despreze	A14	3.6 cde	4 ab	4.7 b
Baraca	A15	4.6 bcd	4 ab	3.9 bc
Sultan	A16	4.3 bcde	1.6 b	1.5 d
Amile	A17	3.6 cde	1.6 b	1.4 d
Eg. 2701	A18	4.6 bcd	4 ab	3.3 c
SP. 270	A19	4 cde	4ab	3.4 c
C. 39	A20	6.3 ab	4 ab	4.3 bc
Athos poly	A21	3.6 cde	4 ab	3.8 bc
Eg. 27	A22	2.3 e	1.6 b	1.5 d
Monte Bianco	A23	3.3 de	1.67 b	1.3 d
Eg. 26	A24	4.6 bcd	4 ab	3.9 bc
Type	A25	4 cde	7 a	6.7 a
Eg. 6	A26	4.6 bcd	7 a	6.9 a
Armure	A27	5.3 abcd	7 a	7.1 a
L.S.D. 0.01		1.76	2.63	0.997

Table 3. Sugar beet varieties and their reaction after nematode inoculation (HS) highly sensitive, (S) sensitive, (MR) moderate resistant and (R) resistant

Varieties reaction	Sugar beet varieties	Code	Computed damage index (DI)
HS	FD 9902	A13	7.2
S	Rhist	A9	6.7
	02-99	A6	6.5
	Toro	A12	6.5
	Armure	A27	6.4
	DS 9004	A3	6.2
	Rosanna	A5	6.1
	Eg.6	A26	6.1
	Type	A25	5.9
	Glorius	A1	5.9
MR	Helwes	A2	4.9
	C.39	A20	4.8
	05-99	A10	4.6
	01-99	A11	4.4
	Baraca	A15	4.1
	Eg.26	A24	4.1
	Francesca	A4	4
	LP-10	A7	4
	Eg-2701	A18	3.9
	SP-270	A19	3.8
	Asthos poly	A21	3.8
	Despreze	A14	3.7
	LP-13	A8	3.7
R	Sultan	A16	2.4
	Amile	A17	2.2

Monte Bianco	A23	2
Eg.27	A22	1.8

Figure 1. Uprooted sugar beet seedlings after 45 days of inoculation

Figure 2. Sugar beet inoculated seedlings after 45 days of nematode inoculations

Figure 3. Infected roots after 45 days of nematode inoculation. Photographs show different size of galls

Glorius, Toro and Type) and one breeding material (Eg.6).

2.3- Moderate resistant genotypes (MR):

The obtained data indicate that there were thirteen genotypes considered as moderate resistant (MR), five of them are monogerm commercial variety (01-99, 05-99, Lp-10, Lp-13 and Francesca), and four polygerm commercial varieties (Helwes, Baraca, Athos poly and Despreze), while there were four breeding materials (Eg.26, Eg.2701, Sp-270 and C.39).

2.4- Resistant genotypes (R):

There were four genotypes in this category two of them were monogerm commercial varieties (Amile and Monte Bianco) and one polygerm commercial variety (Sultan), while the best of them was the breeding material (Eg.27). These results are in agreement with those reported by Gohar (2003) who used 21 sugar beet commercial varieties to study their susceptibility to root-knot nematode, *Meloidogyne incognita*, and the relationships between plant parasitic nematodes of sugar beet and other soil fauna. He found four commercial varieties were resistant to *Meloidogyne incognita*, two of them were polygerm (Kawemira and Sultan) and two were monogerm (Marathon and Emma). Muller (1992), reported that nematode resistant material is now incorporated into commercial breeding lines, so the appearance of nematode resistance cultivars is at last in prospect, although reports from Germany on resistance breaking pathotypes indicate that there are continuing problems in store for the plant breeder if resistant varieties become widely used. Ismail *et al.* (1996) screened twenty-six varieties of sugar beet for their susceptibility to *Meloidogyne incognita* under greenhouse conditions. They found that Carat variety could be ranked as a tolerant host and HM Hill2 and Maribo marine poly as a highly susceptible. Ten varieties out of 26 were rated as moderately susceptible, whereas the remaining varieties were as low as susceptible.

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الملخص العربي

تقييم سبعة وعشرون تركيب وراثى من بنجر السكر من حيث مقاومتها لنيماتودا تعقد الجذور (ميلودوجين جفنيكا)

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أجرى هذا البحث بغرض تقييم ٢٧ من الطرز الوراثية لبنجر السكر ضد نيماتودا تعقد الجذور وذلك لمعرفة الطرز الوراثية المقاومة والحساسة لنيماتودا تعقد الجذور(ميلودوجين جفنيكا) التى تعتبر ذات أنتشار واسع فى الأراضى الجديدة مثل النوبارية والبستان والتى من أهم أهداف التوسع الأفقى فى زراعة محصول بنجر السكر فى مصر. وقد تم الحصول على الطرز الوراثية الخاصة بنباتات بنجر السكر من خلال معهد بحوث المحاصيل السكرية وكانت هذه الطرز الوراثية السبعة والعشرون عبارة عن ٢١ صنفا تجاريا يوجد منهم ١٣ صنفا وحيد الأجنة وثمانية أصناف عديدة الأجنة، أما بقية الطرز الوراثية السبعة والعشرون فقد كانت عبارة عن ستة من مواد تربية بنجر السكر تم الحصول عليها من خلال برنامج تربية بنجر السكر المقام فى مصر منذ أكثر من عشرون عاما من خلال العاملين فى قسم التربية والوراثة بمعهد بحوث المحاصيل السكرية- مركز البحوث الزراعية- مصر.

وقد أجرى هذا البحث فى محطة بحوث الصبحية بالإسكندرية فى موسم الزراعة ٢٠٠٧- ٢٠٠٨ حيث تم زراعة السبع وعشرون طراز وراثى فى أصص فخارية من خلال تجربة تحتوى على ثلاثة مكررات، ثم تم ملء الشوالى الفخار ذات قطر ٢٥سم بالتربة الخفيفة بعد أجراء التعقيم للتربة بالبخار وقد كانت التربة عبارة عن (٢جزء رمل إلى ١جزء طمى).

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 بحوالى ٤٥ يوم تم أخذ القياسات الخاصة بتقدير مدى حساسية أو مقاومة النباتات لنيماتودا تعقد الجذور.

وقد أظهرت النتائج مايلى:

تم تقسيم السبعة وعشرون طراز وراثى 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 هى كما يلى (ريست و أرمور و روزانا و 99 -02) كما أحتوت هذه المجموعة على أربعة أصناف عديد الأجنة هم (تايب و تورو وجلوريا و DS – 9004) بالأضافة إلى أحدى 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 و LP-10 و LP- 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 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) وواحدة من مواد التربية كانت هى

أكثرهم من حيث صفة المقاومة مما يعطى أنطباع جيد إلى أنه يمكن عن طريق الأستغلال الجيد لمواد التربية هذه يمكن زيادة درجة المقاومة لها مع أدخالها فى برامج تربية تؤدى فى النهاية إلى الحصول على صنف يتميز بالمحصول العالى ونسبة السكر الجيدة بالأضافة إلى درجة المقاومة المناسبة التى تجعلها تتميز بالزراعة فى مناطق التوسع الجديدة لزراعة بنجر السكر مثل النوبارية والبستان والتى تتميز بأنتشار نيماتودا تعقد الجذور (*ميلودوجين انكوجنيتا*) فى معظم أراضيها ومادة التربية التى يعقد عليها الكثير من الأمال فى هذا المجال هى (Eg.27).