

Susceptibility of some Sugar Beet Genotypes to Root-Knot Nematode, *Meloidogyne incognita*



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ABSTRACT

Screening of fifteen sugar beet genotypes, obtained from research program funded from Desert Research Center, entitled St. Catherine's Breeding and Conservation Program for Plants with Special Environmental Needs, was carried out to estimate their susceptibility towards infection with root-knot nematode (RKN) *Meloidogyne incognita*. The response of these inbred lines was varied between moderate susceptible (MS) and highly susceptible (HS) based on egg masses (E.M) index, also egg production was a dependable factor. One genotypes called SKG58-642 was found to be moderate susceptible (MS) as they possessed 25 egg mass. Seven genotypes viz., SKG73-311, SKH44-412, SKH44-452, SKC59-422, SKH44-422, SKT48-411 and SKH44-462 (possessed 43.3, 57.3, 63, 73, 74.3, 83.3 and 86.3 egg masses, respectively) were found to be moderately susceptible. The rest seven genotypes were highly susceptible; SKH44-482, SKC59-622, SKH44-442, SKC59-522, SKH44-472 SKH44-432 and SKG73-412 (recorded 103.3, 106, 113.3, 130, 132, 156.3 and 169.3 egg masses per plant, respectively). Data obtained from this research were paving the way to further screenings of other sugar beet genotypes to obtain the highly resistant genotypes vs RKN. Sugar beet breeding programs must also be continued, including introducing genotypes resistant to RKN and improving genotypes that were less susceptible to infection resulting from the previous program, to make them available in the Egyptian market.

Keywords: Root-knot nematode, *Meloidogyne*, sugar beet, genotypes

INTRODUCTION

Sugar beet (*Beta vulgaris* L.) belongs to the family Chenopodiaceae, and it is a biennial crop as it needs two years to complete its life cycle. It has a haploid chromosome number of nine, ($2n = 18$) and contain 758 Mbp per haploid genome (Arumuganathan and Earle, 1991). Sugar beet is an essential crop for providing world with sugar, it come after sugar cane, it shared with about 35% of sugar production globally (Harveson et al., 2009) and increased to 38%, it represents 12.5% from total world sugar crops area (Kenawy and Nagib, 2024), these sharing is due to it can be planted in a varied climatic conditions and many types of soils including arid regions (Tomaszewska et al., 2018 and Bayomi et al., 2019). Egyptian sugar beet production was growing at an average annual rate of 12.54%, as it raised form zero tonnes in 1973 to 16.0 million tonnes in 2024 (Kim, 2024). Recently, sugar beet is considered the first sugar crop in Egypt cultivated in 594.248 feddans, contributing 62.2% of sugar production with an average production of 21.3 tonnes feddan-1 (GAIN, 2019) the area was slightly increased during 2023 to 597923 fed. (Kenawy and Nagib, 2024).

Plant parasitic nematodes (PPNs) are considered one of the dangerous pests in Egypt and many countries especially in tropical regions. Root-knot nematodes (RKNs) *Meloidogyne* spp.

represent the most widespread and polyphagous genus of PPNs, about 100 species were recognized (Subbotin et al., 2021). Under this genus, the major species are *M. arenaria*, *M. hapla*, *M. incognita*, *M. javanica* and *M. enterolobii* (Rusique et al., 2023), they were considered one of the most serious pests of many economic crops (Abad et al., 2003; Ibrahim et al., 2010 and Elling, 2013).

Sugar beet crop is subject to damage by many pests and diseases besides other abiotic stresses. Phytonematodes are one of the most damaging pathogen to sugar beet worldwide, PPNs-infested sugar beet fields causing yield losses varied between 10 and 80% (Hafez 1998). On the other hand, the inclemency of damage caused by nematodes can differ depending on the occurred species the field and their density. It was reported that sugar beet fields can infested by more than thirty seven species of plant parasitic nematodes; however, a little number of nematodes species can cause considerable injury to sugar beet production (Harveson et al. 2009; Karegar, 2006). PPNs can be ordered according their importance and effect on sugar beet production; *Heterodera*, *Meloidogyne*, *Nacobbus*, *Ditylenchus*, *Trichodorus*, *Paratrichodorus* and *Longidorus* (Harveson et al., 2009; Khan et al., 2016; Bridge and Starr 2019; Westerdahl et al., 2023 and Hassan et al., 2024). In Egypt the RKN is highly distributed and the serious nematode in sugar beet that cause considerable damage, the damage can occur by the two common species *M. incognita* or *M. javanica* (Saleh et al., 2009; Korayem et al., 2012 and Hassan et al., 2024)

Nematicides are the most effective control methods of PPNs, due to their hazardous effects to many living organisms in the ecosystem they were restricted in a wide scale globally and many researchers focused on alternative management strategies or non-genetic methods viz., physical, cultural, and biological control (Afia and El-Nuby, 2016; El-Nuby and Alam, 2020; El-Nuby et al., 2020 and El-Nuby, 2021) Producing genotypes having high degree of resistance to specific nematode pest mainly depends genetic elements, which includes determining the sources of resistance then using them in breeding programs. Increasing resistance in sugar beet cultivar against nematode may reduce pollution in environment also minimize the costs of crop production (Kim and Yang, 2019; El-Saadony et al., 2021 and Elkobrosy et al., 2022). So introducing of new varieties that bear new genetic structure enabling them to tolerate these stresses are targeted by plant breeders.

The aim of this research is to evaluate the reaction of new sugar beet genotypes, obtained from breeding program of Desert Research Center (DRC), against root- knot nematode (RKN), *Meloidogyne incognita* under greenhouse conditions.

MATERIALS AND METHODS

Root-knot nematode (RKN) culture, previously identified as *Meloidogyne incognita*, used as inocula in this study were obtained from infected roots of eggplant (*Solanum melongena*) cultivar long white grown in the greenhouse of Plant Protection Department, Desert Research Center (DRC), Cairo, Egypt. Fifteen genotypes of sugar beet, *Beta vulgaris*, (two monogerm and thirteen multigerm) were obtained from the research program entitled “Breeding and preserving plants with special environmental needs” which were shown in Table (1). Plastic pots (15 cm diameter) were filled with soil mixture (3 sand: 1 clay), soil was sterilized using an autoclave for 3 h at 85°C. Three seeds of each sugar beet genotypes were sown in each pot. The plants growing were placed on greenhouse bench, four pots for each genotype served as replicates, all pots were arranged in a randomized complete block design. Plants were watered as needed and received the same fertilization. Fourteen days after emergence vigorous and healthy seedlings of sugar beet genotypes were thinned to one plant per pot. Each pot was inoculated with about two thousands infective stage juveniles of root knot nematode (*M. incognita*), all pots were wetted immediately following inoculation. Three months after

nematode inoculation, all plants were uprooted, by soaking pot in pan filled with water to easily separate the roots from soil, and the root system of each plant was washed under stream of tap water to remove the adhering soil particles.

Nematode parameters; galls, egg masses, eggs per egg mass were counted, also total eggs per root were calculated. To assay the various nematode criteria; lactophenol acid fuchsin solution were used to stain roots (Franklin and Goodey, 1959). By the aid of stereo-zoom microscope knots and egg masses were enumerated. Eggs per egg mass were counted under compound microscope by picking ten egg masses randomly from roots then calculate the mean number of eggs. The final population was calculated by summation of egg masses plus total eggs (number of egg masses multiplied by mean number of egg per egg mass) and the nematode reproduction factor ($RF = Pf/Pi$) was calculated.

To rank the tested sugar beet plants as susceptible or resistant to RKN we used the scale depending on numbers of egg masses/root system or egg mass index (EMI) in this scale the genotypes was categorized in 0 to 5 scale according to Taylor and Sasser (1978). Plants with no egg masses were considered immune (0); 1-2 egg masses/plant were considered resistant (1); 3-10 egg masses/plant were considered moderately resistant (2); 11-30 egg masses/plant were moderately susceptible (3); 31-100 egg masses/plant, susceptible (4) and more than 100 egg masses/ root system were highly susceptible (5).

Data Analysis

The plants were randomly arranged in greenhouse in blocks; so the randomized complete block design (RCBD) was used as a statistical for the experiment in which the replicate number for each treatment were four. Data were analyzed by (ANOVA) using the statistical program (Costat program) and significant differences among the means were partitioned by Duncan's multiple range test at probability level of 5% (Steel et al., 1997).

Table1: Genotypes used.

No.	Genotypes	Seeds
1	SKC59-522	Monogerm
2	SKG58-642	Monogerm
3	SKC59-622	Multigerm
4	SKG73-412	Multigerm
5	SKH44-412	Multigerm
6	SKH44-422	Multigerm
7	SKH44-432	Multigerm
8	SKH44-442	Multigerm
9	SKH44-452	Multigerm
10	SKH44-462	Multigerm
11	SKH44-472	Multigerm
12	SKC59-422	Multigerm
13	SKG73-311	Multigerm
14	SKH44-482	Multigerm
15	SKT48-411	Multigerm

RESULTS AND DISCUSSION

Data in Table (2) showed the reaction of fifteen genotypes either monogerm, SKC59-522 and SKG58-642, or multigerm, for rest genotypes, of sugar beet which screened against RKN infection. One genotype called SKG58-642 was found to be moderately susceptible (MS) which recorded an egg mass index of 3.0 (formed 25 EM). Seven sugar beet genotypes viz., SKG73-311, SKH44-412, SKH44-452, SKC59-422, SKH44-422, SKT48-411 and SKH44-462 which possessed EM index of 4.0 (formed 43.3, 57.3, 63, 73, 74.3, 83.3 and 86.3 egg masses, respectively) were categorized as susceptible (S). The rest seven genotypes; SKH44-482, SKC59-622, SKH44-442, SKC59-522, SKH44-472 SKH44-432 and SKG73-412 (recorded 103.3, 106, 113.3, 130, 132, 156.3 and 169.3 egg masses per plant, successively) were highly susceptible (HS) hosts, their EM index were 5.0. The total population, which represent the total laid eggs and the females inside the egg mass, was varied among sugar beet tested genotypes. The highest population of nematode was recorded in the line SKG73-412 (78983.93) with reproduction factor (RF) of 39.49, followed by the genotype SKH44-432 (70268.67) with RF equal 35.15 and 60408.20 individuals in genotype SKC59-522 with RF equal 30.20 and 59454.17 individuals in line SKH44-442 with RF of 29.37. The lowest population has been built by genotype SKG58-642 also the lowest RF3 .91 was 7820.33 individuals, followed by line SKG73-311 17641.0 individuals & 8.82 RF and 23329.33 individuals in genotype SKH44-412 associated with 11.66 RF.

The compatible reaction of the susceptible sugar beet genotypes towards *M. incognita* infection proved that all tested genotypes lack genes conferring the resistant, so juveniles were able to penetrate the roots of these hybrids, developed normally and females laid eggs. In this study we screened 15 inbred lines obtained from breeding program in occurred in DRC against *M. incognita* that represents a serious challenge stress on sugar beet in Egypt.

Our results were in accordance with previous work of Abd- El- Khair et al. (2013) who tested the reaction of some sugar beet varieties against *M. incognita* and proved that their reaction against the RKN ranged from susceptible (S) to highly susceptible (HS) depending on their damage index. Similarly, Youssef et al. (2016) estimated the resistance index of some sugar beet varieties against *M. incognita*, using the damage index besides the host vigor, they found that all examined varieties were susceptible with varied degree either S or HS. Gohar et al. (2013) categorized the reaction of sugar beet varieties, based on gall index and reproduction factor (Pf/Pi), as susceptible, tolerant and hyper susceptible as a result of field evaluation.

Other investigators also found various degree of susceptibility or resistance in sugar beet genotypes versus RKN challenge; Saleh et al. (2009) found that $\approx 34\%$ of tested sugar beet genotypes were S, $\approx 3\%$ HS and 48% were moderate resistant (MR). While only 15% were resistant (R) to root knot nematode. They also noted diminishing in plant growth due to nematode infection as well as total soluble sugar and total soluble solids. Youssef and El-Nagdi (2015) evaluated the reaction of ten sugar beet varieties (2 monogerm and 8 multigerm) towards root-knot nematode, they found only one 10% variety reacted as a highly resistant varieties, three were moderately resistant 30% and the rest 60% of the tested varieties were susceptible and highly susceptible. Some investigation not reported the high susceptibility degree of certain sugar beet genotypes. Maareg et al. (2018) screened the reaction of ten genotypes of sugar beet towards *M. incognita*; they found that 40% were low susceptible, 40% were moderately susceptible and 20% were tolerant. Other screening estimated the resistance of eight sugar beet varieties for their susceptibility to *M. incognita* under greenhouse conditions, three varieties were susceptible and one hyper susceptible, three tolerant and one moderately resistant depending on gall index and reproduction factor (Gohar et al., 2023)

Table 2: Screening of sugar beet genotypes to root- knot nematode *Meloidogyne incognita* under greenhouse conditions.

Sugar beet genotypes	Galls	Egg Mass	E.M index	Egg /E.M	Total eggs	Total Pop.	RF	SR
SKC59-522	418.7 b	130.0 c	5	463.8 bc	60278.2 c	60408.2 c	30.20	HS
SKG58-642	164.0 j	25.0 i	3	311.5 f	7795.3 k	7820.3 k	3.91	MS
SKC59-622	356.0 c	106.0 d	5	507.2 a	53619.7 d	53725.7 d	26.86	HS
SKG73-412	575.0 a	169.3 a	5	465.4 b	78814.6 a	78983.9 a	39.49	HS
SKH44-412	217.7 gh	57.3 g	4	406.0 e	23272.0 ij	23329.3 ij	11.66	S
SKH44-422	251.0 f	74.3 ef	4	430.9 cde	32056.4 fg	32130.7 fg	16.07	S
SKH44-432	318.0 d	156.3 b	5	448.3 bcd	70112.3 b	70268.7 b	35.13	HS
SKH44-442	299.0 de	113.3 d	5	524.0 a	59340.8 cd	59454.2 cd	29.73	HS
SKH44-452	231.7 fg	63.0 fg	4	404.0 e	25350.0 hi	25413.0 hi	12.71	S
SKH44-462	284.3 e	86.3 e	4	441 bcd	38078.7 f	38165.0 f	19.08	S
SKH44-472	582.7 a	132.0 c	5	514.1 a	67857.3 b	67989.3 b	33.99	HS
SKC59-422	131.7 k	73.0 ef	4	422.2 de	30840.9 gh	30913.8 gh	15.46	S
SKG73-311	177.3 ij	43.3 h	4	406.5 e	17597.7 j	17641.0 j	8.82	S
SKH44-482	207.3 ghi	103.3 d	5	443.3 bcd	45757.7 e	45861.0 e	22.93	HS
SKT48-411	188.7 hij	83.3 e	4	423.7 de	35314.7 fg	35398.0 fg	17.70	S

Means with the same letter (s) in each column for each cultivar are not significantly different at $p = 0.05$. E.M.I= egg mass index; Plants with 0=no egg masses (Immune), 1= 1-2 egg masses/plant were considered resistant -R-; 2=3-10 egg masses/plant, moderately resistant -MR-; 3=11-30 egg masses/plant, moderately susceptible (MS); 4=31-100 egg masses/plant, susceptible -S-; and 5= > 100 egg masses/ root system, highly susceptible -HS- (Taylor and Sasser, 1978). RF = Reproduction factor (Pf= final population/ Pi= initial population), SR= Susceptible rating.

Searching for new RKN-resistant germplasms must be frequent to neutralize nematode injury to plants, many researchers stated that natural nematode resistance genes present in gene pools of crop species and their relatives have long been manipulated with the aim of transferring these characters to certain economic crops where effective resistance is lacking, so resistance is crucial in crop management to obtain sustainable and satisfactory production (Matsuo et al., 2012; Fosu-Nyarko and Jones., 2015; Ukpene and Oduma, 2024). The aforementioned breeding program may be expanded and developed via genetic engineering, to expand the gene pools of sugar beet genotypes available within the advanced plant mechanisms used to perceive and combat nematode invasion.

CONCLUSION

Current study introduces a moderate susceptible, or moderately resistant, genotype (SKG58-642) that possessed the lowest number of egg masses and the lowest rate of build-up of RKN. Also other 7 genotypes (SKG73-311, SKH44-412, SKH44-452, SKC59-422, SKH44-422, SKT48-411 and SKH44-462) that considered susceptible with varied reproductive index, while the rest were highly susceptible. This research represented a step in the road leading to produce more resistant genotypes of sugar beet through breeding programs, which are essential, as the expansion in cultivation of sugar beet in newly reclaimed desert lands in Egypt has been carried out against the threat of root knot nematode, the most serious problem against sugar beet, to beet production will increase, so the control strategies in particular using resistant of low susceptible varieties will represent an urgent need. Furthermore, it is necessary to detect the relationship between the RKN-resistance character and other desirable characters especially high productivity. Also identify the RKN-resistance genes in sugar beet and try to transfer these genes to susceptible sugar beet varieties is a long term target.

DECLARATION

The authors declare that they do not have any actual or potential conflict of interest.

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الملخص العربي

حساسية بعض التراكيب الوراثية لبنجر السكر تجاه نيماتودا تعقد الجذور *Meloidogyne incognita*

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^١ قسم وقاية النبات – مركز بحوث الصحراء – القاهرة- مصر

^٢ قسم الاصول الوراثية – مركز بحوث الصحراء – القاهرة - مصر

تم دراسة حساسية خمسة عشر تركيب وراثي من بنجر السكر، التي تحصل عليها من البرنامج البحثي بعنوان " تربية وصون النباتات ذات الإحتياجات البيئية الخاصة بسانت كاترين" والممول من مركز بحوث الصحراء، ضد الإصابة بنيماتودا تعقد الجذور *Meloidogyne incognita*. أظهرت النتائج تباين بسيط في رد فعل تلك السلالات تجاه الإصابة بالنيماتودا المختبرة، و إعتقادا علي مؤشر كتل البيض المكونة علي الجذور فقد رتبنا تلك السلالات طبقاً لمقاومتها إلي متوسطة الحساسية كما في السلالة SKG58-642 (حيث تكون عدد ٢٥ كيس بيض)، سبع سلالات الحساسية كانت SKG73-311, SKH44-412, SKH44-452, SKC59-422, SKH44-422, SKT48-411 and SKH44-462 حيث كونت ٤٣,٣، ٥٧,٣، ٦٣، ٧٣، ٧٤,٣، ٨٣,٣، ٨٦,٣ كتلة بيض علي التوالي. التراكيب السبعة المتبقية وهم (SKH44-482, SKC59-622, SKH44-442, SKC59-522, SKH44-472 SKH44-432 and SKG73-412) كانت عالية الحساسية مكونة ١٠٣,٣، ١٠٦، ١١٣,٣، ١٣٠، ١٣٢، ١٥٦,٣، ١٦٩,٣ كيس بيض علي التوالي. في النهاية فإن تلك النتائج تفتح الباب لمزيد من الإختبارات والتقييم لتحديد التنوع الجيني للسلالات الأكثر مقاومة لتحسين سلالات تحمل صفات مرغوبة أكثر وعلي الأخص صفة الإنتاجية العالية المتعلقة بالمقاومة أو التحمل لنيماتودا تعقد الجذور وتحديد الجينات المتحكمة في المقاومة لمحاولة نقلها للتراكيب الحساسية من بنجر السكر.