Faculty of Agriculture (Saba Basha)
Agricultural Botany Department

Using molecular biology techniques for nematode resistance in sugar beet

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Presented by

El-Shaimaa Abdelmowla El-Sayed Mohamed

B.Sc. Science (Plant production department, Faculty of Agriculture, Saba Basha, Alexandria University, 2005)

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Sugar beet (*Beta vulgaris* subsp. *vulgaris*) is the most economically valuable crop species in the order Caryophyllales, *B. vulgaris* subsp. the genus *Beta* L. of the family Amaranthaceae (formerly, Chenopodiaceous). Sugar beet is considered one of the most well-known sugar crops. It is a temperate crop; though, it can be cultivated in a wide range of climatic conditions.

In Egypt, Sugar beet, *Beta vulgaris* L. is deliberated as the first sugar crop cultivated in 954.248 feddans contributing with 62.2% of sugar production. In intensive commercial production, where sequential cropping of one susceptible crop after another is practiced, lack of effective root-knot nematodes management strategies has led to total crop failure. Furthermore, problems associated with nematicides application occur, and nematode management mostly relies on the availability and use of adequate sugar beet varieties. It is obvious that farmers need clear information on the host status of the available sugar beet varieties.

The objective of this study was to establish a rapid and effective screening procedure to detect numbers of sugar beet genotypes with resistance to root-knot nematodes through modified conventional susceptibility assessments confirmed by customizing of molecular markers as respective detection platforms, i.e. markers based on single nucleotide polymorphisms (SNPs). In addition, nanotechnology was employed experimentally to assist a given susceptible sugar beet variety to combat RKN infestation.

Therefore, three outdoors pots experiments were carried out at Sabahia Research Station in the ordinary sugar beet growing season of Egypt at autumn of 2018 and 2019, one was carried out for short-run period (60 days) to assess host suitability of sugar beet genotypes for root-knot nematode, *Meloidogyne incognita* according to adapted quantitative scheme for assignment of Canto-Saenz's host suitability (resistance) designations, whereas the two other experiments were carried out in full growing season of sugar beet (180 days).

One of them was conducted to determine the susceptibility or tolerance degree of screened sugar beet varieties by modified host parasite index (MHPI) and the second was carried out to evaluate quantitative and qualitative effects of different concentrations of nanoparticles (Ag-NPs) on the pathogenicity and reproductive of *M. incognita* infecting susceptible sugar beet variety, Polat.

Host suitability after adapted quantitative scheme of Canto-Saenz's (AQSCS) for sugar beet genotypes (*Beta Vulgaris Saccharifera*) designed for root-knot nematode, *M. incognita:*

Eight varieties of sugar beet , i.e. Natura KWS, FARIDA, Lammia KWS, SVH 2015, Lilly, Halawa KWS, Polat and Capella were subjected to root-knot nematode, *M. incognita* host suitability test according to quantitative system for assignment of Canto-Saenz's host factor suitability (resistance). The measurements by gall index (GI) as an indicator for plant damage, and host efficiency (RF) as an indicator for nematode reproduction were the baselines for linkage between them to deduce the host suitability as designed. The resulting classification for the eight investigated sugar beet varieties encompassed four different

groups; differentiated into moderately resistant category (MR); with one variety (SVH 2015), that did not support nematode reproduction (RF ≤1), and root damage in them was minimal (GI ≈2).

The following category was the tolerant group (T), involving two varieties (Lilly and Halawa KWS), which supported relatively high nematode reproduction (RF >1) with fair plant damage(GI≤2). Whereas susceptible category (S) encompassed four varieties (FARIDA, Lammia KWS, Polat and Capella); these varieties supported nematode reproduction factor (RF >1) with high plant damage (GI >2), and highly susceptible category (HS), with one variety (Natura KWS), which did not support nematode reproduction (RF≤1) yet had very high plant damage (GI>2) and was therefore rated as hyper susceptible. In spite of the defined four categories of host suitability, i.e. MR, T, S and HS, it can be noticed that within each category there are significant differences at $P < 0.05$ for Root Damage (gall index), number of $J_2/250\ cm^3$ of Soil and R-factor (host efficiency).

Assessment of sugar beet varieties for their susceptibility to root-knot nematode, *Meloidogyne incognita*, by modified host parasite index (MHPI):

The susceptibility or tolerance degree of the eight screened sugar beet varieties was determined by modified host parasite index (MHPI) as a new susceptibility/ resistance value, which states the amount of reduction in yield and technological characters caused by nematode infection. Sugar beet varieties with MHPI ≤ 4.0 is considered as tolerant (T), 4.1-6.0 as low susceptible (LS), 6.1- 8 as moderately susceptible and ≥ 8.1 as highly susceptible (HS). Least significant differences (LSD) and a paired T- test at 0.05 were performed for all data. According to that data, different sugar beet varieties have a great variation in their susceptibility/resistance to infection with *M. incognita*. Protocol used in this study was (MHPI) at $P < 0.05$, and statistical differences are found among infected and non- infected plants within varieties of sugar beet in the studied yield and quality characters. Results exposed that the yield and quality characters of such investigated sugar beet plant varieties were obviously diminished by *M. incognita* infection to a great level.

Additionally, the screened varieties, which were infected with *M. incognita* showed significant differences in symptoms of root galling, damage index, final population, reproduction factor and susceptibility rate. Thus, the varieties could be classified according to MHPI scale into four significantly separated groups, one variety, SVH 2015 is considered as moderately resistant (MR), three varieties, Lammia KWS, Lilly and Halawa KWS varieties are considered tolerant (T), as well as three other varieties, which are considered as susceptible varieties, FARIDA, Polat and Capella, while, Natura KWS variety, which is ranked as highly susceptible.

The analysis of resistance levels (categories) according to two screening procedures, i.e. Adapted quantitative scheme of Canto–Saenz (AQSCS) and modified host parasite index (MHPI). AQSCS procedure's classification of the eight tested sugar beet varieties into four distinguished categories; moderately resistant, tolerant, susceptible and hyper-susceptible, almost matched with MHPI procedure except for the numbers of tolerant and susceptible varieties for each. Since AQSCS is a short period technique (45 -60 days; it is capable of

detecting susceptible ones more accurately; however, it could not do the same with tolerant ones, because tolerant and resistant varieties were not dependent on initial population (Pi), but were more dependent on yield assessment, sugar beet tolerant varieties are expected to produce high yield regardless of nematode infection, thus 60 days test cannot measure yield performance. On the other hand, MHPI procedure is capable of detecting tolerant sugar beet varieties more accurately because of long period test (180 days) and accomplishing of yields.

In conclusion, the MHPI scale could be considered as standard procedure of host suitability method and reporting of resistance or tolerance of sugar beet to root-knot nematodes. Whereas AQSCS technique is suitable for quick sugar beet plants host suitability test especially for the susceptible sugar beet varieties.

Impact of Ag-NPs concentrations on root-knot nematode; *Meloidogyne incognita* number in soil, reproductive factor and knot disease severity % of roots sugar beet in pots experiment:

Root knot nematodes disrupt the physiology of the plant and can cause great economic losses in production and quality of sugar beet crop. Chemical nematicides are usually preferred for their effective control; the problems associated with nematicides application turned the workers vision to focus on new alternative agents for nematode management programs. In this study, high throughput microcrystalline cellulose decorated silver nanoparticles (Ag-NPs) by different concentrations were evaluated as a nematicidal substance in outdoors pots experiment.

The chosen tested concentrations were 20, 30, 40, 50, and 60 ppm/ml of Ag-NPs with four replicates, each concentration along with two methods of application [one time application (AT1) and application two times (AT2)], applied to sugar beet pots infested with *Meloidogyne incognita*. Applying Ag-NPs directly to infested sugar beet pots achieved significant suppression at $p \leq 0.05$ of root-knot nematode, *M. incognita* in terms of reducing numbers in soil, reproductive factor, and knot disease severity %. Efficacy % that related to untreated pots and relative efficacy % that proportionated with pots treated with Ethoprop 10% G get higher potential as of Ag-NPs of concentrations get higher. Time of application AT2 enhanced the efficacy of Ag-NPs at low concentration (under 50 ppm/ml) and occasionally above 50 ppm/ml.

Effects of different Ag-NPs concentrations and times of application on yield components i.e. root yield plant-1(g), top yield plant-1(g) and sugar yield plant-1(g) of infested sugar beet plant with root-knot nematode, *M. incognita*, were related to degree of Ag-NPs concentration to suppress nematodes activity.

Different concentrations of Ag-NPs increased yield components i.e. root yield plant-1(g), top yield plant-1(g) and sugar yield plant-1(g) even at low concentration (20 ppm/ml) in comparison with control treatment (0.0 ppm/ml). The same trend for quality as sucrose, total soluble solids (T.S.S) and purity percentages of sugar beet infested with root-knot nematode, *Meloidogyne incognita* in pots experiment. Avoidable loss percentage in roots and sugar yields plant-1(g) as an economic expression responded positively to different levels of Ag-

NPs concentrations and to time of application AT2 in low concentration < 50 ppm/ml. This study has demonstrated a potential environmentally friendly alternative for the management of root-knot nematodes.

SNP markers

Single nucleotide polymorphism is the most wonderful molecular marker found frequently in the genome showing a variable distribution among species. Genomic DNA was productively extracted and the specific primer was used in this work effectively amplified a fragment of 650 bp of a eight sugar beet varieties assessed for their resistant to the root-knot nematode using combination of primers Nem06FWD and Nem06REV. Alignment analysis through online blast reveals similarity of 76% to 99 % One of the interesting novel results of this paper, our sequence of eight sugar beet varieties deposited in GenBank. (https://ddbj.nig.ac.jp/submission).

The length of Natura KWS a SNP marker genomic sequence is 657 and consisted of G+C content (39.27%), A+T content (59.06%). In addition, the length of FARIDA a SNP marker genomic sequence is 814 and consisted of G+C content (39.33%), A+T content. Furthermore, the length of Lammia a SNP marker genomic sequence is 566 and consisted of G+C content (39.40%), A+T content (60.42%).

Besides, the length of SVH a SNP marker genomic sequence is 579 and consisted of G+C content (38.86%), A+T content (59.41%). Moreover, the length of Lilly a SNP marker genomic sequence is 793 and consisted of G+C content (41.36%), A+T content (55.74%).

In comparison, the length of Halawa a SNP marker genomic sequence is 793 and consisted of G+C content (40.87%), A+T content (56.61%). On the other hand, the length of Polat a SNP marker genomic sequence is 566 and consisted of G+C content (38.87%), A+T content (60.95%). Finally, the length of Polat a SNP marker genomic sequence is 566 and consisted of G+C content (38.53%), A+T content (61.28%).

The sequences of these amplified products in the varieties Natura KWS, FARIDA, Lammia KWS, SVH 2015, Lilly, Halawa KWS, Polat and Capella have been submitted to the Genbank Database. The Maximum-Likelihood tree revealed that the eight varieties of *Beta vulgaris* were grouped together and were closely related to the two genotype *Beta vulgaris* genotype 2_SB34 and *Beta vulgaris* genotype 6_SB33. On the other hand, these varieties were distant related to all the predicted sequence of *Beta vulgaris* subsp. *vulgaris* transcription factor

Relative value of instantaneous "*r"* should be considered when evaluating them. For simplicity, sum of " *r"* values is made equal to 100, The amino acid frequencies are 7.69% (A.), 5.11% (R.), 4.25 % (N.), 05.13 % (D.), 02.03 % (C.), 4.11 % (Q.), 6.18 % (E.), 7.47 % (G.), 2.30 % (H.), 05.26 % (I.), 09.11 % (L.), 05.95 % (K.), 02.34 % (M.), 04.05 % (F.), 5.05 % (P.), 6.82 % (S.), 05.85 % (T.), 1.43 % (W.), 03.2 3 % (Y.), and 6.64 % (V.). For estimating ML values, a tree topology was automatically computed. The maximum Log likelihood for this computation was -49914.525. This examination involved 38 amino acid sequences. There was a total of 13569 positions in the final dataset

A 600 bp DNA fragment was simply amplified in the eight sugar beet genotypes using combination of primers Nem06FWD and Nem06REV. Based on the homology search on GenBank database of this fragment with sequence (KF303133.1) showed 99.47% similarity among the sequences. Alignment and amino acids composition prediction of the DNA sequences by MEGA-X and DNAMAN software's revealed the presence of five SNPs among them.

The genotype Lamiaa sequence had a transversion in site number 12 (T→C) That lead to an insertion of isoleucine. In site number 13 there was an insertion of C in SV1697, Halawa, Natura, Bolat, and Lilly and an insertion of T in genotype Lamiaa. This insertion Lead to insertion of serine in SV1697, Natura, Bolat, Lamiaa and Lilly. An insertion of C was detected in site number 14 of Lamiaa sequence. In site number 232 there was a transversion (G→C) in SV1697, Bolat, Kabel, Natura and Lilly sequences that replaced glycine with Arginine. A Guanine base was transioned with Adenine in site number 377 in Lamiaa and Lilly sequences with no predicted amino acid changing in response to this transion