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Differentiate between Sugar Beet (*Beta vulgaris* L.) Genotypes Resistance to Root-knot Nematode, (*Meloidogyne incognita*) by Molecular Markers

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ABSTRACT

The application of molecular methods as complementary or alternative methods can help identifying the plants containing the resistance gene at genotypic level. Therefore, DNA markers can be useful tools for selecting resistant genotypes and can save the evaluation time and improve the precisions. Selection takes productive and genetic lines resistant to nematodes have been known in sugar beet genotypes by Polymerase chain reaction (PCR). Sugar beet (*Beta vulgaris* L.) is the greatest vital crops that standing following to sugar cane as sugar crop in the world, later it produces about 20 % of sucrose production annually in the world. In Egypt, Sugar beet is cultivated in 523188 faddans with an average production of 20.7 tons per faddan. 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 its considered as the extended area for sugar beet production in Egypt. The most serious problem against sugar beet extension in new lands is root-knot nematode, *Meloidogyne incognita* and *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 2015 - 2016 at pots experiment in Sabahia Agricultural Research Station, Alexandria, Egypt, for evaluating the reaction of seventeen sugar beet genotypes against the most serious nematode, (*Meloidogyne incognita*). The seventeen sugar beet genotypes tested in this test were twelve sugar beet commercial varieties, three sugar beet inbred lines and two sugar beet breeding materials. Computed damage index classified the seventeen sugar beet genotypes into five categories according to the varietal assessment. (Four were resistant(R), three were moderately resistant (MR), six were tolerant (T), two were susceptible (S) and two were hyper susceptible (HYS)). The results for conventional PCR indicated that genes of *Hs1pro-1*and *HSPRO2* shows resistance to, (*Meloidogyne incognita*) beside the known function of these genes as resistant against cyst nematode (*Heterodera schachtii*). *Mi-1.2* gene consider resistance to root knot nematode (*Meloidogyne incognita*) was found in (Mi-3) inbred line.

Keywords: *Beta vulgaris L.*, *Meloidogyne incognita*, molecular marker, PCR.

INTRODUCTION

Sugar beet (*Beta vulgaris L.*) is a herbaceous dicotyledonous plant, and has a haploid chromosome number of nine, (2n = 18) and a nuclear DNA content of 758 Mbp per haploid genome (Arumuganathan and Earle, 1991).

It belongs to the family *Chenopodiaceae*, and it is a normally biennial crop which completes its life cycle in two years. Sugar beet is an important crop that supplies around 20% of the sugar consumed worldwide. It is cultivated in over 50 countries (FAOSTAT 2016).

In Egypt the total area cultivated with sugar beet in (2016/2017) season was 523188 feddan; the average productivity of feddan is 20.7 tons roots (Sugar Crops Council 2018). Sugar beet as a second sugar crop in Egypt needed a good and safe source of seeds to insure the row materials for six or seven beet sugar factories. For this reason (Breeding Program) of sugar beet has started in Egypt during the last two decades of past century by some Egyptian investigator's and breeders, in the Agric. Res. St., Alexandria, Sugar Crops Res. Inst. Agric. Res. Center, Egypt, the data was very encouragement. (Yonan 1984, El-Manhaly et.al. 1987, Saleh 1993, Ghura 1995 and Saleh and Ghura (2013).

The crops are damaged by biotic (insects, bacteria, nematodes, fungi, etc.) and abiotic (temperature, moisture, salinity, etc.) stresses and the development of new varieties that are tolerant under adverse conditions is one of the main breeding challenges (Biancardi *et al* 2010). About 42% of the potential world crop yield is lost due to biotic stresses (Pimentel 1997). Nematodes are one of the most serious biotic stress caused a large loss in crops yields. Nematodes are the principal animal parasites of plants, causing annual crop losses of more than US$100 billion worldwide (Cagla 2014). Root-knot nematodes (RKNs) (*Meloidogyne spp.*) are among nematode pathogens to sugar beet, especially in tropical and subtropical regions. Sugar beet is highly susceptible to root-knot nematode (*Meloidogyne spp.*) and plants grown in nematode-infested soil inevitably suffer root gall symptoms, which lead to damage and reduced production.

In Egypt, the root-knot nematode is the greatest serious problem against sugar beet expansion which was reported as major nematode pests of sugar beet, (Ibrahim, 1982; Abd-El –Massih *et al*.,1986;Maareg *etal*., 1998;El-Nagdi *etal*.,2004;Korayem,2006 and Saleh *et al*., 2009).

Root-knot nematodes , *Meloidogyne spp.* are highest plant parasite nematodes species affecting the quantity and quality of the crop manufacture in several annual and perennial crops .In Egypt , these nematodes are considered to be one of the main problems in sugar beet cultivation, at West Nubaryia district (Ibrahim ,1982; Dteifa and El-Gindi,1982;Abd-El –Massih *eta*l.,1986; Maareg *et al*., 1988a,1998;Gohar,2003 and Gohar &Maareg,2005). The loss in roots and sugar yields of sugar beet caused by *M.incognita* in West Nubaryia were assessed to be between (0.7-50.8%) and (11.8-68.4%), respectively. Decrease of crop damages due to nematodes is the method of increasing crop yields.

Chemical control is generally used for the controlling of root–knot nematodes. However, synthetic nematicides are currently being re-evaluated with respect to environmental hazard , high costs , unavailability in many developing countries and there reduced effectiveness following frequent applications .There for, some additives such as using natural enemies , improving cultural practices and cultivating resistant cultivars were tested contrary to root-knot nematodes on sugar beet to decrease environmental pollution and save controlling processes economical.

Many investigations evaluated different sugar beet verities for resistance/ susceptibility toward *M. incognita* and *M. javanica* in field and/or green house conditions (Abd El Massih *et al*,1985 ; Maareg *et al* .,1988b , 1998,2005, 2009 and 2018;Gohar,2003;El-Nagdi *et al* ;2004; Salah *et al*,2009;Abd El-khair *et al*.,2013 Gohar etal.,2013) .They reported that the tested sugar beet varieties were categorized in to different groups for their susceptibility / resistance degree.

Development of post-resistance sugar beet verities can play an important role towards sustainable crop production while decreasing environmental effect. the use of transgenic technology is investigated with regard to biodiversity and food safety (Zhang etal.,2008).

Management of root-knot nematode in infested fields is, therefore, critical to sugar beet growers. At present, due to lack of effective and environmentally safe control measures, development of sugar beet using resistance to root-knot nematode is highly desirable (Yu,1995).

The application of molecular methods as complementary or alternative methods can help identifying the plants containing the resistance gene at genotypic level. Therefore, DNA markers can be useful tools for selecting resistant genotypes and can save the evaluation time and improve the precisions. Intensive Beta genotypes screening has been fruitful and genetic lines resistant to nematodes have been identified by Polymerase chain reaction (PCR) (Norouzi 2003).

Molecular markers are integrated widely in nematode resistance breeding programs, particularly for resistance to root knot and cyst nematodes (Hussey and Janssen, 2002; Young and Mudge, 2002; Xu *et al*., 2013).

In this study seventeen sugar beet genotypes were used to differentiate between the genotypes against the degree of resistance to root knot nematode (*Meloidogyne incognita*). Three genes were investigated in this work to detect molecular marker used for nematode resistance in sugar beet (*Hs1pro-1* and *HSPRO2*) discusses resistance to beet cyst nematode *(Heterodera. Schachtii*). and (*Mi-1.2*) gene confer resistance to root knot nematode *(Meloidogyne* spp.).

MATERIALS AND METHODS

1-Sugar beet materials:-

Sugar beet materials used in the present work were kindly obtained from Sugar Crops Research Institute (SCRI) and Egyptian Sugar Beet Breeding Program (ESBBP). The seventeen sugar beet genotypes were used in this study classified as.

a-Twelve sugar beet commercial varieties (BTS 301, BTS 302, Beta 382, Beta 401, Melode, Sultan, Rizoble, Frida, Athos poly, Maripella, Helios poly, and Nada)

b-Three sugar beet inbred lines (Mi-3, C39 and SP-022)

c-Tow sugar beet breeding materials (Eg.6 and Eg.26)

The three sugar beet lines used in this study (Mi-3, C39 and SP-022) were obtained from United States Department of Agriculture (USDA) and registrations of them were recognized by (Lewellen and Skoyen 1988 ; Lewellen, 1994; Doney 1995; Lewellen, 1995). Mi-3 line was classified as resistanr to *Meloidogyne* ssp(YU 2002) these lines were grown in this work as checks. Sugar beet tested materials are listed in (Table 1).

2-pot experiments:-

This study was carried out during 2015/2016 seasons at el sabhia agriculture research station ,Alexandria ,Egypt. Seeds of sugar beet genotypes tested were planted separately in 35 cm dictated clay pots occupied with 3.5 kg stem sterilized sandy loam soil (collected from sugar beet fields of west Nubaria). At two weeks old seedling were thinned to one vigorous plant per pot. For each sugar beet genotype . eight pots with similar in their .growth were selected , four of these were inculcated with 4000 eggs of *M.incoginta* per pot (approximately 400 eggs pre cm^3 soil) according to Gohar and Maareg 2009. Inoculum was distributed in two holes 2-3 deep and protected with soil the other four vessels were kept with out inoculation as control . All pots were retained on screen house bench in experimental strategy as random complete design (RCBD) with a strip -pots arrangement of treatments. Pots were irrigated immediately flowing inculcation .The plants were watered and 15 g of multiple fertilizer (15:15:15) was added at the three weeks old plants.

Table 1. Sugar beet studied materials and there description.

Code	Sugar beet Genotypes	Genotypes handling category	Seed type
1	BTS 301	Commercial var.	Poly
2	BTS 302	Commercial var.	Poly
3	Beta 382	Commercial var.	Poly
4	Beta 401	Commercial var.	Poly
5	Melode	Commercial var.	Mono
6	Soltanes	Commercial var.	Poly
7	Helios poly	Commercial var.	Poly
8	Rizoble	Commercial var.	Poly
9	Frida	Commercial var.	Poly
10	Athos poly	Commercial var.	Poly
11	Maripella	Commercial var.	Poly
12	C39	Inbred line	Poly
13	Mi-3	Inbred line	Poly
14	SP-022	Inbred line	Poly
15	Nada	Commercial var.	Poly
16	Eg.6	Breeding material	Poly
17	Eg.26	Breeding material	Poly

3- The nematode:-

Nematode eggs of *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood 1949 were collected from the heavily infected roots of eggplant (*Solanum melongena,* 'Black beauty'). The eggplant plants were up-rooted and the egg masses were selected as defined by (Hartman and Sasser 1985). One hour earlier inoculation, Nematode inoculums' of 4000 *M. incognita* eggs per pot according to (Gohar and Maareg 2009) - approximately 400 eggs 250 cm^{-3} soils. Inoculum was dispersed into two holes (approximately 2.5 cm deep) and protected with soil. Pots were watered proximately after inoculation. The plants were then watered repeatedly and 15 g of multiple fertilizer (15:15: 15) was added to the 3 weeks old plants.

Fourty five days next nematode eggs inoculation, the plants were up-rooted by insertion the small pots in a slanting location into a big pot containing water, while being shaken softly until the soil was moved into the pot and roots were washed. The roots were surveyed and valued for galling responses on a scale; 1 = 1 – 2 galls; 2 = 3 – 10 galls; 3 = 11 – 30 galls; 4 = 31 100 galls; 5 = 101 galls and above according to (Taylor and Sasser 1978). Before uprooting the plants, 250 cm3 of soil nearby each plant was collected up to a depth of 10 – 15 cm. From each of the soil samples using a changed Bearman's tray method as defined by (Barker 1985), second juvenile larvae (J2s) were extracted. From 2 mL aliquots of each extracts, J2s were calculated under a dividing microscope and this was repeated 10 times (20 mL) to assessment its population in 250 cm-3 of soils.

The host efficiency (reproduction factor 'RF') was calculated, where 'RF' = Pf/Pi, with Pf being final

population in 250 cm3 of soil and Pi being the original inoculums. An "RF' of less than or equal to one (1) indicates no seeming increase in the nematode population (Nwauzor, 1998). Final assessment of the different genotypes was based on modified Canto-Saenz's host resistance descriptions scheme (Gohar *et al.* 2013) as given in (Table 2).

Table 2. Assignment for Adapted Quantitative scheme of Canto - Saenz's host suitability (resistance) Descriptions (Gohar *et. al.* 2013).

Degree of resistance (DR)	Host efficiency [Z] (R-factor)	Plant Damage (Gall index)[Y]
Resistant (R)	≤1	≤2
Moderately Resistant (MR)	≤1	≈2
Tolerant (T)	>1	≤2
Susceptible (S)	>1	>2
Hyper susceptible (HYS)	≤1	>2

[Z] reproductive factor: RF = Pf/Pi where Pi = initial population mass and Pf = final population mass, [Y] Gall index: 0 = no gall formation; 5 = heavy gall formation basis: Sasser *et al* (1984).

4-DNA Extraction

Leaves of sugar beet genotypes were collected for DNA extraction. DNA extraction was done by the method CTAB according to (Doyle and Doyle 1990). The samples were dried by liquid Nitrogen.

CTAB buffer (100Mm Tris, 1.4 M NaCl, 20 Mm EDTA, 2% (w/v) CTAB (hexa- decyltrimethy lammonium bromide) and 1%- (Mercaptoethnol) were added to samples (incubated in water bath 65˚C prior to use). Samples were put in water bath for 30 min. After that, 1 volume of phenol : Chloroform : Isoamylalcol (PCI) was added, mixture by reversing the tubes moderately, centrifuge at 12000 rpm at 22˚C for 10 min, the higher layer were occupied in new tubes, 1 volume Chloroform : Isomamylalcol (24:1 v/v) was added, mixture the tubes moderately, centrifuge with the last circumstance. The higher layer were collected in a new eppendorf, 2/3 volume of ice cold Isopropanol and 1/10 volume of 5M ammonium acetate was added, Samples were left in fridge for 1 h to overnight. Samples were centrifuged for 15 min at 4˚C (12,000 rpm), bolt the supernatant, 70% ETOH were added to pellet, centrifuged for 2 min.

Samples were saved in the incubator until there was no ETOH, 50 ml of water were added and saved on the incubator for 10 min to melt all DNA in the water. 1 ml of RNase were added to all tube .Samples were kept at -20˚C.

5- Primer design

Three specific primers were designed in this study to search for specific three genes known as resistance to nematodes. The three genes were (*Hs1pro-1* and *HSPRO2*) discusses resistance to beet cyst nematode (*Heterodera. Schachtii*) from (*Beta procumbens* and *Beta vulgaris*) respectively and (*Mi-1.2*) gene confer resistance to root knot nematode (*Meloidogyne* spp.) from tomato (*Solanum lycopersicum*), Smith (1944).The specific primers used in this investigation were designed using the National Center for Biotechnology Information (NCBI) data Base (http://www.ncbi.nlm.nih.gov/gene/) according to the sequences listed in Table (3).

6-Molecular analysis

Enzymatic amplification by PCR was done using Taq DNA polymerase. The 25 µl reaction mixture consisted of master mix H2O 14 µl, buffer 1.5 µl,Mgcl2 0.5 µl ,dNTPs 0.5 µl ,Tag 0.5 µl (5000 unit) and 1ml of each primer.

Table 3. Primers and PCR programmers for three genes.

Gene Symbol	Gene bank Accession Number	Description	Product size (pb)	Primer sequence	PCR conditions
HS1Pro-1	U79733	Beta procumbens nematode resistance	941	Forward 5′ GGTACCCTACGCGGTTGAATCTGC3′ Reverse 5′ TCTAGACATTACTCAGCCGAGTCAG3′	1 cycle at 95 c˚ for 5 min ,30 cycles at 95c˚ for 45s,annealing at 76c˚ for 30s,72c˚ for 30 s and a final extension at 72 c˚ for 5 min
HSPRO2	LOC104903543	nematode resistance protein- [*Beta vulgaris subsp. vulgaris*]	474	Forward 5′ CATGCCGTTGAAGTGGACCC 3′ Reverse 5′ TAACCCACGTCTCACCAACG 3′	1 cycle at 95 c˚ for 5 min ,30 cycles at 95c˚ for 45s,annealing at 60c˚ for 30s,72c˚ for 30 s and a final extension at 72 c˚ for 5 min
Mi-1.2	AF039682.1	root-knot nematode resistance protein	262	Forward 5′ AGTATTTGTGGCGACCTCATGT3′ Reverse 5′ ACACGGCCTAGCTTTTGTGA 3′	1 cycle at 95 c˚ for 5 min ,30 cycles at 95c˚ for 45s,annealing at59,9 c ˚for 30s,72c ˚for 30 s and a final extension at 72 c˚ for 5 min

RESULTS AND DISCUSSION

1-Preliminary screening for Root-Knot Nematode (*Meloidogyne incognita*) resistance to sugar beet genotypes tested:-

The resistance and susceptibility of 17 sugar beet genotypes was measured by gall index (GI) as an indicator for plant damage, and host efficiency (RF) as an indicator for nematode reproduction according to Quantitative system for assignment of Canto-Saenz's host factor suitability (resistance) (Canto-Saenz 1983).

The result of the varietal assessment is presented in (Table 4). Four genotypes of the sugar beet tested; BTS 301, Melode, Sultan and Mi-3 were found resistant. These genotypes did not support nematode reproduction (RF ≤1), and root damage by them was minimal (GI ≤2). The following category of genotypes reaction implies three genotypes that were found moderately resistant; C39, Eg.6 and Eg.26. In these genotypes, although nematode reproduction was permitted (RF≤1), damage was equal (GI≈2). Sugar beet genotypes; Beta 382, Beta 401, Rizoble, Farida, Athos poly and Maripella .supported relatively high nematode reproduction (RF >1) with fairly plant damage (GI≤2) and was therefore rated as tolerant. The subsequent category implied two sugar beet genotypes; BTS 302 and SP-022 supported nematode reproduction factor (RF >1) with high plant damage (GI >2) and was therefore rated as susceptible. While, there were two sugar beet genotypes; Helios poly and Nada did not supported nematode reproduction (RF≤1) but with very high plant damage (GI>2) and was therefore rated as hyper susceptible.

The result of the varietal assessment indicated that some of the sugar beet genotypes tested was resistant suggesting that these materials resistance to root-knot nematodes, *Meloidogyne spp.* could be a good materials as

parent stock for sugar beet breeding program, and in crop rotation schemes since they will not encourage nematode build up so that high yielding but susceptible crop genotypes could profitably be cultivated thereafter. They could also be used in localities with high incidence of root-knot nematodes. Some other genotypes were also found to be tolerant suggesting that despite the load of nematodes; these genotypes still possess the potential for high yield and internal mechanisms for compensating for the effect of the nematodes. These genotypes are desirable since most of our agricultural soils in newly reclaimed area harbor large nematode populations. However, it is recommended that resistant or non-host crops should follow these genotypes in a rotation scheme. This is to bring down the nematode population that had built up under the tolerant sugar beet genotypes. A susceptible variety is not encouraged as an intercrop.

Table 4. Host Suitability of sugar beet genotypes (*Beta Vulgaris Saccharifera*) tested for root-knot nematode, *M. incognita.*

Genotypes reaction	Sugar beet varieties	Code	Root gall index* (GI)	J2/250 cm3 of Soil	R-factor host efficiency** (RF)
Resistant	BTS 301	1	1.4	160	0.4
	Melode	5	1.3	120	0.3
	Sultan	6	1.9	350	0.9
	Mi-3	13	1.5	240	0.6
Moderately Resistant	C39	12	1.9	361	0.9
	Eg.6	16	2.0	397	1.0
	Eg.26	17	2.0	377	0.9
Tolerant	Beta 382	3	1.7	960	2.4
	Beta 401	4	2.0	920	2.3
	Rizoble	8	1.9	880	2.2
	Frida	9	1.8	921	2.3
	Athos poly	10	1.8	1042	2.6
	Maripella	11	2.0	800	2.0
Susceptible	BTS 302	2	3.6	1040	2.6
	SP-022	14	4.2	1650	4.1
Hyper susceptible	Helios poly	7	5.0	280	0.7
	Nada	15	4.6	440	1.1

***Gall Index Scale: 0 = 0 galls; 1 = 1 - 2 galls; 2 = 3 galls; 3 = 11 – 30 galls; 4 = 31 -100 galls; 5 = 100 + galls.**

****The R factor is calculated as the middling final egg count distributed by 400 eggs (number of eggs with which every pot was inoculated nearly 400 eggs 250 cm3soils).**

2- Molecular detection

Conventional PCR

Currently there are numerous approaches developed biotechnologically for nematode resistance, PCR with a species-specific primer can be used positively for species variation and establishes a main step forward in rising DNA diagnostics. This methodology allows detecting one or a number of nematode species by using a single PCR test and reductions the diagnostic time and costs (Subbotin *et al*., 2001). DNA markers can be useful tools for selecting resistant genotypes and can save the evaluation time and improve the precision (Norouzi 2003).

Three genes Known as nematode resistant genes were examined in this study to select resistant sugar beet genotypes by conventional PCR analysis. These genes were (*Hs1pro-1*and *HSPRO2*) confer resistance to beet cyst nematode (*H.* schachtii) and (*Mi-1.2*) gene confer resistance to root knot nematode (Yu, *et al*, 1999).

HS1Pro-1 GENE

The data indicated that the resistance gene *HS1pro-1* gene was found in nine genotypes{1-3-4-5-8-10-11-12-17} and was absence in eight genotypes {2-6-7-9-13-14-15-16} showed in figure (1).

$Hs1^{pro-1}$ gene, which was isolated from the wild species of sugar beet (*Beta procumbens*) has been proven to confer resistance to beet cyst nematode (Cagle *et al* 2014).in this work the gene cleared resistant to root knot nematode spices (*Meloidogyne incognita*).

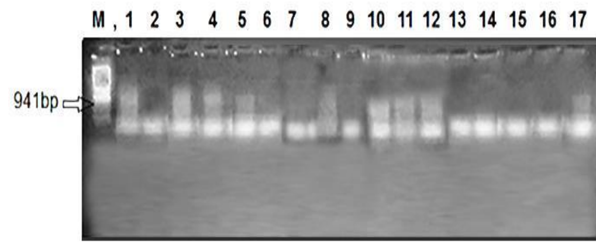

Figure 1. PCR for specific gene *HS1Pro-1* which indicate 941 bp, for genotypes from 1-17.

HSPRO2 GENE

After the discovery of $Hs1^{pro-1}$ in sugar beet, orthologs of this gene were identified in several other plant species. The Arabidopsis (*Arabidopsis thaliana*) genome encodes for 2 homolog's of *B. procumbens* $Hs1^{pro-1}$gene HSPRO1 and HSPRO2 and these two genes have been categorized as general stress signaling genes (Baena-González and Sheen, 2008).

The second investigated gene (HSPRO2) which indicate 474bp, was presence in twelve genotypes {1-3-4-5-6-8-9-10-11-12-16-17} and was absence in five genotypes {2-7-13-14-15} showed in figure (2).

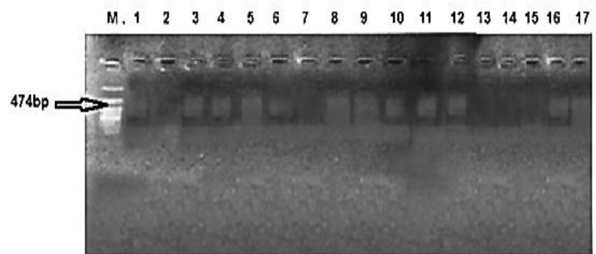

Figure 2. PCR for specific gene *HSPRO2* which indicate 474 bp , for genotypes from 1-17.

Mi-1.2 GENE

Root-knot nematode resistance gene *Mi-1.2* proved that it was resistance to root knot nematode (*Meloidogyne incognita*), which indicates 262 bp. The data showed that this gene was presence in seven genotypes {1-3-5-9-11-13-16} and was absence in ten genotypes {2-4-6-7-8-10-12-14-15-17} Figure (3). *Mi-1.2* gene was found in *M1-3* line (Reg. no. GP-221, PI 628749) was advanced by the USDA-ARS, Salinas (Yu 2002). M1-3 line be responsible for resistance to root-knot nematode that may be suitable as resistance source for breeding program.

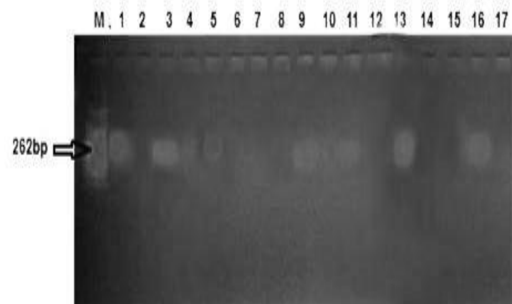

Figure 3. PCR for specific gene *Mi-1.2* which indicate 262 bp , for genotypes from 1-17.

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التفرقة بين التراكيب الوراثية لنباتات بنجر السكر المقاومة للنيماتودا (تعقد الجذور) بإستخدام المعلمات الوراثية

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يمكن ان يساعد تطبيق الطرق الجزيئية كوسائل تكميلية أز بديلة فى تحديد النباتات اليى تحتوى على جين المقاومة فى المستوى الجينى لذلك ،يمكن أن تكون معلمات الحمض النووى أدوات مفيدة لاختبار الطرز الوراثية المقاومة ويمكن أن تقلل وقت التقييم وتحسن من دقة التقييم . التحاليل الجينية المكثفة كانت مثمرة وقد تم تحديد السلالات الوراثية المقاومة للنيماتودا بواسطة تفاعلات أنزيم البلمرة المتسلسل (PCR). يعتبر نبات بنجر السكر واحد من أهم المحاصيل فى العالم لإنتاج السكر والذى يأتى فى المرتبه الثانية بعد قصب السكر، حيث انه يساهم بنسبة ٢٠% من إنتاج السكر فى العالم سنوياً. ويزرع بنجر السكر سنوياً فى مصر على مساحة تصل إلى ٥٢٣١٨٨ فدان مع متوسط إنتاجية للفدان تصل إلى ٢٠.٧ طن جذور للفدان. وحديثاً تم الأتجاه إلى زراعة بنجر السكر فى منطقة النوبارية والبستان حيث حقق زراعة بنجر السكر نجاح كبير فى هذه المنطقة وأعتبرت أنها الأمتداد الأمثل لزراعة بنجر السكر فى مصر. ولكن يعتبر أصابة مثل هذه المناطق بنيماتودا تعقد الجذور هو التحدى الأكبر لإنتشار وأستمرار زراعة بنجر السكر فى مثل هذه المناطق الجديدة، حيث سجلت نيماتودا تعقد الجذور (ميلودوجين إنكوجينيتا وميلودوجين جافينيكا) على أنها الأكثر أنتشاراً فى هذه المنطقة. وللتغلب على هذه المشكلة الكبيرة فإن الإتجاه إلى أستخدام أصناف مقاومة للنيماتودا سيكون هو الحل الأمثل. أجرى هذا البحث فى محطة بحوث الصبحية بالإسكندرية فى موسم (٢٠١٥- ٢٠١٦) حيث تم زراعة ١٧ طراز وراثى من بنجر السكر وذلك لمعرفة الطرز الوراثية المقاومة لنيماتودا تعقد الجذور (ميلودوجين إنكوجينيتا) التى تعتبر ذات أنتشار واسع فى الاراضى الجديدة مثل النوبارية و البستان. ونتيجة لأختبار درجة مقاومة هذه الطرز الوراثية السبعة عشر لنيماتودا تعقد الجذور (ميلودوجين إنكوجينيتا) قسمت هذه التراكيب الوراثية إلى خمس مجاميع تبعا لدرجة مقاومتها وهى: ١-المجموعة الاولى "مقاومة" والتى أحتوت على ٤ طرز وراثية ٢-المجموعة الثانية "متوسطة المقاومة" أحتوت على ٣ طرز وراثية ٣-المجموعة الثالثة "متحملة" أحتوت على ٦ طرز وراثية ٤-المجموعة الرابعة "الحساسة" أحتوت على طرازين وراثين ٥-المجموعة الخامسة "شديدة الحساسية" أحتوت على طرازين وراثين. ونتيجة لأختبار تكنيك تفاعلات أنزيم البلمرة (PCR) أظهر أن بعض الطرز الوراثية تحتوى على جينات Hspro2 &Hs1pro-1 والمعروفة بمقاومتها لنيماتودا الحويصلات المنتشرة فى الأراضى الأوربية (والتى سجلت على بنجر السكر فى مصر فى الفترة الاخيرة) وأثبتت هذه الطرز الوراثية مقاومتها لنيماتودا تعقد الجذور، كما أن السلالة النقية (Mi-3) المعروفة بمقاومتها لنيماتودا تعقد الجذور والتى أستخدمت فى هذا البحث كأختبار وجد أنها تحتوى على الجين (Mi-1.2) المعروف بمقاومته لنيماتودا تعقد الجذور.