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 Nubaria 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 Hspro-1and HS5PRO2 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 raw 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 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-Manahly 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 et al., 1999;El- Nagdi et al.,2004;Karayem,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 Nubaria district (Ibrahim ,1982; Dtifa and El-Gindi,1982;Abd-El –Massih et al.,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 Nubaria 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 varieties 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). 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 varieties 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 *et al.*, 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 *HSMPRO2*) discusses resistance to 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:

- Twelve sugar beet commercial varieties (BTS 301, BTS 302, Beta 382, Beta 401, Melode, Sultan, Rizoble, Frida, Athos poly, Maripella, Helios poly, and Nada)
- Three sugar beet inbred lines (Mi-3, C39 and SP-022)
- 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; Donkey 1995; Lewellen, 1995). Mi-3 line was classified as resistant to Meloidogyne spp (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 sablia 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 inoculated with 4000 eggs of *M. incognita* per pot (approximately 400 eggs per cm$^2$ 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 inoculation. The plants were watered and 15 g of multiple fertilizer (15:15:15) was added at the three weeks old plants.

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

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$^2$ 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 cm$^3$ of soil nearby each plant was collected in field and/or green house conditions and these were inculcated with 4000 eggs of *H. schachtii* per pot (approximately 400 eggs pre cm$^2$ soil) according to (Hartman and Sasser 1985) and Maareg 2009. Inoculum was dispersed 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 inoculation. The plants were watered and 15 g of multiple fertilizer (15:15:15) was added at the three weeks old plants.
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 (Nwaazor, 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 | Host efficiency (R-factor) x Plant Damage | Gall index
|----------------------|-------------------------------------------|--------------
| Resistant (R)        | ≤1                                        | ≈2           |
| Moderately Resistant (MR) | ≤1                                        | ≤2           |
| Tolerant (T)         | >1                                        | ≤2           |
| Susceptible (S)      | ≥1                                        | >2           |
| Hyper susceptible (HYS) | ≥1                                        | >2           |

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 (100 M M Tris, 1.4 M NaCl, 20 Mm EDTA, 2% (w/v) CTAB (hexa- decyltrimethyl ammonium bromide) and 1% (Mercaptoethanol) 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 : Isoamy nalcol (PCI) was added, mixture by adding the tubes moderately, centrifuge with the last tubes, 1 volume Chloroform : Isomamylylacol (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 1 ml of each primer.

Table 3. Primers and PCR programmes for three genes.

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Gene bank Accession Number</th>
<th>Description</th>
<th>Product size (pb)</th>
<th>Primer sequence</th>
<th>PCR conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>HS1Pro-1</td>
<td>U79733 Beta procumbens nematode resistance</td>
<td>941</td>
<td>Forward 5’</td>
<td>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</td>
<td></td>
</tr>
<tr>
<td>Hspro2</td>
<td>LOC104903543 Beta procumbens nematode resistance protein [Beta vulgaris subsp. vulgaris]</td>
<td>474</td>
<td>Forward 5’</td>
<td>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</td>
<td></td>
</tr>
<tr>
<td>Mi-1.2</td>
<td>AF030682.1 root-knot nematode resistance protein</td>
<td>262</td>
<td>Forward 5’</td>
<td>1 cycle at 95 c˚ for 5 min, 30 cycles at 95c˚ for 45s, annealing at 59c˚ for 30s, 72c˚ for 30 s and a final extension at 72 c˚ for 5 min</td>
<td></td>
</tr>
</tbody>
</table>

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 Qualitative 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.

Some other genotypes were also found to be tolerant and non-host crops should follow these genotypes in a rotation scheme since they will not encourage 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.

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

**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 cm3 soils).

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).

**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 HS1Pro-1) 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 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).

Hs1pro-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 species (Meloidogyne incognita).

**HSPRO2 GENE**

After the discovery of Hs1pro-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 Hs1pro-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).

**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 MI-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.
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REFERENCES


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**The equation in Arabic script is not legible and cannot be accurately transcribed.**