Egypt. J. Chem. **Vol. 66**, No. 8. pp. 519 - 529 (2023)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Pathological Screening and SDS-PAGE Analysis of total Protein of Chilli Pepper Cultivars to Meloidogyne incognita

Mostafa Sayed Mostafa El-Ansary[1], Kamal Fouad Abdellatif [1], Ragaa Abdelfattah Hamouda[2], Waleid Kottb Hegazy[1], Adel El Sayed Hegazy[1], Mohamed El Sayed Abd All[1]

[1] *Department of Plant Biotechnology, Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City, Sadat City, Egypt.*

[2]*1Department of Biology, College of Sciences and Arts Khulais, University of Jeddah,*

Abstract

Chilli pepper (Capsicum annuum L.) is an economically important crop — relatively exclusive to Egypt— that shows moderate and high levels of susceptibility to several pests, including the pepper root-knot nematode, (RKN) Meloidogyne incognita. In this study, M. incognita succeeded in reproducing on all the pepper cultivars such as Balady Mecca, 292, Top Star, Dolma H54, and Lama Star. However, the Lama Star cultivar sustained the highest number of juveniles2 in soil, roots, and the total final number as well the rate of nematode build-up, 3.18 times (highly susceptible, HS). On the other four cultivars the nematode developed and reproduced relatively moderate susceptibility (MS) when compared with the others. With regard to plant health, Dolma H54 was the most susceptible cultivar, while Balady Mecca was the least affected one. Notably, shoot weight was reduced in cultivars infected with nematode when compared with the healthy ones. For instance, the percentages of shoot weight reduction ranged between 50.1 to 67.8 %, cv. Balady Mecca, recorded the lowest reduction while cv. Dolma H54 achieved the highest one. SDS-PAGE for the total storage protein of the current pepper cultivars (control and nematode-treated) has been performed. Current results suggest that nematode infection may trigger some gene expression (appears at molecular weight of about 60 kDa), causing the cultivars 'Balady Mecca', 'Dolma H54', '292' and 'Top Star' to exhibit MS to the nematode infection. While the protein pattern of the cultivar 'Lama Star' indicates that it might be HS for RKN infection.

Keywords: Capsicum annuum, Meloidogyne incognita, Susceptible, Plant health, SDS-PAGE, Protein.

1. Introduction

The pepper crop production has found to be declined in modern years; mostly due to the range of biotic agents affecting pepper plants is very broad and includes viruses, bacteria, fungi, nematode, and insects [1]. With regard to plant-parasitic nematode (PPNs), especially root-knot nematodes (RKNs), *Meloidogyne* spp. are obligate phyto-pathogens causing several economic losses in many crops including *Capsicum* species [2,3]. For instance, eight lines of pepper (*Capsicum annuum* L.) has resistance to root-knot nematode (RKN), *Meloidogyne* spp. The french cultivar "Doux Long des Landes" was susceptible to all RKNs tested. Also, the other seven pepper cultivars were highly resistant to *M. javanica*, *M. arenaria* and one population of *M. hapla*. Whereas, only lines "PM687", "PM217", "Criollo de Morelos334", and "Yolo NR" were resistant to *M. incognita* [4]. In addition, some cultivars of pepper plant, i.e., "Pusa Jwala", "Pusa Sadabahar", "PC-1", "Mathania Local", and "Jaipur Local" were examined against RKN, *M. incognita* infection. However, the cultivar "Pusa Jwala" was measured as moderately resistant with the galls number and cultivar PC-1 was highly susceptible increasing the number of galls. All the other cultivars displayed variable degree of variability between "Pusa Jwala", and "PC-1" [5]. Buena et al., [6] reported that some pepper cultivars such as Spain and Uruguay, were examined with the objective of appraising its potential to the response of *C. annuum* against RKN, *M. incognita* be included in

*Corresponding author e-mail: ragaa.hamouda@gebri.usc.edu.eg.; (Ragaa Abdelfattah Hamouda).
Received date 02 January 2022; revised date 20 January 2022; accepted date 27 January 2022
DOI: 10.21608/ejchem.2022.114271.5197

the plant breeding programs as a source of resistance genes. For instance, Cuarentino variety was resistant to 13.5% of the Spanish varieties, but was not resistant to the Uruguayan varieties of nematode. No statistical differences in susceptible, moderately resistant, resistant, and plant frequencies among the four biotypes and races of the *M. incognita* was noticed. Furthermore, resistance to nematode was detected in six of the examined lines from thirty two lines while 8 cultivars were highly susceptible to the RKNs, *M. incognita* with significantly high number of eggs, juveniles2 (J2s) and root nematode populations. Plant biomass was significantly low at 5% level of probability in susceptible plants. Other varieties revealed varying degrees of virulence to the pepper parasitic-nematodes [7]. Thirty-nine lines of the "Universidade Estadual do Norte Fluminense Darcy Ribeiro" (UENF) gene bank were appraised, being "twelve accessions of *C. annuum*, 11 *C. chinense*, 10 *C. baccatum*, and 6 *C. frutescens*". The trial were significant differences among the lines tested in relation to the traits estimated, representing the existence of variability of resistance among and within *Capsicum* species. In addition, UENF 1730, *C. chinense* accession was reflected resistant against RKN, *M. enterolobii* [8]. The aim of this research was to appraise the resistance of "Huacle and Serrano" pepper cultivars to different RKNs, *M. incognita*. However, except for lines 34-3, 35-5, and 42-2, whereas all the others were resistant to *M. incognita* [9]. Four pepper varieties i.e., California wonder, Boulnder A, Marconi, and Maor were evaluated to RKN, *M. javanica* at inoculum level (1000 J2s) in *in vivo*. However, pepper susceptible cv. Moar played to be the most favorable and susceptible to *M. javanica*. Whereas resistant cultivar "Boulnder A" against *M. javanica* number; egg-masses, and galls number supported the least (1698; 11.7 and 8.0, respectively). Also, the plant growth health enhanced over the rest cultivars [10].

In this research, we investigated SDS PAGE analysis from disease resistant and susceptible of pepper cultivars, *C. annuum* infected by RKNs to detect as a biochemical markers that might be used to improve effective management methods against *M. incognita*.

2. Materials and Methods

2.1. Pure nematode isolation

Eggs of root-knot nematode (RKN), *Meloidogyne incognita* were obtained from tomato (*Lycopersicon esculentum* cv. Castle Rock) roots infected with the RKN using 0.5% NaOCl solution [11]. Second stage juveniles2 (J2s) were collected daily from eggmasses and were stored at 15 °C. The larveal used in the study were less than 5 day-old.

2.1.1. Nematode susceptibility test

Commercially pepper (*Capsicum annuum* L.) verities such as, Balady Mecca (Local line), Dolma H54 and 292 were supplied from seeds Company (MECCA Trade, Company Egypt from Indian origin, NAMDHARI Seeds Co.). While, Top star (Japanese origin, TAKII Seeds) was supplied from seeds Company (SEIF GAARA Seeds Co., Egypt). Lama star (China origin, Ergon Seeds Co.) was supplied from seeds Company (Agrolimited Co., Egypt) for RKNs, *M. incognita* susceptibility tests in *in vivo* (Table, 1). Plantlets were placed in plastic pots (30 cm diameter) already filled with 4.5 kg of sterilized soil (1:3; clay: sand ratio) with a final pH= 7.0. The temperature of the soil was maintained at 28±2 °C during the whole trial period. Seeds were planted in nursery from 40 days ago then transplanted in greenhouse of Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City (USC), Minoufiya Gov., Egypt. Each cultivar was replicated five times in pots. Twenty five pots were inoculated with 3000 active J2s per pot at the time of seedling transplantation. Also, 25 pots (from the same cultivars) were non-inoculated with RKN as a control free. All the pots were placed in a completely randomized design in *in vivo* conditions. Plants were collected after 45 days of inoculation. The roots were washed carefully with tap water to remove the soil fine particles and stained with synthetic “Phloxine B” solution (3.5 g in 750 mL distilled water + 250 mL acetic acid 5%) for 5 min to facilitate the counting of all nematode phases in the root. Nematode variables were noted per root including galls, immature stages J3&J4, females, females with egg-masses, and rate of build-up. The second stage juveniles, J2s per 250 g soil was extracted and calculated according to Cobb’s sieving and decanting method, using sieves (20 meshes, 60 meshes and 325 meshes). The pepper growth health such as shoot length and weight, root length and weight and leaves number were measured and documented.

Notable, the degree of root galling on each infected plant was rated according to a modified rating scale of Taylor and Sasser [12]; 0= 0 gall, resistant; 1= 1-2 galls, moderately resistant; 2= 3-10 galls, moderately susceptible; 3= 11-30 galls, susceptible; 4= 31 galls and above, highly susceptible.

Table. 1. Pepper cultivars and their control applied for RKNs infestation

Cultivars	Infected plants	Control (Check without nematode)
Local (Balady Mecca)	A	A'
Dolma H54	B	B'
292	C	C'
Top Star	D	D'
Lama Star	E	E'

2.2. SDS-PAGE procedure

Fresh leaves samples of the five peppers cultivars have been collected from treated plants (45-days post infection by RKNs, *M. incognita* and their control). All samples squeezed in liquid nitrogen to fine powder and then were used for total protein content isolation according to the method of Laemmli, [13]. Sodium Dodecyle Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) method was used to study the protein banding patterns of the cultivars before and after infection. The protein patterns of the cultivars were compared using SDS-PAGE method to study the genetic diversity of the pepper genotypes and to identify the protein bands associated with nematode resistance in chilli pepper. Protein gels were photographed with digital camera and handled with Adobe Photoshop Software in order to adjust the contrast and the brightness. Comparison has been made between cultivars before and after infection with RKN to detect specific bands correlated with nematode resistance. The protein bands were scored as ''1'' for the presence and ''0'' for the absence of bands. However, specific bands have been determined for specific cultivars. Cluster analysis has been made for the collected data of protein pattern bands of different cultivars. A dendrogram was generated from the similarity matrix using the unweighted pair group method with arithmetic in NTSYSpc 2.0 software [14].

2.3. Statistical analysis

All data were subjected to analysis of variance (ANOVA), [16]. Significance of the variable mean differences was prescribed using Duncan's multiple range tests ($p \leq 0.05$). All data analyses were conducted using SPSS 16 software.

3. Results

3.1. Greenhouse studies

3.1.1. Differential response of some pepper cultivars, C. annuum to RKN, M. incognita

Five cultivars of pepper, *C. annum* were tested for their susceptibility to the infection with RKN, *M. incognita*. Data on the nematode reproduction and the plant growth response were collected and presented in tables (2&3). In general, the data show that RKN developed and reproduced well on all the pepper tested cultivars, whereas Lama Star cultivar sustained the highest number of juveniles (J2s) in soil and roots and consequently, the total final number as well the rate of nematode build-up (Highly susceptible, HS). In addition, it has the highest number of the mature females in root. Therefore, the nematode multiplied very well on such cultivar and folded 3.18 times at the end of the trial (Table, 2). In contrast, Dolma H54 cultivar obtain relatively high numbers of the nematode immature J3s stages (39.4) and came after Top star cultivar in counts of the nematode total number (5673.8). Thus, the nematode folded as much as 1.89 times on this cultivar. On the other two cultivars (Balady Mecca and 292) the nematode developed and reproduced relatively (moderate susceptible, MS) when compared with the others. For example, on 292 the nematode multiplied 1.93 times and Balady Mecca cultivars the nematode reproduced 1.59 times. Notable, Balady Mecca cultivar obtain relatively low numbers of the nematode final population (4762.6**),** fig., 1.

Table. 2. Reproduction and build-up of *M. incognita* on roots of five pepper cultivars

Cultivars	Galls	Embedded stages	Females	Egg-masses	Final*** population	Rate of Build-up****	Host reaction
A Balady Mecca	277.4 ab	84.2 a	142 a	208.4 a	4762.6 a	1.59 a	MS*
B Dolma H54	342 ab	79.6 a	196 b	232 ab	7087.6 b	2.36 b	MS**
C 292	214 a	61.8 a	275.2 c	226 ab	5732.8 ab	1.93 ab	MS*
D Top Star	282 b	79.8 b	254 bc	324 b	5673.8 ab	1.89 ab	MS*
E Lama Star	294 c	85.2 c	370 d	314 b	9525.2 b	3.18 b	HS*

Means followed by the same letter (s) within a column are not significantly different ($p \leq 0.05$) according to Duncan's multiple range test.
MS*= Moderate susceptible, **HS****= High susceptible
***Final population including= developmental stages + females + females with egg-masses + juveniles number in soil.
****Rate of build-up = pf (final population /initial population) Norton, [17].

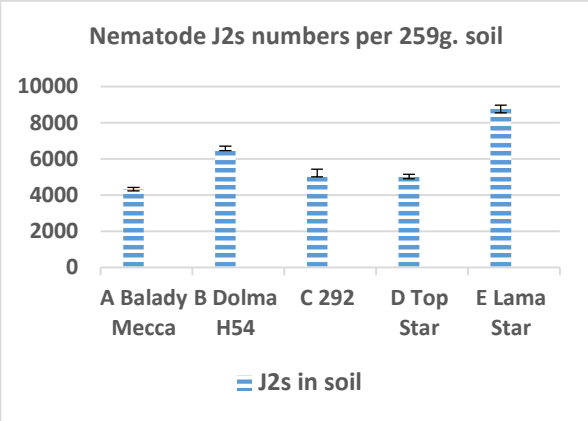

Fig. 1. Effect of some pepper verities on development of Juvenile2 numbers in soil and total populations per plant.

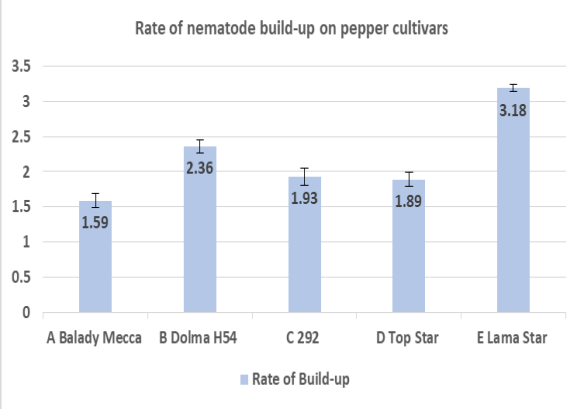

Fig. 2. Rate of nematode build-up, *M. incognita* infecting some pepper cultivars.

3.2. Plant health response

Growth health response of the five pepper verities to the RKNs, *M. incognita* infection was also appraised. Growth criteria as represented by lengths and weights of both shoots and roots, as well the number of leaves were recorded in table (3). Notable, it is valuable to notice that all the pepper growth parameters of the all cultivars affected by *M. incognita*. For example, Dolma H54 was the most susceptible cultivar, while Balady Mecca was the least affected one. However, the nematode affected the shoot, root and weights, whereas variable host reactions were noticed among the five tested cultivars. With regard to, shoot weight was reduced in cultivars infected with nematode when compared with the healthy ones (Fig. 3). For instance, the percentages of shoot weight reduction ranged between (50.1 - 67.8 %), as the lowest reduction was belong to cv. Balady Mecca, while the highest one was for cv. Dolma H54 (Fig., 3).

Accordingly, the tested pepper cultivars could be rated as moderate susceptible hosts, MS (Balady Mecca, 292, Top Star and Dolma H54) or highly susceptible hosts, HS (Lama Star).

Table. 3. Plant growth response of five *capsicum* cultivars to the infection with *M. incognita* comparing with the control without nematode

Cultivars	State	Shoot weight	Shoot length	Root weight	Root length	Leaves number
A Balady Mecca	Infected	5.824	17.4	4.214	13.6	27.2
A' Control	Healthy	8.694	21.4	3.226	17	21.6
LSD 0.05*		0.228*	0.008	0.217*	0.121*	0.078*
B Dolma H54	Infected	4.138	20.4	4.694	9.4	12.2
B' Control	Healthy	6.93	22.6	2.016	10	15
LSD 0.05*		0.454*	0.788*	0.004	0.195*	0.072*
C 292	Infected	6.3	18.6	4.344	10.2	12
C' Control	Healthy	1.33	23.8	3.35	14.8	20.6
LSD 0.05*		0.096*	0.120*	0.156*	0.059	0.97*
D Top Star	Infected	5.248	18.4	4.582	10.8	17.8
D' Control	Healthy	8.512	20.8	2.372	15.2	30.8
LSD 0.05*		0.059*	0.344	0.002	0.529*	0.124*
E Lama Star	Infected	2.834	10.2	4.202	9.6	13.2
E' Control	Healthy	6.05	15.8	2.042	20.4	22.2
LSD 0.05*		0.050*	0.104*	0.000*	0.103*	0.027*

*-Non significant ≥ 0.05 using one way analysis of variance (ANOVA) –significant ≤ 0.05 using one way analysis of variance

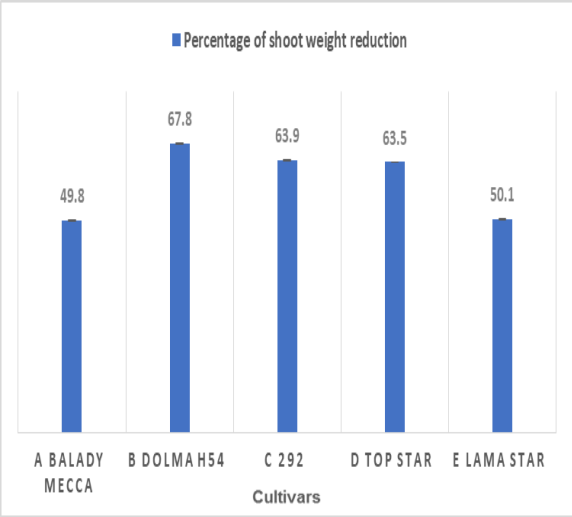

Fig. 3. Effect of root-knot nematode, *M. incognita* on percentage of shoot weight reduction of five *capsicum* cultivars.

3.3. Biochemical SDS-PAGE analysis

SDS-PAGE for the total leaf storage protein of the chilli pepper cultivars such as "Balady Mecca, Dolma H54, 292, Top Star, and Lama Star" (control and nematode-treated) has been performed. Using one-dimensional SDS-PAGE analysis, optical differences were obtained among the five cultivars in their protein patterns. Some protein bands were obtained in the protein pattern of the infected cultivars with RKN, *M. incognita* comparing their control cultivars. For example, a band at molecular weight of about 60 kDa was obtained in the pattern of the nematode infected (treatment) of the cultivars 'Balady Mecca', 'Dolma H54', '292' and 'Top Star'; where it does not appear in the control samples (nematode free) of the same cultivars. This band, however, appears in the protein pattern of both control and treated nematode samples of the cultivar 'Lama Star' (Figure, 4). On the other side, a band at molecular weight of about 25 kDa appears in the pattern of the cultivars 'Top Stars' and 'Lama Stars' (control samples), disappeared in the protein pattern of the same cultivars infected with nematode, and vice versa in the protein patterns of the cultivars 'Balady Mecca' and 'Dolma H54' (Figure, 4).

These results may suggest that nematode infection may triggers some gene expression (appears at molecular weight of about 60 kDa), causing the

cultivars ‘Balady Mecca’, ‘Dolma H54’, ‘292’ and ‘Top Star’ to exhibit moderately susceptible (MS) to the nematode infection. While the protein pattern of the cultivar ‘Lama Star’ suggest that it might be highly susceptible (HS) for nematode infection. Such band may be produced as a defense response from the plant against the nematode infection.

On the other hand, some protein bands may be affected by the nematode infection (such as the protein band at molecular weight of about 25 kDa) and causing the cultivars ‘Top Stars’ and ‘Lama Stars’ to be more susceptible to the nematode infection.

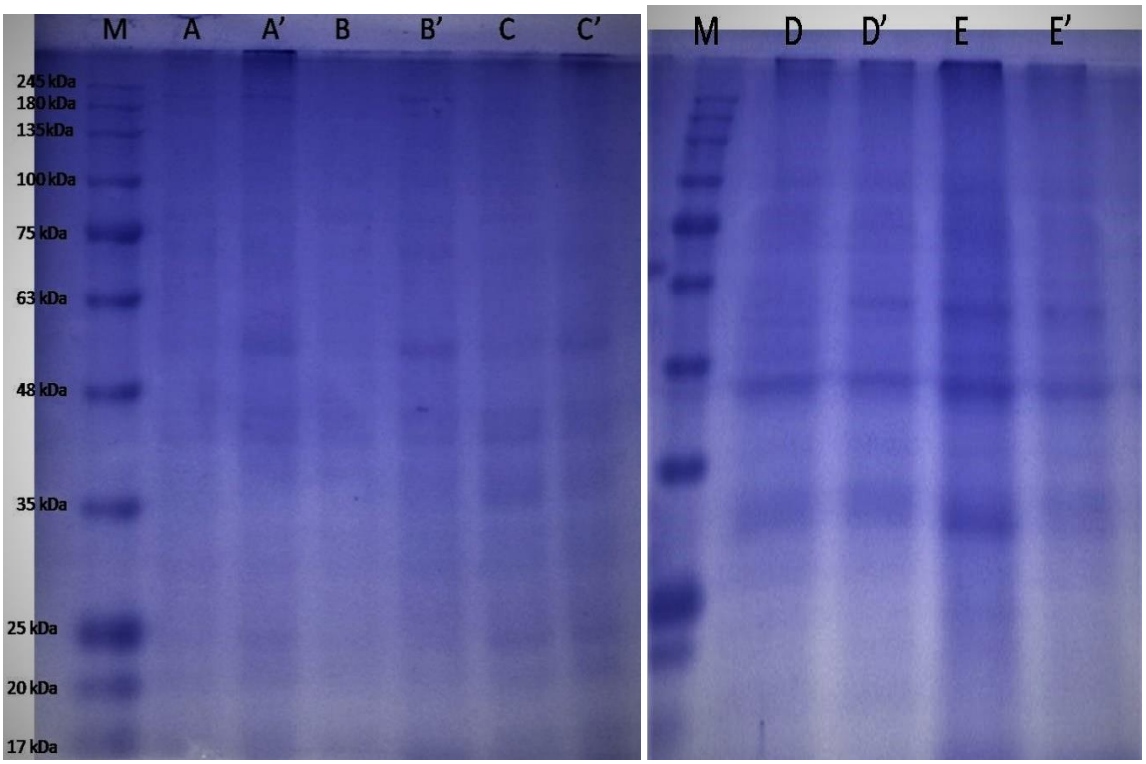

Fig. 4. SDS-PAGE of chilli pepper cultivars before and after nematode infection**.**

According to the cluster analysis of the scored biochemical (total protein separated on SDS-PAGE), the pepper knot-nematode cultivars and their controls (control without nematode) were separated into three clusters. From the above direction of the dendrogram, the first cluster included the control samples of the cultivars ‘Balady Mecca’ and ‘Dolma H54’. The second cluster contained the nematode treated samples of the cultivars ‘Balady Mecca’, ‘Dolma H54’ and ‘292’ along with the control samples of the cultivars ‘292’ and ‘Lama Star’. The third cluster (in the lower most part of the dendrogram) consisted of the treated nematode samples of the cultivars ‘Top Star’ and ‘Lama Star’ along with the control sample of the cultivar ‘Top Star’ (Figure, 5).

In general, the dendrogram results indicated that the treated nematode samples of the cultivars ‘Balady Mecca’, ‘Dolma H54’ and ‘292’ were clustered together in the middle of the dendrogram. The aggregation of these samples together suggests a common pattern among them concerning the bands at molecular weight of 60 and 25 kDa and reflex their interaction with RKN, *M. incognita* (plant growth parameters and reproduction of the nematodes). Thus, it seems that both pathological and biochemical results; are in contiguous and relatively gave almost the same results.

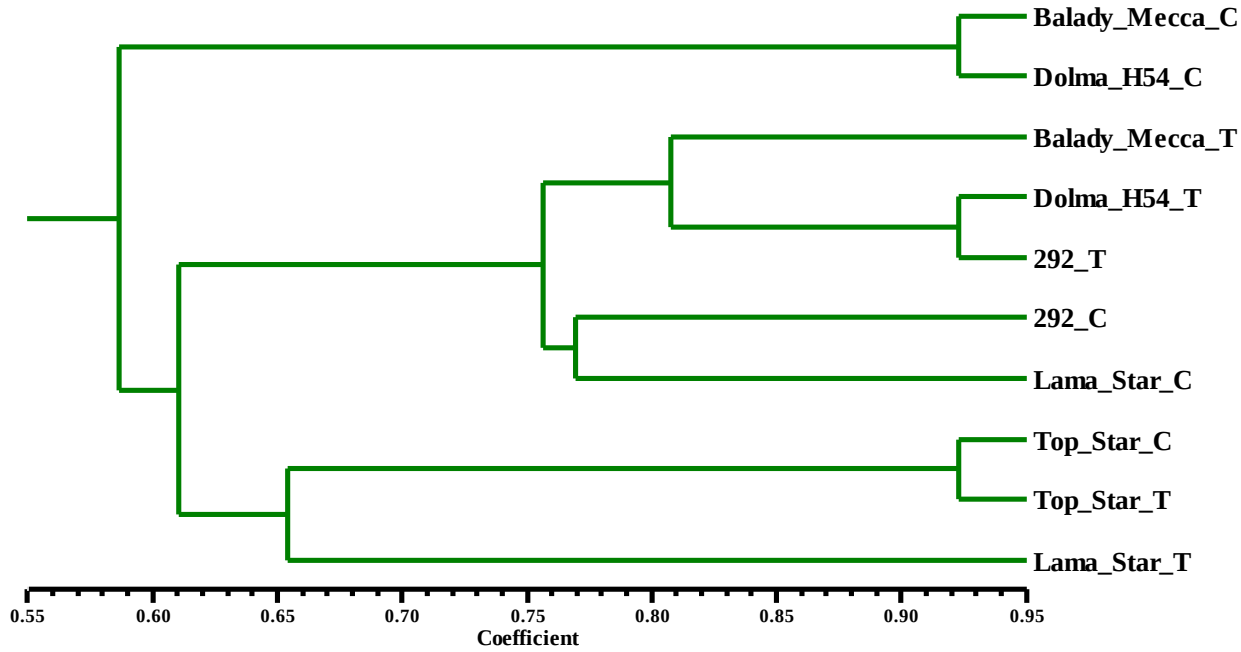

Fig. 5. Dendrogram of cluster analysis of the protein pattern of five pepper cultivars infected with RKN, *M. incognita*.

4. Discussion

4.1. Resistance evaluation

Pepper (*Capsicum annuum* L.) is one of the most important vegetable crop in the world, in terms of world production. Specially, *C. annuum* planted in newly reclaimed areas one located in Western, Eastern, and Southern deserts of Egypt has quickly been increased during the last years [18]. Unfortunately, such increment has been exposed to aggressive attacks by several pathogens and parasitic-nematodes. Plant-parasitic nematode (PPNs), especially root-knot nematodes (RKN), *Meloidogyne* spp. are obligate phyto-pathogens causing several economic losses in many crops including *Capsicum* species [3, 19; 20]. Relative variability of five pepper cultivars 'Balady Mecca', 'Dolma H54', '292', 'Top Star' and 'Lama Star' tested to the infection by *M. incognita* which was experimentally examined in *in vivo* trial. The nematode developed and reproduced well on all the pepper cultivars. Similarly, the aim of this research was to appraise the resistance of Huacle and Serrano pepper cultivars to different RKNs, *M. incognita*. However, except for lines 34-3, 35-5, and 42-2, whereas all the others were resistant to *M. incognita* [9]. Notable, Lama Star cultivar sustained the highest number of nematode juveniles2 (J2s) in soil and roots and consequently, the total final number as well the rate of nematode build-up (Highly susceptible, HS). In addition, it has the highest number of the mature females in root. Therefore, the nematode multiplied very well on such cultivar and folded 3.18 times at the end of the trial. On the other four cultivars such as, 'Balady Mecca', 'Dolma H54', '292' and 'Top Star' the nematode developed and reproduced relatively (moderate susceptible, MS) when compared with the others. Agaba and Fawole [21] study that host suitability of some pepper plant cultivars to *M. incognita*. Whereas, 4 pepper varieties "KNT 204, Tugantashi, Bird's Eye, and Bor-kono-Tsidif" (26.6%) were evaluated resistant, while 5 varieties "Ex-Sam-St, GHA, Prof-fintashi, NHV1F, and California Wonder" (33.3%) were appraised susceptible. For example, on '292' the nematode multiplied 1.93 times and 'Balady Mecca' cultivars the nematode reproduced 1.59 times. Notable, 'Balady Mecca' cultivar obtain relatively low numbers of the nematode final population (4762.6**)**. Root galling (RG) and knot-nematode reproduction was much higher on the 3 susceptible chilli cultivars. One of these susceptible pepper cultivars showed tolerance, as crops were not affected by the RKNs, but nematode reproduction was high [22, 23].

In general, it is concluded that RKNs, *M. incognita* is common pest in cilli pepper production, and that resistant varieties may provide a useful tool in a natural control strategy

4.2. Plant health assessment

Growth health response of the five pepper verities to the RKNs, *M. incognita* infection was appraised. Growth parameters as represented by lengths and weights of both shoots and roots, as well the number of leaves were also recorded. In *in vivo* tests, at constant temperatures between 28 and 30 °C, 'UFRJ107(6)A3' exhibited RKNs, *M. incognita* resistance superior to its parents such as, 'Jalapeno' and 'Round of Hungary', and to the standard resistant bell pepper plant 'Charleston Belle'. Therefore, offering the potential to improve specialty pepper growth and yield, and for use as a parasitic nematode-resistant rootstock for commercial bell *Capsicum* [24]. However, Dolma H54 was the most susceptible cultivar, while Balady Mecca was the least affected one. Hence, the nematode affected the shoot, root and weights. Variable host reactions were noticed among the five tested cultivars. However, pepper susceptible cv. Moar appeared to be the most favorable and more susceptible to *M. javanica* whereas, resistant cultivar (Boulnder A) against *M. javanica* population; egg-masses and galls number supported the least (1698; 11.7 and 8.0, respectively). Also, the plant growth health enhanced over the rest cultivars [10]. With regard to, the percentages of shoot weight reduction ranged between (50.1 - 67.8 %), as the lowest reduction was belong to cv. Balady Mecca, while the highest one was for cv. Dolma H54. The commercial pepper variety grafted on (AR-96023) produced a yield as great as the non-grafted cultivar in the *M. incognita*-free in *in vivo*. Some resistant cultivars and accessions used as rootstocks produced lower productions ($P < 0.01$) than that of the non-grafted cultivar in the check without nematode [25].

Generally, the tested pepper cultivars could be rated as moderate susceptible hosts, MS (Balady Mecca, 292, Top Star and Dolma H54) and highly susceptible hosts, HS (Lama Star).

4.3. Biochemical SDS-PAGE evaluation

SDS-PAGE for the total leaf storage protein of the chilli pepper cultivars such as "Balady Mecca, Dolma H54, 292, Top Star, and Lama Star" (control and nematode-treated) has been performed. Using one-dimensional SDS-PAGE analysis, optical differences were obtained among the previous cultivars in their protein patterns. The results may suggest that nematode infection may triggers some gene expression (appears at molecular weight of about 60 kDa), causing the cultivars 'Balady Mecca', 'Dolma H54', '292' and 'Top Star' to exhibit moderately susceptible to the nematode infection, while the protein pattern of the cultivar 'Lama Star' suggest that it might be highly susceptible for nematode infection. Such band may be produced as a defense response from the plant against the nematode infection. Maracahipes *et al.,* [26] mentioned that upon attack of pepper plant by certain pests, various PR proteins molecules are produced. They observed several low molecular weight proteins the treated samples. On the other hand, some protein bands may be affected by the nematode infection (such as the protein band at molecular weight of about 25 kDa) and causing the cultivars 'Top Stars' and 'Lama Stars' to be more susceptible to the nematode infection. Kumar and Tata [27] obtained twenty protein bands using SDS-PAGE to differentiate among pepper varieties. They stated that the molecular weight of the protein bands ranged between 18.6 to 72 Da and could be distinguished one Varity from the others. Also, Silva and Souza [28] reported that SDS-PAGE analysis indicated distinct band patterns in leaves and roots protein extracts obtained from pepper. According to the cluster analysis of the scored biochemical (total protein separated on SDS-PAGE), the chilli pepper cultivars and their controls were separated into three clusters. From the above direction of the dendrogram, whereas the cluster I included the control samples of the cultivars 'Balady Mecca' and 'Dolma H54'. The cluster II contained the treated nematode samples of the cultivars 'Balady Mecca', 'Dolma H54' and '292' along with the control samples of the cultivars '292' and 'Lama Star'. The cluster III (in the lower most part of the dendrogram) consisted of the treated nematode samples of the cultivars 'Top Star' and 'Lama Star' along with the control sample of the cultivar 'Top Star'. Mena et al., [29] reported that the sixteen (*Capsicum chinense* Jacq.) genotypes displayed considerable variation in protein band number ranging from (15-33). On the basis SDS-PAGE analysis (of total protein) the genotypes were grouped into two major clusters. The first cluster was further sub-

divided to subcluster whereas second cluster has only one genotype (CHFKC-14).

Generally, the dendrogram results indicated that the treated nematode samples of the cultivars 'Balady Mecca', 'Dolma H54' and '292' were clustered together in the middle of the dendrogram. The aggregation of these samples together suggests a common pattern among them concerning the bands at molecular weight of 60 and 25 kDa and reflex their interaction with RKN, *M. incognita* (plant growth parameters and reproduction of the nematodes). Peddakasim et al., [30] constructed dendrogram based on protein profiling score of chilli pepper cultivars using NTSYS software. Total sixteen genotypes were grouped into four clusters. The reported that highly diversified genotypes could be utilized for pepper breeding program.

Thus, it seems that both pathological and biochemical results; are in contiguous and relatively gave almost the same results. However, this study needs more advanced studies emphasizing on the gene expression of the plant proteins affected by the nematode infection that might be used to improve effective control methods against *M. incognita*.

5. Conclusions

Root-knot nematode, *Meloidogyne incognita* succeeded in increase and reproduce on all the tested pepper cultivars. Notable, Lama Star cultivar sustained the highest number of nematode in roots and juveniles (J2s) in soil and consequently, the total final number as well the rate of nematode build-up HS. In addition, it has the highest number of the mature females in root. Therefore, the nematode multiplied very well on such cultivar and copied 3.18 times at the end of the experiment. On the other four cultivars such as, 'Balady Mecca', 'Dolma H54', '292' and 'Top Star' the nematode developed and reproduced relatively MS when compared with the others. Growth health response of the previous pepper cultivars to the RKNs, *M. incognita* infection was assessed. However, Dolma H54 was the most susceptible cultivar, while Balady Mecca was the least affected one. Hence, the nematode affected the shoot, root and weights however, variable host reactions were noticed among the five tested cultivars. In general, the tested pepper cultivars could be rated as MS hosts such as Balady Mecca, 292, Top Star and Dolma H54 and HS hosts like, Lama Star. With regard to, shoot weight was reduced in cultivars infected with nematode when compared with the healthy ones. For instance, the percentages of shoot weight reduction ranged between (50.1 - 67.8 %), as the lowest reduction was belong to cv. Balady Mecca, while the highest one was for cv. Dolma H54. SDS-PAGE for the total storage protein of the current pepper cultivars (control and nematode-treated) has been performed. These results may suggest that nematode infection may triggers some gene expression (appears at molecular weight of about 60 kDa), causing the cultivars 'Balady Mecca', 'Dolma H54', '292' and 'Top Star' to exhibit MS to the nematode infection. While the protein pattern of the cultivar 'Lama Star' suggests that it might be HS for nematode infection. In general, it seems that both pathological and biochemical results; are in contiguous and relatively gave almost the same results.

6. Acknowledgments

The authors gratefully acknowledge Ass. Prof. Dr. Mohamed F. Afifi for critical reading of the manuscript. The authors are also, grateful thanks to all the staff membrane in Tissue culture and Genetic Engineering Lab. (TCGEL)", Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City (USC), Egypt for their help during this work.

7. References

1. Parisi M, Alioto D. Tripodi P. Overview of Biotic Stresses in Pepper (Capsicum spp.): Sources of Genetic Resistance, Molecular Breeding and Genomics. Int J Mol Sci. 21(7): 2587(2020).
2. Gisbert C, Trujillo-Moya C, Sánchez-Torres P, Sifres A, Sánchez-Castro E, Nuez F. Resistance of pepper germplasm to Meloidogyne incognita. Annals of applied biology. 162(1): 110-118 (2013).
3. Bernard GC, Egnin M, Bonsi C. The impact of plant-parasitic nematodes on agriculture and methods of control. Nematology-Concepts, Diagnosis and Control. 10: 121-152 (2017).
4. Caporalino C, Pijarowski L, Januel A, Lefebvre V, Daubeze A, Palloix A, Abad P. Spectrum of resistance to root-knot nematodes and inheritance of heat-stable resistance in in pepper (Capsicum annuum L.). Theoretical and Applied Genetics, 99(3-4): 496-502 (1999).

5. Malhotra A, Agarwal T, Trivedi PC. Screening of some chilli cultivars for their resistance against Meloidogyne incognita. International Journal of Pharma and Bio. (3): 136-142 (2012).
6. Buena AB, López-Pérez JA, Bello A, Robertson L, Escuer L, de León L. Screening of Cuarentino Pepper (Capsicum annum L.) for resistance to Meloidogyne incognita. Nematropica. 36(1): 13-26 (2006).
7. Nwanguma EI, Idowu-Agida OO. Oladigbola AA. Susceptibility of elite cultivars of pepper (Capsicum fruitisens) to the root-knot nematodes, Meloidogyne incognita Race 2, in parts of Southwestern Nigeria. Int. J. Dev. Sustain. 2(4): 2248-2255 (2013).
8. Gonçalves LS, Gomes VM, Robaina RR, Valim RH, Rodrigues R, Aranha. FM. Resistance to root-knot nematode (Meloidogyne enterolobii) in Capsicum spp. accessions. Revista Brasileira de Ciências Agrárias, 9(1): 49-52 (2014).
9. Rodríguez O, Corona-Torres T, Aguilar-Rincón VH. Differential response of pepper (Capsicum annuum L.) lines to Phytophthora capsici and root-knot nematodes. Crop protection, 92: 148-152 (2017).
10. El-Shennawy MZ. Abo-Korah MS. Varietal Susceptibility of some Tomato and Pepper Cultivars against Root–Knot Nematode, Meloidogyne javanica. Journal of Plant Protection and Pathology, 12(7): 481-484 (2021).
11. Hussey RS. Barker RK. A comparison of methods of collecting inocula of Meloidogyne spp. including a new technique, Plant Diseases Report. 57: 1025-1028 (1973).
12. Taylor, AL, Sasser, JN. Biology, Identification and control of root-knot nematode (Meloidogyne species). North Carolina State University Graphics, North Carolina, Raleigh. 111p. (1978).
13. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685 (1970).
14. Rohlf, FJ. NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System Version 2.1. Exeter Publishing Setauket, New York (2000).
16. Sokal RR, Rohlf FJ. Biometry: the principles and practice of statistics in biological research.3rd ed. W.H. Freeman and company New York, 937 (1995).
17. Norton, D.C. Ecology of plant-parasitic nematodes (No. SB998. N45. N6713 1978.). New York: Wiley (1978).
18. Zayed GA, Abdo AA, Hammam HB, Khafagi EY. Cultivation and production of pepper and eggplant in Egypt. Technical issue No. 15, General Administration of Agricultural Culture, Ministry of Agriculture, Egypt (2017).
19. Kihika R, Murungi LK, Coyne D, Nganga M, Hassanali A, Teal PE, Torto B. Parasitic nematode Meloidogyne incognita interactions with different Capsicum annum cultivars reveal the chemical constituents modulating root herbivore. Scientific Reports. 7(1): 1-10 (2017).
20. Soares RS, Silva EHC, Vidal RL, Candido WDS. Franco CA, Reifschneider FJB, Braz LT. Response of Capsicum annuum L. var. annuum genotypes to root-knot nematode infection. Chilean journal of agricultural research, 78(1): 78-85 (2018).
21. Agaba TA, Fawole B. Screening of some pepper cultivars for resistance to Meloidogyne incognita (Chitwood). International Journal of Food, Agriculture and Veterinary Sciences, 5(3): 23-29 (2015).
22. Aguiar JL, Bachie O, Ploeg A. Response of Resistant and Susceptible Bell Pepper (Capsicum annuum) to a Southern California Meloidogyne incognita Population from a Commercial Bell Pepper Field. J. Nematol. 46(4): 346-351(2014).
23. Pinheiro JB., Silva GOD, Macêdo AG, Biscaia D, Ragassi CF, Ribeiro CS, Reifschneider FJB. New resistance sources to root-knot nematode in

Capsicum pepper. Horticultura Brasileira. 38: 33-40 (2020).

24. Maquilan MAD, Padilla DC, Dickson DW, Rathinasabapath B. Improved Resistance to Root-knot Nematode Species in an Advanced Inbred Line of Specialty Pepper (Capsicum annuum). Hortscience. 55(7):1105-1110(2020).
25. Oka Y, Offenbach R, Pivonia S, Pepper Rootstock Graft Compatibility and Response to Meloidogyne javanica and M. incognita. Journal of Nematology, 36(2):137-41(2004).
26. Maracahipes ÁC, Taveira GB, Mello EO, Carvalho AO, Rodrigues RJ, Perales R, Gomes VM. Biochemical analysis of antimicrobial peptides in two different Capsicum genotypes after fruit infection by Colletotrichum gloeosporioides. Bioscience reports, 39(4):1-14(2019).
27. Kumar OA, Tata SS. SDS-PAGE seed storage protein profiles in chili peppers (Capsicum L.). Notulae Scientia Biologicae. 2(3): 86-90 (2016).
28. Silva RDS. Souza CRBD. Extração e análise eletroforética em gel de poliacrilamida (SDS-PAGE) de proteínas totais de folhas e raízes de Piper tuberculatum. Acta Amazonica. 39(2): 255-260 (2009).
29. Mena E, Singh S, Ansari MT, Ramjan M. Genetic diversity in king chilli (Capsicum chinense Jacq.) genotypes through SDS-PAGE. Electronic Journal of Plant Breeding. 10(2): 889-898 (2019).
30. Peddakasim DP, Suneetha SU, Lakshmi R, Srideepthi M, Krishna SR. Seed storage protein profiling and phylogenetic relationships of Capsicum annum L. cultivars using SDS-PAGE. Research Journal of Biotechnology, 10 (2): 1-4(2015).