International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(3): 1434-1438
© 2018 IJCS
Received: 01-03-2018
Accepted: 03-04-2018

Vyomesh Shailesh Patel
Department of Agricultural Biochemistry, AAU, Anand, Gujarat, India

Pitambara
Department of Agricultural Biotechnology, AAU, Anand, Gujarat, India

YM Shukla
Professor & Unit Officer, College of Agriculture, Vaso, AAU, Anand, Gujarat, India

Correspondence
Vyomesh Shailesh Patel
Department of Agricultural Biochemistry, AAU, Anand, Gujarat, India

Screening of various tomato genotypes for resistance to root knot nematode *(Meloidogyne incognita)* infection

Vyomesh Shailesh Patel, Pitambara and YM Shukla

Abstract
Tomato (*Solanum lycopersicum* L., 2n=2x=24) is an important vegetable for human consumption because of its enriched nutritional composition that provide the basic body nutritional requirements. Based on gene expression studies *Mi gene* was considered as a good candidate gene for screening of various tomato genotypes for resistance against root knot nematode. As it was selectively expressed in the resistant genotypes only; two gene specific primers for *Mi 1.2* and *Mi 23* were used for screening of thirteen tomato cultivars procured from MVRS, Anand. The Primer amplified in genotypes ATL-04-62, ATL-11-11, and DVRT-2 (National check) suggesting that these genotypes could be the good candidates for resistance against root knot disease.

Keywords: Gene expression, resistance, root knot nematode

Introduction
Tomato is the world's largest vegetable crop after potato and sweet potato, but it tops the list of canned vegetables. The total global area under tomato is 47.30 lakh ha and the global production is 1639.60 lakh tonnes. Major tomato (*Solanum lycopersicum* L.) producing states are Bihar, Karnataka, Uttar Pradesh, Orissa, Andhra Pradesh, Maharashtra, Madhya Pradesh and Assam. Gujarat is fifth largest producer of tomato (*Solanum lycopersicum* L.) after Madhya Pradesh, Andhra Pradesh, Karnataka, and Orissa. Tomato (*Solanum lycopersicum* L.) is affected by various disease caused mainly by fungi, bacteria and nematodes. Nematodes found to be very fatal infective agents and cause severe yield loses. Root-knot nematodes (*Meloidogyne* spp.) are phytopathogenic obligate endoparasites nematodes that infect many plant species and cause serious damage to agricultural crops per year (Abad *et al.,* 2008) [1]. Root knot nematodes (*Meloidogyne* spp.) are one of the most important polyphagous pathogen in agriculture. Among the top five plant pathogens affecting world's food production, root knot nematodes are one of the most devastating pathogen of crops. Infestation on crops greatly impact their health, yield and quality. Management of plant parasitic nematodes has always been difficult, and the most successful strategy for many years has been the use of toxic fumigant nematicides, such as the most known methyl bromide (Oka *et al.,* 2000b) [6]. Also, effective nematicides such as ethylene dibromide (EDB) and dibromochloropropane (DBCP) have been withdrawn from the market due to their deleterious effects on humans and environment (Oka *et al.,* 2000b) [6]. Thus, new strategies for the control of plant-parasitic nematodes have actively been sought during the last few years, and investigation has been focused more on biological control, organic and inorganic amendments, naturally occurring nematicides and induced resistance (Oka *et al.,* 2000a) [5]. But the safe and eco-friendly approach is to use resistant variety. Even some molecular markers have to be developed for the screening of such resistant varieties.

Many plant enzymes are involved in defence reactions against plant pathogens (Odjakova and Hadjiivanova, 2001) [4]. Most of the studies were confined to model organism fussarium wilt and viruses. Till date very limited information is available for tomato-root knot nematode infection on the biochemical and molecular changes in parameters like protein profile, gene expression profile and isozyme analysis of susceptible and resistant tomato cultivar against the root knot nematode infection. Also very limited resistant genotype of tomato against root knot nematode infection is known. So, this piece of work concentrates on screening of various tomato genotypes for resistance to root knot nematode.

Materials and Methods

The tomato genotypes were procured from the main vegetable research station for screening using *Mi-1.2* and *Mi - 23* genes inorder to find the most promising genotype. (Table 1).

Table 1: List of tomato genotypes procured from MVRS, AAU, Anand

Sr. No.	Genotypes
1	ATL 10-04 (GAT 5)
2	GAT 4
3	ATL 97-26
4	ATL 04-62
5	ATL 11-10
6	ATL 11-11
7	ATL 15-13
8	ATL 16-09
9	ATL 16-06
10	AT 3 (Local Check)
11	JT 3 (Local Check)
12	GT 2 (Local Check)
13	DVRT 2 (National Check)

DNA extraction was done from the leaves of tomato seedling of all genotypes by CTAB method. Quality and quantity of extracted DNA was checked by nanodrop. *Mi gene* was considered as a good candidate gene for screening of various tomato genotypes for resistance against root knot nematode. Two gene specific primers for *Mi 1.2* and *Mi 23* was taken for further screening of thirteen tomato cultivars procured from MVRS, Anand (Table 2).

Table 2: List of primers

Sr. No.	Target Gene		Sequence (5' to 3')
1	*Mi-1.2*	F	AAACCACTGTGGGTCCTCTGTT
		R	TGGATGATTGTATCATAAAGGGACAAATT
12	*Mi-23*	F	TGGAAAAATGTTGAATTTCTTTTG
		R	GCATACTATATGGCTTGTTTACCC

Jaccard's similarity coefficient was calculated from screening data and based on the result obtained Dendrogram was generated by UPGMA software.

Results

In present investigation, the average concentration of DNA extracted from tomato seedlings was 73.4 ng/µl quantified on nanodrop spectrophotometer. The genotype AT 3 showed the highest concentration of 123.8 ng/µl and genotype ATL 97-26 showed the lowest concentration (37.4 ng/µl).

1. Screening of tomato genotype using gene specific primers

***Mi 1.2*:** The gene specific primer for *Mi 1.2* had produced one band with product size of 347 bp (Plate 1). It was found to be amplified in genotypes ATL-04-62, ATL-11-11, ATL-16-09, ATL-16-06 and DVRT-2 (National check) suggesting that these genotypes could be the good candidates for resistance against root knot disease. Clustering pattern of dendrogram generated by UPGMA based on Jaccard's similarity coefficient was shown in figure 1 and table 3. Apart from the above mentioned five promising genotypes rest of eight were clustered in to one group as proposed susceptible genotypes.

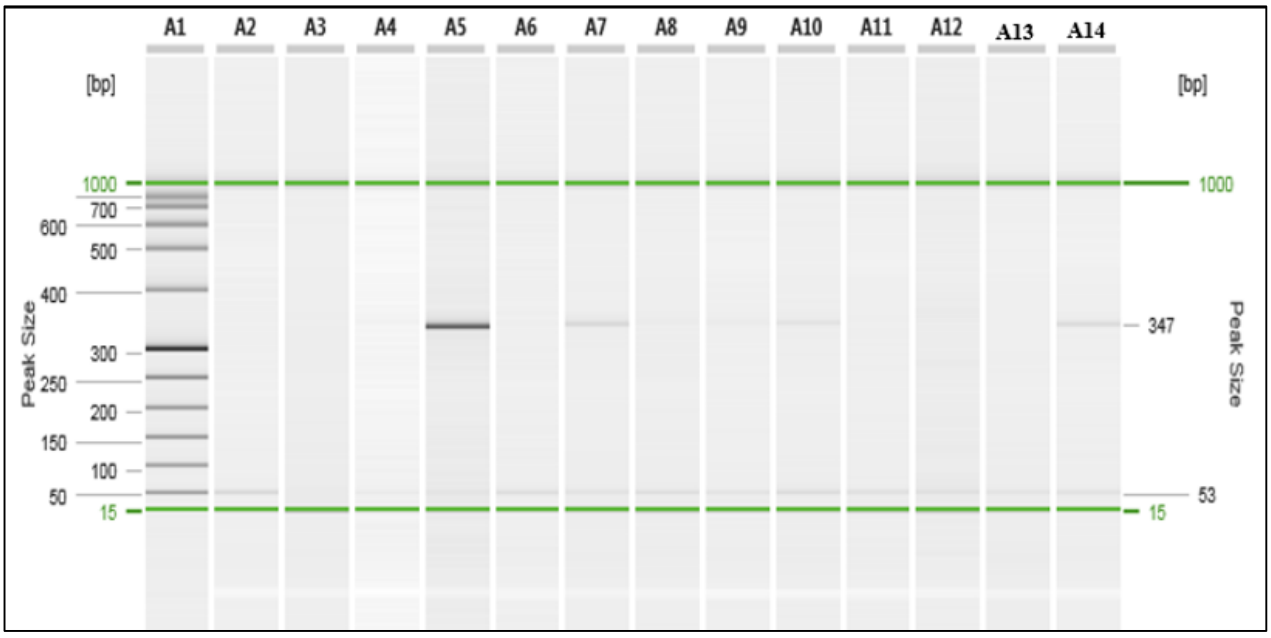

Plate 1: Gene specific primer profile of *Mi – 1.2*

A1	:	1000 bp Marker						
A2	:	ATL 10-04 (GAT-5)	**A3**	:	GAT-4	**A4**	:	ATL 97-26
A5	:	ATL 04-62	**A6**	:	ATL 11-10	**A7**	:	ATL 11-11
A8	:	ATL 15-13	**A9**	:	ATL 16-09	**A10**	:	ATL 16-06
A11	:	AT 3 (Local Check)	**A12**	:	JT-3 (Local Check)			
A13	:	GT-2 (Local Check)	**A14**	:	DVRT-2 (National Check)			

Mi 23: A gene specific primer for *Mi 23* was found to be amplified in genotypes ATL-10-04 (GAT-5), GAT-4, ATL-04-62, ATL-11-11, ATL-15-13, AT 3 (Local check), JT-3 (Local check), GT-2 (Local check) and DVRT-2 (National check) with product length of 455 bp (Plate 2). Clustering pattern of dendrogram generated by UPGMA based on Jaccard's similarity coefficient was shown in figure 2 and table 4. Apart from the above mentioned nine promising genotypes rest of four were clustered in to one group as proposed susceptible genotypes.

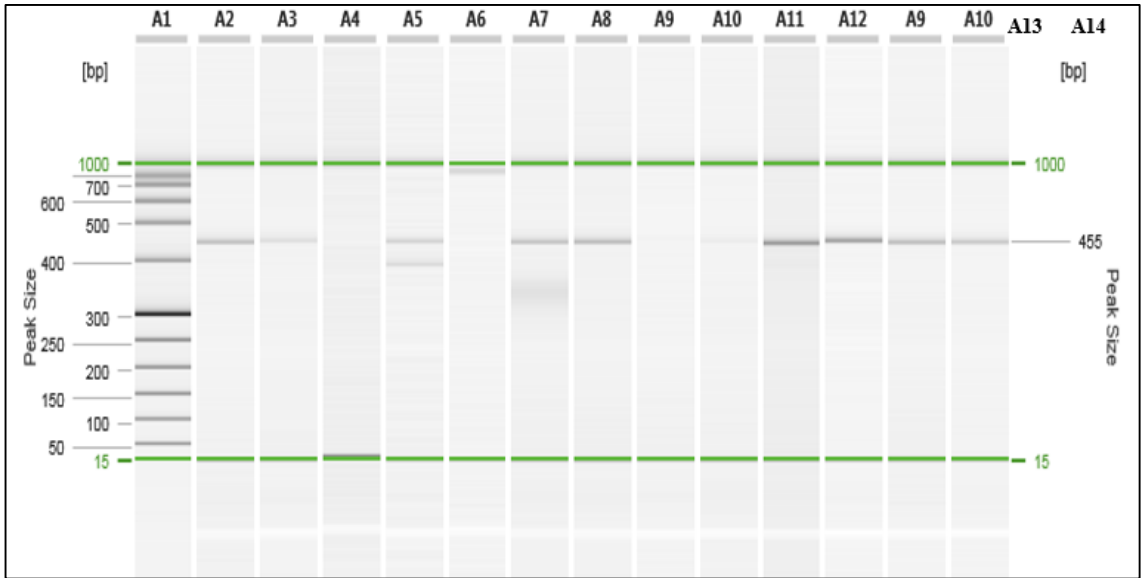

Plate 2: Gene specific primer profile of *Mi – 23*

A1	:	1000 bp Marker						
A2	:	ATL 10-04 (GAT-5)	**A3**	:	GAT-4	**A4**	:	ATL 97-26
A5	:	ATL 04-62	**A6**	:	ATL 11-10	**A7**	:	ATL 11-11
A8	:	ATL 15-13	**A9**	:	ATL 16-09	**A10**	:	ATL 16-06
A11	:	AT 3 (Local Check)	**A12**	:	JT-3 (Local Check)			
A13	:	GT-2 (Local Check)	**A14**	:	DVRT-2 (National Check)			

The result obtained was same as reported by Branch *et al.*, (2003), where it was found that Mi-1 gene of tomato confers resistance against three species of root-knot nematode in tomato *(Lycopersicon esculentum).* Transformation of tomato carrying Mi-1 with a construct expressing NahG, which encodes salicylate hydroxylase, a bacterial enzyme that degrades salicylic acid (SA) to catechol, results in partial loss of resistance to root-knot nematodes thus concluding that SA is an important component of the signalling that leads to nematode resistance and the associated hypersensitive response.

The same was also reported by Jaubert *et al.*, (2004) [3], where cloning of two genes encoding 14-3-3 isoforms from the plant parasitic root-knot nematode *M. incognita*, together with an analysis of their expression. Both genes were shown to be transcribed in unhatched second stage larvae, infective second stage larvae, adult males and females. The Mi-14-3-3-a gene was shown to be specifically transcribed in the germinal primordium of infective larvae, whereas Mi-14-3-3-b was transcribed in the dorsal oesophageal gland in larvae of this stage. The MI-14-3-3-B protein was identified by mass spectrometry in *in vitro*-induced stylet secretions from infective larvae. The stability and distribution of MI-14-3-3 proteins in host plant cells was assessed after stable expression of the corresponding genes in tobacco BY2 cells.

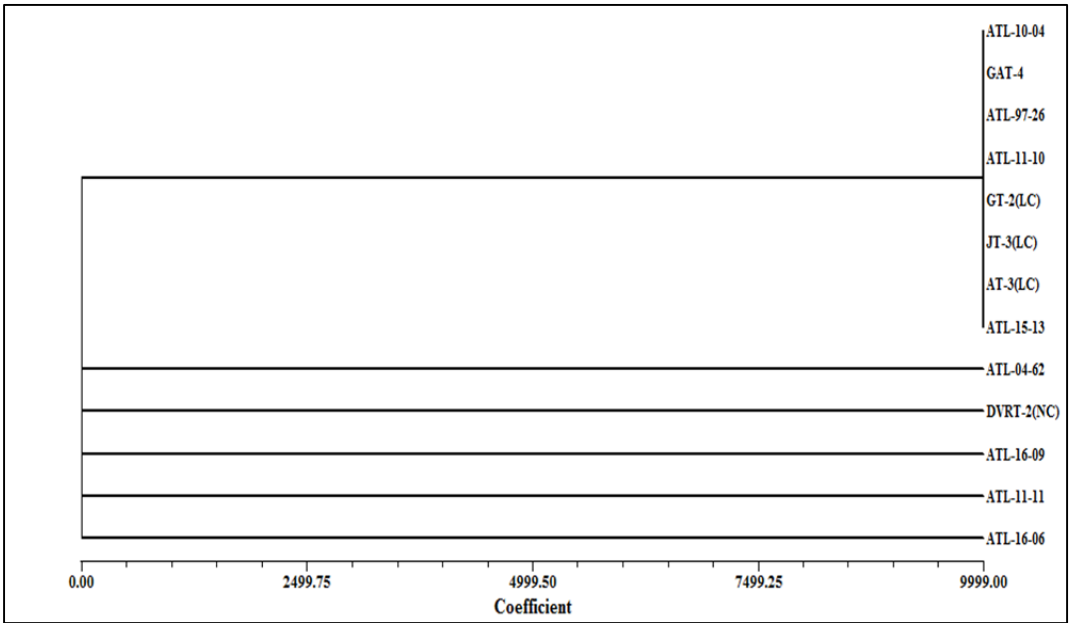

Fig 1: Dendrogram showing clustering of 13 tomato genotypes using UPGMA based on Jaccard's similarity coefficient obtained from gene specific primer *Mi – 1.2* analyses

Note: LC - Local Check; NC - National Check

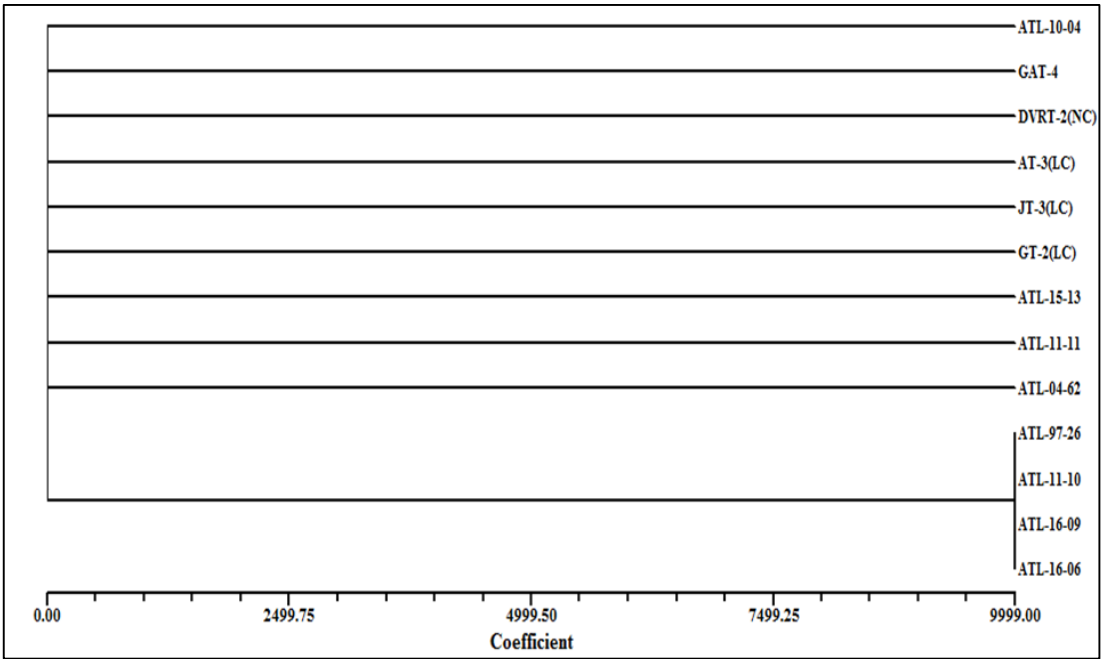

Fig 2: Dendrogram showing clustering of 13 tomato genotypes using UPGMA based on Jaccard's similarity coefficient obtained from gene specific primer *Mi – 23* analyses **Note:** LC - Local Check; NC - National Check

Table 3: Jaccard's similarity coefficient* for *Mi – 1.2*

Genotypes	ATL 10-04	GAT-4	ATL 97-26	ATL 04-62	ATL 11-10	ATL 11-11	ATL 15-13	ATL 16-09	ATL 16-06	AT-3 (LC)	JT-3 (LC)	GT-2 (LC)	DVRT-2 (NC)
ATL 10-04	1.00												
GAT-4	9999.00	1.00											
ATL 97-26	9999.00	9999.00	1.00										
ATL 04-62	0.00	0.00	0.00	1.00									
ATL 11-10	9999.00	9999.00	9999.00	0.00	1.00								
ATL 11-11	0.00	0.00	0.00	1.00	0.00	1.00							
ATL 15-13	9999.00	9999.00	9999.00	0.00	9999.00	0.00	1.00						
ATL 16-09	0.00	0.00	0.00	1.00	0.00	1.00	0.00	1.00					
ATL 16-06	0.00	0.00	0.00	1.00	0.00	1.00	0.00	1.00	1.00				
AT-3 (LC)	9999.00	9999.00	9999.00	0.00	9999.00	0.00	9999.00	0.00	0.00	1.00			
JT-3 (LC)	9999.00	9999.00	9999.00	0.00	9999.00	0.00	9999.00	0.00	0.00	9999.00	1.00		
GT-2 (LC)	9999.00	9999.00	9999.00	0.00	9999.00	0.00	9999.00	0.00	0.00	9999.00	9999.00	1.00	
DVRT-2 (NC)	0.00	0.00	0.00	1.00	0.00	1.00	0.00	1.00	1.00	0.00	0.00	0.00	1.00

Note: *Jaccard's similarity coefficient was analyzed using UPGMA clustering analysis

Table 4: Jaccard's similarity coefficient* for *Mi – 23*

Genotypes	ATL 10-04	GAT-4	ATL 97-26	ATL 04-62	ATL 11-10	ATL 11-11	ATL 15-13	ATL 16-09	ATL 16-06	AT-3 (LC)	JT-3 (LC)	GT-2 (LC)	DVRT-2 (NC)
ATL 10-04	1.00												
GAT-4	1.00	1.00											
ATL 97-26	0.00	0.00	1.00										
ATL 04-62	0.50	0.50	0.00	1.00									
ATL 11-10	0.00	0.00	9999.00	0.00	1.00								
ATL 11-11	1.00	1.00	0.00	0.50	0.00	1.00							
ATL 15-13	1.00	1.00	0.00	0.50	0.00	1.00	1.00						
ATL 16-09	0.00	0.00	9999.00	0.00	9999.00	0.00	0.00	1.00					
ATL 16-06	0.00	0.00	9999.00	0.00	9999.00	0.00	0.00	9999.00	1.00				
AT-3 (LC)	1.00	1.00	0.00	0.50	0.00	1.00	1.00	0.00	0.00	1.00			
JT-3 (LC)	1.00	1.00	0.00	5.00	0.00	1.00	1.00	0.00	0.00	1.00	1.00		
GT-2 (LC)	1.00	1.00	0.00	5.00	0.00	1.00	1.00	0.00	0.00	1.00	1.00	1.00	
DVRT-2 (NC)	1.00	1.00	0.00	5.00	0.00	1.00	1.00	0.00	0.00	1.00	1.00	1.00	1.00

Note: *Jaccard's similarity coefficient was analyzed using UPGMA clustering analysis

Conclusion

Based on gene expression studies *Mi gene* was considered as a good candidate gene for screening of various tomato genotypes for resistance against root knot nematode. As it was selectively expressed in the resistant genotypes only; two gene specific primers for *Mi 1.2* and *Mi 23* were used for screening of thirteen tomato cultivars procured from MVRS, Anand. The gene specific primer for *Mi 1.2* was amplified as one band with product size of 347 bp. It was found to be amplified in genotypes ATL-04-62, ATL-11-11, ATL-16-09, ATL-16-06 and DVRT-2 (National check) suggesting that these genotypes could be the good candidates for resistance against root knot disease. A gene specific primer for *Mi 23* was found to be amplified in genotypes ATL-10-04 (GAT-5), GAT-4, ATL-04-62, ATL-11-11, ATL-15-13, AT 3 (Local check), JT-3 (Local check), GT-2 (Local check) and DVRT-2 (National check) with product length of 455 bp. Apart from the above mentioned nine promising genotypes the rest of four were clustered as proposed susceptible genotypes.

References

1. Abad P, Gouzy J, Aury JM, Castagnone-Sereno P, Danchin EG, Deleury E *et al*. Genome sequence of the metazoan plant-parasitic nematode *Meloidogyne incognita*. Nature biotechnology. 2008: 26:909-915.
2. Branch C, Hwang CF, Navarre DA, Williamson VM. Salicylic acid is part of the *Mi-1*-mediated defense response to root-knot nematode in tomato. Mol Plant Microbe Interact. 2004; 17:351-356.
3. Jaubert S, Laffaire JB, Ledger TN, Escoubas P, Amri EZ, Abada P *et al*. Comparative analysis of two 14-3-3 homologues and their expression pattern in the root-knot nematode *Meloidogyne incognita*. International Journal for Parasitology. 2004; 34:873-880.
4. Odjakova M, Hadjiivanova C. The complexity of pathogen defense in plants. Bulgarian Journal of Plant Physiology. 2001; 27:101-109.
5. Oka Y, Koltai H, Bar-Eyal M, Mor M, Sharon E, Chet I *et al*. New strategies for the control of plant-parasitic nematodes. Pest Management Science. 2000a; 56:983-988.
6. Oka Y, Nacar S, Putievsky E, Ravid U, Yaniv Z, Spiegel Y. Nematicidal activity of essential oils and their components against the root-knot nematode. Journal of Phytopathology. 2000b; 90:710-715.