Vol. 49, No. 2, pp. 131-138 Indian Journal of Nematology December, 2019

Characterization of Tomato Germplasm for Root-Knot Nematode Resistance with the Help of Mi23 Marker

SURESH REDDY YERASU[1*], C. SELLAPERUMAL[1,3], MANJUNTHA T. GOWDA[1], PALLAVI MISHARA[1], SHAILESH KUMAR TIWARI, CHITHRA DEVI PANDEY[2] AND SUSHIL PANDEY[2]

[1]ICAR-Indian Institute of Vegetable Research, Varanasi-221305, [2]ICAR- National Bureau of Plant Genetic Resources, New Delhi-110012
[3]ICAR-Indian Institute of Spices Research, Kozhikode-673012
**Corresponding author;* E-mail: yerasusureshreddy@yahoo.co.in

Received on 05-07-2019 and Accepted on 07-11-2019

ABSTRACT: Tomato is a major vegetable crop and a rich source of micronutrients such as vitamins, antioxidants and minerals for a balanced human diet. Biotic stresses, including Root Knot Nematode (RKN) causes significant yield losses in tomato production worldwide. The resistance gene, *Mi-1* is currently the only source of root-knot nematode resistance in modern tomato cultivars. In total, 397 tomato genotypes received from ICAR-NBPGR were screened for identifying *Mi-1* containing genotypes. A two-step resistance screening protocol involving PCR based molecular screening for *Mi* gene with gene based SCAR marker Mi23 followed by confirmation of the resistance through challenged nematode inoculation under pot condition was followed. Eight genotypes *viz,* EC 705452, EC 699717, EC 759288, EC 002644, EC 035420, EC 054644, EC 129606 and EC 050347 have shown positive band to the marker and gave resistance reaction in screening with *M. incognita* at 2000 second stage infective juveniles per plant under pot condition. The identified resistance genotypes can be used as donors of *Mi* gene in multiple gene pyramiding and also can be tested as parents in nematode resistance hybrid development programmes.

Keywords: Nematode resistance, Root knot nematode, Mi gene, Tomato germplasm, Mi 2.3 marker

Plant Genetic Resources (PGR) forms the basis for any breeding programme intended for development of superior cultivars. Once augmented, utilization of PGR in breeding programmes highly depends on identification of promising accessions. To identify promising accessions for different characters PGR have to be characterized or evaluated by recording data for agronomic traits like yield parameters, quality aspects and tolerance to biotic, abiotic stresses etc

In India, tomato is an introduced crop way back in 18th century and most of the introductions are bred varieties (Seshadri and Srivastava, 2002). Tomato (*Solanum lycopersicum* L.) is most important vegetable crop grown in India after potato with production of19.7 million tonnes (Anonymous, 2017). Quality and quantity of tomato production is hindered by many biotic stresses including root knot nematodes (RKNs). In different parts of the country, tomato crop go through yield loss to the tune of 27.21% with an estimated monetary loss of 2204 million rupees due to root-knot nematode (*Meloidogyne incognita)* infestation alone (Jain *et al.,* 2007). Characterizing PGR for RKN resistance is important as resistance accessions can be used in crop breeding programmes. Phenotypic screening of PGR for nematode resistance is a laborious and time consuming task. The resistance gene, *Mi-1* is currently the only source of root-knot nematode resistance in modern tomato cultivars (Garcia *et al.,* 2007). Many markers were developed for *Mi-1* gene. Among them, Mi23, a codominant SCAR (Sequence Characterized Amplified Region) marker is located within the *Mi-1* locus (Seah *et al.,* 2007).

In the present work, a simple and rapid method for tomato PGR characterization for nematode resistance was proposed. Initially tomato genotypes were screened with Mi23 marker for the presence of *Mi-1* locus. Once

genotypes with *Mi-1* locus identified through marker assays, the identified genotypes were phenotypically screened for the resistance.

MATERIALS AND METHODS

Plant material

The 397 tomato genotypes (Table 1) of the study were received from ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi for seed multiplication in 2015-16. In artificial screening, Kashi Amrit was used as susceptible check. Hissar Lalit and H-88-78-1 were used as resistant checks. The experiment was conducted in ICAR- Indian Institute of Vegetable Research (ICAR-IIVR), Varanasi, Uttar Pradesh, India.

DNA Extraction and Marker Analysis

DNA was extracted from young leaves by using a standard protocol (Prasanna *et al*., 2015). Briefly, the PCR protocol for the markers used in this study was 94 ^{0}C for 4 min, followed by 35 cycles of 94 ^{0}C for 1 min, 55 ^{0}C for 1 min, and 72 ^{0}C for 2 min, with a final extension of 5 min at 72 ^{0}C. PCR was conducted in 25 µL volume consisting of 6.25 µL master mix (2.25 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 U Taq DNA polymerase, 10× PCR buffer and 0.4 µL primers) 2 µLDNA (50 ng/lL) and 16.75 µL of nuclease free water. The PCR products were separated on 2 % agarose gel at 120 V for 60–70 min and DNA bands were visualized with ethidium bromide staining. DNA fragment sizes were determined by using a 50 bp DNA ladder. PCR amplification and gel electrophoresis were carried out twice to confirm the results. Mi23 primer sequences are Mi23F is 5'-TGG AAA AAT GTT GAA TTT CTT TTG-3', and Mi23R is 5'- GCA TAC TAT ATG GCT TGT TTA CCC-3' (Seah *et al.,* 2007).

Screening of Germplasm

Eight tomato germplasm (Table 2) which gave 380 bp amplicon with Mi23 marker were taken along with the resistant (Hissar Lalit and H-88-78-1) and susceptible (Kashi Amrit) checks. All tomato seeds were germinated in sterilized loam soil. Four week old tomato seedlings were transplanted (one seedling per pot) each containing 2 kg of sterilized soil and sand in 3:1 ratio. There were three replicates for each tomato genotype in a completely randomized design and plants were maintained for 90 days.

Sources of Nematode Culture, Extraction and Counting

The pure culture of *M. incognita* was maintained and grown on susceptible tomato plants in pots under net house conditions of ICAR-IIVR, Varanasi. With the help of forceps, egg masses were picked from uprooted roots of heavily infected tomato plants and poured on tissue paper kept on wire mesh in Petri plate containing plain water (Persson 1974). At 24 h interval juveniles were extracted and were concentrated in a beaker. The nematode counting was done under stereo microscope with the help of counting dish and tomato plants were inoculated one week after transplanting at 2000 second stage infective stage juveniles (IJs) per plant.

Plant Reactions to *M. incognita*

Ninety days after inoculation plants were scored for their reaction to RKN. Inoculated plants were uprooted gently and washed to remove the adhering soil. In all the replications root galls were counted and were averaged to subject them to root-knot index (RKI). Root knot indices given by Taylor and Sasser, (1978) on a six-point scale (0–5) were followed and are 0 = no gall or no infection or immune; 1 = 1–2 galls (highly resistant; HR); 2 = 3–10 galls (resistant; R); 3 = 11–30 galls (moderately resistant; MR); 4 = 31–100galls (susceptible; S), and 5 = 100 and above galls (highly susceptible; HS).

Statistical Analysis

Prior to analysis, square root transformation was conducted to normalize the data on number of root galls. Analysis of variance (one way ANOVA) was performed

Table 1. List of germplasm evaluated for presence of *Mi-1* gene

S.No	Accessions	S.No	Accessions	S.No	Accessions	S.No	Accessions	S.No	Accessions
1	EC000482	48	EC007786	95	EC035374	142	EC086501	189	EC161652
2	EC000491	49	EC007787	96	EC035376	143	EC086501-1	190	EC163598
3	EC000493	50	EC007916	97	EC035386	144	EC089252	191	EC163602PI
4	EC000337-1	51	EC008210	98	EC035391	145	EC092788	192	EC163603
5	EC001139	52	EC009018	99	EC035392	146	EC092252	193	EC163615
6	EC002598	53	EC009148	100	EC035413	147	EC095848	194	EC163912
7	EC002611	54	EC009149	101	**EC 035420**	148	EC096403	195	EC000232
8	EC002634	55	EC012528	102	EC035499	149	EC096406	196	EC000276
9	EC002635	56	EC012586	103	EC035503	150	EC099191	197	EC001753
10	EC002640	57	EC013902	104	EC035527	151	EC099926	198	EC002486
11	**EC002644**	58	EC014167	105	EC035530	152	EC099927	199	EC004267
12	EC002645	59	EC016655	106	EC035532	153	EC103614	200	EC005863
13	EC002669	60	EC016789	107	EC035592	154	EC103608	201	EC008939
14	EC002671	61	EC017169	108	EC036604	155	EC104211	202	EC009016
15	EC002672	62	EC017980	109	EC036972	156	EC108764	203	EC012659
16	EC002679	63	EC019720	110	EC036973	157	EC110116	204	EC013112
17	EC002688	64	EC020695	111	EC037163	158	EC110635	205	EC013736
18	EC002689	65	EC026104	112	EC037183	159	EC111086	206	EC015127
19	EC002694	66	EC026105	113	EC037211	160	EC113820	207	EC016652
20	EC002699	67	EC026698	114	EC037250	161	EC114137	208	EC016790
21	EC002839	68	EC026750A	115	EC037267	162	EC114146	209	EC016796
22	EC003103	69	EC027251	116	EC039406	163	EC114504	210	EC018841
23	EC003208	70	EC027904	117	EC039976	164	EC114937	211	EC023528
24	EC003215	71	EC027911	118	EC041028	165	EC115062	212	EC026684
25	EC003216	72	EC027932	119	EC041067	166	EC116872	213	EC027336
26	EC003235	73	EC027932P2	120	EC041278	167	EC117399	214	EC027917
27	EC003236	74	EC027941	121	EC042558	168	EC118282	215	EC027938
28	EC004201	75	EC027950	122	EC042595	169	EC118292	216	EC027945
29	EC004300	76	EC027960	123	EC042596	170	EC119110	217	EC029627
30	EC004302	77	EC027961	124	EC043558	171	EC119200	218	EC029919
31	EC004303	78	EC027964	125	EC050355	172	EC122527	219	EC031346
32	EC004304	79	EC027976	126	EC050358	173	EC125557	220	EC031515
33	EC004522	80	EC027986	127	EC050360	174	EC127171P13	221	EC031767
34	EC004639	81	EC031824	128	EC050362	175	EC128254	222	EC032019
35	EC004707	82	EC032275	129	EC050363	176	EC128769	223	EC032276
36	EC004708	83	EC033276	130	EC052021	177	EC128963	224	EC032404
37	EC004958	84	EC033986	131	**EC 054644**	178	EC129594	225	EC032654
38	EC006050	85	EC035228	132	EC054722	179	EC129595-P3	226	EC033878
39	**EC 006148**	86	EC035230	133	EC054729	180	**EC 129606-PP**	227	EC032240

S.No	Accessions	S.No	Accessions	S.No	Accessions	S.No	Accessions	S.No	Accessions
40	EC006202	87	EC035232	134	EC054893	181	EC129608	228	EC035265
41	EC006509	88	EC035237	135	EC054894	182	EC130046	229	EC032373
42	EC006594	89	EC035273	136	EC057020	183	EC130163	230	EC035393
43	EC006596	90	EC035310	137	EC057440	184	EC137324	231	EC035514
44	EC007210	91	EC035323	138	EC076733	185	EC141827	232	EC036495
45	EC007262	92	EC035338	139	EC085732	186	EC143593	233	EC036888
46	EC007282	93	EC035358	140	EC086494	187	EC144602	234	EC037137
47	EC007317	94	EC035360	141	EC086500	188	EC159959	235	EC037218
236	EC037277	269	EC715380	302	EC721955	334	EC752618	366	EC759259
237	EC038811A	270	EC695037	303	EC721957	335	EC753216	367	EC759260
238	EC041272	271	EC715382	304	EC721958	336	EC753218	368	EC759261
239	EC042295	272	EC715383	305	EC721959	337	EC753219	369	EC759262
240	EC042555	273	EC715384	306	EC721961	338	EC753220	370	EC759263
241	EC042592	274	EC715385	307	EC721963	339	EC753221	371	EC759264
242	EC042885	275	EC715386	308	EC716696	340	EC753223	372	EC759265
243	EC043269	276	EC715387	309	EC759989	341	EC753224	373	EC759266
244	EC048321	277	EC715388	310	EC759991	342	EC753225	374	EC759267
245	EC700930	278	EC715389	311	EC759992	343	EC753226	375	EC759268
246	EC700931	279	EC715391	312	EC759993	344	EC753227	376	EC759269
247	EC700932	280	EC715393	313	EC759997	345	EC753228	377	EC759270
248	EC700933	281	EC715394	314	EC759998	346	EC753230	378	EC759271
249	EC700936	282	EC715396	315	EC759999	347	EC753231	379	EC759272
250	EC700938	283	EC715397	316	EC760002	348	EC753232	380	EC759273
251	EC705436	284	EC715398	317	EC760003	349	EC753233	381	EC759274
252	EC705437	285	EC715399	318	EC760004	350	EC738047	382	EC759275
253	EC705438	286	EC695036	319	EC760005	351	EC738050	383	EC759276
254	EC705439	287	EC695037	320	EC760006	352	EC738054	384	EC759277
255	EC705440	288	EC695038	321	EC760007	353	EC738055	385	EC759278
256	EC705442	289	EC695039	322	EC760008	354	EC739326	386	EC759279
257	EC705443	290	EC695040	323	EC760009	355	EC759243	387	EC759280
258	EC705444	291	EC695041	324	EC760010	356	EC759244	388	EC759281
259	EC705445	292	EC695042	325	EC760011	357	EC759246	389	EC759282
260	EC705446	293	EC695043	326	EC752609	358	EC759247	390	EC759283
261	EC705447	294	EC695044	327	EC752610	359	EC759248	391	EC759284
262	EC705449	295	EC695045	328	EC752612	360	EC759250	392	EC759285
263	EC705450	296	EC699710	329	EC752613	361	EC759251	393	EC759286
264	EC705451	297	EC699714	330	EC752614	362	EC759252	394	EC759287
265	**EC 705452**	298	EC699715	331	EC752615	363	EC759254	395	**EC 759288**
266	EC705453	299	EC699716	332	EC752616	364	EC759255	396	EC759289
267	EC715376	300	**EC 699717**	333	EC752617	365	EC759258	397	EC759290
268	EC715377	301	EC721954						

Note - *Mi-1* gene containing accessions in bold front

Table 2. Reaction of tomato genotypes to root knot nematode *Meloidogyne incognita* inoculated under pot condition

S. No	Genotype	Number of galls per root system (Mean ± SE)	Mean of gall index (0-5 scale)	Resistant reaction
1	EC 705452	7.3 ±0.72[b]	2.0[b]	R
2	EC 699717	8.3 ±0.54[b]	2.0[b]	R
3	EC 759288	5.3 ±0.72[b]	2.0[b]	R
4	EC 002644	1.7 ±0.72[b]	1.0[b]	HR
5	EC 035420	2.3 ±0.72[b]	1.3[b]	R
6	EC 054644	7.7 ±1.19[b]	2.0[b]	R
7	EC 129606-PP	6.7 ±0.72[b]	2.0[b]	R
8	EC 006148	6.0 ±1.25[b]	2.0[b]	R
9	Hisar Lalit	8.3 ±0.98[b]	2.0[b]	R
10	H-88-78-1	1.3 ±0.27[b]	1.0[b]	HR
11	Kashi Amrit	209.3 ±8.49[a]	5.0[a]	HS

Means with different letters indicate statistically difference at $P < 0.05$ using Tukey's HSD test. HR: Highly resistant,R: Resistant, HS: Highly susceptible.

for number of root galls and grouping of tomato genotypes based on root gall index was done by using PROC GLM (SAS version 9.2; SAS institute). When ANOVA was significant ($P < 0.05$) comparisons of relevant means were made using the Tukey's studentized Range (HSD) test at the 5% level of significance.

RESULTS

Mi23 is a sequence characterized amplified region (SCAR) marker and is co-dominant. Out of 397 tomato genotypes only eight genotypes namely, EC 705452, EC 699717, EC 759288, EC 002644, EC 035420, EC 054644, EC 129606-PP and EC 006148 gave amplification of 380bp with Mi23 marker indicating presence of root knot nematode resistance gene *Mi-1,* in them (Fig 1). All the other genotypes gave amplification of 430bp indicating presence of susceptible *mi* gene in them.

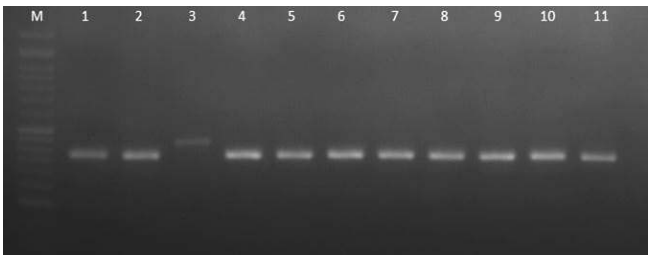

Fig. 1. PCR gel profile of Mi23 marker 1- Hisar Lalit; 2-H-88-78-1; 3 – Kashi Amrit; 4- EC 705452; 5- EC 699717; 6 - EC 759288; 7 - EC 0002644; 8 - EC 0035420; 9 - EC 0054644; 10 - EC 0129606-PP; 11- EC 0006148 and M- 50bp ladder

Phenotypic screening of *Mi-1* containing genotypes was done by inoculating with J2 juveniles at 2000 J2/ plant. As expected all the *Mi-1* containing genotypes gave highly resistant and resistant reaction along with resistant checks Hisar Lalit and H-88-78-1. Susceptible check Kashi Amrit gave highly susceptible reaction (Table 2). Analysis of variance (ANOVA) showed that the number of galls produced by RKN in screened tomato genotypes were significantly ($P< 0.05$) differed compared to susceptible check Kashi Amrit ($F = 178.21$; df=10, 22; $P <0.0001$).

Agro-horticultural characters of *Mi-1* containing genotypes were recorded from field during seed multiplication. Days to 50% flowering was taken (recorded as number of days from sowing date to the date when at least 50% of the plants show flower open. Stigma emergence on the main branch is considered as

Table 3. Agro-horticultural characters of *Mi-1* gene containing genotypes

S. No	Genotype	Days to 50% flowering	Number of locules per fruit	Average fruit weight (-gm)	Pericarp thickness (mm)	TSS
1	EC 705452	78	3	90	4.2	3.4
2	EC 699717	76	3	92	7.1	6.1
3	EC 759288	76	4	92	5.8	4.6
4	EC002644	76	3	16.7	1.4	5.4
5	EC 035420	75	3	37.5	1.2	4.6
6	EC 054644	77	3	44	3	6.4
7	EC 129606	75	3	25.6	4.2	6.2
8	EC006148	78	3	92.5	7.1	6.1
9	Kashi Amrit	81	3	67	4.6	3.9

flowering) on ten plants/row and other characters were average of five observations (Table 3). Pass port data of *Mi-1* containing genotypes is given Table 4.

DISCUSSION

Proper characterization of PGR is very important as it helps in proper utilization of PGR. Characterization of PGR against biotic stresses like RKN is a difficult task. Though several resistant accessions have been identified in the heterogeneous *S. peruvianum* complex (Ammati *et al.,* 1986; Lobo *et al.*, 1988) against RKN in tomato, the *Mi-1* resistance gene that was introgressed from *S. peruvianum* during 1940's (Smith, 1944) is currently the only source of RKN resistance in modern tomato cultivars (Garcia *et al.,* 2007). The *Mi* gene contains three open reading frameworks (ORFs) of which only *Mi1.2* confers resistance to *Meloidogyne incognita, M. javanica* and *M. arenaria* (Arens *et al*., 2010).

Several markers were developed for marker assisted selection of *Mi* gene (Ammiraju *et al.,* 2003; Seah *et al.,* 2007; Arens *et al.,* 2010). Among them, Mi23 is a gene based marker (Seah *et al.,* 2007). Being co-dominant SCAR marker, it has advantage over other PCR-based markers like Rex-1 in that restriction digestion step is not required. It is more reliable as it does not give false positive fragments with the begomovirus resistant breeding lines derived from *S. habrochaites* (Vidavsky and Czosnek, 1998) and *S. chilense* (*Ty-1* locus) (Agrama and Scott, 2006). Reddy *et al.* (2018) and Bhavana *et al.* (2019) reported its use in breeding programmers and germplasm screening for nematode resistance in tomato.

All eight genotypes that gave 380bp amplification with Mi23 marker gave highly resistant and resistant reaction in challenged nematode inoculation under pot condition. Hisar lalit and H-88-78-1 were used as resistant checks and have *Mi-1* with in them (Shrestha *et al.,* 2012; Reddy *et al*. (2018). The identified accessions can be used as source of *Mi-1* gene in disease resistant breeding programmes and also can be tried as parents in nematode resistance hybrid development programmes. The proposed method of characterization of PGR can be used for other characters for which reliable linked markers are available in tomato and in other crops.

ACKNOWLEDGEMENTS

Authors are thankful to Director, ICAR-National Bureau of Plant Genetic Resources, New Delhi for funding under CRP-Agrobiodiversity through which ICAR–IIVR got germplasm for the study. Authors are also thankful to Director, ICAR- Indian Institute of Vegetable Research, Varanasi for providing lab, land facilities and funding for the lab work.

Table 4. Passport data of of *Mi* gene containing genotypes

Accession	Receiving Date	Country	Source	Species	Crop	Variety	Alt_id	Indentor/ Distribution
EC705452	28-Jun-11	Taiwan, Province Of China	Asian Vegetable Research and Development Centre, The World Vegetable Centre P.O.Box 42, Shanhua Tainan-74199	*Solanum lycopersicum*	Tomato	cln2366dc1-58-15-18-23-5-0	AVTO0102	Dr. Arvind Kapur, ACSEN HyVeg Private Limited, Gurgaon
EC699717	25-Apr-11	Taiwan, Province Of China	Asian Vegetable Research & Development Centre, P.O. Box No.42, Shanhua Tainan-74199	*S. lycopersicum*	Tomato	L00671	VI006076	Dr. Hari Har Ram, Krishidhan Vegetable Seeds India Private Limited, Pune
EC759288	8-Nov-12	Jordan	Seminis Vegetable Seeds , Ai-Madinah Al munawarah St. Al-Wafa Commercial Bldg # 225 5th Floor Office # 504 P O Box 830917, Amman	*S. lycopersicum*	Tomato	TJO2 BIS 2289-12	1240	Dr. Sangeeta Dawar Mendiratta, M/s Bayer Biosciences Pvt. Ltd., Gurgaon
EC002644	9-Aug-50	United States Of America	Agricultural experiment station division of truck crops Davis, California USA	*S. lycopersicum*	Tomato	EARLY MARKET		
EC035420	24-Aug-65	Hungary		*S. lycopersicum*	Tomato	Trigrus Keralis		M/s. C. Gregory & Sons Ltd., Chilwell, Nohingham, U.K.
EC054644	4-Oct-68	Canada	Plant Research Inst., Research Branch, Central Expt. Farm, Oltawa, Canada	*S. lycopersicum*	Tomato	ROCKET		
EC129606	19-May-79	New Zealand	Dr.J. Burgmans, Tech. Officer, Min. of Agril. & Fisheries New Zealand- PO Box. No. 1140, Hostings New Zealand	*S. lycopersicum*	Tomato			NBPGR, New Delhi
EC050347	11-Jul-68	Nigeria	Institute For Agriculture Research Samaru Zaria, Nigeria.	*S. lycopersicum*	Tomato	Stekesctale		1. P.I. Division

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