Identification of new stable resistant sources to *Meloidogyne incognita* using conventional and marker assisted selection in tomato (*Solanum lycopersicum*)

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Summary. Forty tomato genotypes were screened through conventional (artificial inoculations in pots) followed by marker assisted selection (MAS) to identify stable resistant sources to the root-knot nematode (RKN) (*Meloidogyne incognita*). 'IIHR-2786' was used as the resistant and 'IIHR-2265' as the susceptible controls. Three lines, 'IIHR-2963', 'IIHR-2964' and 'IIHR-2805', were highly resistant and nine lines, 'IIHR-2965', 'IIHR-2786', 'IIHR-2614', 'IIHR-2785', 'IIHR-2806', 'IIHR-2809', 'IIHR-2810', 'IIHR-2814' and 'IIHR-2815' were found resistant through conventional screening with pot tests. The experiment was conducted twice to confirm the result and to check the stability of resistance source(s). Cluster analysis was done to classify the genotypes into different clusters and the highest inter cluster distance was found between cluster IV and V followed by III and V, which implied that the crosses between those genotypes that were grouped in these clusters may provide maximum heterosis upon hybridisation. In addition, there is a strong probability of obtaining transgressive segregants in further generations. The genotypes together with new hybrid combinations involving resistant × susceptible genotypes were then screened for *Mi* gene using linked molecular markers, which are linked to the *Mi* locus conferring resistance to RKN. The lines 'IIHR-2963', 'IIHR-2964', 'IIHR-2805', 'IIHR-2965', 'IIHR-2614', 'IIHR-2785', 'IIHR-2806', 'IIHR-2809', 'IIHR-2810', 'IIHR-2814' and 'IIHR-2815' plus 'IIHR-2786', amplified the expected product size of the resistant allele. By contrast, 'IIHR-2265', the susceptible control, and all other susceptible lines generated the product size of susceptible allele showing the absence of *Mi* locus. The hybrids generated both size amplicons and were, therefore, heterozygous for the resistant locus (*Mi*) and were also found to be phenotypically resistant, which indicated that the resistance to RKN is governed by dominate gene action and also shows that the use of these co-dominant molecular markers in MAS is effective because of their ability to distinguish between heterozygous and homozygous individuals, resulting in reducing the breeding cycles for developing stable (non-segregating) resistant advance breeding lines.

Key words: breeding cycles, cluster analysis, genotypes, homozygous, hybrids, MAS, *Mi* gene, root-knot nematode, resistance.

Tomato (*Solanum lycopersicum* L.) belongs to Solanaceae family and is universally recognised as a protective food because of antioxidants richness in its fruits and is established as one of the most valuable and indispensable vegetables in the world. It plays a vital and significant role in nutritional supplementation, together with potato and onion. Tomato is grown worldwide under both open field and protected cultivation. Worldwide it is cultivated in an area of 4848.384 thousand ha with the production of 182301.395 thousand tons and 37.60 t ha^{-1} productivity. China is the leading tomato

producing country followed by India and Turkey (http://www.fao.org/faostat/en/#data/OA). Tomato is prone to several diseases and pests, including root-knot nematodes (RKN). The genus of root-knot nematodes, *Meloidogyne*, comprises more than a hundred species among which *M. incognita* is the most prevalent and economically damaging species worldwide, more especially in tropical and sub-tropical regions (Sikora & Fernández, 2005). Its management on commercial fields became almost impossible due to its wide host range (Ntidi *et al.*, 2016; Tariq-Khan *et al.*, 2017), highly sophisticated host hijacking machinery, and the recent ban on some nematicides due to environmental issues and their hazardous effects on health, food and soil (Wu *et al.*, 2009). RKN is also involved in root disease complexes, particularly in association with *Ralstonia* and *Fusarium* in tomato (Abad *et al.*, 2003; Shahbaz *et al.*, 2015). Recently, Fourie *et al.* (2016) reported its involvement in reduction of plant tolerance to abiotic stresses. Together, these factors make RKN a serious threat to world food security and an important pest of tomato (Sasser, 1980; Williamson & Hussey, 1996). The annual crop losses due to RKN infection are more than 100 billion $US dollars (Trudgill & Blok, 2001). In tomato yield losses of 25 to 100% have been reported due to RKN infection (Mahajan & Singh, 2001; Kamalvanshi *et al.*, 2004; Jablonska *et al.*, 2007; Seid *et al.*, 2015).

In addition to that, the area under organic farming is increasing and the use of resistant cultivars is an integral part because of its eco-friendly aspect. For the development of resistant crop cultivars, identification of resistant genetic resources is a prerequisite. The conventional pot-based screening technique is time consuming, labour-intensive and impracticable especially for large populations, and there it difficulty identifying the resistance source(s) based on phenotypic selection. Marker-assisted selection (MAS) is a method by which selection of genotypes can be done on genotypic basis with great precision by using traits-specific molecular markers. The use of molecular markers linked to the RKN resistant gene (*Mi*) has proven to be very effective in resistance breeding programmes for selecting resistant cultivars through MAS.

The present study was conducted to identify new stable resistant sources to RKN through conventional (pot-based assays) followed by MAS for validating the presence or absence of *Mi* gene, and to identify the best parents for crosses to ensure maximum heterosis.

MATERIAL AND METHODS

Experimental site and plant material. The experiment was carried out at ICAR-Indian Institute of horticultural Research (IIHR), Hessaraghatta, Bengaluru, Karnataka, India. The experiment was conducted twice during the years 2016 and 2017. The experimental site is located at 13.58° N and 78° E of the GPS coordinates and 890 m above the mean sea level. Seeds of all tomato genotypes were obtained from the germplasm collections of ICAR-IIHR, Bengaluru. The completely randomised statistical design was used to carry out the experiment (Gomez & Gomez, 1984).

Source of *M. incognita* culture. The culture of *M. incognita* was obtained from the Division of Entomology and Nematology of ICAR-IIHR, Bengaluru and the species was confirmed using perineal cuticular pattern of females. Subsequently, the culture was maintained in the pots on susceptible tomato genotype ('IIHR-2265'). For genotype screening purpose, second-stage juveniles (J2) were obtained from the roots of these infected plants. To collect J2, roots were uprooted and washed thoroughly and then fully developed egg masses were handpicked using forceps. Subsequently, eggs were kept for hatching J2 on wire mess (blotted with sterilised filter paper) in Petri dishes containing distilled water. After 24 h hatched juveniles were collected and transferred to a beaker, where the J2 population was concentrated, and J2 were counted under a stereo zoom microscope to determine the number of active J2 per ml of water. Inoculation of J2 was done around the root zone of each plant with the final population of 6,000 J2 pot^{-1}, which containing 3 kg of sterilised soil (2 J2 g^{-1} of soil) 6 days after transplanting.

Plant reactions to *M. incognita*. For evaluating genotypes in order to identify the new stable resistant sources against RKN, seedlings were transplanted in plastic pots 25 days after sowing. The pots were filled with sterilised growing media (mixture of sand, soil and FYM at the ratio of 1:1:1). Fifteen seedlings were selected from each genotype for screening purpose. 'IIHR-2786' and 'IIHR-2265' genotypes were used as resistant and susceptible controls, respectively. Plants were uprooted 60 days after nematode inoculation and the roots were gently rinsed with water to remove the adhering soil. Thereafter, scoring for number of galls per root and root gall index (RGI) was done as described by Taylor & Sasser (1978) on 0-5 scale (Table 1) for both the seasons by examining the roots individually and independently.

DNA extraction and marker analysis. DNA extraction was done by Silica method using FastPrep®-24 bead-based homogeniser, as previously described by Elphinstone *et al.* (2003) with some minor modification. For MAS, in the present study five co-dominant molecular markers were used (four SCAR and one SSR), for screening of genotypes in order to validating the presence or absence of the *Mi* gene in resistant and susceptible genotypes, respectively (Table 2). The PCR protocol for SCAR markers that were used in the present study was, in brief, initial denaturation at 94°C for 5 min followed by 35 cycles of 94°C for 1 min, 58.3°C for 1 min and 72°C for 2 min followed by final extension of 5 min at 72°C, and for SSR marker 94°C for 5 min for initial denaturation followed by 36 cycles of 94°C for 45 s, 50.7°C for 45 s and 72°C for 45 s followed by final extension of 5 min at 72°C. After amplification, the PCR products were segregated on 2% Invitrogen UltraPure™ agarose and SYBR® Safe DNA Gel Stain was used for visualisation of the resulting amplicons. Invitrogen 50 bp DNA Ladder was used to determine the amplicon size. It was repeated three to four times to confirm the results.

Statistical analysis. SAS-PROC GLM with factorial set up (by taking year as a factor) for two seasons data (with logarithmic data transformation) was carried out and significance was tested based on Duncan multiple comparison test ($P < 0.01$). Mahalanobis D^2 analysis was performed (SAS 9.3, 2012) to group similar genotypes with respect to resistance against RKN. Clustering dendrogram pattern among the 40 genotypes was assessed using Tocher's method by building up suitable SAS codes (SAS 9.3, 2012).

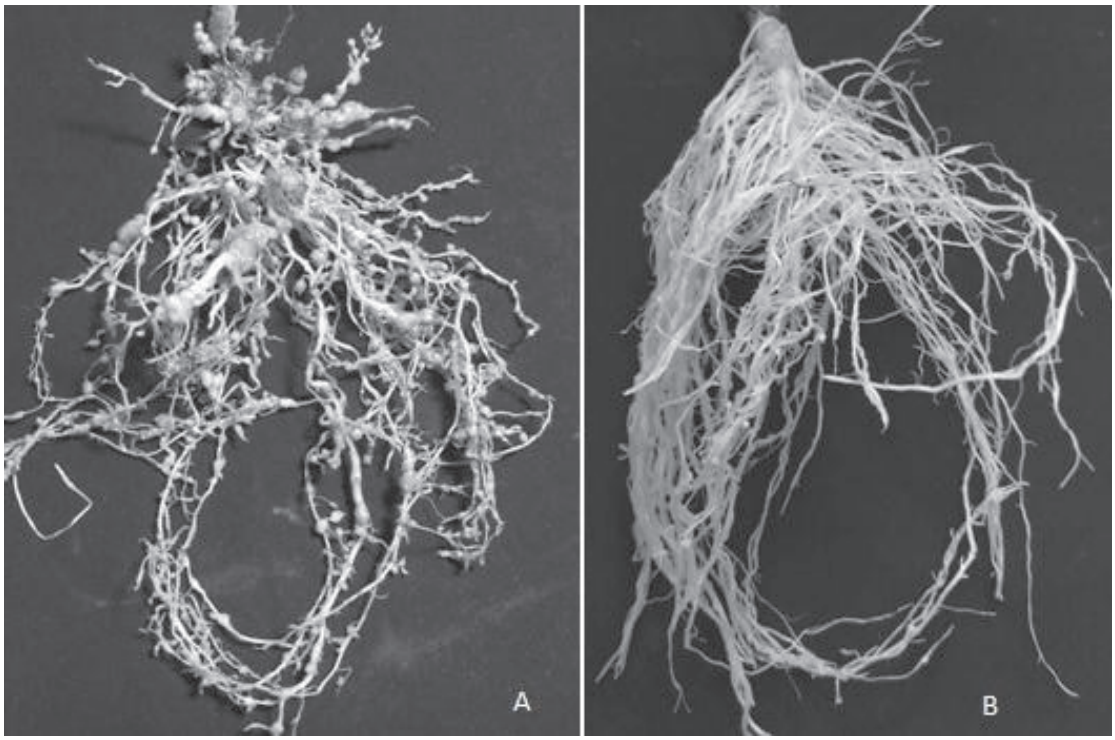

Fig. 1. Root of root-knot nematode susceptible and resistant lines of *Solanum lycopersicum* after 60 days of inoculation: A – susceptible line ('IIHR-2265'); B – resistant line ('IIHR-2963').

RESULTS

Phenotypic screening. Forty genotypes were evaluated during April-June of 2016 and June-August of 2017 for RKN resistance, and based on RGI, genotypes were classified in different classes on a 0 to 5 scale (Taylor & Sasser, 1978). Analysis of pooled data for two seasons under factorial setup (Table 3) revealed that there is a significant difference among the genotypes, seasons, and season × genotypes for the resistance level against *M. incognita*. Out of forty genotypes screened, three ('IIHR-2805', 'IIHR-2963' and 'IIHR-2964') lines were found highly resistant (Fig. 1A) with RGI ranging from 1.2-1.4, and nine lines ('IIHR-2965',

Table 1. Root Gall Index (RGI) scoring for nematodes screening (Taylor & Sasser, 1978).

Serial number	RGI	Number of galls root^{-1}	Reaction	Serial number	RGI	Number of galls root^{-1}	Reaction
1	0	0	I (Immune)	**4**	3	11-30	MR (Moderately Resistant)
2	1	1-2	HR (Highly Resistant)	**5**	4	31-100	S (Susceptible)
3	2	3-10	R (Resistant)	**6**	5	> 100	HS (Highly Susceptible)

Table 2. Molecular markers associated with resistance (*Mi* gene) to root-knot nematodes in tomato.

Sr. No.	Marker	Forward/ Reverse	Product size (bp)		Reference
			R	S	
1	Mi23 (SCAR)	F-TGGAAAAATGTTGAATTTCTTTTG R-GCATACTATATGGCTTGTTTACCC	380	430	Seah *et al.*, 2007
2	PMi (SCAR)	F-GGTATGAGCATGCTTAATCAGAGCTCTC R-CCTACAAGAAATTATTGTGCGTGTGAATG	550	350	Arens *et al.*, 2010
3	TG-180 (SCAR)	F-ATACTTCTTTGCAGGAACAGCTCAC R-CACATTAGTGATCATAAAGTACCAG	1.2 kb	0.9 kb	Yaghoobi *et al.*, 2005
4	TG-263 (SCAR)	F-GCTGAGAAATAAAGCTCTTGAGG R-TACCCTTAATGCTTCGGCAGTGG	0.9 kb	0.75 kb	Yaghoobi *et al.*, 2005
5	W415 (SSR)	F: AAGTCTTATCTAATTGCCTAT R: ATTTCCGTAATGATATGATCT	670	550	Wang *et al.*, 2013

Table 3. Results of ANOVA for data from two seasons under factorial setup.

Source of variation	Degree of freedom (DF)	Sum of squares (SS)	Mean squares (MS)	F Computed	F Tabular
Season	1	0.430	0.430	96.505	6.714
Treatments	39	156.555	4.014	648.427	1.664
Season × treatments	39	0.603	0.015	2.496	1.664
Error	320	1.981	0.006		
Total	399	159.569			

'IIHR-2614', 'IIHR-2785', 'IIHR-2806', 'IIHR-2809', 'IIHR-2810', 'IIHR-2814', 'IIHR-2815' and 'IIHR-2786') plus the resistant control 'IIHR-2786' showed resistance reaction with RGI ranging from 2.0 to 2.4 (Table 4). Among 12 resistant lines, six were from cultivated tomato (*S. lycopersicum*) background and six were from wild (*S. peruvianum*) background. Six lines appeared as moderately susceptible (MS), whereas the remaining 22 lines, including the susceptible control 'IIHR-2265' were regarded as highly susceptible (Fig. 1B), with RGI score of 5.0. However, none of the genotypes showed an immune reaction. The analysis of variance revealed significant differences ($P < 0.01$) between resistant and susceptible genotypes, which indicated that the difference in resistance level in these genotypes is at the genotypic level.

Cluster analysis. Cluster analysis was done to group genotypes to know the level of divergence among the genotypes with respect to resistance against RKN. The genotypes were classified in five different clusters based on distance matrix (Fig. 2; Table 5). The maximum number of genotypes (14) was assigned to the first cluster, followed by fifth and third clusters with 12 and 8 genotypes, respectively. The least number of genotypes (1) was designated as the fourth cluster, followed by five genotypes in the second cluster. The distribution pattern showed clearly that all resistant genotypes were in the same cluster (V), with cluster mean as

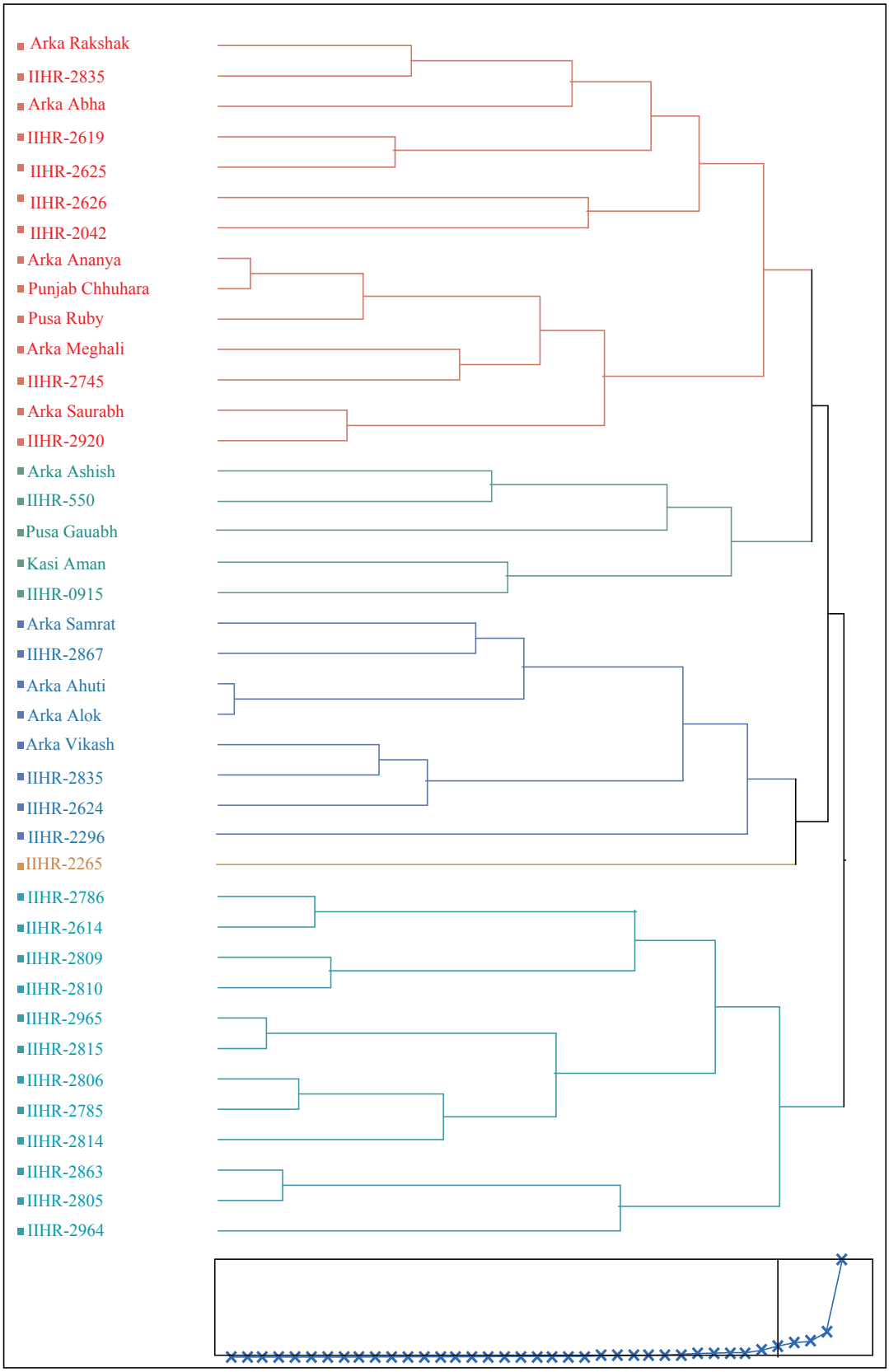

Fig. 2. Dendrogram-clustering pattern of tomato genotypes based of D2 analysis.

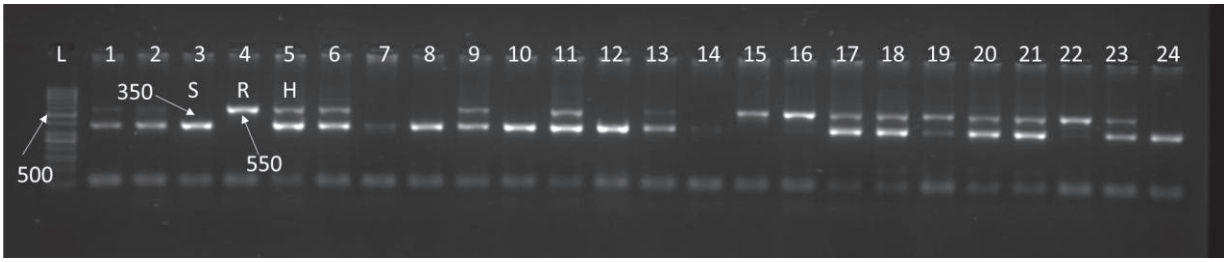

Fig. 3. Genotyping of root-knot nematode resistant and susceptible lines of *Solanum lycopersicum* and newly developed F1 hybrids with molecular marker PMi. L – 50 bp DNA ladder. R – resistant lines: 4-'IIHR-2963', 15-'IIHR-2964', 16-'IIHR-2805' and 22-'IIHR-2786'. S – susceptible lines: 3-'IIHR-2265', 7-'IIHR-2296', 8-'IIHR-2834', 10-'IIHR-2835', 12-'IIHR-2920', 14-'IIHR-2042' and 24-'Pusa Ruby'. H – heterozygous lines and newly developed F_1 Hybrids: 1-'IIHR-2806', 2-'IIHR-2809', 5-'IIHR-2810', 6-'IIHR-2814', 9-'F_1Hybrid', 11-'F_1Hybrid', 13-'F_1Hybrid', 17-'F_1Hybrid', 18-'F_1Hybrid', 19-'F_1Hybrid', 20-'F_1Hybrid', 21-'F_1Hybrid' and 23-'F_1Hybrid'.

Table 4. Tomato genotypes reactions to root-knot nematode (*Meloidogyne incognita*).

Sr. No.	Genotypes	Mean number of galls per root over the season	Mean gall index over the season	Reaction	Sr. No.	Genotypes	Mean number of galls per root over the season	Mean gall index over the season	Reaction
1	'Arka Rakshak'	118.21(2.07)^{ijk}	5.00	HS	**21**	'IIHR-2786'	8.70 (0.97)^{e}	2.30	R
2	'Arka Samrat'	151.3 (2.18)^{lmnop}	5.00	HS	**22**	'IIHR-2963'	2.00 (0.46)^{ab}	1.20	HR
3	'Arka Ananya'	129.41 (2.11)^{klm}	5.00	HS	**23**	'IIHR-2964'	2.20 (0.49)^{b}	1.40	HR
4	'Arka Meghali'	131.30 (2.11)^{klmn}	5.00	HS	**24**	'IIHR-2965'	4.30 (0.71)^{c}	2.00	R
5	'Arka Ahuti'	149.30 (2.17)^{lmnop}	5.00	HS	**25**	'IIHR-2867'	153.60 (2.18)^{lmnop}	5.00	HS
6	'Arka Abha'	123.61 (2.09)^{jkl}	5.00	HS	**26**	'IIHR-2614'	8.00 (0.94)^{e}	2.00	R
7	'Arka Alok'	149.30 (2.17)^{lmnop}	5.00	HS	**27**	'IIHR-0915'	90.40 (1.95)^{fg}	4.60	HS
8	'Arka Vikash'	163.00 (2.21)^{op}	5.00	HS	**28**	'IIHR-550-3'	94.50 (1.97)^{fgh}	4.20	HS
9	'Arka Ashish'	91.60 (1.96)^{fgh}	4.20	S	**29**	'IIHR-2296'	180.90 (2.25)^{p}	5.00	HS
10	'Arka Saurabh'	136.40 (2.13)^{klmno}	5.00	HS	**30**	'IIHR-2042'	103.80 (2.01)^{fghij}	5.00	HS
11	'Pusa Gauabh'	83.10 (1.92)^{f}	4.00	S	**31**	'IIHR-2834'	162.10 (2.21)^{nop}	5.00	HS
12	'Pusa Ruby'	128.70 (2.11)^{klm}	5.00	HS	**32**	'IIHR-2835'	120.00 (2.07)^{ijk}	5.00	HS
13	'IIHR-2620'	129.40 (2.11)^{klm}	5.00	HS	**33**	'IIHR-2920'	135.60 (2.13)^{klmno}	5.00	HS
14	'IIHR-2913'	94.60 (1.97)^{fgh}	4.60	HS	**34**	'IIHR-2805'	1.70 (0.40)^{a}	1.20	HR
15	'IIHR-2619'	112.50 (2.05)^{hijk}	5.00	HS	**35**	'IIHR-2806'	7.90 (0.93)^{e}	2.00	R
16	'IIHR-2626'	97.90 (1.99)^{fghi}	4.60	HS	**36**	'IIHR-2809'	8.50 (0.96)^{e}	2.20	R
17	'IIHR-2745'	133.50 (2.12)^{klmno}	5.00	HS	**37**	'IIHR-2810'	9.30 (1.04)^{e}	2.40	R
18	'IIHR-2265'	242.30 (2.38)^{q}	5.00	HS	**38**	'IIHR-2814'	6.40 (0.83)^{d}	2.00	R
19	'IIHR-2624'	160.91 (2.20)^{mnop}	5.00	HS	**39**	'IIHR-2815'	4.30 (0.66)^{c}	2.00	R
20	'IIHR-2625'	110.90 (2.04)^{ghijk}	5.00	HS	**40**	'IIHR-2785'	8.30 (0.96)^{e}	2.00	R
CD		0.619 (Year), 0.138 (Treatment) and 0.097 (Y × T)							
SEM		0.314 (Year), 0.070 (Treatment) and 0.049 (Y × T)							
CV (%)		4.606							
Range		1.7-242.3 (Average No. of Galls over the year) and 1.2 to 5 (Average Gall Index over the year)							

Note. The log-transformed values are shown in parenthesis and means (mean of two seasons) with different super script letter indicate statistically difference ($P < 0.01$) using Duncan's test based on two factor ANOVA.

Table 5. Distributing pattern of 40 genotypes of tomato into five clusters.

Cluster number	Number of genotypes	Genotype(s) included	Mean number of galls over the year	Mean gall index over the year
I	14	'Arka Rakshak', 'DVRT-2', 'Arka Ananya', 'Arka Abha', 'Punjab Chhuhara', 'Arka Meghali', 'Arka Saurabh', 'IIHR38-7', 'ToLCV-R_4-F_3-38-1-1', 'Vibhav', 'Pusa Ruby', 'H-24', 'IIHR-2042', 'Pant T-3'	122.22	5.00
II	5	'Arka Ashish', 'IIHR-550-3', 'Puas Gaurabh', 'Kashi Aman', 'IIHR-915'	90.84	4.32
III	8	'Arka Samrat', 'Arka Alok', 'IIHR-38-10', 'Arka Ahuti', 'IIHR-12-21', 'Arka Vikash', 'DVRT-1', 'IIHR-1463'	158.8	5.00
IV	1	'IIHR-2265'	242.3	5.00
V	12	'IIHR-2786', 'IIHR-2614', 'BN 10-2', 'IIHR-2963', 'IIHR-2964', 'IIHR-2965', 'IIHR-2805', 'IIHR-2806', 'IIHR-2809', 'IIHR-2810', 'IIHR-2814', 'IIHR-2815'	5.96	1.91

Table 6. Inter- and intra-cluster Euclidean distance.

Cluster	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
Cluster 1	39.47 (0.47)	49.40 (0.70)	63.42 (0.56)	26.03 (1.85)	476.55 (2.83)
Cluster 2	49.40 (0.70)	5.15 (0.51)	46.70 (1.16)	12.00 (2.39)	129.36 (2.15)
Cluster 3	63.42 (0.56)	46.70 (1.16)	10.20 (0.36)	10.34 (1.29)	309.69 (3.22)
Cluster 4	26.03 (1.850)	12.00 (2.39)	10.34 (1.29)	0.00	51.20 (4.26)
Cluster 5	476.55 (2.83)	129.36 (2.15)	309.69 (3.22)	51.20 (4.26)	44.65 (0.67)

Note. The log-transformed values are shown in parenthesis.

5.96 and 1.91 for the average number of galls per root of two seasons and average RGI of two seasons, respectively, and the highly susceptible line ('IIHR-2265') was grouped in a separate cluster (IV) with cluster mean as 242.30 and 5.00 for average number of galls per root and average RGI, respectively. It was also observed from the Euclidean distance (Table 6) that the maximum (4.26) inter cluster distance was between cluster IV and V, followed by III and V, and I and V. The least distance (0.56) was recorded between clusters I and III, followed by between cluster I and II, and II and III. Hence, the genotypes grouped in clusters IV and V may yield maximum heterosis upon hybridisation.

Marker assisted selection. In the present study, we have used five co-dominant molecular markers (four SCAR and one SSR), for screening of genotypes in order to validate the presence or absence of *Mi* gene in resistant and susceptible genotypes, respectively (Table 2). In addition, validation of polymorphic markers was performed in F1 hybrids, which have been developed by crossing between identified resistant and susceptible genotypes. Out of five markers, only three were able to distinguish between resistant, susceptible and F1 hybrids, by producing resistant, susceptible and heterozygous PCR amplicon length, respectively. Two markers (TG-180 and TG-263) could not differentiate between contrasting genotypes. The lines 'IIHR-2963', 'IIHR-2964', 'IIHR-2805', 'IIHR-2965', 'IIHR-2614', 'IIHR-2785' and 'IIHR-2815' plus the resistant control ('IIHR-2786') gave the expected amplicon size of 380 bp, 550 bp and 670 bp with Mi23, PMi and W415, respectively, linked to the *Mi* locus conferring resistance to RKN. 'IIHR-2265', the susceptible control and other susceptible lines generated the 420 bp, 350 bp and 550 bp band size with Mi23, PMi and W415,

respectively, showing the absence of *Mi* locus. The hybrids 'IIHR-2296' × 'IIHR-2963', 'IIHR-2296' × 'IIHR-2964', 'IIHR-2834' × 'IIHR-2963', 'IIHR-2834' × 'IIHR-2964', 'IIHR-2867' × 'IIHR-2963', 'IIHR-2867' × 'IIHR-2964', together with four *S. peruvianum* accessions ('IIHR-2806', 'IIHR-2809', 'IIHR-2810', 'IIHR-2814') generated both bands and were, therefore, heterozygous for the resistant locus (*Mi*) (Fig. 3). Results of marker based screening of resistant lines showed the presence of *Mi* locus in lines showing resistance to RKN.

DISCUSSION

Resistance to root-knot nematodes was incorporated into cultivated tomato (*S. lycopersicum*) from the wild species (*S. peruvianum*) through embryo rescue (Smith, 1944). Resistance to RKN is governed by a single dominant gene, which is designated as the *Mi* gene, which confers resistance against the three major *Meloidogyne* species that infect tomato. *Mi* has been localised to the short arm of chromosome 6 (Kaloshian *et al.*, 1998). This chromosome has been mapped in considerable detail, and multiple markers that are linked to *Mi* have been identified (Williamson *et al.*, 1994; Yaghoobi *et al.*, 2005; Seah *et al.*, 2007; Arens *et al.*, 2010; Wang *et al.*, 2013). The *Mi* gene of tomato belongs to the nucleotide-binding site-leucine rich repeat (NBS-LRR) class of resistant genes (Milligan *et al.*, 1998), which activates effector-triggered immunity (ETI) by recognising the effector protein of RKN. Activation of ETI results in disease resistance by eliciting programmed cell death (PCD) or hypersensitive response (HR) (Jones & Dangl, 2006). The strong HR and resulting resistant phenotype is a product of gene-for-gene interaction, where an effector (Avirulence gene of RKN-Avr) is recognised by the R-protein produced by the resistant plant in the presence of resistant gene (*Mi* gene in tomato) (Dodds & Rathjen, 2010). Application of MAS in RKN resistance breeding programmes increases selection efficiency and precision to a great extent. Several co-dominant molecular markers that are linked to the *Mi-1.2* gene (Milligan *et al.*, 1998; Wang *et al.*, 2013) and located within the *Mi-1* locus (Seah *et al.*, 2007), have been developed and extensively utilised for MAS in the tomato breeding programme for RKN resistance. Utilisation of these co-dominant molecular markers have additional advantage, because of the ability to distinguish between heterozygous and homozygous individuals, which is important for developing stable (non-segregating) resistant inbred line(s) in reduced breeding cycles.

The results of the present study showed that there is a significant difference among the genotypes, seasons, and season × genotypes, indicating that the difference in resistance level in these genotypes is at the genotypic level and is influenced by the environment. Three lines were found highly resistant in both seasons, which indicated that these genotypes may have another *Mi* gene apart from *Mi-1* as reported by Wang *et al.* (2013). Identified resistant lines ('IIHR-2963' and 'IIHR-2964') are stable and could be utilised immediately in the development of resistant tomato cultivars and for undertaking further studies on inheritance and mechanisms of resistance. *Solanum peruvianum* accessions 'IIHR-2805' could be utilised for introgression of the resistance gene into cultivated tomato background by embryo rescue for developing nematode resistance in tomato, pyramiding *Mi* gene with other important genes. This study also demonstrated that the use of *Mi* gene specific marker is a reliable and efficient method for screening tomato germplasm against RKN and for early detection of resistant sources in marker assisted tomato-breeding programmes. The grouping of genotypes in different clusters demonstrated the comparative divergence among the clusters, which demonstrated that the clusters that exhibited maximum inter cluster distance were more divergent and *vice versa*. Therefore, for selecting parents for crossing in hybridisation programmes, preference should be given to the genotypes that were grouped in these particular clusters. Moreover, this enabled plant breeders to select the diverse genotypes during hybridisation programme, which accelerates the exploitation of high heterosis. In addition, there is a strong probability of obtaining transgressive segregants in further generations upon selfing.

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Vinod Jatav, Avverahally Thammanna Sadashiva, Kundapura Venkataramana Ravishankar, Rangarajan Venugopalan, Rajappa Umamaheshwari and Mahendrakar Sreenivasa Rao. Выявление новых стабильных источников устойчивости к *Meloidogyne incognita* с использованием обычной и маркер-опосредованной селекции у томатов (*Solanum lycopersicum*).

Резюме. Сорок генотипов томатов были исследованы для выявления стабильных источников устойчивости к галлообразующим нематодам *Meloidogyne incognita* с помощью обычной (внесение нематоды в горшки с растениями) селекции, после которой применяли маркер-опосредованную селекцию. Генотип ‘IIHR-2786’ был использован в качестве устойчивого и генотип ‘IIHR-2265’ как восприимчивый контроль. Три генетические линии: ‘IIHR-2963’, ‘IIHR-2964’ и ‘IIHR-2805’ были определены как высокоустойчивые, а девять линий: ‘IIHR-2965’, ‘IIHR-2786’, ‘IIHR-2614’, ‘IIHR-2785’, ‘IIHR-2806’, ‘IIHR-2809’, ‘IIHR-2810’, ‘IIHR-2814’ и ‘IIHR-2815’ как устойчивые в процессе скрининга в горшках с зараженными растениями. Эксперимент был повторен дважды для подтверждения результатов и вывления источников устойчивости. Был проведен кластерный анализ для классификации генотипов. Набольшее внутреннее расстояние было обнаружено между кластерами IV и V, после чего следовали кластеры III и V. Эти данные показывают, что скрещивание между генотипами может дать максимальный эффект гетерозиса при гибридизации. К тому же, значительной представляется и возможность получения трансгрессивных фенотипов в последующих поколениях. Был проведен скрининг исследованных генотипов и гибридных комбинаций «устойчивый × восприимчивый» по *Mi* гену используя маркеры, сцепленные с *Mi* локусом. Генетические линии ‘IIHR-2963’, ‘IIHR-2964’, ‘IIHR-2805’, ‘IIHR-2965’, ‘IIHR-2614’, ‘IIHR-2785’, ‘IIHR-2806’, ‘IIHR-2809’, ‘IIHR-2810’, ‘IIHR-2814’ и ‘IIHR-2815’, а также ‘IIHR-2786’ после проведения амплификации, показали присутствие продукта ожидаемой длины. Напротив, генотип ‘IIHR-2265’, применяемый как восприимчивый контроль, и все другие восприимчивые линии дали продукт, указывающий на отсутствие локуса *Mi*. Гибридные формы давали ампликоны двух этих длин, представляя собой формы гетерозиготные по локусу устойчивости *Mi,* и оказались фенотипически устойчивыми. Эти результаты показывают, что устойчивость к *Meloidogyne incognita* определяется доминантным геном. Использование кодоминантных молекулярных маркеров в рамках маркер-опосредованной селекции представлется эффективным методом, позволяющим различать гетерозиготные и гомозиготные особи, что, в свою очередь, позволяет существенно сократить время селекции стабильных (нерасщепляющихся) генетических линий.