

# Relative Response of Four Tomato Species to *Rotylenchulus reniformis* Infestation

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## ABSTRACT

The reniform nematode (*Rotylenchulus reniformis*) is among the most economically damaging plant pathogens in the United States. This nematode is mostly known for its damage to cotton but tomato is also well-within its vast host range that includes 314 plant species across 77 plant families. Nematode-resistant genotypes offer an effective, environmentally safe alternative to agro-chemicals for reniform nematode management. Resistance genes can be introgressed into cultivars through plant improvement efforts. Tomato is a diploid species which is more amenable to identification of resistance genes in contrast to cotton where cultivars are either tetraploid or hexaploid. This greenhouse study examined cultivated and wild *Solanum* species represented by 40 tomato accessions, to identify resistance and susceptibility responses to *R. reniformis*. Accessions were evaluated by using single plants in six replicates. Seeds were germinated in sterile soil and inoculated with mixed vermiform *R. reniformis*. After seven weeks, eggs and vermiform stages were extracted from the root system and counted. A susceptible control *S. lycopersicum* “Rutgers” (LA1090) was included. Seven putatively resistant tomato genotypes were identified. These genotypes in increasing order of resistance are *S. chilense* (LA1029), *S. lycopersicum* (LA1792), *S. chilense* (LA1932), *S. peruvianum* var. *humifusum* (LA0385) *S. pimpinellifolium* (LA2934), *S. peruvianum* f. *glandulosum* (LA1283) and *S. pimpinellifolium* (LA1579).

## KEYWORDS

Nematode-Resistance; *Rotylenchulus reniformis*; Reniform Nematode; *Solanum lycopersicum*; *S. chilense*; *S. peruvianum*; *S. pimpinellifolium*; Tomato

## 1. Introduction

Tomato (*Solanum lycopersicum* L. syn. *Lycopersicon esculentum* Mill.) offers an accessible model system from which to clone a nematode resistance gene [1]. Genes for resistance to the southern root-knot nematode (*Meloidogyne incognita*) and the cyst nematode (*Globodera rostochiensis*) have been successfully isolated and characterized from tomato species *S. peruvianum* L. [formerly *L. peruvianum* (L.) Mill.] and *S. lycopersicum*. Disease symptoms such as galling index cannot be relied on to assess reniform nematode resistance because such

symptoms do not develop in infested plants. Roots infested with reniform nematodes may appear normal unless viewed under a microscope even when aboveground symptoms have been observed [2]. In a susceptible host, the female establishes a specialized feeding site or syncytium and develops to the egg-laying stage. Resistance occurs when the female fails to establish or maintain this feeding site [3,4]. As a result, most evaluation studies either measure egg production as an indicator of nematode reproduction, or vermiform count as an indicator of nematode feeding and survival or both. Often, these counts are expressed as a percentage of a susceptible or

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resistant control [5]. Tomato is a near perfect plant system for basic and applied plant research particularly for discovery of resistance genes, because of its photoperiod, insensitivity and high self-fertility, broad environmental adaptability, and wide difference in fruit size and short life cycle duration [6]. Nematode resistance assays require plants with mature seeds for the next generation, and tomato offers an easier and faster alternative to cotton because of shorter duration to attain maturity (60 - 95 days). Evaluation of 33 tomato genotypes for resistance to root-knot nematodes (RKN), showed tomato Mongal T-11 and tomato Beef Master to be highly resistant to *Meloidogyne* spp. and also with the lowest reproductive factors of 0.71 and 0.53, respectively [7].

Tomato and its wild relatives have good seed yield and ease of controlled pollination and hybridization. This plant is therefore amenable to asexual propagation and *in vitro* plant regeneration [8]. Tomato, a diploid species, has a moderate sized genome (~0.95 pg/1C, 950 Mbp) [9, 10] with minimal gene duplication. A wide array of mutants [11] and diverse genetic stocks are available including a diverse collection of wild species

(<http://tgrc.ucdavis.edu/>; <http://www.sgn.cornell.edu>). The tomato genome encodes an estimated 35,000 genes [12]. The availability of high molecular weight insert genomic libraries, including both Yeast Artificial Chromosome (YAC) and Bacterial Artificial Chromosome (BAC) libraries [13,14], has facilitated map-based or positional cloning. Current genetic maps for tomato include at least 2200 Restriction Fragment Length Polymorphisms (RFLPs), Cleaved Amplified Polymorphic Sequences (CAPs), and Simple Sequence Repeats (SSRs), as well as emerging genetic resources that include a comparative map with Arabidopsis of over 500 Conserved Orthologous Set (COS) markers [15], <http://www.sgn.cornell.edu>. The populations used for generating these maps were derived from crosses between wild relatives of various *Solanum* species and cultivars. This has led to the discovery and introgression of novel alleles for disease resistance [16] and fruit traits into cultivated germplasm. Thus far, resistance to *Meloidogyne incognita*, Fusarium and Verticillium wilts, *Phytophthora infestans*, *Globodera rostochiensis*, and *Pseudomonas syringae* pv. *tomato* has been successfully introgressed into cultivated tomato germplasm. Very few economically important plant species are as well characterized as tomato and therefore strictly from identification of resistance genes to reniform nematode perspective tomato species are also being studied here. Tomato has been recognized as an excellent host of the reniform nematode and infection of tomato has been reported from Puerto Rico, Colombia, India and Pakistan [17]. The nematode causes substantial economic damage to tomato crops throughout tropical and subtropical regions. Systematic evaluation of tomato species and

germplasm for reniform resistance has been reported in older studies and few of these have been undertaken in the past 25 years. Resistance in *S. pimpinellifolium* (PI375937) was first identified by earlier investigators working on tomato [17]. The progeny of *S. pimpinellifolium* (PI375937) × *S. lycopersicum* “Red Rock” cross was tested for resistance to *R. reniformis*, and the presence of one dominant gene in *S. pimpinellifolium* was suggested. Furthermore, in 22 tomato cultivars tested, resistance response to *H. schachtii* and reniform nematode was correlated [18]. In a greenhouse study that provided a 12-day exposure to reniform nematodes, three *S. lycopersicum* selections—Kalyanpur Sel I and III, LA121, and a yellow-fruited *S. pimpinellifolium* accession were categorized as being immune [19]. In this study, we present results from four species comprising 40 genotypes for their responses to reniform nematode infestation.

## 2. Materials and Methods

### 2.1. Growing and Stock Maintenance of the Tomato Genotypes

Seeds of the 40 accessions were obtained from the C.M. Rick Tomato Genetics Resource Center (TGRC), University of California, Davis, CA. These accessions carry LA designation followed by a four digit unique number. In the greenhouse, six seeds of each accession were germinated in trays using Metromix 200 Planting Mix (Sun-gro Horticulture, Bellevue, WA). At the first true leaf stage seedlings were transplanted into one-gallon pots at a single plant per pot. Transplants were watered as needed and fertilized weekly with Miracle Gro 15-30-15 (The Scotts Company, Marysville, OH). Upon maturation, fruits were collected and seeds were extracted and stored for future use.

### 2.2. Reniform Nematode Inocula

Reniform nematode population was collected from an infected field soil and confirmed morphometrically and increased on Delta PineLand 555 B2RF cotton (*Gossypium hirsutum*) in the greenhouse at Auburn University. The population of *R. reniformis* consisted of mixed vermiform stages obtained a day before inoculation. The nematodes were extracted from the cotton roots by gravity sieving followed by sucrose centrifugations. The nematode inoculum was quantified using the Nikon TSX 100 inverted microscope and standardized to apply 2000 vermiform life stages per 3 ml of water.

### 2.3. Reniform Nematode Bioassay in the Greenhouse

Twelve seeds of each tomato genotype were planted in plastic trays filled with Metromix 200 planting medium.

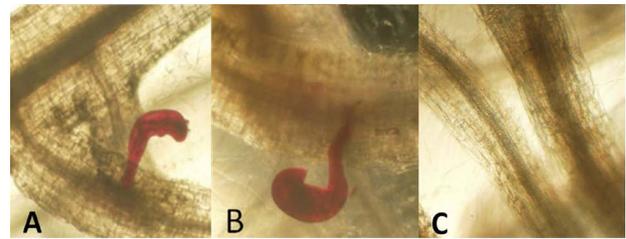
At the first true leaf stage (10 - 14 days after planting), seedlings were transplanted one per pot into 150ml Ray Leach “Cone-tainers” (Stuewe and Sons, Corvallis, OR) filled with 60:40 (v/v) mixture of sand and clay. Tomatoes were screened for nematode response using a modified protocol [2]. The Cone-tainers with the six largest plants of each genotype were infested with nematodes by gently pipetting, the nematode suspension at the base of the tomato stem. Each Cone-tainer received two inoculations 7 days apart each with 2000 mixed vermiform *R. reniformis*. Plants were irrigated daily and fertilized weekly for seven weeks. Eggs were extracted from roots using 0.14 M NaOCl solution [20], followed by centrifugal flotation in 1 M sucrose solution. Vermiform stages were extracted and enumerated at 40 $\times$  with the inverted microscope. A susceptible control *S. lycopersicum* “Rutgers” (LA 1090) was included. This genotype has been shown to support prolific reproduction of reniform nematodes [21]. In all, six randomized replications of 40 genotypes were tested against *R. reniformis*.

#### 2.4. Statistical Analysis

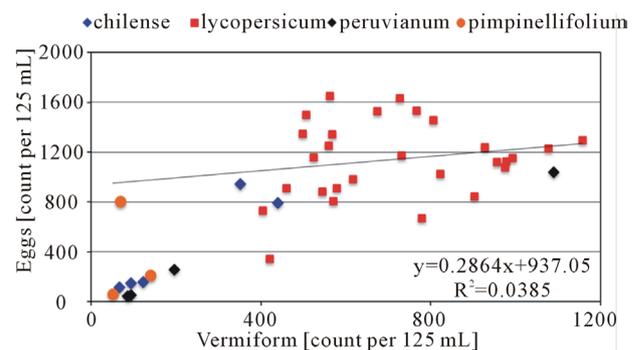
Data were analyzed using generalized linear mixed models procedures as implemented in SAS<sup>®</sup> PROC GLIMMIX (SAS Institute, Cary, NC), employing a lognormal distribution function for counts. The experimental design was a randomized complete block design with six blocks; block was considered to be a random effect. Dunnett’s test was employed to compare accession means to the susceptible control cv. Rutgers. Mean counts and Upper and Lower confidence limits (UCL and LCL) on the log scale were back-transformed to the original scale.

### 3. Results and Discussion

Forty accessions were screened for nematode resistance. All accessions tested supported development and reproduction of reniform nematode. Female nematodes exhibited a preference for penetration of young tender roots generally near root tips (Figure 1). As shown in Tables 1-3, total count nematodes (eggs + vermiform) extracted ranged from a high of 2522/125 mL of soil on *S. lycopersicum* “Moboline” (LA3152) to a low of 122 on *S. pimpinellifolium* (LA1579). Across all accessions, count per gram of fresh root varied from 48 to 1645 on LA1579 and LA3318, respectively. Nematode egg counts were greater than vermiform numbers for majority of the accessions evaluated (Figure 2). However, there was not sufficient evidence to suggest a direct relationship between the two measurements. A strong correlation existed between egg and vermiform counts for the 14 wild species examined ( $r = 0.88, P = 0.00002$ ), the two estimates were somewhat loosely correlated for the 8 background cultivars ( $r = 0.65, P = 0.04$ ) and 18 mutants



**Figure 1.** Reniform nematode infested roots seven weeks post inoculation (wpi). A = Roots of susceptible wild tomato *S. lycopersicum* var. *cerasiforme* (LA 2070). B = Roots of susceptible tomato cultivar *S. lycopersicum* cv. Micro-tom (LA 3911). C = Roots of putatively resistant tomato cultivar *S. lycopersicum* (LA1792).



**Figure 2.** Relationship between vermiform and egg ratios of reniform nematode for 40 accessions belonging to four cultivated and wild species of tomato (*Solanum*).

tested ( $r = 0.35, P = 0.08$ ).

*Solanum lycopersicum* “Rutgers” (LA1090), used in this study as a susceptible check supported abundant nematode reproduction. The average count (including vermiform and eggs) recorded was 1235/125 ml soil. Thirty-two additional accessions registered mean counts comparable to LA1090 and were thus classified susceptible. Seven accessions (~18%) suppressed nematode reproduction significantly (at  $P \leq 0.05$ ) when compared to LA1090 in both counts per 125 mL of soil and gram root fresh weight. These were classified as “putatively resistant”. The egg counts observed on the putatively resistant genotypes were more than 10% but less than 30% of LA1090. Mean counts were not significantly different from the control LA1090 (Table 1). In contrast, 6 of the 7 (~86%) putatively resistant accessions were from the wild species collection with varying geographical origins. These had levels of nematode reproduction that were significantly lower than LA1090 (Table 2). *Solanum pimpinellifolium* (LA2934), *S. chilense* (LA1932), *S. chilense* (LA1029), *S. peruvianum* var. *humifusum* (LA0385), *S. pimpinellifolium* (LA1579), and *S. peruvianum* var. *glandulosum* (LA1283) all supported nematode populations that were significantly smaller than those of the susceptible control. Among the background

**Table 1.** Relative resistance to reniform nematode (RN) by *Solanum lycopersicum* genotypes as compared to susceptible control cv. Rutgers. Data average of six replications.

TGRC Acc. No.	Cultivar	Resistance genes	Total reniform count = vermiform + eggs									
			Count per 125 mL					Count per gram root fresh weight (No. per g RFW)				
			Estimate	95% LCL	95% UCL	% of Rutgers	Dunnett's P-value	Estimate	95% LCL	95% UCL	% of Rutgers	Dunnett's P-value
LA 1090	Rutgers		1235	771	1978	100		718	421	1225	100	
LA 3911	Micro-tom		848	529	1358	69	0.79	785	460	1340	109	1.00
LA 1022	VFN-8	I, Mi, u, Ve	1665	1039	2667	135	0.91	860	504	1467	120	1.00
LA 2838A	Ailsa Craig		1818	1135	2913	147	0.77	695	407	1185	97	1.00
LA 3471	Mogeor	aa, Frl, I, I2, Mi, pyl, Tm-2 <sup>a</sup> , Ve	2130	1329	3412	173	0.45	968	567	1651	135	0.96
LA 3343	Rio Grande		2317	1446	3711	188	0.30	849	498	1449	118	1.00
LA 2400	Castlemart	sp, u	2360	1473	3781	191	0.27	861	505	1470	120	1.00
LA 3342	Unknown		2364	1191	4696	192	0.49	735	339	1594	102	1.00

**Table 2.** Relative resistance to reniform nematode (RN) in wild tomato species compared to susceptible control *Solanum lycopersicum* "Rutgers". Data average of six replications.

TGRC Acc. No.	Species	Total reniform count = vermiform + eggs									
		Count per 125 mL					Count per gram root fresh weight No. per g RFW)				
		Estimate	95% LCL	95% UCL	% of Rutgers	Dunnett's P-value	Estimate	95% LCL	95% UCL	% of Rutgers	Dunnett's P-value
LA 1090	<i>S. lycopersicum</i> "Rutgers"	1235	639	2386	100		718	373	1382	100	
LA 1029	<i>S. chilense</i>	200	97	415	16	0.0068	249	121	514	35	0.2787
LA 1932	<i>S. chilense</i>	301	145	623	24	0.0541	156	76	320	22	0.0316
LA 2747	<i>S. chilense</i>	1266	655	2447	103	1.0000	544	283	1048	76	0.9998
LA 2710	<i>S. lycopersicum</i> var. <i>cerasiforme</i>	1467	759	2835	119	1.0000	615	320	1185	86	1.0000
LA 2081	<i>S. lycopersicum</i> var. <i>cerasiforme</i>	1951	1010	3770	158	0.9770	971	504	1868	135	0.9994
LA 2080	<i>S. lycopersicum</i> var. <i>cerasiforme</i>	2053	1062	3966	166	0.9510	1191	618	2292	166	0.9484
LA 2079	<i>S. lycopersicum</i> var. <i>cerasiforme</i>	2341	1212	4524	190	0.8180	1258	653	2421	175	0.9022
LA 2744	<i>S. peruvianum</i>	524	271	1012	42	0.4872	193	100	371	27	0.0681
LA 3858	<i>S. peruvianum</i>	2314	1198	4472	187	0.8333	794	412	1528	111	1.0000
LA 1283	<i>S. peruvianum</i> var. <i>glandulosum</i>	138	41	463	11	0.0266	86	27	278	12	0.0277
LA 0385	<i>S. peruvianum</i> var. <i>humifusum</i>	186	96	360	15	0.0024	122	63	234	17	0.0044
LA 1579	<i>S. pimpinellifolium</i>	122	63	236	10	0.0001	48	25	93	7	0.0001
LA 2934	<i>S. pimpinellifolium</i>	271	140	524	22	0.0252	91	48	176	13	0.0006
LA 0722	<i>S. pimpinellifolium</i>	916	474	1771	74	0.9996	411	213	790	57	0.9050

genotypes tested and examined, only *S. lycopersicum* (LA1792) was able to meaningfully stifle nematode reproduction (Table 3).

Resistance has been identified by measuring eggs per gram of root [22], vermiform nematodes recovered from soil [5], or a combination of number of eggs and vermiform stages extracted [23,24]. Combining both life stages

to evaluate resistance provides information on nematode reproduction, feeding and survival ability and thus serves as an indicator of the resistance and susceptibility within a host genotype. In this study, our approach involved a combination of eggs and vermiform life stages extracted from roots of forty cotton accessions for resistance evaluation. Near isogenic lines (NIL) are vital genetic stocks

**Table 3. Relative resistance to reniform nematode (RN) in tomato cultivars, isogenic lines (IL), near isogenic lines (NIL) and accessions of unknown hybrid background (UHB) compared to susceptible control Rutgers. Data average of six replications.**

TGRC Acc. No.	Species and cultivar	Gene/ Mutation	Background/ Isogenicity	Estimate	Total reniform count = vermiform + eggs				Dunnnett's <i>P</i> -value	Count per gram root fresh weight No. per g RFW)				
					Count per 125 mL					Estimate	Count per gram root fresh weight No. per g RFW)			Dunnnett's <i>P</i> -value
					95% LCL	95% UCL	% of Rutgers	% of Rutgers			95% LCL	95% UCL	% of Rutgers	
LA 1090	<i>S. lycopersicum</i> "Rutgers"			1235	840	1814	100		718	487	1057	100		
LA 3770	<i>S. lycopersicum</i>	Nor	Ailsa Craig/NIL	1530	1041	2248	124	0.9987	524	356	772	73	0.9525	
LA 3754	<i>S. lycopersicum</i>	mc, rin	Ailsa Craig/NIL	1698	1155	2494	138	0.9428	824	559	1214	115	1.0000	
LA 3537	<i>S. lycopersicum</i>	Nr	Ailsa Craig/NIL	2246	1529	3300	182	0.2729	1001	680	1475	140	0.9276	
LA 3318	<i>S. lycopersicum</i>	sig-1	Castlemart/IL	2169	1476	3186	176	0.3449	1645	1117	2424	229	0.0422	
LA 3152	<i>S. lycopersicum</i> "Moboline"	Ph-2	Monalbo/NIL	2522	1716	3705	204	0.1118	936	635	1379	130	0.9887	
LA 2818	<i>S. lycopersicum</i> "Monalbo"	Ve	Moneymaker/NIL	2396	1570	3657	194	0.2166	966	631	1480	135	0.9796	
LA 2819	<i>S. lycopersicum</i> "Monita"	Mi	Moneymaker/NIL	1928	1312	2833	156	0.6523	929	631	1369	129	0.9913	
LA 3472	<i>S. lycopersicum</i> "Movione"	I, Pto, Ve	Moneymaker/NIL	2224	1514	3268	180	0.2921	965	655	1422	134	0.9701	
LA 3012	<i>S. lycopersicum</i>	mc, rin	Rutgers/NIL	1861	1266	2734	151	0.7519	762	517	1123	106	1.0000	
LA 3001	<i>S. lycopersicum</i>	Nr	Rutgers/NIL	2318	1577	3405	188	0.2182	977	663	1440	136	0.9581	
LA 3013	<i>S. lycopersicum</i>	Nor	Rutgers/NIL	2162	1472	3177	175	0.3515	811	550	1195	113	1.0000	
LA 1792	<i>S. lycopersicum</i>	Hero	UHB	275	187	405	22	0.0001	156	106	230	22	0.0001	
LA 1795	<i>S. lycopersicum</i>	mc, rin	UHB	1336	909	1962	108	1.0000	859	583	1266	120	0.9999	
LA 2009	<i>S. lycopersicum</i> "New Yorker"	Ph	UHB	1388	944	2039	112	1.0000	649	441	957	90	1.0000	
LA 2530	<i>S. lycopersicum</i>	Ora	UHB	1585	1078	2328	128	0.9931	552	375	814	77	0.9900	
LA 2455	<i>S. lycopersicum</i>	Nr-2	UHB	1640	1116	2409	133	0.9770	776	526	1143	108	1.0000	
LA 0159	<i>S. lycopersicum</i>	a, e, mc, t, u, wf, y	UHB	2104	1309	3380	170	0.5714	861	534	1389	120	1.0000	
LA 2089	<i>S. lycopersicum</i>	Epi	VFN-8/IL	2162	1472	3177	175	0.3515	1138	772	1676	159	0.6248	

for investigating the function and regulation of single genes [25], and aid in isolation of genes [26]. Tomato genotypes evaluated in this study were carefully selected to include parental lines, NILs, and *S. lycopersicum* cultivars with considerable common genetic background. Narrow genetic base of cultivars make it imperative to look for novel genes in the wild relatives. The majority of these wild species can be crossed directly to the cultivated tomato, making resistance genes readily transferable.

Seven tomato genotypes supported lower populations of *R. reniformis* than the control LA1090: *S. chilense* (LA1029), *S. lycopersicum* (LA1792), *S. chilense* (LA1932), *S. peruvianum* var. *humifusum* (LA0385), *S. pimpinellifolium* (LA2934), *S. peruvianum* f. *glandulosum* (LA1283), and *S. pimpinellifolium* (LA1579). *Solanum peruvianum*, one of the most distant relatives of the cultivated tomato, originated in central Peru and northern Chile, has been the source of many major resistance genes [27]. An example is the tomato yellow leaf curl virus (TYLCV) that

has been introgressed into the tomato breeding line TY172. The TYLCV resistance in TY172 is controlled by a previously unknown major QTL (*Ty-5*, mapped to chromosome 4), originating from the resistant line, and four additional minor QTLs (mapped to chromosomes 1, 7, 9 and 11) [28]. Several accessions of *S. peruvianum* have been identified with resistance to root-knot nematode controlled by *Mi* gene located on chromosome six [29]. This gene confers resistance to tomato against several *Meloidogyne* species *M. incognita*, *M. javanica*, and *M. arenaria* [30]. One out of the three R gene homologues at the *Mi* locus, (*Mi* 1.2) was found to confer resistance to both a nematode and an aphid [31]. Our results however did not reveal any resistance to the reniform nematode in the cultivated tomato accessions—*S. lycopersicum* "Monita" (LA2819), *S. lycopersicum* "VFN-8" (LA1022), and *S. lycopersicum* "Mogeor" (LA3471) which possess the introgressed *Mi* gene. This suggests a different resistance mechanism for RN than *Mi* gene identified for *Meloidogyne* species.

Plants lack a diversity of receptors associated with their immune system as found in vertebrates, and therefore rely on only a relatively small set of innate immune receptors against pathogenic attacks. Recently, *S. pimpinellifolium* has been observed to possess a plant immune receptor protein Cf-2, providing a dual resistance to fungi and nematode. The Cf-2 protein, previously was identified as an immune receptor for the leaf mold fungus *Cladosporium fulvum*. However, this protein also mediates disease resistance to the root parasitic nematode *G. rostochiensis* pathotype Ro1-Mierenbos [32]. Two *S. pimpinellifolium* accessions, LA2934 and LA1579 suppressed reniform nematode numbers significantly which may be associated with the Cf-2 protein. There are also earlier reports of low reproduction rate of *R. reniformis* on certain accessions of this species [17,19]. The disease resistance gene *Pto* was introgressed into the cultivated tomato species from *S. pimpinellifolium* [33]. The *Pto* gene confers resistance to the bacterial pathogen, *P. syringae* pv *tomato* (*Pst*), the causal agent for bacterial speck disease. *Pst*-infected leaf tissue and fruits develop black specks surrounded by chlorotic halos leading to a reduction in yield [34]. *Pto*, one of the first R-genes to be cloned and sequenced, encodes a serine-threonine protein kinase and is 963 bp long with no introns. This gene belongs to a family of six genes clustered in a 60-kb region of chromosome five of tomato [35]. The *S. lycopersicum* "Movione" (LA3472), with the introgressed *Pto* and other genes did not demonstrate any resistance to the reniform nematode in this study.

*Solanum lycopersicum* (LA1792) is a tomato line with the introgressed *Hero* gene family shown to confer high level (95%) of resistance to all pathotypes of a potato cyst nematode (PCN), *G. rostochiensis* [36], and partial resistance to *G. pallida* [37]. Resistance to PCN in two tomato accessions *S. pimpinellifolium* B6173 and *S. peruvianum* B6001 has been confirmed [38]. The level of resistance in *S. pimpinellifolium* was noted to be greater than that in *S. peruvianum*. As a result of this greater resistance, coupled with ease of hybridization with tomato cultivars, *S. pimpinellifolium* was chosen as a donor parent for imparting PCN resistance into commercial tomato varieties. The resistance of *S. pimpinellifolium* B6173 to the Wren isolate of *G. rostochiensis* was shown to be controlled by a single dominant gene for which the symbol *Hero* was proposed. The *Hero* gene was introgressed into tomato cultivar LA1792 from wild species *S. pimpinellifolium* LA121 [38]. Map-based cloning and structural characterization of the *Hero* gene from tomato and its genomic organization has been reported extensively [39]. *Hero* gene encodes a protein with a nucleotide-binding site (NBS) and a leucine-rich-repeat (LRR) domain. This gene is a member of a family of 14 homologous genes located in a 118 kb region on chromosome

four. A great majority of resistance (R) genes are organized in gene clusters [40], and the *Hero* gene is no exception. In our study, *S. lycopersicum* (LA1792) was among the seven genotypes with the highest resistance to reniform nematode. Further studies are needed to ascertain which of the 14 R gene homologues at the *Hero* gene cluster is most responsible for reniform nematode resistance. In cotton, where resistance to reniform nematode is a critical economic necessity, tomato germplasm identified here provides a simpler approach for identification of resistance genes. Arduous efforts in cotton have shown the introgression and back crossing of resistance from *G. longicalyx* to *G. hirsutum* [41,42] and in other *Gossypium* species [22] including *G. aridum* [43] and from *G. arboreum* and a *G. hirsutum*/*G. aridum* bridging line [44]. Genetic engineering of resistance utilizing oryzacystatin gene for *R. reniformis* has been demonstrated in *Arabidopsis thaliana* [45], in a species with a well-characterized genome. The nature of RN resistance mechanism in tomato at present is unknown.

#### 4. Conclusion

This study provides a comprehensive comparative evaluation of resistance in four *Solanum* spp. to *R. reniformis*. Among these NILs, near NILs, and parental checks within which, specific accessions and cultivars were identified with resistance to *R. reniformis*. Furthermore, a protocol for undertaking rapid detection of RN *in vivo* in tomato field soils is currently being pursued. In the root samples of resistant *Solanum* cultivars and accessions, the female nematodes failed to penetrate and therefore were unable to develop any further while the relatively less resistant cultivars facilitated greater penetration and subsequent development of the RN.

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