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Evaluation of Some Tomato Genotypes to *Meloidogyne incognita* Resistance

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Abstract: The reaction of fourteen tomato genotypes to the root-knot nematode (RKN), *Meloidogyne incognita*, Giza population (G1P) infection was studied in two summer seasons (2013 & 2014) under greenhouse conditions. Five weeks old tomato seedlings were grown in clay pots filled with sterilized loamy soil and inoculated with 3000 freshly hatched J_2, which obtained from galled roots of tomato cv. Super Strain B. After 45 days from infestation, plants were evaluated to nematode resistance. *M. incognita* was able to infect, cause root galling and reproduced only on nine tomato genotypes. Meanwhile, another five genotypes (LA2819, LA2820, LA2822, CGN14387 and LA1221) were ranked as immune. The nine genotypes varied in susceptibility to nematode infection. Super Strain B was highly susceptible with build up (Pf/Pi) of 3.10, while the genotypes, Agyad 7 and Nemaguard were susceptible with Pf/Pi of 2.56 and 2.27, respectively. Agyad 16, Aziza and Peto 86 were tolerant with Pf/Pi of 1.62, 1.66 and 1.30, respectively; while other three genotypes namely, Castlerock, Flora-Dade and GS 12 were highly tolerant.

Key words: *Solanum lycopersicum* · Root-knot nematode · Resistance

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is an important solanaceous fruit vegetable grows in both field and greenhouses around the world for its nutritional and economic value. It is a major part of the income of small and large farmers [1]. Egypt is one of the major tomato production countries and ranked fourth with production about 8, 533, 803 tons with an average of 16.83 tons/fed grown on 507, 014.3 feddens in 2013 (http://faostat.fao.org/). Generally, plant parasitic nematodes are one of the important biotic constraints in crop production especially, tomato which cause enormous crop losses [2]. In Egypt, root knot nematodes (*Meloidogyne* spp.) have been considered a limiting factor in successful intensive tomato cultivation especially for small holders and commercial producers. *Meloidogyne incognita* is the major root knot species of cosmopolitan distribution in tomato-growing areas of the world, including Egypt [3-5]. Charchar *et al.* [6] reported about 30-40% lose in yield of highly susceptible tomato cultivars in tropical and sub-tropical regions due to root knot disease. Vegetable growers are keen to decrease the use of nematicide regimes due to increased health and environmental consciousness. The use of resistant cultivars is a very good alternative for controlling plant parasitic nematodes. Ammati *et al.* [7] claimed that resistance is often triggered by one or more genes in tomato cultivars. Wide variations in resistance and susceptibility responses in tomato cultivars to *M. incognita* were obtained by many research workers [8-12]. Therefore, the present research was conducted to evaluate the response of some tomato genotypes against the root knot nematode, *M. incognita* (G1P) and elect the most promising ones as rootstocks or for the future genetically research work.

MATERIALS AND METHODS

Plant Material: Seeds of fourteen tomato genotypes were obtained from several sources as shown in Table 1. Seeds were sowed in speedling trays filled with 1:1 mixture of peat-moss and vermiculate enriched with macro and micro elements under net-house conditions on the first March of the two seasons.

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Table 1: A list of the evaluated tomato genotypes and their origins

Genotypes	Origin
1- Cultivar	
LA 2819 cv. Monita*	Tomato Genetic Resource Center, University of California, USA.
LA 2820 cv. Motabo*	
LA 2822 cv. Mossol*	
S. lycopersicum var. *cerasiforme* LA 1221 cv. VFNT cherry*	
LA 3242 cv. Flora-Dade	
CGN 14387 cv. VFN-8*	The center of Genetic Resources (CGR), Wageningen, Netherlands.
Castlerock	Castle seeds, USA.
Nemaguard	Namdhari seeds, India
Peto 86	Peto seed, USA.
Super Strain B	Sun seed, Parma, Idaho, USA.
2- Hybrids	
Aziza	Apollo seeds, USA.
GS 12	Northrup King Seed Company, USA
Agyad 7	Horticulture Research Institute, ARC, Egypt.
Agyad 16	Horticulture Research Institute, ARC, Egypt.

* Genotype carries *Mi* gene

Nematode Inocula: A culture of the root-knot nematode, *M. incognita* was obtained from Giza governorate and propagated in pure cultures on tomato cv. Super Strain B at Nematology Division, Faculty of Agriculture, Cairo University and referred as Giza population (G1P).

Experimental Procedure: Five weeks old seedlings of each genotype were transplanted into 15 cm diameter clay pots filled with sterilized loamy soil (sand 85.8%, silt 8.2% and clay 6 %). Seven days after transplanting all the pots, except the check, were inoculated with 3000 J_2 of *M. incognita,* G1P per seedling by pipetting the nematode suspension in 3-4 holes which were then filled with sterilized soil and immediately watered. The pots were labeled and arranged on a clean greenhouse bench in a completely randomized design with five replications. Another 5 un-inoculated pots of each genotype were served as check. All plants were horticultural treated the same.

Data Collection: Tomato plants evaluated to RKN resistance after 45 days from inoculation. Nematode soil population of each pot was extracted by means of the technique of Hooper *et al*. [13] and was then counted. Number of galls, developmental stages, egg masses per root system, final population and nematode multiplication (Pf/Pi) were estimated. Plant growth criteria (length, fresh weight and shoot dry weight) were measured. The following values were calculated according to formulae of:

- % of Fecundity = (number of eggs/egg-mass on a genotype ÷ the highest number of eggs/egg-mass) × 100.
- % of Egg production = (total number of eggs/root of a genotype ÷ the highest total number of eggs/root) × 100
- % of Reproduction = (final population of a genotype ÷ the highest final population) × 100
- % of Root population = (Total nematodes in root tissue ÷ Nematode number inoculated, Pi) × 100
- % of Females = (Total females in root tissue ÷ Total nematodes in root tissue) × 100
- % of Mature females = (Total egg-laying females ÷ Total females) × 100

The experiment was repeated in the following season (summer 2014).

Statistical Analysis: Data were analyzed by analysis of variance (ANOVA) and compared by Duncan's Multiple Range Test [14] at the 5% level of probability using MSTAT version 4.

RESULTS AND DISCUSSION

The fourteen tomato genotypes varied widely in their susceptibility to *M. incognita* (G1P) infection. The population of *M. incognita* (G1P) reproduced only on nine genotypes (Agyad 7, Agyad 16, Aziza, Castlerock, Flora-Dade, GS 12, Nemaguard, Peto 86 and Super Strain B) as indicated by number of galls, egg masses, developmental stages, build up (Pf/Pi), final population and eggs/egg-masses. In contrast, no embedded stages, females and egg-masses were recovered from five genotypes (LA2819, LA2820, LA2822, CGN14387 and LA1221) and consequently, their pots were free from soil population (Table 2).

Table 2: Tomato genotypes reactions to root-knot nematode, *Meloidogyne incognita* (G1P) after 45 days during summer 2013

Genotypes	Galls/Root	Developmental stages	Egg-masses/ Root	Soil population	Final population	Pf/Pi	Eggs/ Egg-mass	Hostcategory**
LA 2819*	0 h	0 h	0 g	0	0 h	0.00 h	0 h	I
LA 2820*	0 h	0 h	0 g	0	0 h	0.00 h	0 h	I
LA 2822*	0 h	0 h	0 g	0	0 h	0.00 h	0 h	I
CGN 14387*	0 h	0 h	0 g	0	0 h	0.00 h	0 h	I
Agyad 7	417 c	569 c	246 c	6853	7668 b	2.56 b	537 a	S
Agyad 16	371 d	480 e	285 b	4095	4860 d	1.62 d	210 d	T
Aziza	686 a	520 d	217 d	4231	4968 d	1.66 d	299 b	T
Castlerock	264 e	176 g	161 e	1823	2160 g	0.72 g	56 g	HT
LA 1221*	0 h	0 h	0 g	0	0 h	0.00 h	0 h	I
Flora-Dade	215 f	279 f	221 cd	2092	2592 f	0.86 f	101 f	HT
GS 12	135 g	145 g	53 f	1530	1728 g	0.58 g	50 g	HT
Nemaguard	256 e	739 b	299 b	5766	6804 c	2.27 c	288 b	S
Peto 86	451 c	521 d	208 d	3159	3888 e	1.30 e	169 e	T
Super Strain B	580 b	921 a	464 a	7903	9288 a	3.10 a	256 c	HS

*Genotype carries *Mi* gene.

**HS (highly susceptible) ≥ 3.01, S (susceptible) = 3.00-2.01, T (tolerant) = 2.00-1.01, HT (highly tolerant) = 1.00-0.01 and I (immune) = 0

Means with in a column sharing the same letter are not significantly different from each other at $P = 0.05$ according to Duncan Multiple Range Test.

In summer 2013, the ninth tomato genotypes showed different reactions to *M. incognita* (G1P) infestation. Super Strain B was ranked as highly susceptible (HS) having a build up value of 3.10. Agyad 7 and Nemaguard were categorized as susceptible (S) having build up values of 2.27 and 2.56, respectively. Agyad 16, Aziza and Peto 86 were tolerant (T) genotypes with build up values of 1.62, 1.66 and 1.30 respectively, whereas Castlerock, Flora-Dade and GS 12 were highly tolerant (HT) with Pf/Pi values of 0.72, 0.86 and 0.58 respectively.

The greatest number of galls per root was obtained in Aziza (686) followed by Super Strain B (580), while lowest number of galls was recorded in GS 12 (135). The maximum number of egg masses per root system was obtained in tomato genotype, Super Strain B (464) followed by Nemaguard (299) and Agyad 7 (246), while the minimum number of egg masses was recorded in GS 12 (53). Number of eggs per egg mass was significantly increased in Agyad 7 (537) followed by Aziza (299) and Nemaguard (288) as compared to Castlerock (56) and GS 12 (50).

Number of developmental stages per plant was significantly increased in Super Strain B (921) followed by Nemaguard (739), Agyad 7 (569), but it was decreased in Peto 86 (521) and Aziza (520) followed by Agyad 16 (480) and Flora-Dade (279) as compared to Castlerock (176) and GS 12 (145). Meanwhile, nematode final population was significantly increased in Super Strain B (9288) followed by Agyad 7 (7668) while minimum decrease was obtained by GS 12 (1728). No nematodes penetrated, developed or reproduced in roots of genotypes (LA2819, LA2820, LA2822, CGN14387 and LA1221) which were ranked as immune genotypes.

The relative percentages of fecundity, egg production and relative reproduction of *M. incognita* (G1P) on tomato genotypes are illustrated in Table (3). The lowest fecundity, egg production and reproduction ratios were recorded on GS 12 (9.3, 2.0 and 18.6%, respectively), while the highest ratios of female fecundity and egg production were found in Agyad 7 and reproduction was in Super Strain B. The percentages of root population, females and mature females were also illustrated in Fig. (1). % of root population was increased in Super Strain B (34.5 %) followed by Nemaguard (33.1 %) while the lowest in Castlerock (5.8 %). Moreover, the highly percentage of females was observed in all infected roots of tomato genotypes, the maximum in GS 12 (100 %) followed by Peto 86 (99.8 %), Flora-Dade (99.4 %) and Agyad 7 (99.2 %), but percentage egg-laying females was the highest in Castlerock roots (95.2 %) and the lowest in roots of GS 12 (36.3 %).

Results in Table 4 indicate that plant length was decreased in all inoculated tomato genotypes except that of GS 12, Agyad 16, Flora-Dade or LA1221 which insignificantly by 25.2, 9.4, 7.1 or 5.2%, respectively. In contrast, nematode infection significantly decreased total plant length of LA2819, LA2820, CGN14387, Castlerock and Super Strain B. Plant fresh weight of all genotypes were substantially decreased owing to nematode infection with significant differences in case of LA2819, LA2822 and CGN14387. Almost the same results were observed in case of shoot dry weight. Nematode infection reduced shoot dry weight of some genotypes but increased it in Agyad 16 (3.2 g) and LA2820 (6.2 g). Insignificant reductions in shoot dry weight were recorded in almost genotypes. In summer 2014, almost the same trend in the obtained results were observed (Tables 5, 6 & 7 and Fig. 2) except the host category of Agyad 16 and Aziza which reacted as susceptible (S) instead of tolerant (T) in season, 2013.

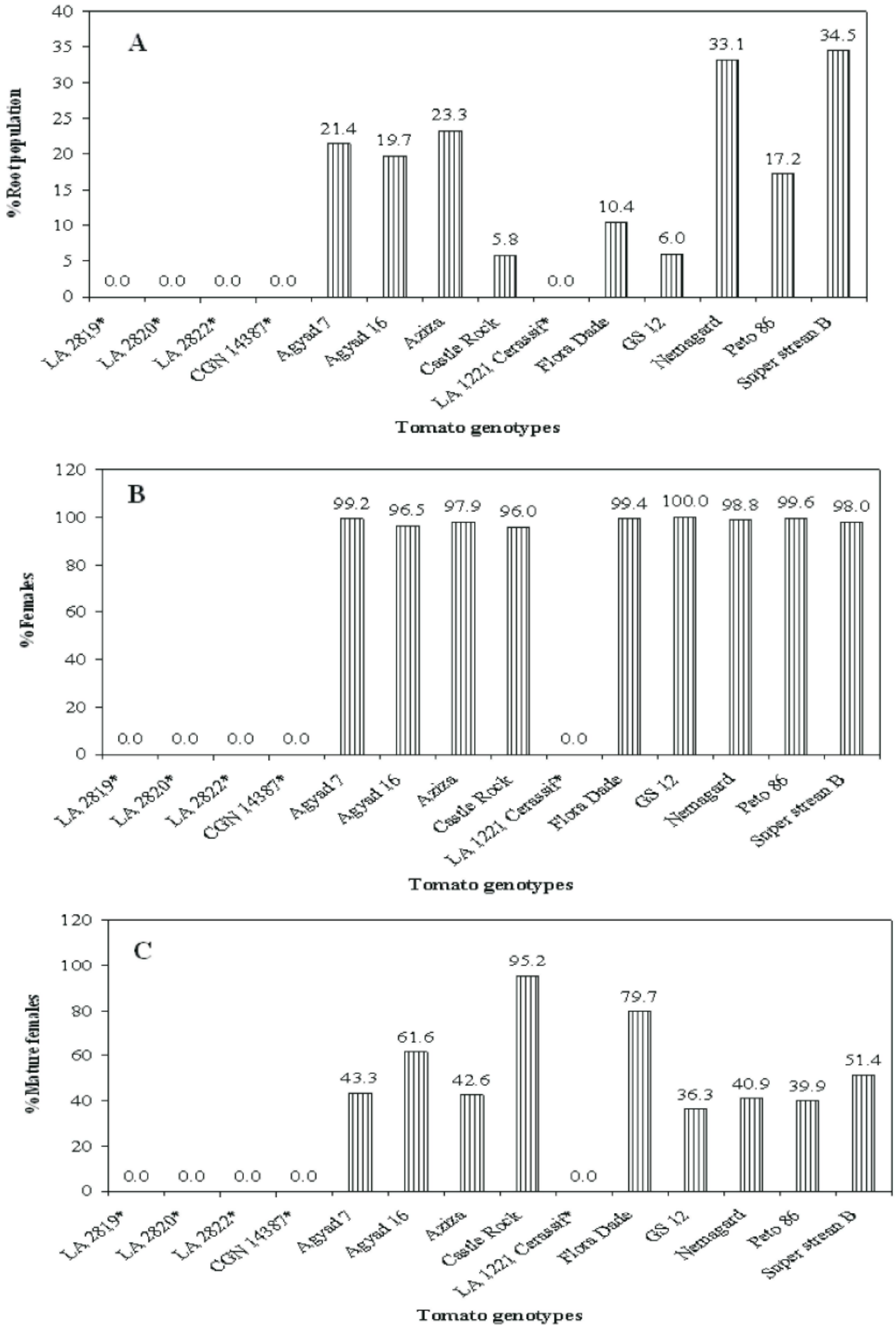

Fig. 1: Percentage of root population, females and mature females of *M. incognita* (G1P) on tomato genotypes during summer 2013

Table 3: Relatives fecundity, egg production and reproduction of *Meloidogyne incognita* (G1P) on tomato genotypes during summer 2013

Genotypes	Fecundity	Egg production	Reproduction
LA 2819*	0.0	0.0	0.0
LA 2820*	0.0	0.0	0.0
LA 2822*	0.0	0.0	0.0
CGN 14387*	0.0	0.0	0.0
Agyad 7	100.0	100.0	82.6
Agyad 16	39.1	45.3	52.3
Aziza	55.7	49.1	53.5
Castlerock	10.4	6.8	23.3
LA 1221*	0.0	0.0	0.0
Flora-Dade	18.8	16.9	27.9
GS 12	9.3	2.0	18.6
Nemaguard	53.6	65.2	73.3
Peto 86	31.5	26.6	41.9
Super Strain B	47.7	89.9	100.0

* Genotype carries *Mi* gene.

Table 4: Tomato genotypes growth response to *Meloidogyne incognita* (G1P) infection during summer 2013

	Plant Length (cm)			Plant fresh weight (g)			Shoot dry weight(g)		
Genotypes	Inoculated	Un-inoculated	Change (%)	Inoculated	Un-inoculated	Change (%)	Inoculated	Un-inoculated	Change (%)
LA 2819*	81.2	106.7	- 23.9	12.4	20.8	- 40.4	3.1	3.5	- 11.4
LA 2820*	93.5	116.3	- 19.6	27.5	28.9	- 4.8	6.2	6.1	+ 1.6
LA 2822*	59.0	71.0	- 16.9	8.6	14.4	- 40.3	1.2	2.6	- 53.8
CGN 14387*	67.0	106.0	- 36.8	18.2	33.1	- 45.0	2.7	2.9	- 6.9
Agyad 7	78.0	87.3	- 10.7	15.4	14.9	+ 3.4	2.1	2.3	- 8.7
Agyad 16	92.7	84.7	+ 9.4	18.7	20.2	- 7.4	3.2	2.8	+ 14.3
Aziza	67.7	69.0	- 1.9	17.9	16.5	+ 8.5	1.2	1.5	- 20.0
Castlerock	75.0	106.0	- 29.2	19.0	22.6	- 15.9	2.1	2.9	- 27.6
LA 1221*	94.3	89.7	+ 5.1	17.4	17.7	- 1.7	1.3	2.8	- 53.6
Flora-Dade	70.3	65.7	+ 7.0	14.4	19.8	- 27.3	1.5	2.3	- 34.8
GS 12	82.0	65.5	+ 25.2	9.5	8.7	+ 9.2	1.3	1.9	- 31.6
Nemaguard	50.3	57.0	- 11.8	7.4	9.4	- 21.3	1.4	1.8	- 22.2
Peto 86	102.7	112.0	- 8.3	29.7	25.1	+ 18.3	2.6	3.9	- 33.3
Super Strain B	63.3	93.7	- 32.4	14.6	22.6	- 35.4	1.8	2.8	- 35.7

* Genotype carries *Mi* gene, - = % decrease, + = % increase.

Table 5: Tomato genotypes reactions to the root-knot nematode, *Meloidogyne incognita* (G1P) after 45 days during summer 2014.

Genotypes	Galls/Root	Developmental stages	Egg-masses/ Root	Soil population	Final population	Pf/Pi	Eggs/ Egg-mass	Host category**
LA 2819*	0 g	0 i	0 h	0	0 g	0.00 h	0 h	I
LA 2820*	0 g	0 i	0 h	0	0 g	0.00 h	0 h	I
LA 2822*	0 g	0 i	0 h	0	0 g	0.00 h	0 h	I
CGN 14387*	0 g	0 i	0 h	0	0 g	0.00 h	0 h	I
Agyad 7	468 c	643 d	276 d	7721	8640 b	2.88 b	603 a	S
Agyad 16	458 c	592 e	352 c	5104	6048 c	2.02 d	259 d	S
Aziza	923 a	700 c	292 cd	5704	6696 c	2.23 c	402 b	S
Castlerock	261 e	174 h	159 f	246	579 f	0.68 g	55 g	HT
LA 1221*	0 g	0 i	0 h	0	0 g	0.00 h	0 h	I
Flora-Dade	241 e	313 g	248 d	2355	2916 e	0.97 f	113 f	HT
GS 12	167 f	179 h	65 g	1808	2052 f	0.68 g	62 g	HT
Nemaguard	344 d	994 ab	402 b	7784	9180 ab	3.06 ab	387 bc	HS
Peto 86	445 c	516 f	205 e	3167	3888 d	1.30 e	167 e	T
Super Strain B	651 b	1034 a	521 a	8813	10368 a	3.46 a	287 d	HS

*Genotype carries *Mi* gene.

**HS (highly susceptible) $\geq$ 3.01, S (susceptible) = 3.00-2.01, T (tolerant) = 2.00-1.01, HT (highly tolerant) = 1.00-0.01 and I (immune) = 0

Means with in a column sharing the same letter are not significantly different from each other at $P = 0.05$ according to Duncan Multiple Range Test.

Table 6: Relatives fecundity, egg production and reproduction of *Meloidogyne incognita* (G1P) on tomato genotypes during summer 2014

Genotypes	Fecundity	Egg production	Reproduction
LA 2819*	0.0	0.0	0.0
LA 2820*	0.0	0.0	0.0
LA 2822*	0.0	0.0	0.0
CGN 14387*	0.0	0.0	0.0
Agyad 7	100.0	100.0	83.3
Agyad 16	43.0	54.8	58.3
Aziza	66.7	70.6	64.6
Castlerock	9.2	5.3	19.8
LA 1221*	0.0	0.0	0.0
Flora-Dade	18.8	16.9	28.1
GS 12	10.2	2.4	19.8
Nemaguard	64.2	93.6	88.5
Peto 86	27.7	20.5	37.5
Super Strain B	47.7	90.0	100.0

* Genotype carries *Mi* gene.

Table 7: Tomato genotypes growth response to *Meloidogyne incognita* (G1P) infection during summer 2014.

	Plant Length (cm)			Plant fresh weight (g)			Shoot dry weight (g)		
Genotypes	Inoculated	Un-inoculated	Change (%)	Inoculated	Un-inoculated	Change (%)	Inoculated	Un-inoculated	Change (%)
LA 2819*	80.1	99.4	- 19.4	11.6	20.6	- 43.7	2.9	3.5	- 17.1
LA 2820*	91.3	109.7	- 16.8	26.0	28.2	- 7.8	5.9	6.0	- 1.7
LA 2822*	56.9	67.7	- 16.0	8.2	13.9	- 41.0	2.1	2.5	- 16.0
CGN 14387*	63.9	102.3	- 37.5	17.6	31.5	- 44.1	2.6	2.8	- 7.1
Agyad 7	73.6	85.2	- 13.6	14.1	15.0	- 6.0	2.1	2.2	- 4.5
Agyad 16	86.4	83.6	+ 3.3	18.5	18.8	- 1.6	2.6	3.2	- 18.8
Aziza	62.3	62.8	- 0.8	15.2	16.3	- 6.7	1.1	1.4	- 21.4
Castlerock	68.3	97.6	- 30.0	17.5	20.6	- 15.0	2.0	2.6	- 23.1
LA 1221 *	83.6	93.1	- 10.2	16.2	17.5	- 7.4	2.2	2.8	- 21.4
Flora-Dade	61.9	68.9	- 10.2	13.6	19.3	- 29.5	1.4	2.2	- 36.4
GS 12	62.5	79.1	- 21.0	8.4	9.0	- 6.7	1.2	1.9	- 36.8
Nemaguard	48.0	55.0	- 12.7	7.1	9.0	- 21.1	1.4	1.7	- 17.6
Peto 86	96.8	109.3	- 11.4	23.7	29.0	- 18.3	2.5	3.7	- 32.4
Super Strain B	59.0	92.4	- 36.1	14.4	21.0	- 31.4	1.8	2.6	- 30.8

* Genotype carries *Mi* gene, - = % decrease, + = % increase

The number of galls, egg masses, developmental stages and nematode final population were directly related to nematode build up except eggs/egg-mass. As the nematode final population increases the rate of reproduction also increase [15-17]. Due to genetic variability among the tested genotypes *M. incognita* reproduced variably on 9 tomato genotypes [18-21]. Compatible reactions lead to differential plant responses to nematode infection [22-25].

Our results categorized the tested genotypes to 5 immune, 3 highly tolerant and 3 tolerant genotypes. Williamson and Kumar [25] reported that the nematode resistant plants are characterized by failure of the nematodes to produce functional feeding sites in the host after invasion and to develop subsequently as reproducing females, including hypersensitive responses. Also, the level of susceptibility of tomato to the root-knot nematodes is controlled by the presence of resistance genes such as *Mi* gene and the genetic background of tomato cultivar [3 and 26]. The homozygous or heterozygous state of the *Mi* locus has been found to affect the degree of resistance to the root-knot nematode, with the cultivars having the heterozygous form of the *Mi* gene being more susceptible than homozygous cultivars. Such genetic variations between cultivars may explain the variation in the numbers of galls and egg-masses on their roots. Factors other than genetic variation are those related to populations and resistant breaking pathotypes [27].

Two types of mechanisms for nematodes resistance in plants have been reported, including pre-infection resistance, where the nematodes cannot enter the plant roots due to the presence of toxic or antagonistic chemicals in root tissue [28] and post-infection resistance

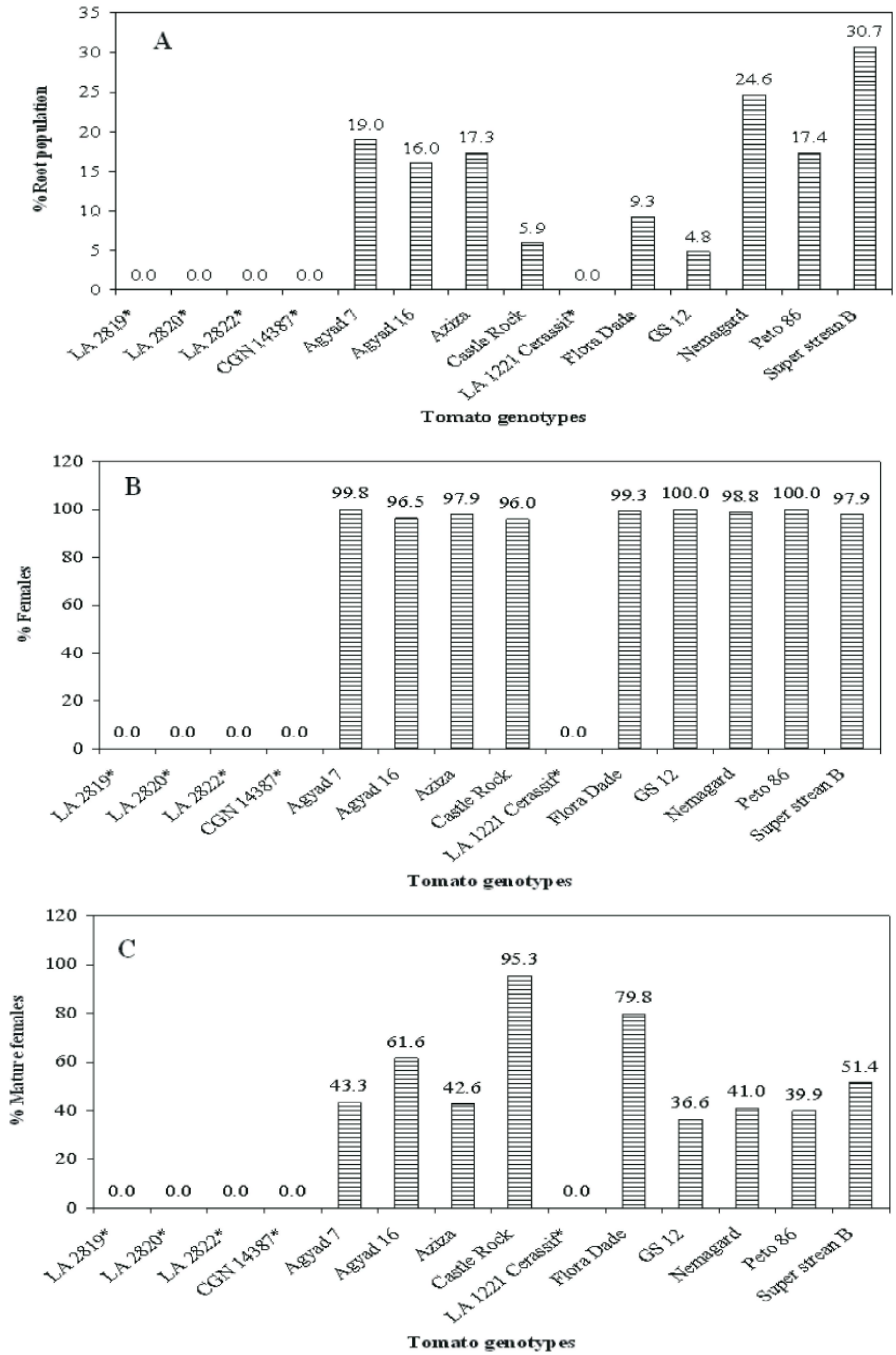

Fig. 2: Percentage of root population (A), females (B) and mature females (C) of *M. incognita* (G1P) on tomato genotypes during summer 2014

in which nematodes are able to penetrate roots but fail to develop [29]. Post-infection resistance is often associated with an early hypersensitive reaction (HR), in which rapid localized cell death in root tissue around the nematode prevents the formation of a developed feeding site, leading to resistance. Tomato plants that are resistant show typical HR upon avirulent RKN infection [22]. Boiteux and Charechar [30] reported that resistant genotypes have gene of resistance in their gene pool which confers resistance to *M. incognita*. Resistant roots always reacted to root knot nematodes attack by decreasing catalase activity. There are some alkaloids or phenolics which have the capability of inhibiting these enzymes and act as an elicitor of resistance in tomato plant attacked by *Meloidogyne* species. Resistance and susceptibility to *M. incognita* reflect the effect of the plant on the nematode's ability to reproduce [31] as our results indicated on genotypes; LA2819, LA2820, LA2822, CGN14387 and LA1221 (which carry resistant *Mi* gene), nematodes can not penetrated or reproduced as compared to other genotypes. The compatible reaction of all the nine tomato genotypes to *M. incognita* infection indicated that they lack resistant genes so genotypes were unable to stop the penetration, development and reproduction.

According to the result of plant growth criteria, nematode infection reduced almost all of these criteria, but in some cases the opposite was observed. In these cases, increase in some plant growth criteria may be due to the presence of galls which increase the root fresh weight and/or to stimulate the root to produce new rootlets to compensate the infected useless ones which resulted in increasing plant fresh weights. Our results in agreement with those of Farahat *et al*. [32], Kamran *et al*. [10] and Khanzada *et al*. [11]. This upraises the need to transfer resistant genes to our marketing tomato genotypes to avoid the infection by nematodes, which is essential for the management of root knot nematodes.

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