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Retention of Resistance to *Meloidogyne incognita* in *Lycopersicon* Genotypes at High Soil Temperature[1]

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Abstract: *Lycopersicon glandulosum* and *L. peruvianum* clones and *L. esculentum* cultivars 'VFN8' (resistant) and 'Rutgers' (susceptible) were tested for their resistance to *Meloidogyne incognita* (race 1) at soil temperatures of 25 and 32 C. *L. esculentum* cv. VFN8 and *L. peruvianum* Acc. No. 128657, both of which possess the Mi gene, were resistant at 25 C but were susceptible at 32 C. *L. glandulosum* Acc. No. 126443 and *L. peruvianum* Acc. No. 270435, with combined resistance to *M. hapla* and *M. incognita*, and *L. peruvianum* Acc. Nos. 129152 and LA2157, with resistance to *M. incognita*, were highly resistant at both temperatures. In a second experiment three of these accessions under heat stress simulated by 32 C ambient and soil temperature retained a high level of resistance. Two clones of *L. glandulosum* Acc. No. 126440, with resistance to *M. hapla*, were moderately susceptible to *M. incognita* at 25 and highly susceptible at 32 C. *M. incognita* produced significantly ($P = 0.01$) more eggs on *L. esculentum* cv. Rutgers at 32 than at 25 C. This study supports the existence of genes other than the Mi gene that confer resistance to *M. incognita* and are functional at high soil temperatures.

Key words: root-knot nematodes, resistance, *Lycopersicon*, soil temperature, tomato.

High soil temperature (> 28–30 C) is a major limiting factor to the use of root-knot nematode (*Meloidogyne* spp.) resistance in tomato in some areas of the world (2,3,5–7,13). A recent review of the history of tomato resistance (9) revealed that all resistant tomato stocks now in use derive their resistance from one hybrid plant obtained from a *Lycopersicon esculentum* (L.) Mill. × *L. peruvianum* (L.) Mill. (PI 128657) cross made by Smith (12). Because of the breakdown in expression of the Mi gene resistance at high soil temperature and the appearance of naturally occurring Mi gene resistance-breaking biotypes of the main species of *Meloidogyne* associated with tomato, the development of tomato cultivars with additional resistance genes is desirable. A preliminary screening of many exotic germplasms of tomato was conducted, and clones with good resistance to *Meloidogyne* species were identified (1). There is some evidence to suggest that resistance gene(s) other than the Mi gene may be present in some of these clones (1).

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We report here on evaluations of the expression of resistance to *M. incognita* (race 1) at high soil temperature in *Lycopersicon* clones selected from the previous study.

Materials and Methods

The clones used in these experiments were *L. peruvianum* USDA Acc. Nos. 128656, 129152, LA2157, 270435, and 128657; and *L. glandulosum* C. H. Mull USDA Acc. Nos. 126440 and 126443. The original source of the Mi gene, *L. peruvianum* Acc. No. 128657, was included as a reference for comparison with the selected clones. *L. esculentum* cv. Rutgers and cv. VFN8 were used as susceptible and resistant controls, respectively.

M. incognita (race 1) was used in both experiments. The inoculum source was established from a single egg mass and increased on Rutgers.

Greenhouse experiment: The experiment was carried out in water-bath temperature tanks in a greenhouse. Soil temperature in the pots was maintained at 25 or 32 C. Air temperature ranged from 20 to 32 C. The test used 1-month-old seedlings of Rutgers and VFN8 and rooted cuttings of *L. glandulosum* and *L. peruvianum*.

The plants were grown singly in 600-cm^3 fiber pots (Western Pulp Products Co., Upland, Calif.) containing steamed (full steam for 1 hour at 100 C) loamy sand. Three pots were buried to their rims in sandy soil in a plastic bucket, and the buckets were set in a water bath. Inoculum was prepared

TABLE 1. Host suitability of *Lycopersicon* clones to *Meloidogyne incognita* (race 1) at 25 and 32 C soil temperature, on the basis of reproduction factor (R) and mean gall index.

Clone or cultivar	25 C			32 C		
	Mean gall index	Host efficiency (mean R* factor)	Suitability designation (resistance)	Mean gall index	Host efficiency (mean R* factor)	Suitability designation (resistance)
L. esculentum						
Rutgers	3.3	41.0	Susceptible	3.3	50.0	Susceptible
VFN8	0.6	0.3	Resistant	1.3	12.4	Susceptible
L. glandulosum						
126443-1-MH	0	0.0	Resistant	0	0.0	Resistant
126443-5-MH	0	0.0	Resistant	0.3	0.5	Resistant
126443-MI	1	0.9	Resistant	2	3.5	Susceptible
126440-1-MH	1.3	5.5	Susceptible	2.3	25.8	Susceptible
126440-9-MH	1.3	1.7	Susceptible	3.6	23.0	Susceptible
L. peruvianum						
LA2157	0.6	0.8	Resistant	0.6	0.2	Resistant
128656-2-MI	0	0.0	Resistant	2	3.5	Susceptible
129152-6-MI	0.3	0.0	Resistant	0.6	0.6	Resistant
270435-3-MH	0.3	0.1	Resistant	0.6	1.4	Susceptible
128657-8-MI	0.6	0.4	Resistant	2	19.9	Susceptible
128657-3-MI	1	0.7	Resistant	1	4.3	Susceptible

* The R factor is calculated as the average final egg count divided by 5,000 eggs (initial inoculum). Results were the average of development on three plants.

by the Hussey and Barker method (8). A suspension of 5,000 eggs and infective juveniles was pipetted into holes in the soil around the roots in each pot. Experimental periods were 41 days at 32 C and 59 days at 25 C after inoculation. These periods were chosen on the basis of 18,000 degree-hours accumulation above the developmental threshold temperature of 10 C which has been shown to allow the maximum reproduction of first-generation females of *Meloidogyne javanica* (Treub) Chitwood on Rutgers (4). The information on degree-hour accumulation, although developed for *M. javanica,* was used in these tests with *M. incognita* because experience has shown that it takes the two species about the same length of time to complete the life cycle at 25–30 C. At the end of the experiment, plants were removed from the pots and their roots were gently washed free of soil with running water, weighed, scored for galling, and processed to recover eggs using the Hussey and Barker method (8). A galling index was calculated and total eggs per root system determined. Data were analysed statistically using the ANOVA procedure. Resistance was assessed as recommended by Sasser et al. (11), combining the galling index and the reproduction factor, R = Pf/Pi, with Pf being the average final egg count of each genotype and Pi being 5,000 eggs and J2 used as initial inoculum.

Growth chamber experiment: A test was conducted in a growth chamber maintained at 32 C to test the effect of heat stress, both soil and air temperatures, on root-knot nematode resistance in the selected clones. Light intensity was 14,700 lux under 16-hour photoperiod. Five replicates of selected *L. glandulosum* and *L. peruvianum* clones and Rutgers were used. The experimental period was reduced from 41 to 30 days (15,840 degree-hours) after inoculation because of root damage on some clones and cultivars under the stress of both high soil and air temperature. All other techniques were similar to those described for the greenhouse experiment.

RESULTS

Greenhouse: L. esculentum Rutgers, the susceptible control, showed root galls at 25 and 32 C soil temperatures (Fig. 1A). Larger galls, resulting from multiple infections, were seen at 25 C, but numerous galls were also present at 32 C. Numerous galls were observed on *L. esculentum* VFN8, carrying the Mi gene, only at 32 C (Fig. 1B). Some

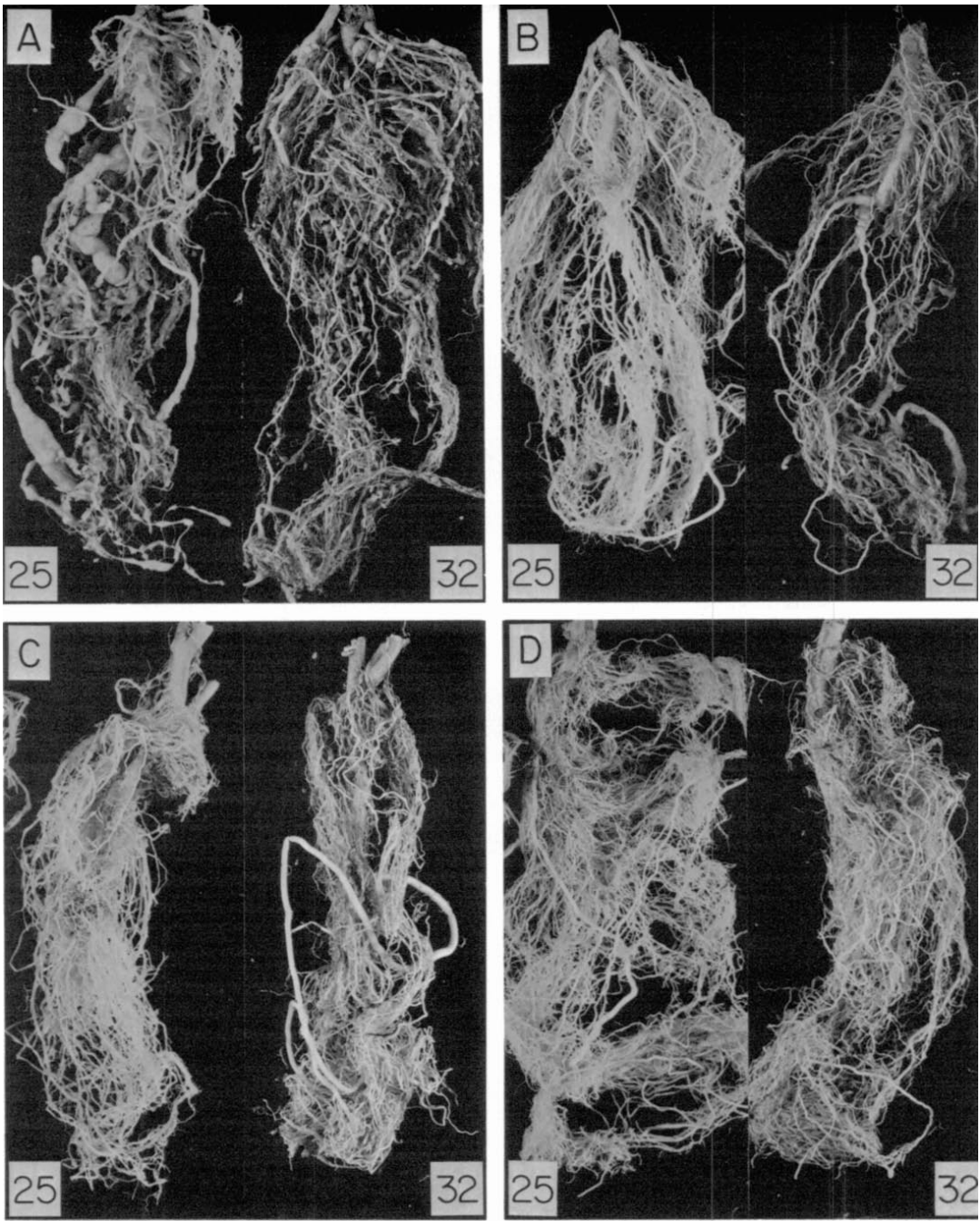

FIG. 1. Root systems of *Lycopersicon* species following exposure to *Meloidogyne incognita* (race 1) for 59 days at 25 C or 41 days at 32 C. A) *L. esculentum* cv. Rutgers. B) *L. esculentum* cv. VFN8. C) *L. glandulosum* Acc. No. 126443-5-MH. D) *L. peruvianum* Acc. No. 270435-3-MH.

L. glandulosum and *L. peruvianum* clones did not show any root galls and retained resistance at 32 C (Fig. 1C, D). The reproduction factor and galling index, calculated on different germplasms at two soil temperatures, are presented in Table 1. At 25 C, *L. glandulosum* Acc. No. 126443 and all *L. peruvianum* clones were resistant. *L. glandulosum* Acc. No. 126440 was susceptible. At 32 C, a breakdown in resistance of most germplasms was observed. Only *L. glandulosum* Acc. Nos. 126443-1-MH and

TABLE 2. Mean gall index, reproduction factor, and egg production observed on *Lycopersicon* clones exposed for 30 days to *Meloidogyne incognita* (race 1) at 32 C soil and ambient temperature.

Clone or cultivar	Mean gall index	Host efficiency (mean R factor)	Mean egg production per gram root
L. esculentum			
Rutgers	4.0	5.2	9,156 a
L. glandulosum			
126443-5-MH	0.0	0.06	247 b
126443-1-MH	0.2	0.004	14 b
L. peruvianum			
270435-3-MH	0.4	0.04	134 b
129152-6-MI	0.4	0.08	251 b

Means of five replicates per clone or cultivar.

Means followed by the same letter within a column are not significantly different at 1% level according to Duncan's multiple-range test.

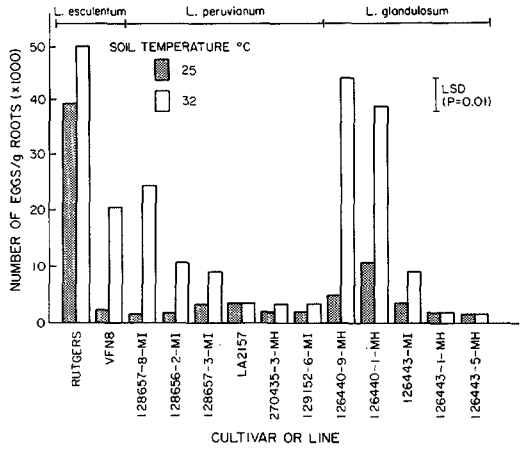

FIG. 2. Resistance of *Lycopersicon* genotypes to *Meloidogyne incognita* (race 1) at soil temperatures of 25 and 32 C, on the basis of nematode reproduction.

126443-5-MH and *L. peruvianum* Acc. Nos. 129152-6-MI and LA2157 retained resistance at 32 C (Fig. 1, Table 1).

Growth chamber: On the basis of galling index, reproduction factor, and egg mass production (Table 2), resistance in *L. glandulosum* 126443-1-MH and 126443-5-MH and *L. peruvianum* 270435-3-MH and 129152-6-MI clones was not affected by heat stress. Reproduction by *M. incognita* on these clones was significantly less ($P = 0.01$) than on Rutgers, the susceptible control.

DISCUSSION

Stability of root-knot nematode resistance under heat stress found in several *Lycopersicon* genotypes has potential use in cultivars for tropical and subtropical areas where root-knot nematodes are a major constraint on tomato productivity. On VFN8 and *L. peruvianum* Acc. No. 128657, similar levels of reproduction of *M. incognita* were observed at each of the temperatures tested (Table 1, Fig. 2). This suggests that nematode resistance conferred by the Mi gene is apparently not affected by the *L. esculentum* background. Thus transfer of other resistance genes from *Lycopersicon* species into the *L. esculentum* background could provide additional useful resistance.

Among *L. glandulosum* accessions, only clones 126443-1-MH and 126443-5-MH, which combined resistance to *M. hapla* and *M. incognita*, were resistant at high soil temperature (Table 1) and under heat stress (Table 2).

L. peruvianum Acc. Nos. 129152 and LA2157 do not have any resistance to *M. hapla* but retained their resistance to *M. incognita* at 32 C. *L. peruvianum* Acc. No. 129152 does not have the variant allele in locus 1 of the acid phosphatase, a natural marker for the Mi gene (1). This additional information and the evident retention of resistance at high soil temperature support the hypothesis that new resistance gene(s) have been identified. Although *L. peruvianum* Acc. No. 270435, with resistance to *M. hapla*, was classified as susceptible (Table 1) at 32 C, it showed significantly less egg production (Figs. 1D, 2; Table 2) when compared to any other susceptible genotype and could be a good source of *Meloidogyne* resistance. The genetic variability in resistance to *M. incognita* seen both between accessions of *L. peruvianum* and *L. glandulosum* and within accessions (1), and also in response to the nematode at high (32 C) soil temperature, is consistent with genetic variation reported by Rick (10) within the *L. peruvianum* complex. Considering the extent of variation observed in *L. peruvianum*, it is perhaps not surprising that a gene(s) that confers resistance to *M. incognita* and that is operative at high soil temperature has been found.

Significantly ($P = 0.01$) more eggs were produced on Rutgers than on any other genotype at 25 C, and egg production on

this variety was higher at 32 than at 25 C (Table 1, Fig. 2). Our results do not agree with those reported by Arajo et al. (2), who observed similar egg production on *L. esculentum* cv. Floradade, a susceptible genotype, at 25 and 32.5 C soil temperatures.

Literature Cited

1. Ammati, M., I. J. Thomason, and P. A. Roberts. 1985. Screening *Lycopersicon* species for new genes imparting resistance to root-knot nematodes (*Meloidogyne* spp.). Plant Disease 69:112–115.

2. Arajo, M. T., D. W. Dickson, J. J. Augustine, and M. J. Basset. 1982. Optimum initial inoculum levels for evaluation of resistance in tomato to *Meloidogyne* spp. at two different soil temperatures. Journal of Nematology 14:536–540.

3. Arajo, M. T., D. W. Dickson, J. J. Augustine, and M. J. Basset. 1983. Reproduction of two *Meloidogyne incognita* races in tomato grown at high temperatures. Journal of Nematology 15:640–641.

4. Bost, S. C., and A. C. Triantaphyllou. 1982. Genetic basis of the epidemiologic effect of resistance to *Meloidogyne incognita* in the tomato cultivar Small Fry. Journal of Nematology 14:540–544.

5. Dropkin, V. H. 1969. The necrotic reaction of tomatoes and other hosts resistant to *Meloidogyne*: Reversal by temperature. Phytopathology 59:1632–1637.

6. Fassuliotis, G. 1979. Plant breeding for root-knot nematode resistance. Pp. 425–453 *in* F. Lamberti and C. E. Taylor, eds. Root-knot nematodes (*Meloidogyne* species) systematics, biology and control. London and New York: Academic Press.

7. Holtzman, O. V. 1965. Effect of soil temperature on resistance of tomato to root-knot nematodes (*Meloidogyne incognita*). Phytopathology 5:990–992.

8. Hussey, R. S., and K. R. Barker. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Disease Reporter 57:1025–1028.

9. Medina-Filho, H., and S. D. Tankesley. 1983. Breeding for nematode resistance. Pp. 904–923 *in* D. A. Evans, W. R. Sharp, P. V. Ammirato, and Y. Yamada, eds. Handbook of plant cell culture techniques for propagation and breeding, vol. 1. London: Collier MacMillan.

10. Rick, C. M. 1986. Reproductive isolation in the *Lycopersicon peruvianum* complex. *In* W. D'Arcy, ed. Proceedings of the second conference on the Solanaceae. New York: Academic Press. In press.

11. Sasser, J. J., C. C. Carter, and K. M. Hartman. 1984. Standardization of host suitability studies and reporting of resistance to root-knot nematodes. A cooperative publication of the North Carolina State University Department of Plant Pathology and the United States Agency for International Development. Raleigh: North Carolina State Graphics.

12. Smith, P. G. 1944. Embryoculture of a tomato species hybrid. Proceedings of American Society of Horticultural Science 44:413–416.

13. Zacheo, G., and T. Bleve-Zacho. 1984. Influence of different temperatures on resistance of tomato plants to *Meloidogyne incognita*. Nematologia Mediterranea 12:221–230.