SCREENING FOR ROOT-KNOT NEMATODE RESPONSE IN SOMACLONAL VARIANTS OF *SOLANUM QUITOENSE* LAM.

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ABSTRACT

Santamaria, L., R. P. Mulrooney and S. L. Kitto. 2004. Screening for root-knot nematode response in somaclonal variants of *Solanum quitoense* Lam. Nematropica 34:73-82.

Solanum quitoense, a perennial herbaceous plant native to the tropical regions of Ecuador and Colombia, is susceptible to root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood. The objectives of this study were to develop protocols for screening regenerants of *S. quitoense* for response to root-knot nematode. Internodal stem segments cultured on MS medium supplemented with BA (4.4 to 44 μM) were the most regenerative explants. Growth regulators were not required to proliferate, root and reestablish *S. quitoense*. Sterile cultures of *M. incognita* were initiated and maintained on *S. quitoense* root cultures. Root-knot nematode response for the regenerants was evaluated by screening greenhouse- and *in vitro*-rooted microcuttings. Screening in the greenhouse of 248 regenerants of *S. quitoense* 'Dulce' inoculated with 1,000 eggs of *M. incognita* resulted in 21 regenerants having five or fewer galls. Further screening of these 21 regenerants in the greenhouse determined that nine had greater fresh weights and one regenerant produced more eggs per plant when compared to the susceptible control. Reproductive factor (Rf) values and Host Resistance Classification (HRC) percentages suggest that five regenerants had reduced nematode reproduction.

Key words: Meloidogyne incognita, naranjilla, resistance, *in vitro*, tissue culture, greenhouse.

RESUMEN

Santamaria, L., R. P. Mulrooney y S. L. Kitto. 2004. Seleccionemiento da la respuesta al nematodo formador de agalla en variants somaclonales de *Solanum quitoense* LAM. Nematropica 34:73-82.

Solanum quitoense, una planta herbacea perenne nativa de las regiones tropicales de Ecuador y Colombia, es susceptible al nematodo formador de nudo, *Meloidogyne incognita* (Kofoid & White) Chitwood. Los objetivos de este estudio fueron desarrollar protocolos para el seleccionamiento de regenerantes somaclonales de *S. quitoense* y su respuesta al nematodo formador de agalla. Secciones internodales de tallo cultivadas en medio MS suplementado con BA (4.4 to 44 μM) fueron los explantes mas regenerativos. Reguladores de crecimiento no fueron necesarios para proliferación, enraizamiento y adaptación de *S. quitoense*. Cultivos estériles de *M. incognita* fueron iniciados y mantenidos en cultivos de raíces de *S. quitoense*. La respuesta de los regenerantes a los nematodos formadores de agallas fue evaluada seleccionando plantulas de *in vitro* enraizadas en el invernadero, y microplantas y raices *in vitro*. En el seleccionamiento de invernadero 248 regenerantes de *S. quitoense* 'Dulce' fueron inoculadas con 1,000 huevos de *M. incognita* que determinó 21 regenerantes con cinco o menos nudos (agallas). Adicionales evaluacciones en invernadero de estos 21 regenerantes determinaron que nueve de los regenerantes tuvieron mas alto peso fresco y un regenerante produjo mas huevos por planta comparado con el control susceptible. El Factor de Reproduccion (Rf) y los porcentajes de Clasificacion de Resistencia del Huesped (HRC) sugieren que cinco regenerantes han reducido la reproducción de nematodos.

Palabras claves: Meloidogyne incognita, naranjilla, resistencia, *in vitro*, cultivo de tejidos, invernadero.

INTRODUCTION

Naranjilla, *Solanum quitoense* Lam. (Solanaceae), is native to Ecuador and Colombia and has been described as "the golden fruit of the Andes" and "the nectar of the gods" (Vietmeyer and Dafforn, 1989) because its fruit has a slightly acid flavor and exotic taste. 'Dulce', the variety having the best flavor, is rarely available in the marketplaces (Santamaria, personal observation). An under-exploited plant, commonly used in juice, ice cream, jellies, and other cooked confections, naranjilla has considerable economic potential as a high value crop (Heiser, 1993).

Farmers have cultivated naranjilla since the middle of the seventeenth century (Patiño, 1962). One of the major challenges for growers is its susceptibility to root-knot nematodes, *Meloidogyne* spp. (Scheerens, 1994). To cope with root-knot nematodes, farmers in Ecuador have increased planting in virgin areas ('slash and burn') believed to be nematode-free or have grafted naranjilla scions onto nematode-resistant stocks of other solanaceous species (Heiser, 1984). However, in tropical and subtropical regions, the use of nematode-resistant cultivars may be the most economical and environmentally sound method of controlling root-knot nematodes (Castagnone-Sereno *et al.*, 1993). There has been little effort to improve naranjilla through breeding (Camacho and Rodriguez, 1984). While naranjilla has a narrow genetic base (Whalen and Bailey, 1984) wide crosses between cultivated and wild species have resulted in some root-knot nematode resistance (Heiser, 1993); however, fruits from these hybrids have poor flavor. One alternative to breeding is the production of nematode-resistant somaclonal variants from high quality cultivars (Hashmi *et al.*, 1995).

Hendrix *et al.* (1987) have developed regeneration protocols with leaves of naranjilla and Heiser (1993) has studied naranjilla's resistance to nematodes in the field. The main objective of this research was to produce and identify root-knot nematode resistant plants from the high value cultivar 'Dulce', by developing protocols for: 1) regenerating and micropropagating naranjilla, 2) maintaining root-knot nematodes on naranjilla root cultures and 3) screening regenerants for response to root-knot nematodes in the greenhouse and/or *in vitro*.

MATERIALS AND METHODS

Plant Material

This research was conducted from 1994 to 1996 at the University of Delaware in the Department of Plant and Soil Sciences, Newark, DE. Germplasm for all experiments came from seeds of *S. quitoense* cultivar 'Dulce' provided by Dr. Heiser (Indiana University, Bloomington, IN). Seeds were surface disinfested with a solution of 1% sodium hypochlorite (20% household bleach) and 0.1% Tween® 20 (v/v) (Fisher Scientific Co., NJ) for 30 minutes, washed three times with sterile water, and placed one each into sterile Petri plates containing moist filter paper. A one-month-old seedling was dissected into nodal segments (Barna and Wakhlu, 1995) and multiplied to obtain 20 stock cultures each with 4 microcuttings. Stock cultures, placed under high light (62 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 16-h photoperiod) maintained monthly by subculturing single-node explants to basal medium, were the source of shoot and root explants for further experiments.

Medium

Micropropagation: Basal medium contained MS salts (Murashige and Skoog, 1962), vitamins (0.4 $mg \cdot liter^{-1}$ thiamine·HCl, 0.5 $mg \cdot liter^{-1}$ nicotinic acid, 0.5

mg·liter^{-1} pyridoxine·HCl, 100 mg·liter^{-1} *i*-inositol), 3% sucrose, and 0.7% Phytagar. This medium, pH adjusted to 5.7-5.8 before agar was added, was autoclaved at 121°C, 124 kPa for 20 minutes and dispensed as 25 ml into jars (55 × 72 mm).

Root culture: Root medium contained Gamborg's B_5 (Gamborg *et al.*, 1968) salts, vitamins (10 mg·liter^{-1} thiamine·HCl, 1 mg·liter^{-1} nicotinic acid, 1 mg·liter^{-1} pyridoxine·HCl, 100 mg·liter^{-1} *i*-inositol), 2% (w/v) sucrose, and 1% Phytagar (Life Technologies, Inc., New York). The medium, adjusted to pH 6 prior to adding agar, was dispensed (50 ml each) into 100 × 15 mm Petri plates.

Regeneration

Stems and petioles: Internodal-stem segments and petioles (ca. 0.5 cm) were cultured on basal medium containing benzyladenine (BA) (4.4, 1, 11, 22 or 44 µM) with or without naphthaleneacetic acid (NAA) (0.44 µM). Stock plants were placed under low light (10 µmol·m^{-2}·s^{-1}, 16-h photoperiod) one week prior to obtaining explants.

Nematode Maintenance and Inoculum

In vitro: One-cm root tips of 'Dulce', cultured on basal medium under high light or dark conditions, were measured weekly over three weeks. Sterile nematode inoculum of *Meloidogyne incognita* (Kofoid & White) Chitwood was obtained from in-vitro root cultures of tomato (*Lycopersicon esculentum* Mill. 'Rutgers') provided by the Nematology Department, USDA-ARS, Beltsville, MD. Root cultures, initiated from 1-cm-long root tips of 'Rutgers' tomato or *S. quitoense*, were inoculated with egg masses and were maintained on root medium.

Greenhouse: Inoculum for greenhouse experiments was obtained from 'Rutgers' tomato plants that had been inoculated with sterile root-knot nematode eggs. Nematode eggs were extracted from infested roots according to Hussey and Barker (1973) and counted to determine the number of eggs per ml (Sasser *et al.*, 1984).

Inoculum concentration: Microcuttings (apical tip plus one or two nodes) of 'Dulce' were placed in sand in 6 × 10 cell-flats (4 × 4 × 5 cm^3/cell). Flats were covered with humidity domes, placed under intermittent mist (6 sec every 15 light-sun-accumulation units [1 unit = Watt·m^2] or every 6 min whichever was sooner from 6:30 a.m. to 6.30 p.m.; however the mist came on every 3 min if light was between 20-50 Watt·m^2) for one week after which the humidity domes were removed. Flats stayed in the mist for one or two more weeks, and then were moved to a greenhouse bench and placed on a heating mat that maintained the soil temperature at 28°C.

To determine the best inoculum level, naranjilla plants were inoculated with *M. incognita* 0, 1,000, 3,000, 5,000 or 10,000 eggs. Inoculum was applied 1-cm deep, close to the roots using a micropipette, the same day that the eggs were extracted. There were five plants per treatment. Plants were irrigated carefully each day during a five-week period. Two weeks after inoculation, plants were fertilized weekly with Peters Excel 21-5-20 (Scotts-Sierra Horticultural Products, OH).

Screening Regenerants for Resistance:

Experiment 1.—Screening of 'Dulce' regenerants: Microcuttings containing the apical tip plus one node were taken from 248 regenerants established in MetroMix 510 (Scotts-Sierra Horticultural Products, OH) and were rooted for 2 wk in sand in individual pots [60 mm^2, 32 pots/flat (Kord Products Ltd., Bramalea, Ontario-Canada)]. One stock plant (positive control) was placed at random in each flat. Each

regenerant was inoculated with a suspension of 1,000 nematode eggs and evaluated after 5 wk of inoculation by counting the number of galls.

Experiment 2.—Greenhouse: Regenerants having 5 or fewer galls in Experiment 1 were re-screened as described above. Five weeks after inoculation, the data collected from each plant were number of galls, number of egg masses and number of eggs, root fresh weight, and shoot fresh and dry weight. Four replications of each regenerant were screened and the experiment was repeated once. While each experiment had a balanced design, plant death resulted in unequal plant numbers for evaluation.

Experiment 2.—In vitro: Fourteen of the 21 somaclonal variants (14, 47, 57, 79, 122, 138, 151, 197, 199, 204, 232, 282, 285, 312) having 5 or fewer galls in Experiment 1 were screened *in vitro*. One-week-old rooted microcuttings (1-1.5 cm tall with two or three roots 1-1.5 cm long, grown on basal medium with ¼ strength MS [Huettel and Hammerschlag, 1986]) and 2-week-old root cultures (1.5 cm long, grown on Gamborg's B5 medium) were inoculated by placing two *M. incognita* egg masses, obtained from stock naranjilla root cultures, near root tips. After five weeks, data collected were number of galls, number of egg masses, and number of eggs from each explant. The microplant and root culture experiments were each repeated one time.

Response to nematodes was evaluated in four ways:

1. *Egg masses index* (EMI): Egg masses were stained brilliant red by placing galled roots in a solution of phloxine B (0.15 g·liter^{-1} tap water) for 15 minutes and then rinsing in tap water before counting (Daykin and Hussey, 1985).
2. *Egg number:* Extracted eggs were placed in 20 ml of tap water and 0.5 ml of staining solution (3.5 g acid fuchsin, 250 ml acetic acid, and 750 ml distilled water) (Byrd *et al.*, 1983), heated to boiling and then allowed to cool to room temperature before counting.
3. *Reproduction factor* (Rf): To evaluate the reproductivity of nematodes, a reproduction factor was calculated (Rf = Pf/Pi), where Pf is the final population of eggs recovered from the roots of infested plants and Pi is the initial population of eggs with which the plants were inoculated (Sasser *et al.*, 1984). For the in vitro experiments, Pi was calculated based on 300 eggs/egg mass (Sassanelli *et al.*, 2000).
4. *Host resistance classification* (HRC): HRC evaluates nematode reproduction [eggs per gram of root fresh weight (egg/fw)] compared to a known susceptible cultivar, in this experiment the original stock plant (Taylor and Sasser, 1978).

Data analysis: All the experiments were repeated at least once unless stated otherwise and were completely randomized. Data were statistically analyzed by analysis of variance with means separated by Duncan's Multiple Range Test using the procedures of SAS (SAS Institute, Inc., 1989). A mixed model analysis of the final experiment was done to contrast individual regenerant lines with the control. Two separate replicates of a randomized complete block design with 4 blocks and 24 treatments were run. Analysis combined both replicates. Replicate Block and Replicate x Treatment interactions were regarded as random. Each individual line was compared to the average of the control and the Kenward and Roger's (1997) method for degrees of freedom in the denominator was used. Computations were carried out using the mixed procedure of SAS (SAS Institute, Inc., 1999).

RESULTS

Micropropagation

One single-node microcutting produced an average of 8.3 ± 1.5 (SD) nodes every four weeks *in vitro*. After three weeks in sand, microcutting average root length was 24 mm ± 10 (SD) and average root number was 5.3 ± 2 (SD).

Regeneration

Regenerants initiated from the cut surfaces of internodal-stem segments and petioles; however, there was no gradient effect (distal versus proximal) (data not shown). There was significantly more regeneration from explants cultured on medium supplemented with BA alone (289 regenerants from stem segments on BA media compared to 2 regenerants on BA + NAA media), although there was no significant BA-concentration effect.

Nematode Maintenance and Inoculum

Greenhouse: Five weeks after inoculation, 100% of the plants had developed galls (Table 1). Nematode egg number did not influence leaf color, shoot dry weight, root fresh weight, or number of galls; however, plants inoculated with 0 or 1,000 eggs had significantly greater shoot fresh weights compared to the plants inoculated with 10,000 eggs. Plants inoculated with 1,000 eggs had significantly more roots and greater root dry weight compared to the other treatments, for this reason inoculation with 1,000 eggs was chosen for regenerant screening experiments. Eighty percent of the control stock plants (not inoculated) had galls in the 6 × 10 communal cell-flats; therefore, all future nematode screening experiments were carried out using individual 60 mm^2 pots.

In vitro: Root length increased approximately 1 cm each week (data not shown). Dark-grown roots (6.5 ± 1.7 cm) grew significantly longer than light-grown roots (4.7 ± 1.5 cm) and roots cultured in the dark were observed to be wider and to have more root hairs (personal observation). A preliminary nematode inoculation experiment determined that 'Dulce' roots were susceptible to *M. incognita* based on

Table 1. Determination of optimal nematode egg inoculum (0, 1,000, 3,000, 5,000, or 10,000 eggs/plant) for *S. quitoense* 'Dulce'.

Nematode inoculum conc[z]	N[y]	Shoot means[x]			Root means[v]			
		Leaf color	FW	DW	No. roots	FW	DW	No. galls
0	10	2.0 a[w]	0.7 a	0.06 a	8.3 b	0.4 a	0.03 b	11.3 a
1,000	10	2.1a	0.7 a	0.06 a	9.6 a	0.4 a	0.04 a	44.3 a
3,000	10	1.8a	0.4 ab	0.03 a	7.9 b	0.3 a	0.03 b	53.8 a
5,000	10	1.8a	0.4 ab	0.03 a	7.1 c	0.3 a	0.02 c	60.7 a
10,000	10	1.7a	0.3 b	0.03 a	6.0 d	0.2 a	0.02 c	35.2 a

[z]Number of eggs of *Meloidogyne incognita* inoculated per plant.
[y]Number of plants per treatment, experiment repeated once.
[x]Shoot means: leaf color: 1 = green, 2 = yellow green, 3 = yellow; FW = gm fresh weight/shoot; DW = gm dry weight/shoot.
[w]Means within a column separated using Duncan's multiple range test, $p < 0.05$.
[v]Root means: No. of roots = number of primary roots; FW = gm fresh weight/root system; DW = gm dry weight/root system.

the presence of galls and egg masses after five weeks of inoculation.

Screening Regenerants for Resistance

Greenhouse Experiment 1: Number of galls ranged from 0 to 87 for the regenerants five weeks after inoculation (data not shown). Twenty-one regenerants, each having 5 or fewer galls, were selected for further screening. In Experiment 2, the number of egg masses and number of eggs from the control were no different than those from the regenerants (Table 2). Nine of the regenerants (57, 79, 122, 124, 138, 199, 204, 249, and 285) had greater plant fresh weights and one regenerant (122) produced more eggs per plant compared to the control. All the regenerants and the control produced comparable numbers of egg masses per plant. However, the Rf values indicated nematode reproduction was reduced in regenerants 57, 178, 293, 312, and 323 (Fig. 1). Based on the HRC, four regenerants, 57, 178, 293 and 312, were slightly 'resistant' and

Table 2. Comparison of *S. quitoense* 'Dulce' regenerants to the control stock plants.

Plant ID	N	Avg. no. egg masses	Avg. no. eggs	Avg. FW[z] (gm)
Control	24	9 (4.1)[y]	2964 (1365)	3.6 (0.8)
14	6	20 (10.5)	4655 (2107)	5.2 (1.6)
47	7	11 (3.1)	4051 (1137)	4.6 (0.1)
57	5	7 (2.8)	1354 (565)	5.2 (1.4)*
79	8	11 (3.3)	5915 (1924)	5.3 (0.7)*
122	7	27 (13.2)	13062 (8640)	6.0 (0.8)*
124	4	15 (4.0)	4679 (1734)	6.9 (1.1)*
138	8	13 (3.0)	4745 (1385)	5.1 (0.9)*
151	8	14 (3.1)	3882 (1121)	4.7 (0.9)
178	6	5 (1.4)	1364 (438)	3.5 (0.8)
197	6	16 (5.5)	4349 (1176)	5.0 (0.9)
199	8	12 (4.4)	3548 (1208)	5.2 (1.1)*
204	8	14 (4.4)	5765 (1911)	5.1 (1.0)*
229	5	14 (7.7)	6477 (4148)	5.1 (0.7)
232	6	6 (2.1)	2519 (1271)	3.9 (0.8)
249	5	15 (2.9)	5059 (1662)	4.7 (0.9)*
250	7	8 (2.7)	3947 (1767)	4.5 (0.7)
282	7	11 (4.2)	3661 (1680)	5.0 (1.1)
285	8	8 (3.1)	3033 (1066)	5.1 (0.9)*
293	7	6 (1.8)	1546 (451)	4.6 (1.2)
312	5	10 (2.9)	2490 (873)	5.6 (1.2)
323	5	14 (12.1)	4889 (4177)	5.5 (1.5)

[z]FW = Plant Fresh Weight.
[y]Numbers in parentheses are standard errors.
*Numbers followed by an * are significantly different from the control within a column.

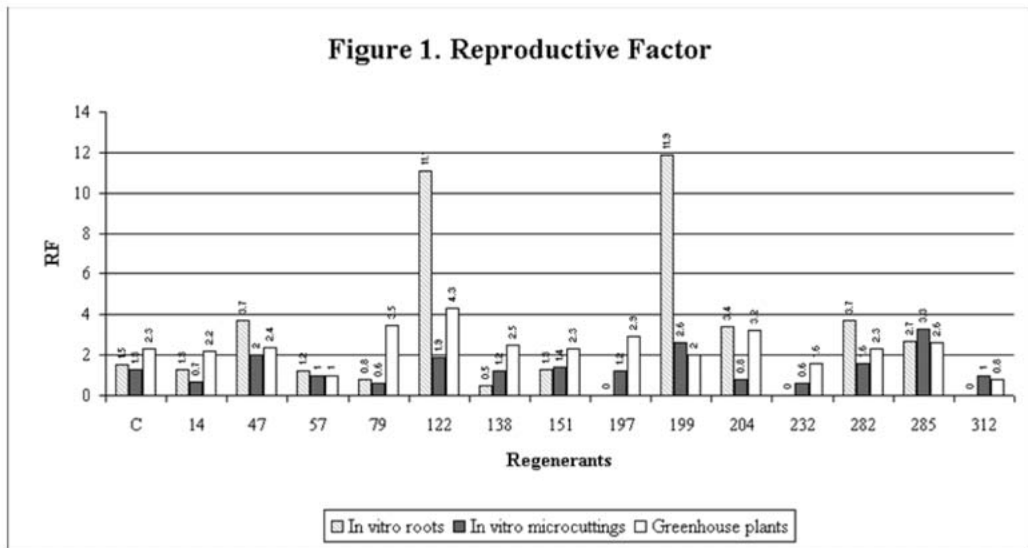

Fig. 1. Comparison of Reproductive Factor (Rf) values for *in vitro* roots, *in vitro* microcuttings and greenhouse plants [Rf = Pf/Pi; Reproductive Factor = Final egg Population/Initial egg Population; Pi for *in vitro* roots and microcuttings based on 300 eggs/egg mass (Sasanelli *et al.*, 2000).]

regenerant 323 was moderately 'resistant' (Fig. 2). Also, ten regenerants had higher HRC values than the control plants. Although regenerant 323 was promising based on the Rf and HRC classification systems, 'resistance' was questionable because line 323 had one plant that produced 62 egg masses having >20,000 eggs.

In vitro: Galls were evident after 4 weeks (personal observation) but experimental data was collected after 5 weeks. Roots from regenerants 79, 138, 197, 232, 312 and rooted microcuttings from regenerants 14, 57, 79, 204, 232, and 312 had reduced Rf values compared to the control (Fig. 1). Rooted microcuttings of three regenerants, 14, 79, and 232, appeared to be slightly 'resistant' based on HRC (Fig. 2).

The results of the three screening experiments, greenhouse-rooted microcuttings, *in vitro* roots, and *in vitro*-rooted microcuttings, were compared in every two-way combination and no correlation was found (data not shown).

DISCUSSION

Somaclonal variation has been generated for desirable traits such as herbicide resistance, abiotic stresses, and disease resistance (Duncan, 1997). Production of somaclonal variants has not been reported for *Solanum quitoense* but a wide range of somaclonal variation has been reported in its Solanaceous relatives (Mohan, 2001). The present research was initiated to generate and assess somaclonal variation in *S. quitoense* conferring resistance to *Meloidogyne incognita*.

S. quitoense responded exceptionally well to all of the *in vitro* and *ex vitro* manipulations reported herein. To ensure clonal integrity during the micropropagation phases, we wanted, if at all possible, to develop growth regulator-free protocols. Shoot proliferation and rooting *in vitro* and rooting and reestablishment in the greenhouse did not require growth regulators.

Hendrix *et al.* (1987) regenerated *S. quitoense* from leaf explants of an unknown cul-

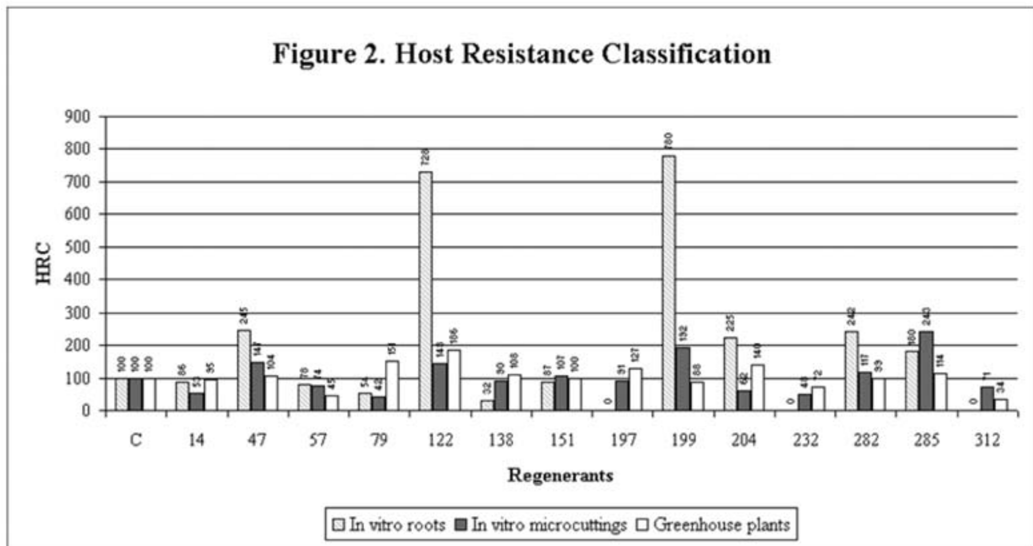

Fig. 2. Comparison of Host Resistance Classification (HRC) percentages for *in vitro* roots, *in vitro* microcuttings and greenhouse plants [HRC = (eggs/regenerant root FW)/(eggs/control root FW) × 100%, <1% = highly resistant, 1-10% = very resistant, 10-25% = moderately resistant, 25-50% = slightly resistant.]

tivar on medium with BA plus NAA where the highest concentration of NAA resulted in the initiation of callus and adventitious roots. In the present research, no callus was generated and regenerants appeared to initiate directly from the cut surfaces (Takeuchi *et al.*, 1984). When 'Dulce' stem and petiole explants were cultured on medium containing BA and NAA, regenerants were obtained basically only in medium with BA suggesting that the essential growth regulator for regeneration in naranjilla is cytokinin. Regeneration to obtain somaclonal variants with useful agronomic traits has been initiated from calli (Bertin and Bouharmont, 1997), adventitious buds (Chevreau *et al.*, 1998), immature embryos (Arun *et al.*, 2003), and cotyledonary nodes (Hossain *et al.*, 2002) and, herein, we report on regeneration from internodal-stem segments.

One thousand eggs per greenhouse-maintained clonal stock plant appeared to be the best inoculum based on root number and dry weight after 5 wk (Huettel and Hammerschlag, 1986, Hashmi *et al.*, 1995). For greenhouse experiments with *Capsicum annuum*, 36-day-old seedlings were each inoculated with 1,500 eggs to evaluate response to *M. incognita* (Zamora and Bosland, 1994) while *in vitro*-generated plants of *Prunus cerasifera* were evaluated for nematode response by inoculating each plant with 1,500 to 4,500 freshly hatched second-stage juvenile (J2) *Meloidogyne* spp. (Esmenjaud *et al.*, 1993).

Sand, as a rooting substrate, allowed *M. incognita* to become established on naranjilla roots. Five weeks post-inoculation with eggs, root galls and egg masses were present on the 21 selected somaclones of naranjilla. Use of sand as a substrate when screening for nematode resistance in a greenhouse also has been reported in *Prunus cerasifera* (Esmenjaud *et al.*, 1993) and *Ipomoea batatas* (Cervantes-Flores *et al.*, 2002).

Work with olive (*Oleo europaea)* root cultures demonstrated that 'sterilizing' egg masses and eggs collected from greenhouse-

infested plants for use as *in vitro* inoculations was challenging (Sasanelli *et al.*, 2000). There was a fine line between injury and sterilization of nematode eggs during the 'sterilization' procedure. *In vitro* maintained roots of naranjilla served as a source of sterile nematodes due to *M. incognita*'s ability to readily reproduce. Five weeks after inoculation, both galls and egg masses were present.

Determination of a plant's response to nematodes commonly has been based on (1) the capability of nematodes to reproduce (Rf) and (2) comparison of new genotypes with genotypes known to be susceptible (HRC) (Sasser *et al.,* 1984). Based on Rf values and HRC percentages derived from greenhouse-rooted microcuttings, five regenerants (57, 178, 293, 312 and 323) of naranjilla were found to be highly promising for field evaluation response to *M. incognita*.

In vitro-rooted microcuttings of naranjilla, inoculated with two egg masses, became infested as has been reported in *in vitro*-maintained microplants of olive (Sasanelli *et al.*, 2000); however, gall data was collected from naranjilla after 5 weeks and from olive after 2 months. Naranjilla root cultures inoculated with two egg masses also became infested and galls were observed after 4 weeks. Alternatively, cassava root cultures became infested after inoculation with eggs and galls were observed after 2 weeks (Jansen van Vuuren and Woodward, 2001).

While the data from the greenhouse and *in vitro* screening protocols did not correlate in this study, *in vitro* techniques for evaluating plant response to nematode infestation and reproduction proved to be a valid alternative to greenhouse screening protocols with *Prunus persica* (Hashmi *et al.*, 1995).

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