# EVALUATING ROOT-KNOT NEMATODE INFECTION IN WILD POTATOES

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## ABSTRACT

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Cultivated potato varieties are highly susceptible to root-knot nematodes (*Meloidogyne* spp.). Resistance in wild relatives could be transferred to breeding lines as an environmentally friendly control strategy. We evaluated 28 genotypes of three putatively resistant wild potato species (*Solanum spegazzinii*, *S. kurtzianum*, and *S. vernei*) from Argentina for reaction to one of the most damaging potato root-knot nematodes, *Meloidogyne arenaria*. Two screening assays found great variability among and within species of potato in response to nematode infection. The nematode reproductive efficiency was significantly reduced in two genotypes of *S. spegazzinii*, which exhibited low to moderate resistance to *M. arenaria*.

*Key words:* central west Argentina, *Meloidogyne*, *Solanum*, variability, wild germplasm.

## RESUMEN

García, L. E., D. M. Segura, R. W. Masuelli, and M. V. Sanchez-Puerta, 2014. Evaluación de la infección por nematodos del nudo en papas silvestres. Nematropica 44:31-36.

Las variedades de papa cultivada son altamente susceptibles a nematodos del nudo (*Meloidogyne* spp.). La resistencia en especies silvestres emparentadas podría ser utilizada en programas de mejoramiento como una estrategia ecológica de manejo. En este trabajo se evaluó la respuesta a la infección por uno de los nematodos del nudo más dañinos, *Meloidogyne arenaria*, de 28 genotipos de tres especies de papas silvestres (*Solanum spegazzinii*, *S. kurtzianum* and *S. vernei*) de Argentina. Los dos ensayos realizados mostraron gran variabilidad de respuesta a la infección por el nematodo entre y dentro de las especies de papas silvestres. La eficiencia reproductiva del nematodo fue significativamente reducida en dos genotipos de *S. spegazzinii*, los cuales exhibieron baja a moderada resistencia a *M. arenaria*.

*Palabras clave:* Argentina, germoplasma, *Meloidogyne*, resistencia, *Solanum*.

The cultivated potato (*Solanum tuberosum* L. ssp. *tuberosum*) originated in the Andes in South America and has more than 200 wild relatives (Hawkes, 1994). Cultivated potato varieties show a wide range of agronomic traits but are susceptible to several pathogens (Ross, 1986). Root-knot nematodes, *Meloidogyne* spp., cause great losses of potato crops worldwide. Around 95% of the damage caused by root-knot nematodes is due to three species (*M. incognita*, *M. arenaria*, and *M. javanica*). In Mendoza (Argentina), 7,000 to 9,000 ha are cultivated for potato. *Solanum tuberosum* cv. Spunta is the most widely consumed variety in Argentina (Fundación Instituto de Desarrollo Rural, Argentina) and is highly susceptible to root-knot nematodes, including *M. arenaria*.

Wild tuber-bearing *Solanum* spp. are a great reservoir of resistance genes for a number of diseases (Ross, 1986; Gebhardt and Valkonen, 2001; Song *et al*., 2003; Suarez *et al*., 2009) that could be exploited in breeding programs, reducing the need for pesticide applications, lowering production costs and minimizing environmental damage. The first steps in the development of resistant cultivars include screenings of wild relatives for reaction to pathogens (Sasser *et al.*, 1984). Resistance to root-knot nematodes was previously reported for wild tuber-bearing *Solanum* spp., including *S. kurtzianum*, *S. spegazzinii,* and *S. vernei* (Nirula *et al*., 1967; Nirula *et al*., 1969; Bamberg *et al*., 1994; Berthou *et al*., 2003). However, only a few nematode-resistant

genotypes were clearly registered and are readily available from germplasm banks. The objectives of this study were: i) to evaluate putatively resistant wild potatoes from germplasm banks for reaction to the root-knot nematode *M. arenaria*; and ii) to identify potentially useful germplasm for breeding programs.

Screening experiments to evaluate 28 genotypes of three putatively resistant wild potato species (*S. spegazzinii*, *S. vernei,* and *S. kurtzianum*) for reaction to the root-knot nematode, *M. arenaria,* were performed. Seeds were obtained from germplasm collections (Table 1). Plants of *S. spegazzinii* and *S. vernei* were cultivated in pots with autoclaved loamy soil and multiplied by cuttings. Due to the low survival rate of cuttings, plants of *S. kurtzianum* were maintained and multiplied *in vitro* in MS medium (Murashige and Skoog, 1962) and plantlets with 4-6 leaves were transplanted to sterilized loamy soil. Plants of *S. tuberosum* ssp. *tuberosum* cv. Spunta were included as a susceptible control. Plants were fertilized as needed and were arranged in a completely randomized design with up to 10 replicates. The inoculum originated from a well-characterized population of *M. arenaria* race 2, which was isolated from a production field in the province of Mendoza, Argentina (García and Sanchez-Puerta, 2012). The nematode population was maintained on susceptible tomato plants (*Solanum lycopersicum* cv Roma). Egg masses from infected tomato roots were manually isolated from the roots and placed in a Petri dish with distilled water. Juveniles (J2) were collected for inoculum.

Two rounds of resistance screenings were conducted. A suspension of 100 J2 was delivered into a hole in the soil next to the roots of a plant with 4-6 leaves in each pot. An average of 75 d (first screening) and 60 d (second screening) after inoculation, plants were harvested and evaluated. The root system of each plant was washed with tap water and weighed. Gelatinous egg masses were stained by immersion in cold, yellow eosin solution (0.1g/l in water) and stirred for 30 min (Daykin and Hussey, 1985). The number of galls and egg masses on the root system were counted using a stereoscopic microscope. Egg masses were isolated and placed in 1% NaOCl solution to dissolve the gelatinous matrix. Second-stage juveniles were isolated from the soil of each pot by the centrifugal-flotation method (Jenkins, 1964). Second-stage juveniles and eggs were counted with a microscope at 40×. Data collected included: i) egg masses per gram of root; ii) root galls per gram of root; iii) J2 in the soil; and iv) number of eggs per egg mass. Eggs per egg mass and egg masses per gram of root were $\sqrt{(x+2)}$ and Ln(x+5) transformed, respectively, to attain a normal distribution. Normalized data were subjected to ANOVA using the computer program InfoStat (Di Rienzo et al., 2008). The significance of the differences among genotypes was estimated with Duncan´s test at $P < 0.05$.

The first resistance assay included 18 genotypes of *S. kurtzianum*, *S. spegazzinii*, and *S. vernei*, which produced either lower or higher average egg masses, root galls, and J2 per gram of root than *S. tuberosum*

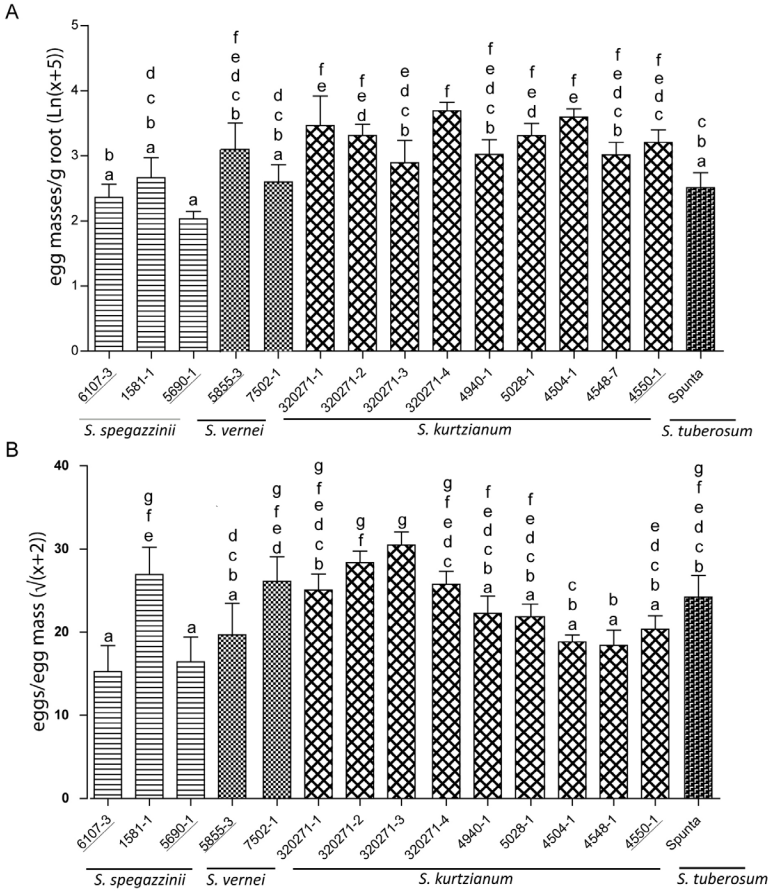


Fig. 1. Response of several genotypes of three wild tuber-bearing *Solanum* to the nematode *Meloidogyne arenaria*. A: Number of egg masses per gram of root; B: Number of eggs per egg mass. Same letters express no statistical difference ($P$ <0.05).

Table 1. List of wild potato accessions examined in this study, indicating the accession number, genotype, geographic origin, altitude, and source.

| Species | Accession number | Genotype | Geographic origin | Altitude (meters) | Source |
|---|---|---|---|---|---|
| *S. spegazzinii* | Oka6107 | 6107-1 | Villa Vil, Belén (Catamarca, Argentina) | 2340 | [y]INTA Balcarce |
| *S. spegazzinii* | Oka6107 | 6107-2 | Villa Vil, Belén (Catamarca, Argentina) | 2340 | INTA Balcarce |
| *S. spegazzinii* | Oka6107 | 6107-3 | Villa Vil, Belén (Catamarca, Argentina) | 2340 | INTA Balcarce |
| *S. spegazzinii* | Oka6147 | 6147-1 | Condorhuasi, Belén (Catamarca, Argentina) | 1920 | INTA Balcarce |
| *S. spegazzinii* | Oka6147 | 6147-2 | Condorhuasi, Belén (Catamarca, Argentina) | 1920 | INTA Balcarce |
| *S. spegazzinii* | Oka6142 | 6142-1 | Pozo de Piedra, Belén (Catamarca, Argentina) | 1900 | INTA Balcarce |
| *S. spegazzinii* | Ol4930 | 4930-1 | Campo de Arenal, Santa María (Catamarca, Argentina) | 2220 | INTA Balcarce |
| *S. spegazzinii* | Ol4930 | 4930-2 | Campo de Arenal, Santa María (Catamarca, Argentina) | 2220 | INTA Balcarce |
| *S. spegazzinii* | Oka5690 | 5690-1 | Tafí del Valle (Tucumán, Argentina) | 2014 | INTA Balcarce |
| *S. spegazzinii* | CIS1765 | 1765-1 | Lara, Tafí del Valle (Tucumán, Argentina) | 2929 | INTA Balcarce |
| *S. spegazzinii* | CIS1765 | 1765-2 | Lara, Tafí del Valle (Tucumán, Argentina) | 2928 | INTA Balcarce |
| *S. spegazzinii* | CIE1581 | 1581-1 | Chicoana (Salta, Argentina) | 3005 | INTA Balcarce |
| *S. spegazzinii* | CIE1561 | 1561-1 | Ayuyerada, La Poma (Salta, Argentina) | 2762 | INTA Balcarce |
| *S. vernei* | OKA7502 | 7502-1 | Cuesta del Obispo, Chicoana (Salta, Argentina) | 3450 | [z]ARS, USDA |
| *S. vernei* | OKA5855 | 5855-1 | Lizoite, Santa Victoria ( Salta, Argentina) | 3400 | ARS, USDA |
| *S. vernei* | OKA5855 | 5855-2 | Lizoite, Santa Victoria (Salta, Argentina) | 3400 | ARS, USDA |
| *S. vernei* | OKA5855 | 5855-3 | Lizoite, Santa Victoria (Salta, Argentina) | 3400 | ARS, USDA |
| *S. kurtzianum* | 320271 | 320271-1 | Alijilan, Santa Rosa (Catamarca, Argentina) | 486 | ARS, USDA |
| *S. kurtzianum* | 320271 | 320271-2 | Alijilan, Santa Rosa (Catamarca, Argentina) | 486 | ARS, USDA |
| *S. kurtzianum* | 320271 | 320271-3 | Alijilan, Santa Rosa (Catamarca, Argentina) | 486 | ARS, USDA |
| *S. kurtzianum* | 320271 | 320271-4 | Alijilan, Santa Rosa (Catamarca, Argentina) | 486 | ARS, USDA |
| *S. kurtzianum* | ORH4285 | 4285-1 | Peñasquito (San Juan, Argentina) | 2700 | INTA Balcarce |
| *S. kurtzianum* | OKA4940 | 4940-1 | Cuesta de Belén (Catamarca, Argentina) | 1100 | INTA Balcarce |
| *S. kurtzianum* | OKA4996 | 4996-1 | Gral. Lavalle (La Rioja, Argentina) | 1300 | INTA Balcarce |
| *S. kurtzianum* | OKA5028 | 5028-1 | Cuesta de Zapata, Tinogasta (Catamarca, Argentina) | 1800 | INTA Balcarce |
| *S. kurtzianum* | SCI4504 | 4504-1 | Las Higueras, Las Heras (Mendoza, Argentina) | 1180 | INTA Balcarce |
| *S. kurtzianum* | SCI 4548 | 4548-1 | Pampa del Leñador, Las Heras (Mendoza, Argentina) | 1600 | INTA Balcarce |
| *S. kurtzianum* | SCl 4550 | 4550-1 | Quebrada del Toro ( Mendoza, Argentina) | 1770 | INTA Balcarce |

[y]Potato and Forage Germplasm Bank of the Estación Experimental Agropecuaria (EEA) Balcarce (INTA, Argentina).
[z]USDA, ARS, National Genetic Resources Program. Germplasm Resources Information Network - (GRIN). [Online Database] National Germplasm Resources Laboratory, Beltsville, Maryland.

Table 2. Response of several genotypes of three wild tuber-bearing *Solanum* to the nematode *Meloidogyne arenaria* in the first screening experiment.

| | | | Egg masses/g root | | Galls /g root | | Juveniles/g root | |
|---|---|---|---|---|---|---|---|---|
| Species | Genotype | n[x] | Average | SD[y] | Average | SD | Average | SD |
| *S. spegazzinii* | *spg1765-1* | 6 | 111.4 | 65.3 | 362.4 | 89.9 | 783.3 | 480.7 |
| *S. spegazzinii* | *spg1765-2* | 4 | 81.2 | 51.8 | 347.3 | 111.0 | 1667.1 | 1952.9 |
| *S. spegazzinii* | *spg6107-1* | 5 | 98.8 | 76.3 | 235.5 | 134.2 | 2280.9 | 1532.4 |
| *S. spegazzinii* | *spg6107-2* | 5 | 123.1 | 27.4 | 283.1 | 115.9 | 309.7 | 379.3 |
| *S. spegazzinii* | *spg6107-3* | 6 | 23.7 | 33.2 | 111.2 | 179.6 | 4413.2 | 9185.6 |
| *S. spegazzinii* | *spg5690-1* | 4 | 14.1 | 6.4 | 30.4 | 7.8 | 189.1 | 115.9 |
| *S. spegazzinii* | *spg1561-1* | 4 | 90.6 | 47.2 | 288.6 | 127.2 | 1587.7 | 978.3 |
| *S. spegazzinii* | *spg6147-1* | 5 | 34.9 | 23.3 | 138.8 | 92.2 | 721.3 | 555.6 |
| *S. spegazzinii* | *spg6147-2* | 5 | 68.0 | 64.6 | 179.5 | 83.8 | 351.1 | 278.3 |
| *S. spegazzinii* | *spg6142-1* | 5 | 44.0 | 54.2 | 122.3 | 148.7 | 44.8 | 29.8 |
| *S. spegazzinii* | *spg4930-1* | 5 | 108.0 | 97.5 | 263.6 | 196.1 | 725.3 | 816.9 |
| *S. spegazzinii* | *spg4930-2* | 6 | 149.6 | 137.5 | 346.2 | 383.0 | 1126.5 | 814.6 |
| *S. vernei* | *Vrn5855-1* | 5 | 14.4 | 13.8 | 40.1 | 29.8 | 191.7 | 194.1 |
| *S. vernei* | *Vrn5855-2* | 5 | 12.4 | 21.0 | 23.3 | 44.8 | 144.4 | 285.8 |
| *S. vernei* | *Vrn5855-3* | 6 | 2.6 | 4.7 | 8.1 | 15.7 | 35.5 | 46.2 |
| *S. kurtzianum* | *ktz4285-1* | 9 | 16.5 | 5.8 | 45.0 | 17.4 | Not tested | Not tested |
| *S. kurtzianum* | *ktz4996-1* | 8 | 25.2 | 14.1 | 64.3 | 49.6 | Not tested | Not tested |
| *S. kurtzianum* | *ktz4550-1* | 4 | 10.0 | 9.2 | Not tested | Not tested | Not tested | Not tested |
| *S. tuberosum* | *Spunta* | 10 | 40.8 | 52.6 | 94.7 | 148.4 | 584.3 | 1250.7 |

[x]n: number of plants analyzed
[y]SD: standard deviation

(Table 2). All parameters were highly variable within and among species. Genotypes with fewer egg masses/g of root than the cultivated potato included three genotypes of *S. spegazzinii* (spg6107-3, spg5690-1, spg6147-1) and all genotypes of *S. vernei* and *S. kurtzianum*. The same genotypes, except for spg6107-3 and spg6147-1, also had fewer root galls/g of root than the cultivated potato. The fewest juveniles/g root were observed for spg6107-2, spg5690-1, spg6147-2, spg6142-1, and all three genotypes of *S. vernei*.

The second resistance assay included another 10 genotypes belonging to the three wild potato species (*S. kurtzianum*, *S. spegazzinii,* and *S. vernei*) plus four genotypes identified as putatively resistant in the first assay (underlined in Fig. 1). The number of egg masses/g root (Fig. 1A) and the number of eggs/egg mass (Fig. 1B) for each genotype varied considerably among populations within a single species. Significant differences in eggs/egg mass were observed for populations within *S. spegazzinii* and within *S. kurtzianum* (Fig. 1B). Interestingly, all genotypes of *S. kurtzianum*, which were not different than the cultivated potato, had been previously reported as resistant to root-knot nematodes (Bamberg *et al*., 1994; Segura *et al*., 2008). In addition, we found differences in the response to infection among wild potatoes and the cultivated potato (Fig. 1). Two genotypes of *S. spegazzinii* (spg5690-1 and spg6107-3) had significantly fewer eggs/egg mass than *S. tuberosum* cv. Spunta (Fig. 1B). The genotype spg5690-1 also had, on average, fewer egg masses/g root than the cultivated potato, but this difference was not statistically significant (Fig. 1A).

Overall, significant variation in the reaction to *M. arenaria* was observed among individual members of a single species of wild potatoes. Other studies have also reported large differences among populations of *Solanum* species in response to root-knot nematode infection (Ammati *et al*., 1985; Janssen *et al*., 1996; Janssen *et al*., 1997). These findings emphasize the importance of sampling diverse populations of a few wild potato species, instead of a single representative from a large number of different species, when screening for pathogen resistance or other relevant traits. In addition, two genotypes of *S. spegazzinii* exhibited low to moderate resistance to *M. arenaria* race 2 but not at a sufficient level for a breeding program. The mechanism responsible for the diminished nematode reproductive efficiency in these plants is unknown. Further studies are needed to distinguish whether the resistance mechanism affects root penetration (Araya and Caswell-Chen, 1994), entrance to the vascular cylinder (Potenza *et al*., 1996), establishment of the feeding site (Das *et al*., 2008), or female development (Fasque and Starr, 2009). Finally, great variability in response to nematode infection exists in wild potato germplasm banks.

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