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Genetic Analysis of Resistance to *Meloidogyne chitwoodi* Introgressed from *Solanum hougasii* into Cultivated Potato

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Abstract: An accession of *Solanum hougasii,* a wild tuber-bearing potato species native to Mexico, was found to be resistant to races 1 and 2 of *Meloidogyne chitwoodi.* A resistant selection was selfed and its progeny possessed the same combined resistance uniformly. A selected resistant seedling from the selfed progeny was crossed to cultivated tetraploid potato (*S. tuberosum)* to form an F_1 hybrid, and was backcrossed to cultivated tetraploid potato to form a BC_1 population in which resistance to the two races segregated. Progeny of the BC_1 were tested in inoculation experiments with four replicates for each progeny genotype for each race of nematode. Resistance was evaluated on the basis of extracted egg counts from the entire root system of pot-grown plants. Considering resistance to each race separately, for race 1, non-host ($Rf \leq 0.1$) status was exhibited by approximately half of the BC_1. About one-third of the progeny showed non-host status to race 2. Egg production among progeny that showed non-host status for both races was higher with race 2 than with race 1. Analysis of co-segregation established that genetic control for the two races appears to be independently segregating. Although genes for resistance to race 1 derived from *S. bulbocastanum* and *S. fendleri* were previously described, this report is the first analysis showing independent genetic control in *Solanum* spp. for resistance to race 2 of *M. chitwoodi* only.

Key words: breeding, Columbia root-knot nematode, inheritance, introgression, *Meloidogyne chitwoodi,* nematode, potato, resistance, *Solanum bulbocastanum, Solanum fendleri, Solanum tuberosum,* wild species.

Columbia root-knot nematode (*Meloidogyne chitwoodi* Golden, O'Bannon, Santo & Finley) is a major pest of potato (*Solanum tuberosum* L.) in the Pacific Northwest of North America and the Netherlands (Evans and Trudgill, 1992). Second-stage juveniles (J2) invade tubers through the lenticels and produce brown spots visible on peeled tubers, where females have produced egg masses. Tubers also become galled, a condition that becomes more accentuated the longer infested tubers are allowed to remain unharvested in the field, or in storage before processing. The damage can be pronounced enough to make potatoes unmarketable in the fresh market and to processors. Production fields are chemically fumigated to reduce nematode populations. Genetic resistance, a highly desirable trait to incorporate into future cultivars, is unknown within cultivated potato. Clearly, cultivars possessing resistance at the non-host level would be highly desirable, as damage to the tubers is proportional to the population of J2 available to infest the tubers. In addition, environmentally friendly partial control measures, such as green manures (Mojtahedi et al., 1992, 1993), will be economically more viable when the suitability of the host is much lower than the current level. Such a level of resistance to reproduction on the roots is found only in wild *Solanum* spp. The introgression of resistance requires a full assessment of the genetics of resistance at the outset. Resistance has been described in *S. bulbocastanum* Dun. and *S. hougasii* Corr. (Brown et al., 1989, 1991, 1994). Other germplasm surveys have reconfirmed resistance in these species and discovered it in other species, e.g., *S. fendleri* Asa Gray, *S. stoloniferum* Schlecht. et Bché, *S. chacoense* Bitt., *S. brachistrotrichum* (Bitt.) Rydb., and *S. cardiophyllum* Lindl. (Janssen et al., 1995, 1996, 1997a, 1997b, 1997c).

Infraspecific variation in virulence of *M. chitwoodi* is important. Alfalfa is a non-host for race 1, but a good host for race 2 (Mojtahedi et al., 1988). The *S. bulbocastanum* accession SB22 (285187), one source of resistance already incorporated into breeding efforts in the United States, is a non-host to

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races 1 and 2 (Brown et al., 1989) while being a good host to a resistance-breaking biotype of race 2 (formerly known as race 3) (Mojtahedi et al., 1994, 1998). In the Netherlands, a taxon similar to *M. chitwoodi* is given unique specific status as *M. fallax* Karssen (Karssen, 1996). Generally, wild *Solanum* accessions resistant to *M. chitwoodi* race 1 are also resistant to *M. fallax* (Janssen et al., 1997c). Certain accessions of *Solanum* spp. and infraspecific selections within accessions have shown differences in host status when challenged with races 1 and 2 (Brown et al., 1989, 1991, 1994, 1995).

Solanum bulbocastanum has served as a useful source of resistance to race 1 of *M. chitwoodi*. The introgression of this trait was initiated by the introduction of resistance into cultivated potato breeding pools through protoplast fusion of the diploid *S. bulbocastanum* with tetraploid cultivated potato (Austin et al., 1993). Introgression has been carried out by backcrossing. In the BC_2, assortment and recombination of *S. bulbocastanum* chromosomes were sufficient to permit mapping and assignation of the location of resistance factor, R_{Mc1}, to chromosome 11 (Brown et al., 1994, 1995, 1996). Continued backcrossing has led to the rapid incorporation of this trait into increasingly horticulturally superior materials.

Interest in accession 161726.7 of *S. hougasii* was accentuated by the discovery that it is also resistant to the late blight pathogen (*Phytophthora infestans*) (unpubl. data, Inglis). There are several genes controlling resistance to *M. chitwoodi* in the Solanaceae. The genes R_{Mc1} from *S. bulbocastanum* (Brown et al., 1996), R_{Mc2} from *Solanum fendleri* (Janssen et al., 1997a) and *Mi* of tomato (*Lycopersicon esculentum* L.) (Brown et al., 1997) contribute to an array of resistance gene options for potato cultivar improvement. There is also the possibility that the resistance to race 2 derived from *S. hougasii* will be more substantial in advanced backcrosses than that found with R_{Mc1}. The purpose of this study is to analyze the genetic control of resistances to races 1 and 2 of *M. chitwoodi* in a segregating population derived from introgression of resistance from *S. hougasii* into cultivated potato. Resistance in the host, as used in this context, is synonymous with low reproductive factor (Rf) of the nematode.

Materials and Methods

Meloidogyne chitwoodi race 1 isolate WAMc1 and race 2 isolate WAMc30 (Mojtahedi et al., 1988) were maintained on wheat to prevent contamination by *M. hapla,* for which it is a non-host. *Meloidogyne chitwoodi* race 1 was then cultured on carrot (*Daucus carota* L., cv. Red Core Chantenay), and race 2 was propagated on alfalfa (*Medicago sativa* L. cv. Thor) (Mojtahedi et al., 1988). After four propagative cycles on these differentials, all inocula were multiplied on tomato (cv. Columbia, an *mi* cultivar, i.e., susceptible to *M. chitwoodi*). Progenies from a self were screened for resistance, and seven progeny were found to possess resistance to both races comparable to 161726.7 (Table 1). A single seedling resulting from the self-pollination of 161726.7, resistant to both races (95A2.8), was selected for crossing

Table 1. Description of germplasm used in this study.

Identity	Description	Source	Characteristics
161726.7	Resistant seedling selected out of *S. hougasii* PI accession	NRSP-6, Sturgeon Bay, WI	Resistant to *M. chitwoodi*
95A2.8	*S. hougasii* derived from selfing 161726.7	USDA/ARS, Prosser, WA	Resistant to *M. chitwoodi*
A77715.6	*S. tuberosum*	USDA/ARS, Aberdeen, ID	Resistant to corky ringspot
A89875.5	*S. tuberosum*	USDA/ARS, Aberdeen, ID	Resistant to corky ringspot
96A2.1 (F_1)	*S. hougasii* (95A2.8) × *S. tuberosum* (A77715.6)	USDA/ARS, Prosser, WA	Not tested
BC_1	*S. hougasii* × *S. tuberosum* (96A2.1) × *S. tuberosum* (A89875.6)	USDA/ARS, Prosser, WA	Segregating for resistance to *M. chitwoodi*

with cultivated tetraploid potato (*S. tuberosum*). A single F_1 hybrid was obtained from a cross with an accession resistant to corky ringspot (A77715.6). The F_1 was crossed with a second corky ringspot resistant breeding clone (A89875.5) to give the BC_1 population (Fig. 1).

Progeny seedlings from the BC_1 generation were maintained in in vitro culture (Brown et al., 1988). Cuttings propagated in vitro were transplanted as rooted cuttings into 15-cm-diam. plastic pots containing loamy sand soil (84% sand, 10% silt, 6% clay) fumigated with methyl bromide. Cuttings were transplanted into this soil mix when they were approximately 6 cm high. Seven days after transplanting, 5,000 eggs in 5 ml water were pipetted into holes made in the soil around the root system of four replicate pots of each genotype-nematode type combination. The pots were arranged in a completely randomized design on greenhouse tables. Plants were regularly watered and fertilized with slow-release pellets (Osmocote 14-14-14, Scotts, Marysville, OH) at 24 ± 3 C for 55 days, at which time eggs were extracted and counted (Hussey and Barker, 1973). Reproductive factor (Rf = final population [Pf] ÷ initial population [Pi]) depends on the fecundity of a proportion of the primary inoculum and of intermediate generations that successfully infest the host and reach reproductive maturity. Rf is one measure of resistance of a host crop species to *Meloidogyne* spp. (Oostenbrink, 1966; Sasser et al., 1984). Host status was divided into three categories on the basis of Rf values as follows: Rf ≥ 1.0, suitable host; 0.1 < Rf <1.0, poor host; Rf ≤ 0.1, non-host (Sasser et al., 1984). A one-way analysis of variance was performed on the ln (× + 1)-transformed egg counts. Rf values were calculated from geometric means. Means were separated using least significant differences ($P < 0.05$), or orthogonal comparisons between groups, and the test of independence of host status of races 1 and 2 was a chi-square test of a 3 × 3 contingency table (Steel and Torrie, 1980).

Results and Discussion

Accession 161726.7 was found in a previous study to be a non-host for races 1 and 2 (Brown et al., 1991). Selection 95A2.8 from the progeny derived from self-pollination of 161726.7 (Table 1) had non-host responses to both races. Attempts to cross *Solanum hougasii* accession 161726.7 with *S. tuberosum* were not successful, while 95A2.8 was successfully crossed. Table 2 shows the Rf values of the parental materials involved in the pedigree of the segregating BC_1 progeny. The recurrent cultivated tetraploid parents were good hosts, as was tomato. The differentials showed the typical responses for *M. chitwoodi*. Pepper was a non-host for both races, while alfalfa was a good host for race 2 and a non-host for race 1 (data not shown).

Figure 2 shows the distribution of egg production of the BC_1 progeny tested for race 1. A total of 73 progeny were evaluated for

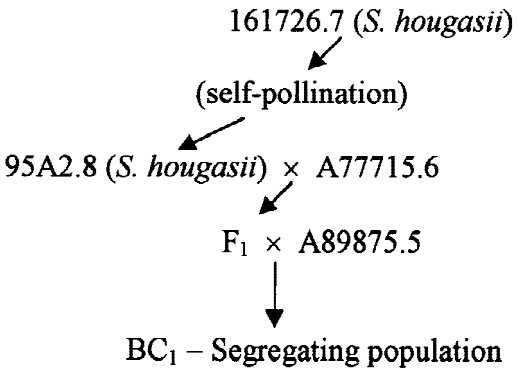

Fig. 1. Diagram of the introgression of nematode resistance into the cultivated potato gene pool.

Table 2. Reproductive factors of *Meloidogyne chitwoodi* races 1 and 2 on parental materials used in developing the BC_1 segregating population and on susceptible standard tomato cv. Columbia.

Parental Identity	Reproductive factor (Rf)	
	Race 1	Race 2
Tomato[b]	6.8b	3.9 b
S. hougasii[c]	0.0a	0.03a
A89875.5	25.6c	1.9 b
A77715.6	22.5c	3.0 b

a Means not sharing the same letter are different at $P < 0.05$ by least significant difference test.
b cv. Columbia.
c 95A2.8.

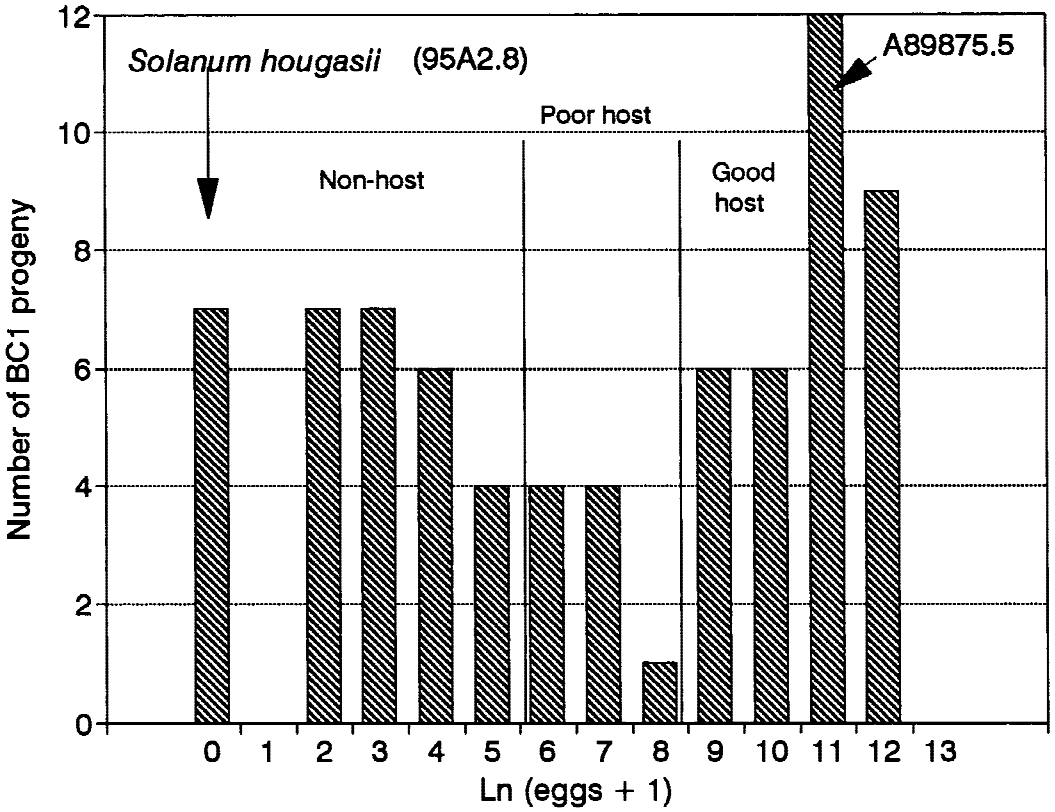

FIG. 2. Distribution of ln (eggs + 1)-transformed values for race 1.

race 1 host status. Approximately one-half (53.4%) fell within the non-host category, while 5.5 and 41.2% were classified as poor and good hosts, respectively. The analysis of variance of ln (× + 1)-transformed egg numbers identified genotypes as a significant source of variation ($F_{(78,\ 234)} = 7.02$, $P < 0.001$, $LSD_{(0.05)} = 3.4$).

The distribution of 72 progeny screened for race 2 host status is given in Fig. 3. A smaller percentage were classified as non-hosts (29.2%) than with race 1. A comparatively larger portion, 38.9%, fell into the poor-host category, while good hosts comprised 31.9% of the total. Non-host responses for race 1 were characterized by seven failures in egg production, while this occurred with only one progeny for race 2. The analysis of variance of ln (× + 1)-transformed egg numbers determined that genotypes were a significant source of variation ($F_{(80,\ 241)} = 3.55$, $P < 0.001$, $LSD_{(0.05)} = 3.4$).

A separate analysis of variance was performed including only those progeny that were classified as non-hosts to both races. Interaction between genotype and nematode race was a significant source of variation ($F_{(27,\ 83)} = 2.625$, $P < 0.001$). An orthogonal comparison revealed that the mean ln (× + 1)-transformed egg number was higher for the race 2 than for race 1 ($F_{(1,\ 83)} = 8.33$, $P < 005$). Race 1 had significantly higher Rf values than race 2, suggesting that this group of 14 progeny showing double resistance was more resistant to race 1 than to race 2.

Co-segregation of host status of the BC_1 progeny when challenged by races 1 and 2 is shown in Fig. 4. The host status vs. both races is represented by nomenclatural codes where the host suitability of race 1 is the first letter and that of race 2 the second letter (e.g., G-N= good host for race 1 and non-host for race 2). The test for independence of resistance to races 1 and 2 indicated that

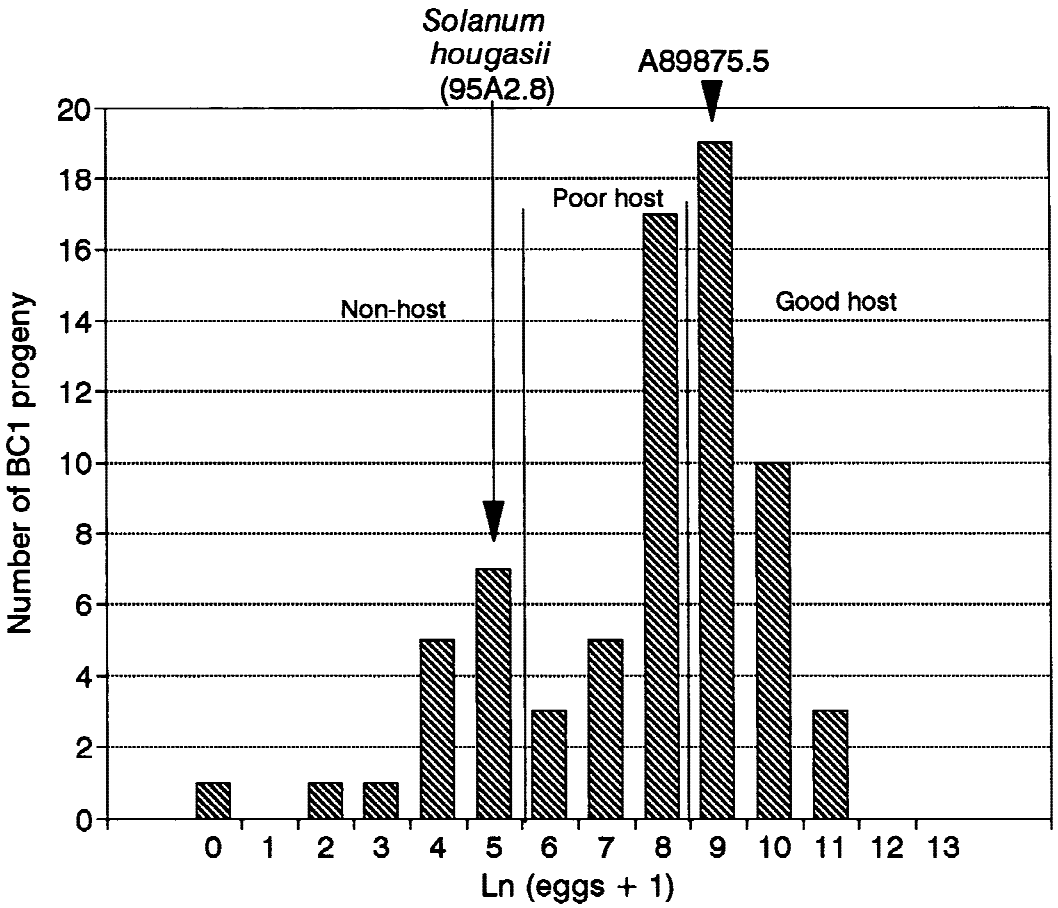

FIG. 3. Distribution of ln (eggs + 1)-transformed values for race 2.

there was no statistically supported association between resistance to races 1 and 2 [chi-square (4 degrees of freedom) = 6.26, P = 0.18]. or (chi-square [4 degrees of freedom] = 6.26, P = 0.18).

Solanum hougasii is thought to be an amphihexaploid comprised of three distinct genomes, A, A^d, and B. Although A and A^d are hypothesized to be similar versions of the cultivated potato genome present in South and North American species of *Solanum,* the B genome is considered to have evolved in isolation in North American *Solanum* spp. and is further conjectured to lack affinity to all types of the A genome (Hawkes, 1990; Matsubayashi, 1981). The primitive diploid species that have been found to be resistant to *M. chitwoodi,* i.e., *S. bulbocastanum, S. brachistrotrichum,* and *S. cardiophyllum,* are probably B-genome species. They are reproductively isolated from A-genome species and cross with them only with difficulty. For this reason they have been frequently hybridized somatically (Helgeson et al., 1993). These circumstances provide indirect evidence that the resistance genes controlling resistance to *Meloidogyne* spp. in *Solanum* may reside only on the B-genome in the above-mentioned diploids and in the B-genome homeologues in the amphiploids of the Series Longipedicellata (allotetraploid, 2n =48) and Demissa (allohexaploid, 2n = 72). Confirmation of this hypothesis awaits the development of genome-specific molecular markers. Following this line of reasoning, the F_1 would be expected to have the pentaploid genomic composition of $AAAA^dB$. The apparent 1:1 segregation for resistant non-host status of race 1 is consistent with a single gene present on only one of the ho-

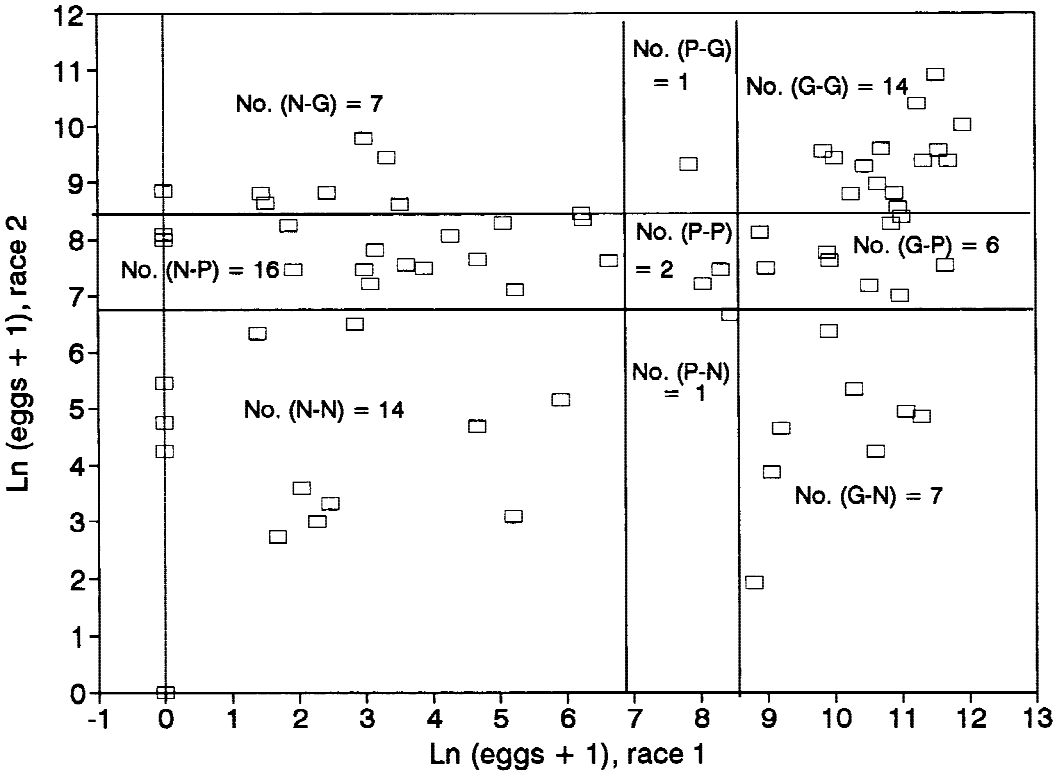

FIG. 4. Co-distribution of ln (eggs + 1)-transformed values for races 1 and 2. Each open rectangle represents the performance of a single BC_1 progeny. Letter codes followed by numbers indicate the number of progeny that fell into the host suitability category of good, poor, or non-host denoted by G, P, and N, respectively, with race 1 listed first. The statistical independence means that the two resistances are controlled by different genes located in unlinked portions of the genome.

meologues present in *S. hougasii* and, as stated above, there is circumstantial evidence that this might be the B-genome homeologue. The gene controlling this resistance is apparently fully dominant over susceptibility. The gene symbol $R_{Mc1(hou)}$ is introduced to represent this. Previously, a single gene, R_{Mc1}, was named for resistance to race 1 derived from *S. bulbocastanum.* In another study the resistance gene R_{Mc2}, derived from *S. fendleri,* was established (Janssen et al., 1997a). Since the resistance in this case was to race 1 of *M. chitwoodi* and to *M. fallax,* which behaves like race 1 of *M. chitwoodi,* it would be more descriptive to denote this gene as $R_{Mc1(fen)}$. It appears that a series of genes has now been identified, and that a nomenclatural system that will be sustained over time is necessary. It is proposed, therefore, that the race of the nematode to which resistance is found, followed by the germplasm source, be appended as subscripted suffixes. With these rules in mind, R_{Mc1} Brown et al., 1996) should be modified to $R_{Mc1(blb)}$. If further studies establish that there is a monogene-controlling resistance to race 2 derived from *S. hougasii,* then the gene symbol would be $R_{Mc2(hou)}$.

The selfed progeny of 161726.7 did not segregate for resistance, indicating that the genotype at this locus was probably homozygous in the original accession. The amphihexaploid would be expected to function disomically with bivalent pairing during gametogenesis. Resistance to race 2 is less clear-cut. The reduced percentage of individuals showing non-host status may indicate polygenic control, although it may also be consistent with a single gene that shows less expressivity or dominance, as reflected in the larger percent of the progeny occupying the poor-host category than was the case with

race 1. Alternatively, a monogene resistance to race 2 may be located on a wild species chromosome that is lost at a higher frequency during the meiosis of the pentaploid F_1. The independence of resistances to race 2 and race 1 is, however, a definitive result, indicating separate genetic controls. This may eventually lead to the identification of a specific genetic factor governing resistance solely to race 2.

Resistance to both races 1 and 2 in terms of percent recovery and level of expression in the BC_1 are encouraging for the use of *S. hougasii* as a source of resistance. Approximately 20 percent of the progeny displayed a non-host level of resistance for both races, a recovery rate that would be quite feasible in a practical breeding effort.

Literature Cited

Austin, S., J. D. Pohlman, C. R. Brown, H. Mojtahedi, G. S. Santo, D. S. Douches, and J. P. Helgeson. 1993. Interspecific somatic hybridization between *Solanum tuberosum* L. and *S. bulbocastanum* Dun. as a means of transferring nematode resistance. American Potato Journal 70:485–495.

Brown, C. R., S. Kwiatkowski, M. W. Martin, and P. E. Thomas. 1988. Eradication of PVS from potato clones through excision of meristems from in vitro, heat-treated shoot tips. American Potato Journal 65:633–638.

Brown, C. R., H. Mojtahedi, and G. S. Santo. 1989. Comparison of reproductive efficiency of *Meloidogyne chitwoodi* on *Solanum bulbocastanum* in soil and in vitro tests. Plant Disease 73:957–959.

Brown, C. R., H. Mojtahedi, and G. S. Santo. 1991. Resistance to Columbia root-knot nematode in *Solanum* spp. and in hybrids of *S. hougasii* with tetraploid cultivated potato. American Potato Journal 68:445–452.

Brown, C. R., H. Mojtahedi, and G. S. Santo. 1995. Introgression of resistance to Columbia and northern root-knot nematodes from *Solanum bulbocastanum* into cultivated potato. Euphytica 83:71–78.

Brown, C. R., H. Mojtahedi, G. S. Santo, and S. Austin-Phillips. 1994. Enhancing resistance to root-knot nematodes derived from wild *Solanum* species in potato germplasm. Pp. 426–438 *in* G. W. Zehnder, M. L. Powelson, R. K. Jansson, and K. V. Raman, eds. Advances in potato pest biology and management. Minneapolis, MN: APS Press.

Brown, C. R., H. Mojtahedi, G. S. Santo, and V. Williamson. 1997. Effect of the *Mi* gene on the reproductive efficiency of *Meloidogyne chitwoodi* and *M. hapla*. Journal of Nematology 29:416–419.

Brown, C. R., C. -P. Yang, H. Mojtahedi, G. S. Santo, and R. Masuelli. 1996. RFLP analysis of resistance to Columbia root-knot nematode derived from *Solanum bulbocastanum* in a BC_2 population. Theoretical and Applied Genetics 92:572–576.

Evans, K., and D. Trudgill. 1992. Pest aspects of potato production, part 1: The nematode pests of potatoes. Pp. 438–480 *in* P. Harris, ed. The potato crop. London: Chapman and Hall.

Hawkes, J. G., 1990. The potato: Evolution, biodiversity, and genetic resources. Washington, DC: Smithsonian Institution Press.

Helgeson, J. P., G. T. Haberlach, M. K. Ehlenfeldt, G. Hunt, J. D. Pohlman, S. Austin. 1993. Fertile somatic hybrids of potato and wild *Solanum* species: Potential for use in breeding programs. American Potato Journal 70:437–432.

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.

Janssen, G. J. W., A. von Norel, R. Janssen, and J. Hoogendoorn. 1997a. Dominant and additive resistance to the root-knot nematodes *Meloidogyne chitwoodi* and *M. fallax* in Central American *Solanum* species. Theoretical Applied Genetics 94:692–700.

Janssen, G. J. W., A. von Norel, B. Verkerk-Bakker, and R. Janssen. 1995. Detecting resistance to the root-knot nematodes *Meloidogyne hapla* and *M. chitwoodi* in potato and wild *Solanum* spp. Potato Research 38:353–362.

Janssen, G. J. W., A. von Norel, B. Verkerk-Bakker, R. Janssen. 1996. Resistance to *Meloidogyne chitwoodi, M. fallax,* and *M. hapla* in wild tuber-bearing *Solanum* spp. Euphytica 92:287–294.

Janssen, G. J. W., A. von Norel, B. Verkerk-Bakker, R. Janssen, and J. Hoogendoorn. 1997b. Introgression of resistance to root-knot nematodes from wild Central American *Solanum* species into *S. tuberosum* ssp *tuberosum*. Theoretical Applied Genetics 95:490–496.

Janssen, G. J. W., A. von Norel, B. Verkerk-Bakker, R. Janssen, and J. Hoogendoorn. 1997c. Intra- and interspecific variation of root-knot nematodes, *Meloidogyne* spp., with regard to resistance in wild tuber-bearing *Solanum* species. Fundamental and Applied Nematology 20:449–457.

Karssen, G. 1996. Description of *Meloidogyne fallax* n. sp. (Nematoda: Heteroderidae), a root-knot nematode from The Netherlands. Fundamental and Applied Nematology 19:593–599.

Matsubayashi, M. 1981. Species differentiation in tuberous *Solanum* and the origin of cultivated potatoes. (In Japanese.) Recent Advances in Breeding 22:86–106.

Mojtahedi, H., G. S. Santo, C. R. Brown, H. Ferris, and V. M. Williamson. 1994. A new host race of *Meloidogyne chitwoodi* from California. Plant Disease 78:1010.

Mojtahedi, H., G. S. Santo, and R. E. Ingham. 1993. Suppression of *Meloidogyne chitwoodi* with sudangrass cultivars as green manure. Journal of Nematology 25: 303–311.

Mojtahedi, H., G. S. Santo, and J. H. Wilson. 1988.

Host tests to differentiate *Meloidogyne chitwoodi* races 1 and 2 and *M. hapla*. Journal of Nematology 20:468–473.

Mojtahedi, H., G. S. Santo, J. H. Wilson, and A. N. Hang. 1992. Managing *Meloidogyne chitwoodi* on potato with rapeseed as green manure. Plant Disease 77:42–46.

Mojtahedi, H., J. G. Van der Beek, G. S. Santo, and C. R. Brown. 1998. Reassessment of host race concept for Columbia root-knot nematode, *Meloidogyne chitwoodi*. Journal of Nematology 30:506–507 (Abstr.).

Oostenbrink, M. 1966. Major characteristics of the relation between nematodes and plants. Mededelingen Landbouwhogeschool Wageningen 66:3–46.

Sasser, J. N., C. C. Carter, and K. M. Hartman. 1984. Standardization of host suitability studies and reporting of resistance to root-knot nematodes. Raleigh, NC: Crop Nematology. Research and Control Project, NCSU/USAID, North Carolina State University.

Steel, R., and J. Torrie. 1980. Principles and procedures of statistics: A biometrical approach. New York: McGraw-Hill.