Association between Solavetivone Production and Resistance to *Globodera rostochiensis* in Potato

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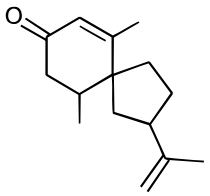
High ratios of solavetivone to total sesquiterpenes were previously associated with derivation from a Bolivian accession of potato (*Solanum tuberosum* ssp. *andigena* CPC1673) that also confers the H1 gene for resistance to *Globodera rostochiensis* (golden nematode). To test the relationship between solavetivone production and nematode resistance, the inheritance of these traits was determined using four crosses among potato clones that are nematode-susceptible homozygotes or nematode-resistant heterozygotes. Progeny from each cross were screened for tuber sesquiterpene production after treatment with arachidonic acid and for nematode resistance by counting individual cysts on roots of plants inoculated in the greenhouse. Nematode resistance exhibited dominant, single-gene segregation. A wide range of sesquiterpene levels and ratios of solavetivone to total sesquiterpenes was recovered among the progeny, indicating that these are complex traits. There was no correlation between sesquiterpene levels and nematode resistance, but ratios of solavetivone to total sesquiterpenes of nematode-susceptible and nematode-resistant progeny were significantly different in all four crosses ($p < 0.01$ by t tests of least-squares means). These data indicate that a gene or genes that control solavetivone accumulation are located on potato chromosome V close to the H1 locus for resistance to *G. rostochiensis*.

Keywords: *Solanum tuberosum; potato breeding; sesquiterpenes; Globodera rostochiensis*

INTRODUCTION

Globodera rostochiensis, the golden nematode, attacks the roots of potato (*Solanum tuberosum*) plants and causes serious crop losses in many potato-growing regions of the world (Brodie et al., 1993). Efforts to control *G. rostochiensis* include strict quarantine practices that restrict movement of potatoes from infested areas. Only pathotype Ro1 of *G. rostochiensis* has been a major problem in the United States, and this race is confined to New York state. Once established in potato field soils, nematodes are difficult to eradicate, requiring high doses of fumigants. Concerns about the cost and potential environmental impact of soil fumigants have stimulated ongoing efforts to develop potato cultivars with enhanced resistance to *G. rostochiensis*. In 1948, nematode resistance was identified in several Bolivian lines of the primitive cultivar, *S. tuberosum* ssp. *andigena* (Ellenby, 1952). One of these cultivars, CPC1673, was the source of a single dominant gene, designated H1, which has been used to introgress durable and high-level resistance to *G. rostochiensis* pathotype Ro1 into a wide variety of commercial potato cultivars (Brodie and Mai, 1989). The H1 gene for nematode resistance has been mapped to potato chromosome V, but the gene has not yet been isolated (Gebhardt et al., 1993; Pineda et al., 1993). Furthermore, the biochemical function of the H1 gene is unknown.

In a previous study (Desjardins et al., 1995), we investigated sesquiterpene production by 46 potato clones, including many newer cultivars and breeding selections that incorporate germplasm from primitive cultivars and wild species of potato. Rishitin, lubimin, and solavetivone (the structure of solavetivone is shown in Figure 1) were the major sesquiterpenes produced in tuber slices after treatment with the elicitor arachidonic acid, but many newer clones differed significantly from older cultivars in concentrations and ratios of these three sesquiterpenes. The most remarkable difference was the accumulation in 10 clones of high ratios of solavetivone (75−98% of total sesquiterpenes), a biosynthetic precursor of lubimin and rishitin (Murai et al., 1982) that typically comprises <15% of total sesquiterpenes in standard cultivars. Analysis of their pedigrees indicated that all 10 of these potato clones carried the H1 gene derived from *S. tuberosum* ssp. *andigena* CPC1673. The association of high solavetivone ratios with the H1 gene, after up to 6 generations of backcrossing into a nematode-susceptible breeding pool, was unexpected and suggested that solavetivone might be involved in H1-mediated resistance and/or closely linked to the H1 locus. The present study was undertaken to test this association by analyzing the inheritance of sesquiterpene production and nematode resistance among F_1 progeny potatoes segregating for these traits.



Solavetivone

Figure 1. Structure of solavetivone.

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Table 1. Total Sesquiterpenes and Solavetivone Ratio of Potato Clones Used as Parents in Crosses in This Study

cross number	parents	total sesquiterpenes[a] (μg/g)	solavetivone ratio[a] (%)	nematode resistance response[b]
R15	Atlantic	12	95	R
	E11-45	17	93	R
R17	Pike	20	95	R
	E11-45	17	93	R
R18	Andover	12	92	R
	E11-45	17	93	R
R170	Pike	20	95	R
	Q155-3	11	45	S

[a] Concentration and percent composition of sesquiterpenes (rishitin plus lubimin plus solavetivone, micrograms per gram fresh weight) in arachidonic acid-treated potato tubers were assayed as described under Materials and Methods. [b] Data on nematode response are from tests performed as described under Materials and Methods. R, resistant, <6 cysts per plant; S, susceptible, ≥6 or more cysts per plant.

MATERIALS AND METHODS

Sesquiterpene Elicitation and Analysis. Sesquiterpenes were elicited in potato tuber slices and analyzed as previously described (Desjardins et al., 1995). Tubers were brought from 8 °C storage to room temperature 1 day before testing. Slices were aged for 1 day, and then each slice was treated with 100 μL of arachidonic acid (Sigma Chemical Co., St. Louis, MO) 20 mM in absolute ethanol. For each test, 10 treated slices were incubated at 18 ± 1 °C for 4 more days, then extracted with diethyl ether, and analyzed by gas−liquid chromatography (GLC). The sesquiterpene detection limit was equivalent to 0.5−1.0 μg/g of tuber fresh weight. The concentrations of sesquiterpenes in each extract were determined by standard curves of rishitin, lubimin, and solavetivone prepared from arachidonic acid-treated tubers.

Assessment of Nematode Resistance. To assess the resistance of potato clones to *G. rostochiensis*, clay pots (7.6 cm in diameter) were filled with potting soil that was artificially infested with sufficient nematode cysts to provide an inoculum level of 5000 viable eggs per pot. Each pot was planted with an individual tuber or tuber piece of the desired clone and placed in a greenhouse maintained at 25−27 °C during the day and at 23−25 °C at night. Supplemental lighting was used to provide 14 h of light per day. To allow each plant equal time for cysts to develop on its roots, the date of emergence was noted for each individual plant. Eight weeks after emergence, the individual plants were carefully removed from the pots, leaving the root ball intact. The number of cysts visible on the outside of the roots was recorded for each plant. Plants with 0−5 cysts per root ball were rated resistant, and plants with >5 cysts per root ball were rated susceptible. The number of cysts on the root balls of resistant clones was usually 0, but occasionally a few cysts were formed. In contrast, susceptible clones generally had more than 100 cysts. Well-grown susceptible plants under controlled conditions always had >6 cysts. Each potato genotype (clone), including all parents and F_1 progeny, was replicated at least twice in each test. The rare occasions on which the ratings of the two replicates did not agree was interpreted as a mixture of genotypes.

Statistical Analysis. Sesquiterpene analyses and nematode response tests were compared using SAS software (SAS Institute, 1990). The total sesquiterpenes and the ratio of solavetivone to total sesquiterpenes were analyzed by least-squares analysis of variance techniques in a model that included cross (crosses C15, C17, C18, and C170) and nematode response (susceptible or resistant) as main effects plus the cross × nematode response interaction. Least-squares (i.e. adjusted) means were used for comparisons because of the unequal observations per subcell. The means comparisons were by pairwise *t* tests at $p < 0.01$.

RESULTS

Segregation of Nematode Resistance. Four progenies were utilized in this study (Table 1). The parents of these progenies included the potato cultivars Andover, Atlantic, and Pike and breeding selection E11-45, all of which are known to be resistant to *G. rostochiensis* pathotype Ro1. These resistant clones appear to be simplex (single copy of the dominant allele) in other progenies. F_1 progeny plants were available from crosses between nematode-resistant clones: between selection E11-45 and cultivar Atlantic (cross R15), between selection E11-45 and cultivar Pike (cross R17), and between selection E11-45 and cultivar Andover (cross R18). In crosses R15, R17, and R18, the segregation ratios for resistant and susceptible progeny fit the 3:1 ratio expected for the segregation of a trait controlled by a single dominant gene, with both parents being simplex for the gene (Table 2 and Figure 2).

The tetraploid breeding selection Q155-3 is nulliplex (homozygous recessive) for the H1 gene (Table 1) and came from a cross that segregated for the H1 gene. F_1 progeny were available from cross R170 between Q155-3 and cultivar Pike. The segregation ratio of 25 resistant progeny and 21 susceptible progeny fit the 1:1 ratio expected for a trait controlled by a single dominant gene, with one parent having a single copy of the gene and the other parent lacking the gene (simplex × nulliplex) (Table 2 and Figure 2).

Segregation of Sesquiterpene Levels and Ratio of Solavetivone to Total Sesquiterpenes. The four F_1 populations tested for nematode response were analyzed for sesquiterpene production in tuber slices after treatment with the elicitor arachidonic acid. Among all of the potatoes tested, both parents and progeny, the major sesquiterpenes produced were rishitin, lubimin, and solavetivone. No other sesquiterpenes were detected at concentrations >0.5 μg/g of tuber fresh weight.

Sesquiterpene production exhibited complex inheritance among the progeny of the four test crosses. In each cross, both sesquiterpene levels and solavetivone ratios varied continuously among the progeny, suggesting the interaction of many genes or environmental factors (Figure 2). To test the association of nematode response with sesquiterpene level and solavetivone ratio, progeny from each cross were divided into two classes by

Table 2. Segregation of Nematode Response, Total Sesquiterpenes, and Ratio of Solavetivone to Total Sesquiterpenes

cross number	progeny response to nematode R:S[a]			total sesquiterpenes[b] (μg/g)			solavetivone ratio[b] (%)		
	predicted	observed	χ^2	resistant progeny	susceptible progeny	P^d	resistant progeny	susceptible progeny	P^d
R15	3:1	30:15	1.92 NS[c]	10 ± 8	6 ± 4	0.38	61 ± 23	36 ± 15	<0.01
R17	3:1	40:11	0.44 NS	15 ± 12	16 ± 10	0.86	88 ± 12	69 ± 13	<0.01
R18	3:1	36:14	0.43 NS	4 ± 2	8 ± 4	0.11	65 ± 15	24 ± 11	<0.01
R170	1:1	25:21	0.35 NS	13 ± 15	9 ± 6	0.16	87 ± 8	53 ± 19	<0.01

[a] Nematode response data are as in Table 1 footnote *b*. [b] Sesquiterpene data are as in Table 1 footnote *a*, mean ± standard deviation. [c] NS, not significant. [d] Probability of *t* value.

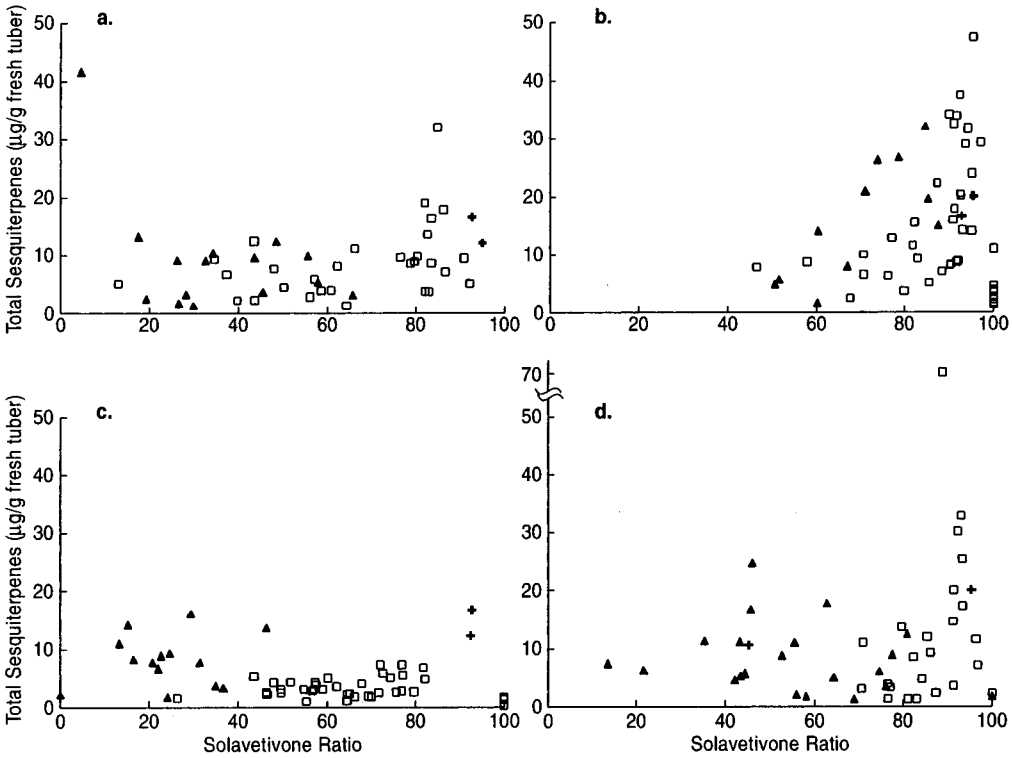

Figure 2. Relationship between nematode response, sesquiterpene level, and solavetivone ratio in F_1 progeny from crosses R15 (a), R17 (b), R18 (c), and R170 (d). Each point represents one clone. Crosses indicate the two parents of each segregating population. Solid triangles indicate nematode-susceptible progeny, and open squares indicate nematode-resistant progeny. Assays are as described in Table 1.

nematode response (as resistant or susceptible). If resistant and susceptible progeny differ significantly in sesquiterpene production, then a gene or genes affecting sesquiterpene production are involved in the activity of H1 or are linked to the H1 locus used to subdivide the population. A total of 192 clones from four crosses were analyzed for nematode response and sesquiterpene production. Three of the 48 clones from cross R15 and 4 of the 54 clones from cross R18 were deleted from the statistical analysis because their total sesquiterpene levels were <1 μg/g, and thus the solavetivone ratios could not be accurately determined.

Sesquiterpenes in Crosses between Nematode-Resistant Genotypes. In crosses R15, R17, and R18, breeding selection E11-45 was crossed with the cultivars Atlantic, Pike, and Andover, respectively. All four of these parents were nematode-resistant heterozygotes and produced high ratios of solavetivone (Table 1). Nematode-susceptible progeny were recovered in crosses R15, R17, and R18. Furthermore, in each cross, the majority of nematode-susceptible progeny produced significantly lower ratios of solavetivone than the nematode-resistant parents and the majority of nematode-resistant progeny from that cross. In contrast, the total sesquiterpene level did not differ between nematode-resistant and-susceptible subgroups (Table 2 and Figure 2a−c).

In cross R18, nematode-resistant and -susceptible progeny segregated into two distinct classes with high (>40%) and low (<40%) solavetivone ratios (Figure 2c). Sufficient tuber material was available to retest sesquiterpene production of two intermediate progeny. Upon retest, the solavetivone ratio of nematode-resistant clone R18-59 increased from 26% to 55%, and the solavetivone ratio of nematode-susceptible clone R18-114 decreased from 46% to 28%. Both of these changes brought these progeny more in line with the other data from this cross, which indicate complete linkage of the H1 gene and a relatively high (>40%) ratio of solavetivone to total sesquiterpenes.

The segregation patterns among progeny of crosses R15 and R17 indicated that a number of progeny that produced relatively low (<60%) solavetivone ratios were nonetheless nematode-resistant (Figure 2a,b). Tuber material was available to retest sesquiterpene production of eight progeny of cross R15. Upon retest, sesquiterpene levels of all eight progeny were consistent with the first test results, but solavetivone ratios of two progeny were not consistent. The solavetivone ratio of nematode-resistant clone R15-84 increased from 35% to 78%, and the solavetivone ratio of nematode-susceptible clone R15-106 decreased from 58% to 14%. Both of these changes brought these progeny more in line with the other data from this cross. Sesquiterpene ratios of the other six progeny were consistent in both tests, including clone R15-104 which was nematode-resistant but low in solavetivone ratio (37% and 39%) in both tests. Clone R15-104 may therefore be a recombinant in which the linkage between the H1 gene and a gene or genes responsible for a high solavetivone ratio has been broken. It is also possible for clones with susceptible genotypes to be scored as resistant due to escape from infection in the nematode assay or from physical mixtures of tubers between clones at harvest. This is unlikely to be the case with clone R15-104, which was tested three times with two plants per test and scored as nematode-resistant on all six plants. In our experience, clones with this record have never been later shown to be susceptible escapes.

To further assess reproducibility of sesquiterpene assays, segregation of sesquiterpene production in cross R17 was first tested on 51 progeny in January and then retested on 20 of these progeny 4 months later. Solavetivone ratios from both tests of these 20 progeny

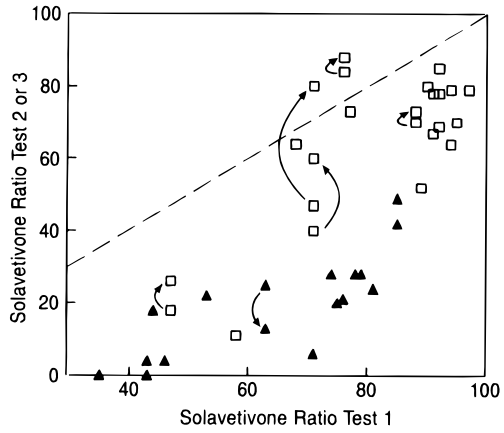

Figure 3. Comparison of solavetivone ratios in replicate tests of potato tubers from 20 selected progeny of cross R17 and 15 selected progeny of cross R170. For each progeny, the solavetivone ratio from test 1 is plotted against the solavetivone ratio from test 2, which was conducted on tubers that had been stored for several months longer. To simplify the figure and because the results from both crosses were similar, crosses R17 and R170 are not distinguished by different symbols. Solid triangles indicate nematode-susceptible progeny from both crosses, and open squares indicate nematode-resistant progeny from both crosses. Arrows indicate the change in solavetivone ratio from test 2 to test 3 for all progeny that were tested three times. The dashed line represents the results expected if the solavetivone ratios from all replicate assays of each progeny are the same. Assays are as described in Table 1.

are included in Figure 3. There was a general decrease in both sesquiterpene levels and solavetivone ratios in the progeny from test 1 to test 2, which may have been due to physiological aging of the tubers in storage. Despite the decrease in its absolute value, however, the solavetivone ratio remained lower in nematode-susceptible progeny than in nematode-resistant progeny of the same tuber age, with two notable exceptions: clones R17-135 and R17-140. Clone R17-140 and four other clones from cross R17 were replanted, and tubers from these second-generation plants were tested for nematode response and sesquiterpene production. Changes in solavetivone ratios of these progeny from test 2 to test 3 are indicated by the open circles with arrows in Figure 3. Clone R17-140 remained nematode-resistant and relatively low in solavetivone ratio (47%, 18%, 26%) in all three tests. Clones R17-135 and R17-140 may therefore be recombinants resulting from a crossover event between H1 and a gene or genes responsible for a high ratio of solavetivone to total sesquiterpenes.

Sesquiterpenes in a Cross between Nematode-Resistant and -Susceptible Genotypes. In cross R170, nematode-resistant cultivar Pike was crossed with nematode-susceptible selection Q155-3 (Table 1). Nematode-resistant and -susceptible progeny segregated with little overlap into two discrete classes with high (>75%) and low (<75%) solavetivone ratios, with one apparent exception (Figure 2d). Clone R170-86 was nematode-susceptible but high in solavetivone ratio, producing 1.7 μg/g solavetivone and no detectable rishitin or lubimin. Because sesquiterpene production by this progeny was so low and tuber material was not available for retesting, the phenotype assigned to this progeny is tentative.

Reproducibility of sesquiterpene assays was assessed by retesting tubers of 15 progeny of cross R170 4 months after the first test. Solavetivone ratios from both tests of these 15 progeny are included in Figure 3. There was a general decrease in both sesquiterpene levels and solavetivone ratios in the progeny from test 1 to test 2; however, the solavetivone ratio remained lower in nematode-susceptible progeny than in nematode-resistant progeny of the same tuber age. One progeny was replanted, and the nematode response and solavetivone ratio of tubers from second-generation plants were consistent with the previous two tests (Figure 3, solid triangles with arrows).

In summary, the total amount of sesquiterpenes produced was affected ($p < 0.01$) by cross differences. Cross R17 had higher total sesquiterpenes than crosses R15 and R18, and cross R170 was intermediate. Neither the nematode response nor the cross × nematode response interaction had a significant effect on the total sesquiterpenes. The solavetivone ratio was affected by cross ($p < 0.01$), with crosses R17 and R170 having higher ratios than crosses R15 and R18. For the four test crosses overall, the nematode-susceptible progeny had a solavetivone ratio of 0.45, while the nematode-resistant progeny had a solavetivone ratio of 0.75, a significant ($p < 0.01$) difference. The cross × nematode response was also significant ($p = 0.02$). In the crosses with the highest overall solavetivone ratios, crosses R17 and R170, the nematode-resistant progeny had a solavetivone ratio approximately 1.5 times the ratio of the nematode-susceptible progeny. In the crosses with the lower overall solavetivone ratios, crosses R15 and R18, the nematode-resistant progeny had a solavetivone ratio approximately 2.5 times the ratio of the nematode-susceptible progeny.

DISCUSSION

Our analysis of segregating F_1 potato populations has shown that a gene or genes that control the ratio of solavetivone to total sesquiterpenes are tightly linked to the H1 locus for resistance to *G. rostochiensis*. The apparent importance of the sesquiterpene ratio rather than the total sesquiterpene level may seem somewhat puzzling. In fact, similar differences in ratios of structurally related components have been shown to strongly affect specificity in insect pheromone systems (Tumlinson and Teal, 1987).

Solavetivone can accumulate to high levels in a variety of potato tissues, including stems, stolons, and tubers, as well as roots (Abenthum et al., 1995), the major invasion site of *G. rostochiensis* (Evans and Stone, 1977). Although solavetivone itself does not appear to have been tested, the related sesquiterpene rishitin has been shown to be toxic to certain species of nematodes in vitro (Alphey et al., 1988). The classical genetic analysis in the present study and the limited biochemical data provide evidence that solavetivone may play a role in H1-mediated resistance, but unequivocal proof will require isolation and characterization of the H1 gene and of the gene product. Despite their agricultural importance, little is known about the mode of action of H1 or other nematode resistance genes. The $Hs1^{pro-1}$ gene for resistance to the beet cyst nematode was recently cloned (Cai et al., 1997). Its nucleotide sequence, which is similar to that of some plant disease resistance genes, suggests that $Hs1^{pro-1}$ may participate in a cascade of defense reactions.

The genetic analysis indicates that loci controlling nematode resistance and a high solavetivone ratio are closely linked. However, two progeny of cross R17 are consistently nematode-resistant and low in solavetivone ratio. There may be several reasons for this result. Progeny may occasionally be misclassified as nematode-

resistant due to poor root growth or other problems during the bioassay. On the other hand, these progeny may be recombinants resulting from a crossover event between H1 and a gene responsible for a high solavetivone ratio. If this interpretation is correct, then H1 and a sesquiterpene biosynthetic gene or genes are tightly linked on chromosome V but are not at the same locus, and solavetivone is thus not directly involved in resistance to nematodes.

Solavetivone and related sesquiterpenes have been correlated with resistance to a number of potato pathogens, including the fungi *Phytophthora infestans*, which causes late blight (Preisig and Kuc, 1987), and *Gibberella pulicaris*, which causes tuber dry rot (Desjardins and Gardner, 1991), and the bacterium *Erwinia*, which causes blackleg (Abenthum et al., 1995). It may, therefore, be more than simple coincidence that genes involved in the biosynthesis of solavetivone and other sesquiterpenes are located in a region of chromosome V that appears to be particularly rich in disease resistance loci, including a gene for virus resistance and genes for resistance to *P. infestans* (Gebhart et al., 1993; Pineda et al., 1993) and genes for glandular trichomes involved in insect resistance (Yencho, unpublished data), as well as a bacterial resistance gene on the homologous tomato chromosome (Tanksley et al., 1992).

An important outcome of this study is that selection of nematode-resistant H1 genotypes might be facilitated by selection of tubers with high solavetivone ratios. Tuber sesquiterpene assays are simple and rapid, and if a gas chromatograph is not available, sesquiterpenes can be analyzed by thin layer chromatography. If solavetivone ratios are to be used as an aid in potato breeding, then the causes of the between-test variability observed in our study need to be considered. Many environmental factors, including physiological changes in tubers during storage, undoubtedly contribute to variation between tests. Nevertheless, this biochemical screening method could still be of use to potato breeders in most areas of the United States and Canada, where nematode bioassays cannot be conducted due to quarantine restrictions on *G. rostochiensis*. Because nematode response is difficult and time-consuming to score, using solavetivone assays to eliminate even one-third or one-half of the clones early in a breeding program could be a significant saving of time and effort.

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