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New sources of resistance to race Ro1 of the Golden nematode (*Globodera rostochiensis* Woll.) among reputed duplicate germplasm accessions of *Solanum tuberosum* subsp. *andigena* in the VIR (Russian) and US Potato Genebanks

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Abbreviations:
USPG: US Potato Genebank (see Bamberg's affiliation)
PCN: Golden potato cyst nematode, *Globodera rostochiensis* Woll.
RAPD: Random Amplified Polymorphic DNA

Abstract

Cultivated *Solanum tuberosum* subsp. *andigena* is well known as a rich source of valuable traits for potato breeding, especially for resistance to diseases and pests. The Potato cyst nematode, *Globodera rostochiensis* Woll., is considered to be one of today's most serious hindrances to potato production in Europe and North America. Thus, the breeding of new cultivars that have resistance to PCN is of great importance. The USPG (USA) and VIR (Russian) potato genebanks, as well as others, maintain many samples of primitive cultivated and wild potato species originating from Latin America. Many of these samples are assumed to be genetically duplicate because the material in both genebanks came from the same original source. A joint investigation of new genotypes of subsp. *andigena* forms resistant to Potato Cyst Nematode (PCN) was carried out on samples of subsp. *andigena* at VIR with reputed duplicate samples at USPG. After

careful screening, 14 samples which possessed resistance to PCN were identified. A high level of this resistance was transmitted to sexual progeny at a high frequency for all of the selections. Eleven of the accessions found to be resistant have reputed duplicates in USPG that were not previously known to be resistant. Thus, this research not only broadens the choice of parents available for resistance breeding, but identifies model materials for future research testing the parity of PCN resistance among reputed duplicate samples in the two genebanks.

Introduction

Potato Cyst Nematode continues to inflict significant damage on potato production in some Eastern European countries. Control is very difficult and expensive because PCN lives and overwinters in soil where chemical control is difficult and expensive. Thus, the best method known for controlling PCN is to create potato cultivars with genetic resistance.

A practical method of breeding potatoes with resistance became possible after the work of C. Ellenby (1954), who first began to evaluate the potato germplasm in the Commonwealth Potato Collection (CPC) in the UK. He was the first to find resistance to nematodes in *S. tuberosum* subsp. *andigena*, a tetraploid species cultivated in Latin America. Resistant accessions were CPC 1673, 1685, 1692, and 1595.

In the decades following, further investigations were carried out in different countries (Rothacker and Stelter 1957, Ross 1986) regarding the nature of resistance in *subsp. andigena.* An active form of immunity was found in which larvae hatch on roots, but are unable to complete the cyst development cycle.

Resistance to pathotype Rol in *subsp. andigena* is determined by a single dominant gene, H1 (Cole and Howard 1957, Rothacker and Stelter 1957, Toxopeus and Huijsman 1952 & 1953, Huijsman 1955, Huijsman 1960). However, resistance genetics may be much more diverse (Ross 1969). Resistance to other nematodes has also been derived from *subsp. andigena* (Brodie et al. 1991).

Resistance from the H1 gene has been incorporated into several commercial varieties (e.g., Plaisted et. al 2001) that are available as parents for breeding. Germplasm with resistance to multiple races of PCN has also been developed (Brodie et al. 2000).

During the last three decades more than 40 samples possessing resistance to PCN were discovered among the collection of 2,690 *subsp. andigena* accessions at the N. Vavilov Research Institute (VIR)(Kiru and Sdvizhkova 1999). However, of the approximately 850 accessions of *subsp.*

andigena at the US Potato Genebank (USPG), only 9 have been reported to be resistant (Hanneman and Bamberg 1987, Bamberg et al. 1994). Identifying a broader array of resistance sources opens the door for research to determine if useful variation in Ro1 resistance is present in these materials.

The USPG and VIR potato genebanks, as well as others, maintain many samples of primitive cultivated and wild potato species originating from Latin America (Hijmans and Spooner 2001). In many cases, genebanks have reputed duplicates (Huaman et al. 2000). Such accessions originated from the same initial source population and are identified as being the same material, so evaluation data from one genebank is often attributed to the duplicated sample in other genebanks. Such sharing of evaluation data across genebanks is a great benefit to breeders since it lessens the need for duplicate screening. The duplicate sample within a breeder's own country is also much more readily accessible, since quarantine testing of potato germplasm from other countries is usually required. However, since duplicate samples have been stored and propagated sexually under different conditions, they may not be true duplicates in the genetic sense. Indeed, significant differences in the presence of DNA markers have been demonstrated for *subsp. andigena* from VIR and USPG (Bamberg et al. 2001).

The main objective of this study was to screen accessions from the VIR *subsp. andigena* collection for resistance to PCN to expand the diversity of parental material available for use in resistance breeding (Howard et al. 1970). In addition, since the accessions tested had reputed duplicates in the USPG, finding resistance would identify materials in USPG with potential resistance which would serve as a model system for testing the parity of reputed duplicates with respect to expression of an economic trait.

Materials and Methods

The evaluation was conducted at VIR using 115 of the 144 *subsp. andigena* accessions in the VIR potato genebank with reputed duplicates in USPG (Bamberg et al. 1996). The 115 seed populations tested in this experiment included 34 different forms originating in Argentina, Peru, Bolivia, Colombia, Mexico and Ecuador. Plants were evaluated for resistance to PCN race Ro1 after artificial infection. Inheritance of resistance was then tested in the progeny of the selected tuberlings.

The plant materials were evaluated in a greenhouse with 14 h light (2000 lux) at 20-23ºC. They were grown in pots with a diameter of 10 cm.

Each pot was filled with soil, and infected with 500 cysts with viable larva. Each of the 115 populations was represented by 5 tuberlings in the initial evaluation. Accessions were considered resistant only if all 5 clones were resistant. In this way, 14 accessions were found to be resistant. Clones within each resistant accession were selfed and the seeds bulked. Then, 30 of these seedling progeny were tested again by the same method. The susceptible cultivar Nevsky and its self progeny were used as susceptible controls in the initial and progeny tests, respectively. Finally, the 14 selected clones were crossed with susceptible subsp. *tuberosum* cultivars (Table 3), and F_1 seedling progeny were also evaluated by the same method.

The presence of root cysts was visually detected on the entire root ball after two months. Plants were classified as resistant if the number of viable cysts they produced were less than 2, susceptible if 2-50 cysts were produced, and very susceptible if more than 50 cysts were produced.

Results and Discussion

Table 1 lists by country of origin, the accessions with plants determined to be resistant (less than two viable cysts produced) in the initial test. Five different South American countries and Mexico are represented, showing that genotypes possessing resistance to PCN may be found not only among the Bolivian and Peruvian forms of *subsp. andigena*, as is sometimes assumed (Howard et al. 1970) but also from Argentina, Mexico, and Colombia. The Argentine samples examined that were found to be resistant confirm the assumption of Brücher (1954) that there is a high probability of finding resistant forms among wild and cultivated potato species originating in any provinces of Argentina infected by the nematode.

Our results do not support the conclusions of some authors (Kameraz and Ponin, 1974) that diversity in the number of *G. rostochiensis* resistant forms of subsp. *andigena* is limited.

The result of many tests over three years shows that subsp. *andigena* is a rich source of race Ro1 PCN resistant genotypes useful for breeding. Of the 115 screened samples, 14 (about one-eighth) expressed strong resistance. A high proportion of self seedlings derived from clones of these 14 resistant accessions were also resistant (Table 2). None of the self seedling progeny listed in Table 2 are less than 50% resistant at $p<=0.05$. Resistance of the self progeny not only confirms the resistance of parental clones from the 14 selected accessions, but demonstrates that the inheritance of resistance is likely simple and dominant. When 10 of the 14 selected clones were crossed with susceptible cultivars, 65% of the progeny were resistant (Table 3).

One of the accessions determined to be highly resistant was PI 205624 / VIR 23696. This result might be expected since this accession is a hybrid of CPC 1673. Samples from PI 205624 / VIR 23696 and PI 230457 / VIR 23704 were reported resistant in both genebanks (Table 2), although reputed duplicate samples of these accessions in the two genebanks had only about 90% of (Random Amplified Polymorphic DNA) RAPD bands in common (Bamberg et al. 2001).

This screening identified new resistance to PCN in *subsp. andigena* from various countries. Particularly interesting is the discovery of numerous resistant accessions from Mexico, from which no resistant accessions have been previously reported. Eleven of the accessions found to be resistant have reputed duplicates in USPG that were not previously known to be resistant.

The work described here does not prove that the new sources of PCN resistance possess any breeding value beyond that already widely deployed in the H1 gene. However, a search for useful allelic diversity at the H1 locus or other potentially useful modifier loci would logically be conducted within germplasm in which resistance had naturally evolved. Our work identifies such germplasm for future breeding and genetic studies.

Thus, this research not only potentially broadens the choice of parents available for resistance breeding, but identifies model materials for future research to test the parity of PCN resistance among reputed duplicate samples in the two genebanks.

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Table 1. Country of origin of selected *S. ssp. andigena* Juz. et Buk. accessions resistant to *G. rostochiensis* race Ro1

Origin	N° of accessions screened	N° of resistant accessions	Percent
Argentina	23	2	9
Bolivia	17	2	12
Colombia	20	1	5
Ecuador	9	0	0
Mexico	19	5	26
Peru	22	4	3
Total	115	14	12

Table 2. Segregation of resistance in seedlings derived from self pollination of resistant *S. ssp. andigena* clones

PI number (USPG)	K number (VIR)	Collector Number	Country of origin	N° of seedlings tested	Number of resistant (R), susceptible (S) and very susceptible (VS) seedlings[b]		
					0-1cysts (R)	2-50 cysts (S)	>50cysts (VS)
160215	23688	COR 14220A	MEX	50	24	26	-
161136	22034	COR 14261	MEX	50	27	23	-
161683	23691	COR 14434	MEX	50	19	31	-
161716	21655	COR 14380	MEX	50	24	19	7
195162	23694	CPC 300	PER	50	34	16	-
205624*	23696	CPC 1673[a]	BOL	50	39	11	-
214427	23699	SMI 454	PER	50	32	18	-
214430	23700	SMI 460	PER	50	37	13	-
230457*	23704	CPC 1464	PER	50	33	8	9
233982	21665	GND 16	BOL	50	28	22	-
243415	17165	CCC 249	COL	50	19	27	4
243430	17172	CCC 330	ARG	50	32	18	-
246516*	23719	COR P204	ARG	50	30	20	-
285017	21683	UGN 1098	MEX	50	26	24	-
Average					28.9	19.7	1.4
Control	Nevsky		RUS	50	-	6	44

[a] hybrid seed

[b] None significantly less than 50% resistant at p=<=0.05

* Reported as resistant in USPG screening records (see Hanneman and Bamberg, 1987 and USPG homepage: http:\\www.ars-grin.gov/nr6).

Table 3. The inheritance of resistance to *G. rostochiensis* Ro1 in progeny of ten selected *S. ssp. andigena* forms crossed with susceptible cultivars

F_1 cross[a]	Total N° of seedlings	Segregation of resistance in seedlings[b]		
		S	R	%R
Lugovskoy x PI 161893	94	22	72	76
Romashka x PI 214427	87	25	62	71
Nevsky x PI 160215	79	20	59	74
Rozhdestvenskii x PI 195162	87	26	61	70
Orbita x PI 205624	89	29	60	67
PI 214430 x Zarevo	83	17	66	79
PI 230457 x Peterburgsky	77	18	59	76
PI 246516 x Gybrydny14	90	41	49	54
Udacha x PI 243430	82	19	63	76
Peterburgsky x PI 233982	95	27	54	70
Average				65
Nevsky x PI 243384*	97	97	0	0

[a] Resistant parent given as USPG germplasm number. See Table 2. for VIR number

[b] S= susceptible (>2 viable cysts), R= resistant (0-1 viable cysts).

*Control cross of susceptible cultivar Nevsky x susceptible *Solanum* subsp. *andigena*