Mendelian proof for a gene-for-gene relationship between virulence of *Globodera rostochiensis* and the H_1 resistance gene in *Solanum tuberosum* ssp. *andigena* CPC 1673

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SUMMARY

A virulent and an avirulent inbred line of *G. rostochiensis* were crossed to determine the genetics of virulence to the resistance gene H_1 of *Solanum tuberosum* ssp. *andigena* CPC 1673. The 3:1 segregation in avirulent and virulent larvae of the F_2 generation, obtained by selfing the F_1, showed that virulence to the H_1 gene is controlled by a single major recessive gene. The virulence percentages of the F_1 generations agreed with this finding. Reciprocal crosses showed no evidence of sex-linked inheritance of virulence.

RÉSUMÉ

Preuve mendélienne d'une relation « gène-pour-gène » entre la virulence de Globodera rostochiensis *et le gène de résistance H_1 chez* Solanum tuberosum *ssp.* andigena *CPC 1673*

La génétique de la virulence vis-à-vis du gène H_1 de *Solanum tuberosum* ssp. *andigena* CPC 1673 est étudiée par croisement réciproque de deux lignées, virulente et avirulente, de *Globodera rostochiensis*. La F1 est avirulente. Multipliée sur elle-même, elle donne une F2 dont la virulence des larves est étudiée. La ségrégation observée est 1:3 entre individus virulents et avirulents. Ceci montre que la virulence est sous la dépendance d'un gène unique majeur récessif, non lié au sexe.

Breeding of potato varieties with resistance against potato cyst nematodes (*e.g.* Toxopeus & Huijsman, 1952, 1953; Huijsman, 1955) was initiated in the fifties with the discovery of the H_1 resistance gene in *Solanum tuberosum* ssp. *andigena* CPC 1673 by Ellenby (1952). The monogenetic inheritance resulted in successful breeding programmes which stimulated the search for more sources of resistance. Major genes for resistance were identified in *S. multidissectum* : H_2 (Dunnett, 1961), *S. tuberosum* ssp. *andigena* CPC 2775 : H_3 (Howard, Cole & Fuller, 1970), *S. kurtzianum* : A, B (Huijsman, 1960) and *S. spegazzinii* : Fa, Fb (Ross, 1962). Resistance in *S. vernei* (*e.g.* Goffart & Ross, 1954; Ross, 1969; Huijsman & Lamberts, 1972; Kort, Jaspers & Dijkstra, 1972; Huijsman, 1974), *S. spegazzinii* and *S. oplocense* (Ross, 1972) seemed to be caused by a complex of major and minor genes. A number of these sources of resistance, including *S. vernei*, are used in the international pathotype test assortment. With this assortment, five pathotypes of *G. rostochiensis* and three pathotypes of *G. pallida* can be recognized on basis of multiplication rates (Kort *et al.*, 1977).

The variations in the virulence spectra of potato cyst nematode populations show similarities with host-parasite systems in which resistance and virulence operate in a gene-for-gene relationship. Howard (1959) and Jones and Parrott (1965) suggested that, similar to the relationship in *Linum usitatissimum-Melampsora lini* (Flor, 1956), the interaction between *G. rostochiensis* and the H_1 gene of *S. tuberosum* ssp. *andigena* CPC 1673 is controlled by a gene-for-gene mechanism. Females developing on a resistant clone are thought to be homozygous recessive; males can have any genetic constitution : AA, Aa or aa (Jones, Parrott & Ross, 1967). Studies supporting this hypothesis (Parrott & Berry, 1974; Parrott, 1981), lacked the experimental and numerical base necessary for a formal proof according to Flor's criterion (Sidhu, 1975). They were based on mass matings between groups of males and females from field populations. The exact numbers of virulent individuals

in these populations were unknown, sib matings may have occurred, and the testing of the relative numbers of female progeny on hosts with and without resistance was subject to large errors (Jones, Parrott & Perry, 1981).

In this study we have avoided the difficulties associated with genetic studies on potato cyst nematodes by using inbred lines (Janssen, Bakker & Gommers, 1990*b*), controlled single matings and an improved technique to assess the number of virulent phenotypes in the progenies (Janssen, Bakker & Gommers, 1990*a*).

Materials and methods

Analyses of inheritance of virulence in *G. rostochiensis* against the resistance H_1 gene of *S. tuberosum* ssp. *andigena* CPC 1673 were carried out with the avirulent line Ro_1-19 and the virulent line Ro_5-22 (Janssen, Bakker & Gommers, 1990*b*), on the susceptible cultivar Eigenheimer and the resistant cultivar Saturna. Experiments were carried out in Petri dishes and pots, in a controlled environment room at 18 °C and 16 h light.

Reciprocal crosses (Ro_5-22 × Ro_1-19) were made by placing a male on the gelatinous matrix of a female. This was done each time on separate Petri dishes to ensure that the female was not fertilized by more than one male. Males of both lines were reared on cv. Eigenheimer in sandy loam in clay pots (700 ml), inoculated with 50 cysts, and harvested after about 30 days with an elutriator (Oostenbrink, 1960). Virgin females were obtained from potato roots grown on water agar (Mugniéry & Person, 1976) inoculated with one larva per root tip per Petri dish. Females of Ro_1-19 and Ro_5-22 were reared on roots of the cvs Eigenheimer and Saturna respectively.

The virulence of the larvae from 30 F_1 cysts of both crosses were tested in Petri dishes by inoculating 200 larvae (two per root tip), unless stated otherwise (Mugniéry & Person, 1976; Janssen, Bakker & Gommers 1990*a*). Virulence is expressed as a percentage of the number of females that developed on the resistant cultivar relative to the number of females on the susceptible cultivar.

Larvae of another 30 F_1 cysts were selfed by inoculating cv. Eigenheimer with larvae of one cyst per clay pot. Larvae were artificially hatched by cutting cysts in half before inoculation (Janssen, Bakker & Gommers, 1987). Newly formed cysts were recovered from the wet soil with an elutriator (Kort, 1960), using an upstream of 4.5 l min^{-1}, and collected on a 0.30 × 3.65 mm pore sieve. Nine F_2 lines per cross with more than 50 newly formed cysts were selected for virulence tests. Larvae were obtained by artificial hatching.

Results

The virulence percentages of the parent lines Ro_1-19 and Ro_5-22 and their reciprocal crosses are shown in Table 1. Ro_1-19 × Ro_5-22 is fully avirulent for the H_1 resistance gene. Ro_5-22 × Ro_1-19 has a virulence level of 0.2 %; out of the 600 inoculated larvae on the resistant cultivar Saturna one cyst developed.

Table 1

Virulence percentages of virulent phenotypes for the avirulent line Ro_1-19, the virulent line Ro_5-22 and the F_1 of their crosses on cv. Saturna

Parents and F_1	*Virulence percentages*
P Ro_1-19	0
F_1 Ro_1-19 ♀ × Ro_5-22 ♂	0 *
F_1 Ro_5-22 ♀ × Ro_1-19 ♂	0.2*
P Ro_5-22	100.2

* 600 larvae (two larvae per root tip) on cv. Saturna.

Table 2

Numbers of newly formed cysts (F_2) after inoculation of one F_1 cyst per clay pot on cv. Eigenheimer.
E lines : cross Ro_1 19 ♀ × Ro_5-22 ♂; S lines : cross Ro_5-22 ♀ × Ro_1-19 ♂

Line	*Cysts*	*Line*	*Cysts*	*Line*	*Cysts*	*Line*	*Cysts*	*Line*	*Cysts*	*Line*	*Cysts*
E1	22	E11	27	E21	40	S1	38	S11	102	S21	50
E2	74	E12	10	E22	218	S2	100	S12	48	S22	45
E3	8	E13	26	E23	114	S3	59	S13	33	S23	13
E4	1	E14	175	E24	2	S4	49	S14	18	S24	14
E5	25	E15	7	E25	17	S5	30	S15	28	S25	50
E6	62	E16	39	E26	43	S6	156	S16	57	S26	46
E7	59	E17	97	E27	64	S7	114	S17	10	S27	19
E8	45	E18	79	E28	30	S8	(911)*	S18	36	S28	57
E9	45	E19	38	E29	36	S9	199	S19	34	S29	1
E10	62	E20	51	E30	15	S10	251	S20	28	S30	135
Sum					1 531						1 820
$\bar{x}$					51.0						62.8
σ					48.4						58.8

* Not included.

The numbers of newly formed cysts per clay pot after inoculation with one F_1 cyst are shown in Table 2. The multiplication rates varied from 1 to 251. Thirty six percent of the F_1 cysts produced more than 50 new cysts and only 8 % less than 10. Line S8 produced 911 new cysts, an unexpected and extremely large number which may have resulted from the development of a second generation in this pot due to crushed cysts after an inspection of the root ball. This line was omitted from the experiment.

Eighteen of the F_2 lines with 50 cysts or more were considered for a virulence test but only eight lines of the cross Ro_1-19 × Ro_5-22 (E numbers) and seven lines of the cross Ro_5-22 × Ro_1-19 (S numbers) produced more than 400 larvae, the minimum number needed for the virulence test (Tab. 3). One F_2 line did not hatch at all and two other lines gave only 27 and 158 larvae.

Table 3

Numbers of cysts of the F_2 lines in the virulence tests formed on cvs Saturna and Eigenheimer, and the calculated virulence frequencies. E lines : cross Ro_1-19 ♀ × Ro_5-22 ♂ ; S lines : cross Ro_5-22 ♀ × Ro_1-19 ♂

Line no.	*Numbers of cysts formed*		*Virulence frequencies*
	Eigenheimer	*Saturna*	
E2	110	25	22.7
E6	123	31	25.2
E14	143	33	23.1
E17	147	36	24.5
E18	140	32	22.9
E22	141	28	19.9
E23	138	33	23.9
E27	121	34	28.1
S2	116	37	31.9
S6	135	36	26.7
S7	136	40	29.4
S9	120	37	30.8
S10	143	41	28.7
S11	151	42	27.8
S30	138	41	29.7
$\bar{x}$			26.3
σ			3.5

On the basis of the number of cysts developing on cv. Eigenheimer relative to that number on cv. Saturna, frequencies of virulent genotypes were calculated (Table 3). These frequencies in both crosses did not differ significantly (at the 95 % confidence level) from the expected virulence frequency of 25 % belonging to a 3:1 segregation for avirulence (AA and Aa) and virulence (aa).

Assuming a monogenic basis of virulence, the relative frequencies of avirulent (AA and Aa) and virulent (aa) genotypes in the F_2's are 0.75 and 0.25, respectively. The ratio of the numbers of cysts developing on cv. Eigenheimer *vs* the number developing on cv. Saturna equals then 4 (AA + Aa + aa) : 1 (aa). Table 4 presents the Chi-square test. None of the individual lines showed significant deviations from the expected frequencies. Testing for heterogeneity between lines within crosses, and between crosses revealed no heterogeneity between lines within either cross, but a significant difference was found comparing the pooled E and S lines. This difference resulted from constantly higher numbers of observed virulent genotypes in the S lines than expected on basis of the hypothesis. Though the deviation of the pooled S lines is significant, it is small as is confirmed by the absence of overall heterogeneity between lines.

Discussion

Gene-for-gene relationships have been suggested for a wide range of pathogens, including bacteria, fungi, viruses, insects, nematodes and plant parasitic plants. However, the number of host-parasite systems for which Mendelian segregation patterns of both interacting partners have been analyzed, is relatively small (Crute, 1985). A formal proof accordings to Flor's criterion (Sidhu, 1975) has been obtained for associations of only fifteen fungi/plants and one insect/plant. In this study we analyzed the segregation patterns of virulence in potato cyst nematodes for the H_1 gene and showed that the gene-for-gene concept applies to one of the potato cyst nematodes and its host. The avirulence of the F_1 and the 3:1 segregation in the F_2 demonstrates that virulence is inherited at a single locus and is recessive to avirulence. As might be expected from the epigenic nature of the sex determination in potato cyst nematodes (Trudgill, 1967; Mugniéry & Fayet, 1981, 1984; Mugniéry, 1982, 1985), the reciprocal crosses revealed no sex-linked inheritance.

The virulence percentage in the F_1 of the Ro_5-22 (aa) × Ro_1-19 (AA) cross showed a 0.2 % deviation from the expected complete avirulence (Tab. 1). This observation does not invalidate the gene-for-gene hypothesis. A plausible explanation for such a low virulence level may be, as discussed earlier (Janssen, Bakker & Gommers, 1990*b*), that the resistance mechanism conferred by the H_1 gene is not absolute. Arguments for heterozygosity in the Ro_1-19 line lack a numerical basis. If one out of the 30 F_1 cysts in the virulence test had been the result of a Ro_5-22 (aa) × Ro_1-19 (Aa) cross, the virulence percentage should have been 1.7. The absence of cysts in the F_1 of the reciprocal cross further supports the argument of escapers. Another explanation for this deviation may be found in some kind of maternal effect operating in the Ro_5-22 line, which also could explain the small but significant difference from the 4:1 segregation of the pooled S lines in the F_2 (Table 4).

Person (1959) stated that genetic data for both inter-

Table 4

Segregation for virulence in the F_2 generation of the reciprocal crosses of an avirulent (Ro_1-19) and a virulent (Ro_5-22) line for cv. Saturna and the heterogeneity test; E lines : Ro_1-19 ♀ × Ro_5-22 ♂; S lines : Ro_5-22 ♀ × Ro_1-19 ♂

	Line no.	*avirulent*		*virulent*		χ^2_1 *for 4:1*	*P-value*
		observed	*expected*	*observed*	*expected*		
	E2	110	108	25	27	0.1852	0.50-0.70
	E6	123	123.2	31	30.8	0.0016	0.95-1.00
	E14	143	140.8	33	35.2	0.1719	0.50-0.70
	E17	147	146.4	36	36.6	0.0123	0.90-0.95
	E18	140	137.6	32	34.4	0.2093	0.50-0.70
	E22	141	135.2	28	33.8	1 2441	0.20-0.30
	E23	138	136.8	33	34.2	0.0526	0.80-0.90
	E27	121	124	34	31	0.3629	0.50-0.70
Total E		1 063	1 052	252	263	0.5751	0.30-0.50
	S2	116	122.4	37	30.6	1.6732	0.10-0.20
	S6	135	136.8	36	34.2	0.1184	0.70-0.80
	S7	136	140.8	40	35.2	0.8182	0.30-0.50
	S9	120	125.6	37	31.4	1.2484	0.20-0.30
	S10	143	147.2	41	36.8	0.5992	0.30-0.50
	S11	151	154.4	42	38.6	0.3744	0.50-0.70
	S30	138	143.2	41	35.8	0.9441	0.30-0.50
Total S		939	970.4	274	242.6	5.0802	0.01-0.05
Total E + S		2 002	2 022.4	526	505.6	1.0289	0.30-0.50

HETEROGENEITY TEST

Source	χ^2	*d.f.*	*P-value*
Between E-lines	*1.6648*	*7*	*0.95-1.00*
Between S-lines	*0.6957*	*6*	*0.95-1.00*
E versus S	*4.6264*	*1*	*0.01-0.05*
Total	*6.9869*	*14*	*0.90-0.95*

acting partners are not required in evaluating a possible gene-for-gene mechanism. His concept (Sidhu, 1975) is based on the pattern of interactions generated when several host varieties are inoculated with different cultures of the pathogen. The concept presupposes two alternative conditions of expression in the host (resistance *vs* susceptibility) and the parasite (virulence *vs* avirulence). It is tempting to extrapolate this concept to the various sources of resistance against plant parasitic nematodes. However, in plant parasitic nematodes with an amphimictic mode of reproduction isolates which are fixed for virulence alleles are hardly available. Interaction patterns of *e.g.* potato cyst nematodes and their host often result in an array of multiplication rates ranging from 0 to more than 60 (*e.g.* Kort *et al.*, 1977) thus masking possible gene-for-gene relationships. However, regardless of these specific problems in amphimictic nematode species, Person's approach is less precise than Flor's criterion. In this report we have chosen the Mendelian approach to obtain a solid basis for tracing a gene for (a)virulence in potato cyst nematodes.

REFERENCES

CRUTE, R. (1985). The genetic base of relationships between microbial parasites and their hosts. In : Fraser, R.S.S. (Ed.). *Mechanisms of resistance to plant diseases.* Dordrecht, Martinus Nijhoff/Dr W. Junk Publishers : 80-142.

DUNNETT, J. M. (1961). Inheritance of resistance to potato-root eelworm in breeding lines stemming from *Solanum multidissectum* Hawkes. *Rep. Scott. breed. Stn* : 39-46.

ELLENBY, C. (1952). Resistance to the potato root eelworm, *Heterodera rostochiensis* Wollenweber. *Nature,* 170 : 1016.

FLOR, H. H. (1956). The complementary genetic systems in flax and flax rust. *Adv. Genet.,* 8 : 29-54.

GOFFART, H. & ROSS, H. (1954). Untersuchungen zur Frage der Resistenz von Wildarten der Kartoffel gegen den Kartoffel Nematoden (*Heterodera rostochiensis* Wr.). *Zuchter,* 24 : 193-201.

HOWARD, H. W. (1959). Biotypes of potato root eelworm in Great Britain. *TagBer. dt. Akad. LandwWiss. Berl.,* 20 : 95-106.

HOWARD, H. W., COLE, C. S. & FULLER, J. M. (1970). Further sources of resistance to *Heterodera rostochiensis* Woll. in the Andigena potato. *Euphytica*, 19 : 210-216.

HUIJSMAN, C. A. (1955). Breeding for resistance to the potato root eelworm II. *Euphytica*, 4 : 133-140.

HUIJSMAN, C. A. (1960). Some data on the resistance against the potato root-eelworm (*Heterodera rostochiensis* W.) in *Solanum kurtzianum*. *Euphytica*, 9 : 185-190.

HUIJSMAN, C. A. (1974). Host-plants for *Heterodera rostochiensis* Woll. and the breeding for resistance. *EPPO Bull.*, 4 : 501-509.

HUIJSMAN, C. A. & LAMBERTS, H. (1972). Breeding for resistance to the potato cyst-nematode in the Netherlands. In : French, E.R. (Ed.). *Prospects for the Potato in the Developing World.* Lima, Peru, Int. Potato Center : 161-171.

JANSSEN, R., BAKKER, J. & GOMMERS, F. J. (1987). Circumventing the diapause of potato cyst nematodes. *Neth. J. Pl. Path.*, 93 : 107-113.

JANSSEN, R., BAKKER, J. & GOMMERS, F. J. (1990*a*). Assessing intraspecific variations in virulence in *Globodera rostochiensis* and *G. pallida*. *Revue Nématol.*, 13 : 11-15.

JANSSEN, R., BAKKER, J. & GOMMERS, F. J. (1990*b*). Selection of virulent and avirulent lines of *Globodera rostochiensis* for the H_1 resistance gene in *Solanum tuberosum* ssp. *andigena* CPC 1673. *Revue Nématol.*, 13 : 265-268.

JONES, F. G. W. & Parrott, D. M. (1965). The genetic relationships of pathotypes of *Heterodera rostochiensis* Woll. which reproduce on hybrid potatoes with genes for resistance. *Ann. appl. Biol.*, 56 : 27-36.

JONES, F. G. W., PARROTT, D. M. & PERRY, J. N. (1981). The gene-for-gene relationship and its significance for potato cyst nematodes and their solanaceous hosts. In : Zuckerman, B. M. & Rohde, R. A. (Eds). *Plant Parasitic Nematodes. Vol. III.* New York, Academic Press : 23-36.

JONES, F. G. W., PARROTT, D. M. & ROSS, G. S. J. (1967). The population genetics of the potato cyst-nematode, *Heterodera rostochiensis* : mathematical models to simulate the effects of growing eelworm-resistant potatoes bred from *Solanum tuberosum* ssp. *andigena*. *Ann. appl. Biol.*, 60 : 151-171.

KORT, J. (1960). A technique for the extraction of *Heterodera* cysts from wet soil and for the estimation of their eggs and larval content. *Versl. Meded. Plziektenk. Dienst Wegeningen*, 233 : 3-7.

KORT, J., JASPERS, C. P. & DIJKSTRA, D. L. (1972). Tesung for resistance to pathotype C of *Heterodera rostochiensis* and the practical application of *Solanum vernei* hybrids in the Netherlands. *Ann. appl. Biol.*, 71 : 289-294.

KORT, J., ROSS, H., RUMPENHORST, H. J. & STONE, A. R. (1977). An international scheme for identifying and classifying pathotypes of potato cyst-nematodes *Globodera rostochiensis* and *G. pallida*. *Nematologica*, 23 : 333-339.

MUGNIÉRY, D. (1982). Influence de l'hôte sur le développement et l'expression du sexe chez un nématode phytoparasite à déterminisme sexuel épigénique, *Globodera pallida* Stone. *C. r. hebd. Séanc Acad. Sci., Paris*, 294, Sér. III : 49-52.

MUGNIÉRY, D. (1985). Résistance des solanacées à *Globodera* et influence de certains facteurs sur les résultats des tests de résistance. *EPPO Bull.*, 15 : 199-206.

MUGNIÉRY, D. & FAYET, G. (1981). Détermination du sexe chez *Globodera pallida* Stone. *Revue Nématol.*, 4 : 41-45.

MUGNIÉRY, D. & FAYET, G. (1984). Détermination du sexe de *Globodera rostochiensis* Woll. et influence des niveaux d'infestation sur la pénétration, le développement et le sexe de ce nématode. *Revue Nématol.*, 7 : 233-238.

MUGNIÉRY, D. & PERSON, F. (1976). Méthode d'élevage de quelques nématodes à kystes du genre *Heterodera*. *Sci. Agron., Rennes*, 217-220.

OOSTENBRINK, M. 1960. Estimating nematode populations by some selected methods. In : Sasser, J. N. & Jenkins, W. R. (Eds). *Nematology*. Chapel Hill, Univ. N. Carolina Press : 85-102.

PARROTT, D. M. (1981). Evidence for gene-to-gene relationships between resistance gene H_1 from *Solanum tuberosum* ssp. *andigena* and a gene in *Globodera rostochiensis*, and between H_2 from *S. multidissectum* and a gene in *G. pallida*. *Nematologica*, 27 : 372-384.

PARROTT, D. M. & BERRY, M. M. (1974). Inheritance of ability to overcome resistance. *Rothamsted Exp. Stn Rep. 1973, Part 1 :* 152-153.

PERSON, C. (1959). Gene-for-gene relationship in host : parasite systems. *Can. J. Bot.*, 37 : 1101-1130.

ROSS, H. (1962). Über die Vererbung der Resistenz gegen den Kartoffel Nematoden (*Heterodera rostochiensis* Woll.) in Kreuzungen von *Solanum famatina* Bitt. *et* Wittm. mit *Solanum tuberosum* L. und *S. chacoense* Bitt. *Zuchter*, 32 : 74-80.

ROSS, H. (1969). Zuchtung von Kartoffelsorten mit Resistenz gegen *Heterodera rostochiensis* Woll. *Mitt. Biol. BundesAnst, Ld-u. Forstw., Berlin-Dahlem*, 136 : 59-64.

ROSS, H. (1972). Improvement of the potato in Germany for resistance to the two species of cyst nematode. In : French, E. R. (Ed.). *Prospects for the Potato in the Developing World.* Lima, Peru, Int. Potato Center : 181-190.

SIDHU, G. S. (1975). Gene-for-gene relationships in plant parasitic systems. *Sci. Prog. Oxford*, 62 : 467-485.

TOXOPEUS, H. J. & HUIJSMAN, C. A. (1952). Genotypical background of resistance to *Heterodera rostochiensis* in *Solanum tuberosum* var. *andigenum*. *Nature*, 170 : 1016.

TOXOPEUS, H. J. & HUIJSMAN, C. A. (1953). Breeding for resistance to potato root eelworm I. *Euphytica*, 2 : 180-186.

TRUDGILL, D. L. (1967). The effect of environment on sex determination in *Heterodera rostochiensis*. *Nematologica*, 13 : 263-272.

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