Selection of virulent and avirulent lines of *Globodera rostochiensis* for the H_1 resistance gene in *Solanum tuberosum* ssp. *andigena* CPC 1673

[redacted]

Ro_5-Harmerz which was obtained from Dr. H. J. Rumpenhorst, Münster, FRG. The cultivar " Eigenheimer " was used as the general susceptible clone. The differential *Solanum tuberosum* ssp. *andigena* CPC 1673 with the H_1 resistance gene was represented by the cultivar " Saturna ".

Virulent lines for the H_1 gene were selected as follows. Adult females for controlled matings were reared in Petri dishes (9 cm diam.) on roots of sprouts of cultivar " Saturna " grown on water agar according to the method of Mugniéry and Person (1976) and Mugniéry (1982). To prevent inbreeding, only one larva per Petri dish was inoculated. Females were fertilized by placing

possible genotypes (AA, Aa, and aa), with the virulent genotype (aa) being present in 2 % of the individuals and the population being in Hardy-Weinberg equilibrium, the frequency of the a allele (q) is 14 % and of the A allele (p) 86 %. This results in 24 % and 74 % of the Aa and AA genotype, respectively. The fraction of cysts with aa larvae is then $(0.24 + 0.02)^2 \times 7$ % and without aa larvae is $100 - 7 = 93$ %. The presence of virulent larvae in the hatching sample is assured when 40 cysts are taken since the chance of cysts without aa larvae is less than 5 %. In this study we always used 40 or more cysts.

All pot experiments were carried out in clay pots

Table 1

Fraction of virulent phenotypes (vp %) of F_3 and F_4 Ro_5-Harmerz lines on cultivar " Saturna ", calculated as percentages of the number of females that developed on the susceptible cultivar " Eigenheimer ".

Line no.	4	7*	8	19	22*	23	34	47	52
vp %	93.1 *b*	91.7 *bc*	87.0 *c*	77.8 *d*	100.2 *a*	—[1]	—[1]	94.0 *b*	—[1]

* F_3 lines.
1. Virulence test not carried out.
Lines with the same letter are not significantly different ($P < 0.05$).

Table 2

Fraction of virulent phenotypes (vp %) of F_3 Ro_1-Mierenbos lines for cultivar " Saturna ", calculated as percentages of the number of females that developed on the susceptible cultivar " Eigenheimer ".

Line no.	1	6	8	11	12	14	15	16	17	18	19	22	24	27	42	44	51	52	58
vp %	2.1	0	2.8	0	3.4	0	0	0	0.2	2.8	0	0.6	0	0	1.0	0	0.5	0	0

lation died out after three subsequent generations of multiplication in pots.

PRODUCTION OF AVIRULENT LINES FOR THE H_1 GENE

Population Ro_1-Mierenbos was chosen because the initial number of virulent phenotypes in population Ro_1-Mierenbos was extremely low (< 0.1 %) (Janssen, Bakker & Gommers, 1990*a*). Inoculating 585 root tips with one larva resulted in the development of 416 females of which 72.4 % were successfully fertilized. Eighty-six F_1 cysts were multiplied in pots on cultivar " Eigenheimer ", producing 63 F_3-lines. The virulence characteristics of nineteen lines were tested. Eleven lines produced no cysts on cultivar " Saturna " (Table 2). Six of these eleven avirulent lines were tested again. Only two of these lines appeared to be avirulent in the second test (Table 3).

Table 3

Retest of 6 Ro_1-Mierenbos lines on virulent phenotypes (vp %) for cultivar " Saturna ".

Line no.	6	11	14	19	24	27
vp %	0	0.03	0	0.12	0.04	0.08

Discussion

Jones, Parrott and Ross (1967) suggested that the gene-for-gene hypothesis is also applicable to *G. rostochiensis* and *S. tuberosum* ssp. *andigena* CPC 1673, with virulence (a) being inherited at a single locus and recessive to avirulence (A). It was also assumed that this interaction is confined to larvae developing into females. Males developing on cultivars having the H_1 gene can have any genotype (AA, Aa, aa). If this hypothesis is correct the estimate of the fraction of virulent phenotypes corresponds with the number of homozygous recessive genotypes (aa). This also implies that the F_1 cysts from population Ro_5-Harmerz able to reproduce on cultivar " Saturna " are the progeny of either an aa × aa or an aa × Aa cross. The first type of cross will result in lines with 100 % virulent genotypes, the second cross, as can be calculated according to Jones, Parrot and Ross (1967), will result in F_1 lines having 50 % virulent genotypes, while the F_2, F_3 and F_4, produced on cultivar " Saturna ", will have virulence levels of 75 %, 88 % and 94 %, respectively. Not all our data match with these calculations. The levels of virulent phenotypes (Table 1) in line 4, 7 and 47, presumed to be derived from an aa × Aa cross, and line 22, from an aa × aa cross, fit the calculated expectations, whereas the levels of virulent phenotypes in line 8 and 19 are significantly lower than the expected 94 %.

Conclusive evidence that the number of virulent genotypes in line 22 is 100 % cannot be inferred from our data. Since no near-isogenic lines with and without resistance are available, the estimates of virulent phenotypes are influenced by the choice of the potato cultivars because these were made by expressing the number of females that developed on the resistant cultivar relative to the number on the susceptible cultivar. However in a parallel report (Janssen, Bakker & Gommers 1990*b*) we showed, in studying segregation patterns of virulence, that line 22 is indeed homozygous for virulence.

Another disparity with the gene-for-gene theory as proposed by Jones, Parrott and Ross (1967), is that the

number of lines having a virulence level of 100 % is relatively low (Table 1). From the initial frequencies of the virulent phenotypes in population Ro_5-Harmerz, it is expected that 84.6 % of the F_1 cysts able to reproduce on " Saturna " are derived from an aa × aa cross and 14.6 % of the F_1 cysts are derived from an aa × Aa cross. The results show that of the nine lines tested only one line has a virulence level of approximately 100 % (line 22). Statistically this outcome does not correspond with the expected percentage. From the gene-for-gene theory, the chance of such a result is less than 5 %. Also the attempts to select virulent lines from Ro_3-C_{133} and Ro_3-C_{129} are puzzling. For example, the number of virulent phenotypes in population Ro_3-C_{129} increased after four generations of multiplication on cultivar " Saturna " from 30.1 % to 50.8 %, whereas an increase to 94.4 % was expected.

Comparable contradictions with the gene-for-gene therory are also observed with the selection of avirulent lines from Ro_1-Mierenbos. First, only 57.9 % of the lines produced no cysts on cultivar " Saturna " (Table 2). Retesting 6 F_3 lines showed that this percentage is actually lower (Table 3). As can be calculated from the initial number of virulent phenotypes in population Ro_1-Mierenbos, the expected percentage of avirulent lines was 87.9 %. Second, the lines producing cysts on cultivar " Saturna " have virulence levels ranging from 0.03 % to 3.4 % (Tables 2 & 3), which is too low to explain by the gene-for-gene theory. According to the theory the lowest intermediate virulence level is 6 %, *i.e.* lines derived from an AA × Aa cross. A possible explanation for the low virulence levels observed, not mutually exclusive with a gene-for-gene theory, is that these lines are derived from an AA × AA cross and that the resistance mechanism conferred by the H_1 gene is not absolute. Definite proof that the females developed on cultivar Saturna are indeed avirulent (AA) can only be obtained by crossing with double recessive males and by studying the virulence characteristics of the progenies.

Several studies have been aimed at studying the genetics of virulence towards the H_1 gene (Parrott & Berry, 1973; Parrott, 1981). Although no definitive proof was obtained for a gene-for-gene relationship, most of the data were in reasonable agreement with the calculated values (Jones, Parrott & Perry, 1981). However, our data show that the selection of virulent and avirulent lines is less straightforward than rexpected from a gene-for-gene relationship. Evidently our data do not exclude such a relationship. Conclusive evidence to accept or reject the hypothesis can only be obtained by making crosses of the virulent and a virulent lines and studying the Mendelian behaviour of the virulence

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