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

Richard JANSSEN, Jaap BAKKER and Fred J. GOMMERS

Department of Nematology, Agricultural University, 10 Binnenhaven, 6709 PD Wageningen, The Netherlands.

SUMMARY

Virulent and avirulent lines of *Globodera rostochiensis* for the H_1 resistance gene in *Solanum tuberosum* ssp. *andigena* CPC 1673 were obtained with controlled single matings. The selection of virulent and avirulent lines was less straightforward than expected from the gene-for-gene theory. First, the number of lines with intermediate levels of virulence was larger than expected and, second, in many of these lines the levels of virulence dit not agree with the genetic interpretation according to the theory.

RÉSUMÉ

Sélection de lignées de Globodera rostochiensis *virulentes et non-virulentes vis-à-vis du gène de résistance H_1 chez* Solanum tuberosum *ssp.* andigena *CPC 1673*

Des lignées de *Globodera rostochiensis* virulentes et non-virulentes vis-à-vis du gène de résistance H_1 présent chez *Solanum tuberosum* ssp. *andigena* CPC 1673 ont été obtenues par fécondation contrôlée d'individus isolés. La sélection de lignées virulentes et non-virulentes s'est révélée moins nette que la théorie du « gène-pour-gène » le laissait espérer. En premier lieu, le nombre de lignées possédant un taux de virulence intermédiaire était plus élevé que prévu et en second lieu, chez nombre de ces lignées, les niveaux de virulence ne correspondent pas à l'interprétation génétique découlant de la théorie.

In the international pathotype scheme the pathotypes of *Globodera rostochiensis* (Ro_1-Ro_5) and *Globodera pallida* (Pa_1-Pa_3) are delineated by their reproduction pattern on sets of differentials (Canto Saenz & de Scurrah, 1977; Kort *et al.*, 1977). The pathotypes were already present in Europe before the relevant resistant cultivars were grown. These variations in virulence are predominantly the result of four processes : 1) the genetic structures of the initial populations introduced from South America, 2) random genetic drift and 3) gene flow (Bakker, 1987). Since the late sixties the frequencies of alleles for virulence have also been affected by selection caused by the cultivation of resistant potato cultivars.

Refined estimates of the virulent phenotypes indicated that these four processes have not led to field populations fixed for avirulence or virulence alleles for the H_1 resistance gene of *S. tuberosum* ssp. *andigena* CPC 1673 (Janssen, Bakker & Gommers, 1990*a*). The lack of such monomorphic populations hampers research aimed at unravelling the genetics of virulence and the molecular and cellular mechanisms underlying virulence.

Earlier genetic research (Parrott, 1981) to verify the theory of a gene-for-gene relationship (Howard, 1959; Jones & Parrott, 1965) did not result in conclusive data because no lines with defined levels of virulence were used. Initiatives to breed the appropriate virulent and avirulent lines were in the past discouraged by the unattractive length of the genetic analysis, the absence of an accurate test to assess levels of virulence, and unreliable techniques for controlled matings. In this research we solved these difficulties by circumventing the diapause (Janssen, Bakker & Gommers, 1987) and by rearing females and cysts on roots of sprouts grown on agar plates (Mugniéry & Person, 1976; Janssen, Bakker & Gommers, 1990*a*). With these techniques we were able to breed lines of *G. rostochiensis* with 0 and 100 % virulent individuals for the H_1 resistance gene.

Materials and methods

The population Ro_1-Mierenbos was used to select avirulent lines and the populations Ro_5-Harmerz, Ro_3-C_{133} and Ro_3-C_{129} were used to select virulent lines. The populations were supplied by the Plant Protection Service, Wageningen, the Netherlands, except for

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 the male on top of the gelatinous matrix of the female. Males were in all cases reared on the cultivar " Eigenheimer " grown in pots. The F_1 cysts were multiplied separately in pots on cultivar " Saturna " after artificial hatching (Janssen, Bakker & Gommers, 1987). Cysts were cut in halves and these halved cysts were inoculated by pouring the suspension in preformed holes in the soil when the plants were approximately 15 cm tall. Multiplication of the F_2 and F_3 lines on cultivar " Saturna " was done in a similar way by inoculating larval suspensions from ten cysts or less per pot. The number of virulent phenotypes in the F_3 or F_4 was estimated by expressing the number of females that developed on the resistant cultivar " Saturna " as a percentage of those that developed on the susceptible cultivar " Eigenheimer ". These virulence tests were carried out in Petri dishes by inoculating two second stage larvae (L_2) per root tip (Janssen, Bakker & Gommers, 1990*a*). Two hundred L_2 were inoculated on each cultivar. To guarantee a random batch of L_2, 100 cysts were used for hatching. When less than 100 cysts were available for testing, the F_3 line was multiplicated again on cultivar " Saturna ". The whole procedure was completed within an average period of 15 months : one generation in Petri dishes, three generations in pots, and the virulence test.

The procedure for the selection of avirulent lines of the H_1 gene was similar, except that for all generations, the susceptible cultivar " Eigenheimer " was used and virulence testing on cultivar " Saturna " was carried out in pots (700 ml) instead of Petri dishes because of the higher detection level for low virulence frequencies in pots. Virulence levels were calculated by expressing the number of females that developed on cultivar " Saturna " as a percentage of those that developed on cultivar " Eigenheimer ". Due to the limited number of cysts, the inoculation density was not the same for the susceptible (P_i = 10 cysts) and the resistent cultivar (P_i = 40 cysts). Evidently this methodology results in an overestimation of the number of virulent phenotypes, but is adequate for this study.

To study the virulence levels in the progeny of single male-female matings a sufficient number of cysts are required. For a monogenic inheritance of virulence as described by Jones, Parrott and Ross (1967) with three 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 (700 ml) with loamy sandy soil and a slow release N-P-K granulate fertilizer (Osmocote R) in a controlled environment room at 18° C with 16 h light. Petri dishes covered with black plastic were stored under the same conditions.

To optimalize the recovery of wet cysts from wet soil, a Kort elutriator (Kort, 1960) was used at the maximum water stream of 4.5 l/min, and a special sieve (pore size 0.30 × 3.65 mm) was used to collect the cysts. Males were reared for all experiments in pots on the susceptible cultivar " Eigenheimer " and recovered from the soil with an Oostenbrink elutriator (Oostenbrink, 1960).

Results

Selection of virulent lines for the H_1 gene

Population Ro_5-Harmerz proved to be suitable for the selection of virulent lines. The initial number of virulent phenotypes in this population was 84.6 % (Janssen, Bakker & Gommers, 1990*a*) and inoculating 579 root tips of the resistant cultivar " Saturna " resulted in the development of 252 females. Mating these virgins with single males resulted in 164 fertilized females. Of these F_1-cysts 87.4 % were found to reproduce on the cultivar " Saturna " in pots. Nine F_2 lines were selected for multiplication. To obtain sufficient cysts for a virulence test seven of the nine F_3 lines were multiplied again on the cultivar " Saturna ". In the virulence tests one line (no. 22) showed a percentage of virulent phenotypes of approximately 100 % (Table 1).

Population Ro_3-C_{133} and Ro_3-C_{129} had low levels of virulence so the virulence level was enhanced by initially rearing these populations in pots for several generations on cultivar " Saturna ". After four generations the percentage of virulent phenotypes of population Ro_3-C_{129} increased from 30.1 % to 50.8 %. Controlled single crossings in Petri dishes resulted in nineteen fertilized F_1 cysts. Unfortunately only one cyst multiplied to a F_2 containing eighteen cysts; this was insufficient for a virulence test and these cysts were not used in further experiments. With the Ro_3-C_{133} population having 1.4 % virulent phenotypes we were unable to select virulent lines for the H_1 gene because the popu-

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 characters.

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