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Dominant and additive resistance to the root-knot nematodes *Meloidogyne chitwoodi* and *M. fallax* in Central American *Solanum* species

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Abstract The inheritance of resistance to *Meloidogyne chitwoodi* and *M. fallax* in *Solanum fendleri*, *S. hougasii* and *S. stoloniferum* was studied assuming disomic behaviour of these polyploid *Solanum* species. Various populations were produced from crosses within the wild *Solanum* species; resistant × susceptible and reciprocal crosses (F_1), self-pollinations (S_1), testcrosses (TC) and self-pollinations (F_2) of resistant hybrids, if possible. For the test crosses with *S. hougasii*, susceptible genotypes of *S. iopetalum* were used. In seedling tests, numbers of egg masses were counted after inoculation with *M. chitwoodi* or *M. fallax*. Almost all seedlings of the F_1 and S_1 populations of *S. fendleri* appeared to be resistant, whereas the TC and F_2 populations of three different resistant hybrid genotypes segregated into resistant (having 1 or no egg mass) and susceptible plants (having more than 1 egg mass) at ratios of 1:1 and 3:1, respectively. The results clearly indicate the action of a single dominantly inherited gene, and the symbol R_{Mc2} is proposed for this gene. In the case of *S. hougasii*, F_1 and S_1 seedlings appeared to be mostly resistant. Difficulties were met in producing TC and F_2 populations, and only four TC populations were obtained, which segregated at a 1:1 ratio. These results also indicate the presence of a simple dominant factor. For both *S. fendleri* and *S. hougasii* no differences were observed between *M. chitwoodi* and *M. fallax*, indicating that resistance genes are the same for both nematode species. The F_1, S_1 and TC populations of *S. stoloniferum* segregated for the square root number of egg masses into normal-like distributions, which deviated between the *Meloidogyne* species used. The patterns indicate the presence of several additive genes and one or more genes effective to *M. fallax* but not to *M. chitwoodi*. The relationship of resistance genes present in various Central American *Solanum* species is discussed.

Key words Introgression · Potato resistance breeding · *Solanum fendleri* · *S. stoloniferum* · *S. hougasii*

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Introduction

The root-knot nematodes, *Meloidogyne* spp., are a potential threat for the cultivation of potato in northwestern Europe and the western states of the USA. *Meloidogyne chitwoodi* Golden et al. and *M. fallax* Karssen especially can cause serious economic losses by reducing yield and inflicting damage on the tubers. The recently described *M. fallax* (Karssen 1996) is thought to be genetically closely related to *M. chitwoodi* (Janssen et al. 1996). Resistance to these pests would be an effective control method but it appears to be absent in the potato cultivars currently used (Brown et al. 1994; Janssen et al. 1995).

Resistance has been identified in the wild tuber-bearing *Solanum* species *S. bulbocastanum* and *S. hougasii* (Brown et al. 1989, 1991; Janssen et al. 1996), and the introgression of the resistance into the potato gene pool is in progress (Brown et al. 1994). More recently, also other promising sources of resistance have been isolated for example in diploid *S. brachistotrichum* and *S. cardiophyllum* and tetraploid *S. fendleri* and *S. stoloniferum* (Janssen et al. 1996). The former two species are genetically closely related to *S. bulbocastanum* and are considered to be primitive species, very distantly related to *S. tuberosum*. The species *S. fendleri*

and *S. stoloniferum* have been classified as belonging to the more advanced series *Longipedicellata* (Hawkes 1990) and are – in contrast to the former species – directly crossable with *S. tuberosum*. The hexaploid species *S. hougasii* from the series *Demissa* is also directly crossable with potato. Therefore, the introgression of resistance from these *Solanum* species into the cultivated potato can be achieved through sexual crosses, although difficulties can be expected due to genomic differences and differences in ploidy levels (Hermsen 1994). However, the success of its introduction into the potato gene pool will largely depend on the inheritance of the resistance.

Although *S. fendleri*, *S. stoloniferum* and *S. hougasii* have a polyploid genome, these species are cytologically disomic and during meosis show regularly 24 and 36 bivalent configurations in the tetraploid and hexaploid species, respectively, and only very low frequencies of multivalents (Swaminathan and Hougas 1954; Marks 1965; Dvorack 1983; Matsubayashi 1991; Watanabe and Orrillo 1994). Moreover, a disomic inheritance of various traits in species from the series *Longipedicellata* and *Demissa* has been suggested and proven in genetic studies (McKee 1962; Cockerham 1970; Everhart and Rowe 1974; Malamud and O'Keefe 1976). Several researchers consider these so-called allopolyploid species to have arisen from natural hybridisations of two (prehistoric) *Solanum* species (e.g. Marks 1965; Hawkes 1990; Spooner et al. 1995). Subsequently, gradual changes in the originally similar chromosomes would have led to diploidisation favouring balanced gamete formation (Matsubayashi 1991). Others suggest a genetic control of the suppression of homoeologous chromosome pairing (Lamm 1945; Dvorack 1983). In the study discussed here, disomic inheritance will be considered for *S. fendleri*, *S. stoloniferum*, as well as *S. hougasii*.

The genetic behaviour of traits like resistance are best investigated using intraspecific populations so as to ensure normal meiotic reduction divisions leading to Mendelian segregation patterns. The use of hybrid populations of distantly related species can result in distorted segregations of simply inherited characters. As an example, a major locus responsible for resistance to *M. chitwoodi* from *S. bulbocastanum* was found using restriction fragment length polymorphism (RFLP) markers, but the mapping population showed a distorted segregation as a result of irregular meiosis in the hybrid parent (Masuelli et al. 1995; Brown et al. 1996).

In the study presented here we describe the inheritance of resistance to *M. chitwoodi* and *M. fallax* of wild *S. fendleri*, *S. stoloniferum* and *S. hougasii* by analysing cross populations within the *Solanum* species or between very related species.

Materials and methods

Plant material

Genotypes of *S. fendleri*, *S. stoloniferum*, *S. hougasii* and *S. iopetalum*, either resistant or susceptible to *M. chitwoodi* and *M. fallax*, had

Table 1 Origin of crossing parents and level of resistance to *M. chitwoodi* and *M. fallax*

Solanum sp.	Genotype	Source	*M. chitwoodi*	*M. fallax*
S. fendleri	93-89-6	BGRC 23568[a]	Resistant[b]	Resistant[b]
S. fendleri	93-89-21	BGRC 23568	Resistant	Resistant
S. fendleri	93-114-11	BGRC 8083	Resistant	Resistant
S. fendleri	93-114-12	BGRC 8083	Resistant	Resistant
S. fendleri	93-115-7	BGRC 8090	Susceptible	Susceptible
S. fendleri	93-115-14	BGRC 8090	Susceptible	Susceptible
S. fendleri	93-115-18	BGRC 8090	Susceptible	Susceptible
S. fendleri	M94-33-3	93-114-12 × 93-115-18	Resistant	Resistant
S. fendleri	M94-51-1	93-114-11 × 93-115-7	Resistant	Resistant
S. fendleri	M94-79-1	93-115-14 × 93-89-21	Resistant	Resistant
S. hougasii	93-71-3	BGRC 55203	Resistant	Resistant
S. hougasii	93-71-6	BGRC 55203	Resistant	Resistant
S. iopetalum	93-108-1	BGRC 8101	Susceptible	Susceptible
S. iopetalum	93-108-11	BGRC 8101	Susceptible	Susceptible
S. hou. × *S. iop.*	M94-11-3	93-71-3 × 93-108-1	Resistant	Resistant
S. hou. × *S. iop.*	M94-11-4	93-71-3 × 93-108-1	Resistant	Resistant
S. iop × *S. hou.*	M94-32-2	93-108-1 × 93-71-6	Resistant	Resistant
S. iop × *S. hou.*	M94-32-5	93-108-1 × 93-71-6	Resistant	Resistant
S. stoloniferum	93-STOL-1	BGRC 7229	Mod. resistant	Resistant
S. stoloniferum	93-STOL-3	BGRC 7229	Mod. resistant	Resistant
S. stoloniferum	93-STOZ-1	BGRC 7230	Susceptible	Susceptible
S. stoloniferum	93-STOZ-2	BGRC 7230	Susceptible	Susceptible
S. stoloniferum	M94-23-1	93-STOL-3 × 93-STOZ-2	Mod. susceptible	Resistant

[a] BGRC-accessions are from the Dutch-German potato gene bank, Wageningen, The Netherlands
[b] Level of resistance based on multiple resistance tests (E. G. Janssen et al. 1997)

Table 2 Populations from crosses within *S. fendleri*. Populations are derived from crosses of resistant parent genotypes (RP) and resistant F_1 genotypes (RF_1) with susceptible parent genotypes (SP), and from various self-pollinations

		RP 93-89-6	RP 93-89-21	RP 93-114-12	RF_1 M94-33-3	RF_1 M94-51-1	RF_1 M95-79-1	SP 93-115-7	SP 93-115-14	SP 93-115-18
RP	93-89-6	M94-34								M94-68
RP	93-89-21		M94-142						M94-146	
RP	93-114-11							M94-51		
RP	93-114-12			M94-29					M94-148	M94-33
RF_1	M94-33-3				M95-241				M95-239	
RF_1	M94-51-1					M95-238			M95-236	
RF_1	M94-79-1						M95-244	M95-242		
SP	93-115-7						M95-243			
SP	93-115-14		M94-79	M94-122	M95-240	M95-237			M94-147	
SP	93-115-18	M94-120								

Table 3 Populations from crosses of *S. hougasii* and *S. iopetalum*. Populations are derived from crosses of resistant parent genotypes (RP) and resistant F_1 genotypes (RF_1) with susceptible parent genotypes (SP), and from various self-pollinations

		RP 93-71-3	RP 93-71-6	SP 93-108-1	SP 93-108-11
RP	93-71-3	M94-31		M94-11	
RP	93-71-6		M94-17		
RF_1	M94-11-3				M95-232
RF_1	M94-11-4				M95-233
RF_1	M94-32-2				M95-234
RF_1	M94-32-5				M95-235
SP	93-108-1	M94-13	M94-32	M94-108	

Table 4 Populations from crosses of *S. stoloniferum*. Populations are derived from crosses of resistant parent genotypes (RP) and resistant F_1 genotypes (RF_1) with susceptible parent genotypes (SP), and from various self-pollinations

		RP 93-STOL-1	RP 93-STOL-3	SF_1 M94-23-1	SP 93-STOZ-2
RP	93-STOL-1	M94-95			M94-131
RP	93-STOL-3		M94-24		M94-23
SP	93-STOZ-1			M95-229	
SP	93-STOZ-2	M94-132	M94-130	M95-228	

been previously selected from resistance screening trials (Janssen et al. 1996). Since no susceptible genotypes of *S. hougasii* had been found, susceptible genotypes of related *S. iopetalum* were used for crosses. Plants were crossed in a glasshouse during the spring and summer of 1994 and 1995. Flowers were emasculated 1 or 2 days before anthesis (except for self-pollinations) and pollinated once they were open. Fruits were harvested 6 weeks after pollination. Resistant genotypes were crossed with susceptible ones and self-pollinated to produce the F_1 and S_1 populations, respectively. Resistant F_1 genotypes were selected from various hybrid populations and used for making testcross population (TC) with susceptible genotypes and self-pollinations (F_2). The level of resistance of the parent and hybrid genotypes was analysed in multiple glasshouse experiments using several nematode populations (Janssen et al. 1996, 1997). The characteristics of the genotypes used are presented in Table 1. The cross combinations and derived populations are described in Tables 2, 3 and 4 for *S. fendleri*, *S. hougasii* with *S. iopetalum* and *S. stoloniferum*, respectively.

Nematode inoculum

The nematode populations 'CHE' of *M. chitwoodi* originating from Heide, The Netherlands, and 'FB' of *M. fallax* originating from Baexem, The Netherlands, were used in the resistance tests. These populations had also been used in previous resistance tests with the crossing parents included (Janssen et al. 1997) and were maintained on tomato plants cv 'Nematex'. Species identity was regularly verified by analysing single females for their esterase and malate dehydrogenase isozyme patterns (Esbenshade and Triantaphyllou 1990). To prepare inoculum, we followed the method of Hussey and Barker

(1973) in which eggs are harvested from the roots by dissolving egg masses in 0.5% NaOCl solution. Juveniles were hatched in water and stored at 4°C for up to 1 month until used as inoculum.

Resistance tests

Resistance tests were performed during 1995 and 1996 to analyse the level of resistance of the seedlings to *M. chitwoodi* and *M. fallax*. For each *Solanum* species two resistance tests were carried out: the first with F_1 and S_1 populations and the second with F_2 and TC populations. Seeds were sown in a potting soil/silver sand (1:1) mixture and transplanted into square plastic tubes of 240 ml filled with moist silver sand and NPK fertiliser. The tubes were put in trays and the trays placed randomly in a temperature-controlled glasshouse ($22° \pm 2°C$). Approximately 3 weeks after transplantation plants were inoculated with 400 juveniles of either *M. chitwoodi* or *M. fallax*. During the experiment, stolons were regularly cut to prevent ingrowth into neighbouring tubes. The plants were harvested 8 weeks after inoculation, the roots were washed free from sand and for each seedling the number of egg masses was counted after staining with Phloxine B (Dickson and Strubble 1965).

Depending on the *Solanum* species used and the number of seedlings available, populations were represented as randomly situated plots of 5–18 seedlings per tray, for each nematode species four to eight trays were used. Each tray contained 4 plants of potato cv 'Nicola' to serve as a susceptible control for possible miscellaneous nematode conditions.

In order to determine whether resistance to *M. chitwoodi* and *M. fallax* was the same or highly linked in *S. fendleri* and *S. hougasii*, 11 genotypes of TC population M95-236 and 15 of TC population M95-235 were tested in four replications to each nematode species. Seeds were sown in vitro on MS medium (Murashige and Skoog 1962) containing 30 g/l sucrose, and shoots were cut until enough clones were available. Two weeks after the last cutting, in vitro plantlets were transplanted into 350-ml stone pots filled with moist silver sand and NPK fertiliser, and the experiment was further carried out as described for the seedling tests. Least Significant Difference (LSD) was analysed with ANOVA using GENSTAT (Payne et al. 1987) after square root transformation of data.

Results

Resistance in *S. fendleri*

Most of the seedlings from the various F_1 and S_1 populations of *S. fendleri* appeared to be completely resistant to *Meloidogyne* spp. Only occasionally was a seedling with more than one egg mass observed. In Fig. 1A the distribution pattern of seedlings from the F_1 population M94-122 and its reciprocal cross M94-148 is shown. For other cross combinations similar patterns were found. The population M94-147, derived from self-pollination of the susceptible genotype 93-115-14, showed a normal-like distribution ranging from 0 to 9 after square root transformation of the number of egg masses (Fig. 1B). One seedling was found having no egg masses and was considered to be an escape. The seedling populations which were obtained from testcrosses of resistant hybrids with a susceptible genotype clearly segregated into groups of

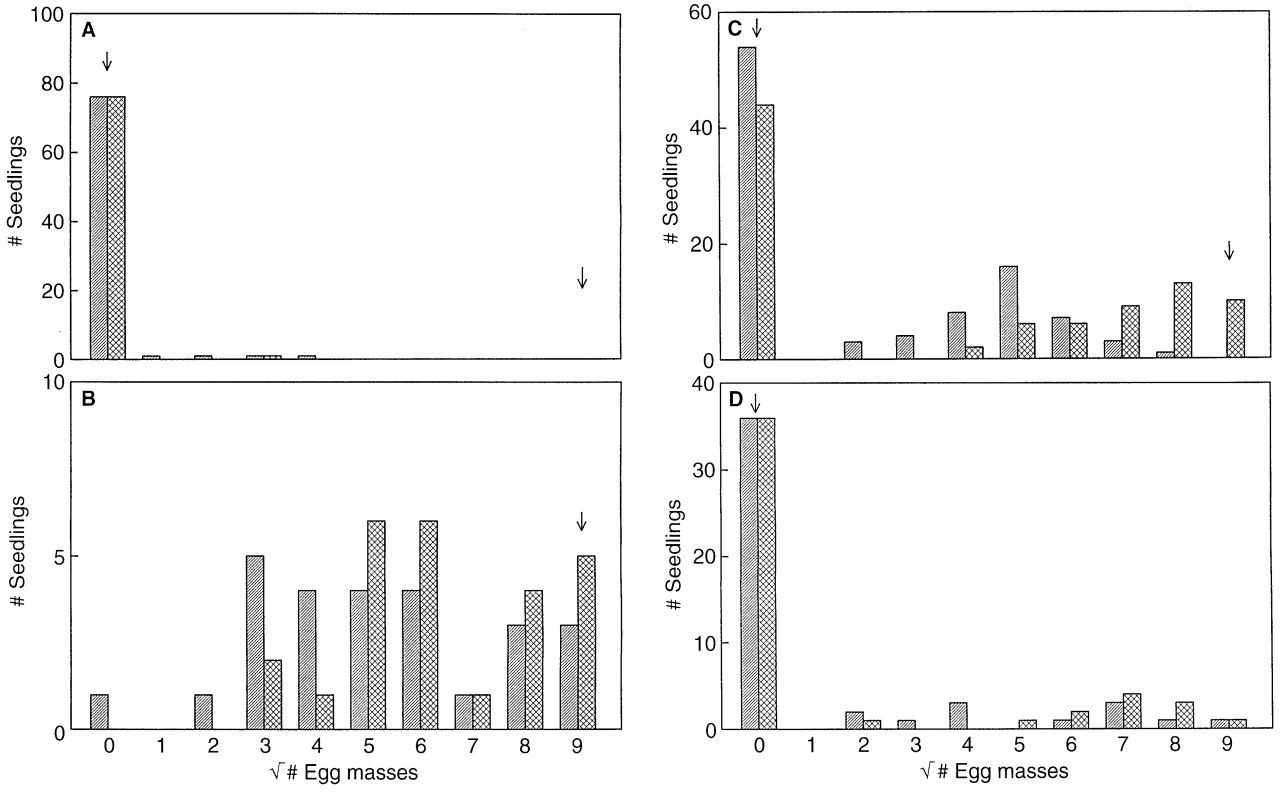

Fig. 1A–D Distribution of levels of infection seedling populations of *S. fendleri* infected with *M. chitwoodi* (striped) or *M. fallax* (*cross hatched*). **A** M94-122 + M94-148 (reciprocal cross), **B** M94-147, **C** M95-239 + M94-240 (reciprocal cross), **D** M95-241. The infection level of the parents is indicated with *arrows*

Table 5 Mean square root number of egg masses of *M. chitwoodi* (CHE) and *M. fallax* (FB) on genotypes of *S. fendleri* M95-236 and (*S. hougassii* × *S. iopetalum*) × *S. iopetalum* M95-235. Means are based on four replications

Population	Nematode	Genotype														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
M95-236	CHE	8.8	0.3	0.0	0.0	0.0	7.7	0.0	0.0	7.8	7.1	9.5				
	FB	8.6	0.0	0.0	0.0	0.0	7.0	0.0	0.0	8.0	7.4	8.9				
M95-235	CHE	7.1	0.0	8.3	6.6	7.3	7.3	0.3	0.3	5.3	6.6	0.0	0.0	0.0	2.4	0.0
	FB	7.3	0.0	7.0	7.1	8.4	6.9	0.0	0.0	5.9	7.4	0.0	0.3	0.0	5.0	0.0
LSD ($P < 0.05$) = 1.2																

resistant and susceptible plants. An example of this segregation is shown with population M95-239 and its reciprocal cross M95-240 in Fig. 1C. The F_2 populations also segregated into distinct groups of resistant and susceptible plants, for example M95-241 (Fig. 1D).

In all of the seedling populations tested no clear deviant pattern was observed between the nematode species *M. chitwoodi* and *M. fallax*. Moreover, the 11 genotypes of M95-236 which were tested against both nematode species were either resistant or completely susceptible to both (Table 5). In other experiments numerous genotypes of *S. fendleri* and the interspecific hybrid- and backcrossed genotypes of *S. fendleri* with *S. tuberosum* were screened, and so far tests have not revealed any genotype with a distinct behaviour towards these nematode species (data not shown).

On the basis of segregation patterns shown in Fig. 1A–D, plants having no or one egg mass were regarded to be resistant (R) and plants having more than one eggs mass as susceptible (S). The observed segregation R : S of tested populations of *S. fendleri* is shown in Table 6. Hardly any segregation was observed for the F_1 and S_1 populations. All TC populations showed a segregation pattern that fitted a 1 : 1 distribution for R : S. The populations from the self-pollinations of the hybrid genotypes fitted a 3 : 1 segregation. Reciprocal differences were not observed in any of the cross combinations. Assuming disomic inheritance, the observed segregation patterns can be explained with a single dominantly inherited gene, which would be present in a homozygous form in the resistant parental genotypes.

Table 6 Segregation patterns of various cross populations of *S. fendleri*

Population	From cross		Expected$_{R:S}$[a]	*M. chitwoodi*		*M. fallax*	
				Observed$_{R:S}$	χ^2-prob	Observed$_{R:S}$	χ^2-prob
M94-79	93-115-14 × 93-89-21	(SP × RP)[b]	1 : 0	35 : 2	–	36 : 1	–
M94-146	93-89-21 × 93-115-14	(RP × SP)	1 : 0	30 : 2	–	31 : 4	–
M94-142	93-89-21 self-poll.	(RP ⊗)	1 : 0	33 : 3	–	39 : 0	–
M94-68	93-89-6 × 93-115-18	(RP × SP)	1 : 0	39 : 1	–	39 : 0	–
M94-120	93-115-18 × 93-89-6	(SP × RP)	1 : 0	30 : 1	–	30 : 2	–
M94-34	93-89-6 self-poll.	(RP ⊗)	1 : 0	32 : 1	–	37 : 0	–
M94-122	93-115-14 × 93-114-12	(SP × RP)	1 : 0	37 : 1	–	38 : 2	–
M94-148	93-114-12 × 93-115-14	(RP × SP)	1 : 0	39 : 0	–	39 : 1	–
M94-29	93-114-12 self-poll.	(RP ⊗)	1 : 0	37 : 2	–	37 : 1	–
M95-236	M94-51-1 × 93-115-14	(RF_1 × SP)	1 : 1	25 : 19	0.37	20 : 27	0.31
M95-237	93-115-14 × M94-51-1	(SP × RF_1)	1 : 1	22 : 24	0.77	23 : 25	0.77
M95-238	M94-51-1 self-poll.	(RF_1 ⊗)	3 : 1	23 : 4	0.22	14 : 3	0.48
M95-239	M94-33-3 × 93-115-14	(RF_1 × SP)	1 : 1	20 : 28	0.25	26 : 22	0.57
M95-240	93-115-14 × M94-33-3	(SP × RF_1)	1 : 1	24 : 19	0.44	28 : 20	0.25
M95-241	M94-33-3 self-poll.	(RF_1 ⊗)	3 : 1	36 : 12	1.0	36 : 12	1.0
M95-242	M94-79-1 × 93-115-7	(RF_1 × SP)	1 : 1	24 : 24	1.0	24 : 24	1.0
M95-243	93-115-7 × M94-79-1	(SP × RF_1)	1 : 1	23 : 23	1.0	24 : 22	0.76
M95-244	M94-79-1 self-poll.	(RF_1 ⊗)	3 : 1	36 : 14	0.24	33 : 14	0.57

[a] Expected segregation based on a monogenic dominant factor present in a homozygous form in the resistant parent genotype
[b] SP, Susceptible parent genotypes; RP, resistant parent genotypes; RF_1, resistant F_1 genotypes; ⊗, self-pollinated

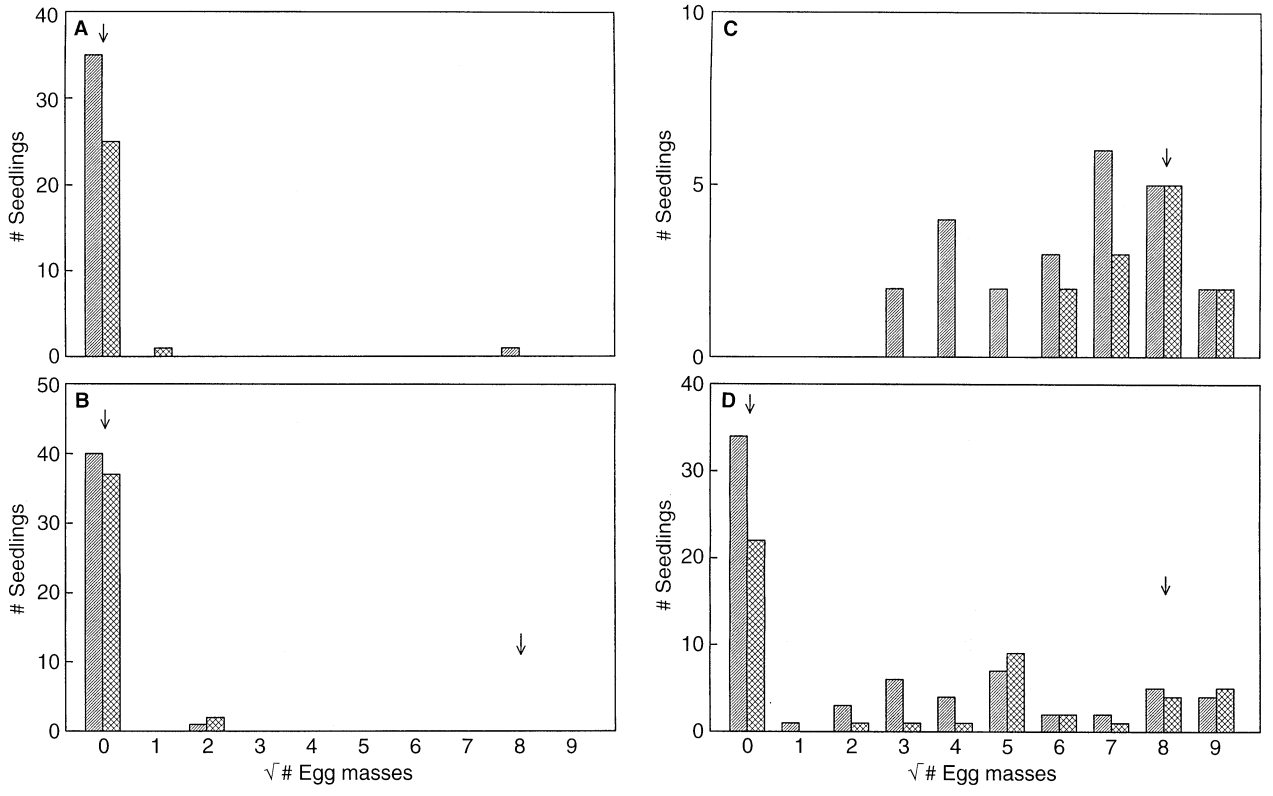

Fig. 2A–D Distribution of levels of infection of seedling populations of *S. hougasii* (× *S. iopetalum*) infected with *M. chitwoodi* (striped) or *M. fallax* (*cross hatched*). **A** M94-31, **B** M94-11 + M94-13 (reciprocal cross), **C** M94-108, **D** M95-234 + M95-235. The infection level of the parents is indicated with *arrows*

Resistance in *S. hougasii*

Like *S. fendleri*, most of the seedlings arising from self-pollination of resistant genotypes of *S. hougasii* as well as from crosses with *S. iopetalum* appeared to be completely resistant. Figure 2A and B expresses the (lack of) segregation of populations from self-pollination of 93-71-3 and from crosses of this genotype with susceptible 93-108-1, respectively. The susceptible status of 93-108-1 is confirmed by the distribution of genotypes obtained from self-pollination (Fig. 2C).

It was difficult to obtain seeds from testcrosses of resistant hybrids with susceptible genotypes of *S. iopetalum*, and only crosses with the hybrid as female produced some seeds. All 4 TC populations segregated, and M95-234 and M95-235 were pooled to obtain the segregation pattern of Fig. 2D. Since very few seeds were derived from self-pollinations, these were not tested. The 15 genotypes of TC population M95-235, which were tested against both *M. chitwoodi* and *M. fallax*, did not reveal any different behaviour between the species (Table 5) and other experiments testing several interspecific genotypes of *S. hougasii* with *S. tuberosum* confirmed that, like in *S. fendleri*, the resistance of *S. hougasii* to *M. chitwoodi* and *M. fallax* is the same or highly linked (data not shown).

Using the same criterion as for *S. fendleri* to distinguish between resistant and susceptible plants, we noticed that there were scarcely any susceptible plants in the F_1 and S_1 populations. The TC populations fitted a 1:1 segregation, but only for M95-234 and M95-235 were the numbers of seedlings sufficient to validate this assumption (Table 7). If *S. hougasii* is assumed to display disomic behaviour, monogenic dominantly inherited resistance is possible; but as the results were inconclusive, several alternative hypotheses can not be excluded.

Resistance in *S. stoloniferum*

In contrast to *S. fendleri* and *S. hougasii*, the level of resistance to *M. chitwoodi* and *M. fallax* in *S. stoloniferum* is not absolute and also different for the two nematode species (Table 1). The actual level of resistance was well-represented by the mean level of resistance of the seedling populations from the self-pollination of 93-STOL-1 (Fig. 3A) and 93-STOL-3 (Fig. 3B), i.e. 1 and 2 for the square root number of egg masses of *M. fallax* and *M. chitwoodi*, respectively. The seedlings from the crosses of these genotypes with susceptible 93-STOZ-2 showed a normal-like distribution for the square root number of egg masses from 0 to 7 with a mean of approximately 2 for *M. fallax* and

Table 7 Segregation of F_1, S_1 and TC populations of *S. hougassi*, crossed with *S. iopetalum*

Population	From cross		Expected$_{R:S}$[a]	*M. chitwoodi*		*M. fallax*	
				Observed$_{R:S}$	χ^2-prob	Observed$_{R:S}$	χ^2-prob
M94-11	93-71-3 × 93-93-108-1	(RP × SP)[b]	1:0	20:0	–	20:0	–
M94-13	93-108-1 × 93-71-3	(SP × RP)	1:0	25:2	–	26:1	–
M94-31	93-71-3 self-poll.	(RP ⊗)	1:0	35:1	–	26:0	–
M94-32	93-108-1 × 93-71-6	(SP × RP)	1:0	18:0	–	27:0	–
M94-17	93-71-6 self-poll.	(RP ⊗)	1:0	23:0	–	25:0	–
M95-232	M94-11-3 × 93-108-11	(RP_1 × SP)	1:1	3:1	–	6:3	–
M95-233	M94-11-4 × 93-108-11	(RF_1 × SP)	1:1	2:3	–	2:1	–
M95-234	M94-32-2 × 93-108-11	(RF_1 × SP)	1:1	14:13	0.84	12:12	1.0
M95-235	M94-32-5 × 93-108-11	(RF_1 × SP)	1:1	21:19	0.75	10:12	0.67

[a] Expected segregation based on a monogenic dominant factor present in a homozygous form in the resistant parent genotype
[b] See footnote to Table 6 for definition

a distribution from 0 to 9 with a mean of 3–4 for *M. chitwoodi* (Fig. 3C, D).

Only one hybrid genotype, which had a reasonable level of resistance to *M. fallax* but was moderately susceptible to *M. chitwoodi*, was used to make test-crosses and the distribution of TC seedlings is shown in Fig. 3E. The level of resistance to *M. fallax* was highly variable, and the mean square root number of egg masses was approximately 5. Self-pollinations of the hybrid genotype were not successful, presumably due to the poor condition of the plant. The results indicate that possibly several additive genes are responsible for resistance and that at least one or more resistance genes are effective against *M. fallax* but not against *M. chitwoodi*.

Discussion

The genetic analysis of *S. fendleri* revealed the likely presence of a monogenic and dominantly inherited factor responsible for resistance to both *M. chitwoodi* and *M. fallax*. Very recently, monogenic resistance to *M. chitwoodi* and *M. hapla* has been identified in *S. bulbocastanum* and designated as R_{Mc1} (Brown et al. 1996), and this resistance also appeared to be effective towards *M. fallax* (CPRO-DLO, unpublished results). The resistance in *S. fendleri* as described here does not suppress the multiplication of *M. hapla* to a significant extent (Janssen et al. 1997), indicating the existence of a different gene in *S. fendleri*. We propose the symbol R_{Mc2} for the gene from *S. fendleri*. Although two different accessions of *S. fendleri* were used in this study, it is expected that the resistant genotypes from these accessions bear the same resistance gene. However, this needs to be confirmed with test crosses.

It has been considered that disomic inheritance is the most likely mode of inheritance in *S. fendleri*. However, when assuming tetrasomic inheritance, a single resistance gene present in a triplex form in the parent genotypes and in a simplex form in the F_1 genotypes would also explain the segregation patterns observed. The chance of selecting three simplex genotypes randomly from a triplex × nulliplex cross would be one in eight. When we take into account the results described, the occurrence of tetrasomic inheritance cannot be excluded. Nevertheless, as already mentioned in the Introduction, disomic behaviour is thought to be most probable.

The results with the hexaploid *S. hougasii* also indicated the presence of simply inherited resistance to *M. chitwoodi* and *M. fallax*, but caution is needed because not enough genotypes and cross combinations were available for testing. Serious difficulties were encountered while making test crosses and self-pollinations of the interspecific hybrid genotypes of *S. hougasii* and *S. iopetalum*, the numerous attempts resulted in only a few successful crosses. Other researchers have also reported successful hybridisation of several interspecific combinations of *Solanum* species within the series *Demissa*, but the high levels of sterility of the hybrids resulted in unsuccessful self-pollinations and backcrosses (Swaminathan and Hougas 1954; Hawkes 1995).

In previous studies the genotypes of *S. hougasii* expressed not only resistance to *M. chitwoodi* and *M. fallax* but also moderate resistance to *M. hapla* (Janssen et al. 1997); similar resistances were expressed by resistant genotypes of backcrossed genotypes of *S. bulbocastanum* with *S. tuberosum* (CPRO-DLO, unpublished results). The hexaploid species *S. hougasii* is only distantly related to the primitive diploid species *S. bulbocastanum*, and gene exchange through natural hybridisation is not likely to have occurred. Nevertheless, the similarity in the working spectrum of resistance could indicate homology of the resistance genes. If the occurrence of resistance genes is not the result of recent

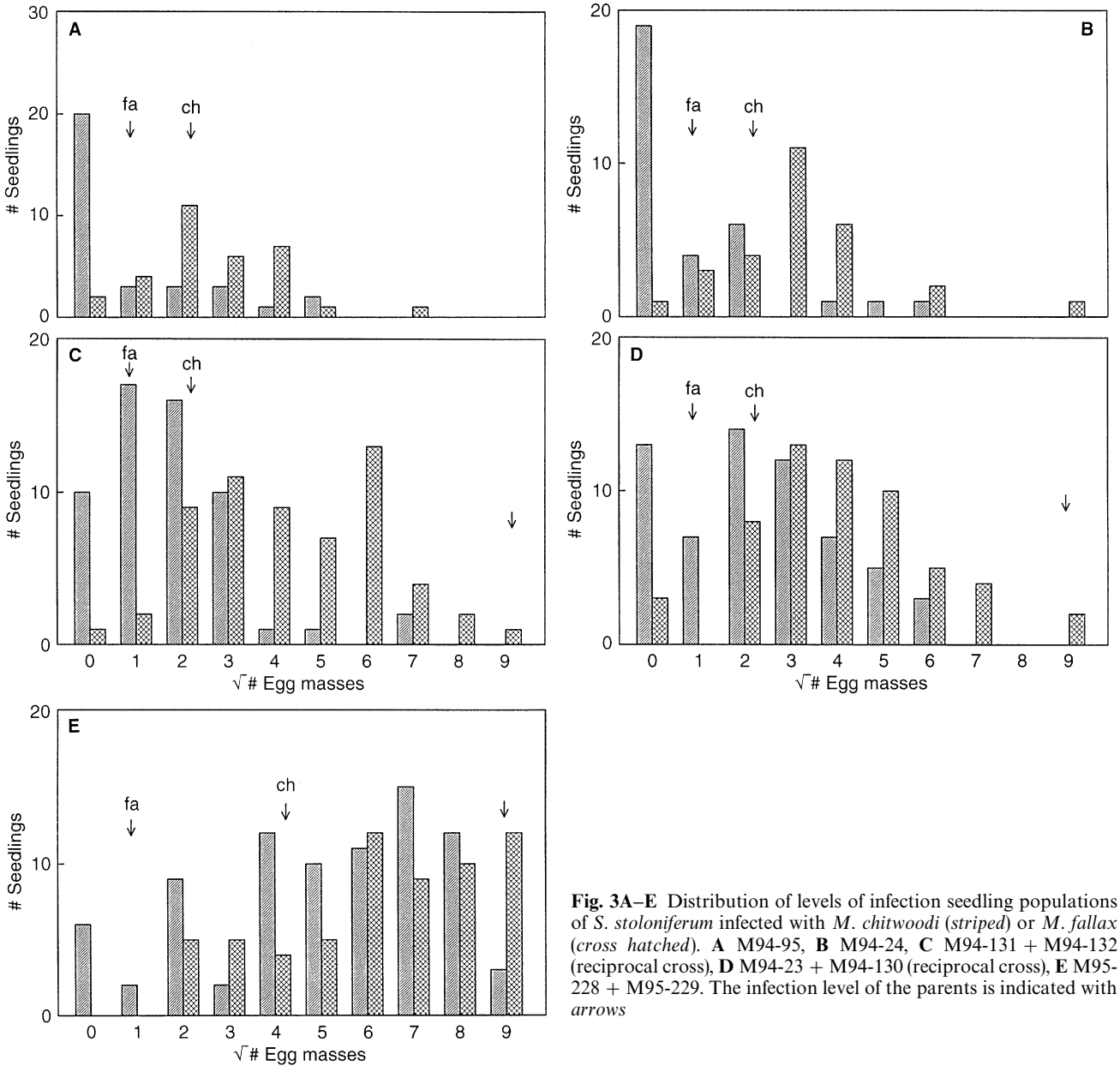

Fig. 3A–E Distribution of levels of infection seedling populations of *S. stoloniferum* infected with *M. chitwoodi* (*striped*) or *M. fallax* (*cross hatched*). **A** M94-95, **B** M94-24, **C** M94-131 + M94-132 (reciprocal cross), **D** M94-23 + M94-130 (reciprocal cross), **E** M95-228 + M95-229. The infection level of the parents is indicated with *arrows*

introgression, resistance genes might have been conserved during the evolution of *Solanum* species, combined with a continuous selection pressure towards resistance, this would explain the presence of resistance to *M. chitwoodi* and related *M. fallax* in various Central American *Solanum* species. All of the *Solanum* species investigated in this study as well as the resistant sources *S. bulbocastanum*, *S. brachistotrichum* and *S. cardiophyllum* have their natural habitat situated in Central America, primarily Mexico (Hawkes 1990), and resistance to *M. chitwoodi* and *M. fallax* seems to be rare in South American *Solanum* species (Janssen et al. 1996). Furthermore, *M. chitwoodi* has been found in different states of Mexico (Cuevas and Sosa Moss 1990) justifying this hypothesis.

Sometimes, most notably in the F_1 and S_1 populations of *S. fendleri* and *S. hougasii*, the cross population was found to be virtually completely resistant, although a susceptible plant was occasionally observed. These positives are regarded as artefacts of the resistance tests due to the ingrowth of susceptible plants into neighbouring tubes. However, they could not have had any effect on the segregation of populations due to the large numbers of genotypes tested.

The resistance of *S. stoloniferum* could not be explained by a simple inheritance, and polygenic inheritance seems likely. Furthermore, resistance factors to *M. chitwoodi* and *M. fallax* were not completely linked, in contradiction with the results observed with the other *Solanum* species, as indicated by the deviant

segregation patterns and the difference in levels of resistance of the parents used. For an investigation of this type of polygenic and incomplete resistance, a simple analysis of seedling populations is obviously not adequate and the use of replications of genotypes is necessary in order to decrease the experimental variation.

The introgression of resistance to root-knot nematodes from *S. fendleri*, *S. hougasii* and *S. stoloniferum* into cultivated potato has been initiated using the information obtained from these inheritance studies. In the case of the earlier found resistance from *S. bulbocastanum*, somatic hybridisations with *S. tuberosum* were necessary as a first step of gene transfer to *S. tuberosum* (Austin et al. 1993). With the *Solanum* species investigated direct sexual crosses with *S. tuberosum* were successful and currently resistant genotypes have been selected from first backcrosses. Future research will concentrate on the localisation of resistance genes to the root-knot nematodes in wild *Solanum* species using molecular markers, which will also enable marker-assisted selection to achieve the rapid introduction of new resistance genes into new commercial potato cultivars.

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References

Austin S, Pohlman JD, Brown CR, Mojtahedi H, Santo GS, Douches D, Helgeson JP (1993) Interspecific somatic hybridization between *Solanum tuberosum* L. and *S. bulbocastanum* Dun as a means of transferring nematode resistance. Am Potato J 70:485–495

Brown CR, Mojtahedi H, Santo GS (1989) Comparison of reproductive efficiency of *Meloidogyne* chitwoodi on *Solanum bulbocastanum* in soil and in vitro tests. Plant Dis 73:957–959

Brown CR, Mojtahedi H, Santo GS (1991) Resistance to Columbia root-knot nematode in *Solanum* ssp. and in hybrids of *S. hougasii* with tetraploid cultivated potato. Am Potato J 68:445–452

Brown CR, Mojtahedi H, Santo GS, Austin-Phillips S (1994) Enhancing resistance to root-knot nematodes derived from wild *Solanum* species in potato germplasm. In: Zehnder GW, Powelson ML, Jansson RK, Raman KV (eds) Advances in potato pest biology and management. APS press, St. Paul, pp 426–438

Brown CR, Yang C-P, Mojtahedi H, Santo GS (1996) RFLP analysis of resistance to Columbia root-knot nematode derived from *S. bulbocastanum* in a BC_2 population. Theor Appl Genet 92:572–576

Cockerham G (1970) Genetical studies on resistance to potato virus X and Y. Heredity 25:309–348

Cuevas OJ, Sosa-Moss C (1990) Host plants of *Meloidogyne chitwoodi* in the states of Tlaxcala and Puebla, Mexico, Curr Nematol 1:69–70

Dickson DW, Struble FB (1965) A sieving-staining technique for extraction of egg masses of *Meloidogyne* incognita from soil. Phytopathology 55:497

Dvorack J (1983) Evidence for genetic suppression of heterogenetic chromosome pairing in polyploid species of *Solanum*, sect. *petota*. Can J Genet Cytol 25:530–539

Esbenshade PR, Triantaphyllou AC (1990) Isozyme phenotypes for the identification of *Meloidogyne* species. J Nematol: 22:10–15

Everhart ER, Rowe PR (1974) Disomic inheritance of anthocyanins and flavonal glycosides in the tetraploid tuber-bearing species *Solanum stoloniferum*. Am Potato J 51:287–294

Gilles A (1955) Recherches cytogénétiques sur les *Solanum* (section *Tuberarium*). Nombres chromosomiques et associations méitoiques. Cellule 57:7–31

Hawkes JG (1955) Hybridization studies on four hexaploid *Solanum* species in series *Demissa* Buk. New Phytol 55:191–205

Hawkes JG (1990) The potato. Evolution, biodiversity and genetic resources. Belhaven Press, London

Hermsen JGTh (1994) Introgression of genes from wild species, including molecular and cellular approaches. In: Bradshaw JE, MacKay G (eds) Potato genetics. CAB Int, Wallingford, UK, pp 515–538

Hussey RS, Barker KR (1973) A comparison of methods of collecting inocula for *Meloidogyne* spp., including a new technique. Plant Dis Rep 57:1025–1028

Janssen GJW, Norel A van, Verkerk-Bakker B, Janssen R (1995) Detecting resistance to the root-knot nematodes *Meloidogyne hapla* and *M. chitwoodi* in potato and wild *Solanum* spp. Potato Res 38:353–362

Janssen GJW, Norel A van, Verkerk-Bakker B, Janssen R (1996) Resistance to *Meloidogyne chitwoodi*, *M. fallax*, and *M. hapla* in wild tuber-bearing *Solanum* spp. Euphytica 92:287–294

Janssen GJW, Norel A van, Verkerk-Bakker B, Janssen R (1997) Intra- and interspecific variation of root-knot nematodes, *Meloidogyne* spp., for resistance from wild tuber-bearing *Solanum* spp. Fund Appl Nematol (in press)

Karssen G (1996) Description of *M. fallax* n. sp. (Nematoda: Heteroderidae), a root-knot nematode from The Netherlands. Fund Appl Nematol 19:593–597

Lamm R (1945) Cytogenetic studies in *Solanum*, sect. *Tuberarium*. Hereditas 31:1–128

Malamud O, O'Keefe RB (1976) Inheritance of the reaction to *Verticillium* and *Fusarium* wilts in tuber-bearing *Solanum* species and species-hybrids. Am Potato J 53:357–358

Marks GE (1965) Cytogenetic studies in tuberous *Solanum* species. III. Species relationships in some South and Central American species. New Phytol 64:293–306

Masuelli RW, Tanimoto EY, Brown CR, Comai L (1995) Irregular meiosis in a somatic hybrid between *S. bulbocastanum* and *S. tuberosum* detected by species-specific PCR markers and cytological analysis. Theor Appl Genet 91:401–408

Matsubayashi M (1991) Phylogenetic relationships in the potato and its related species. In: Tsuchiya T, Gupta PK (eds) Chromosome engineering in plants: genetics, breeding, evolution, part B. Elsevier, Amsterdam, pp 93–118

McKee RK (1962) Identification of R-genes in *S. stoloniferum*. Euphytica 11:42–46

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497

Payne RW, Lane PW, Ainsley AE, Bicknell KE, Digby PGN, Leech PK, Simpson HR, Todd AD, Verrier PJ, White RP, Gower JC, Tunnicliffe Wilson G, Paterson LJ (1987) GENSTAT 5 reference manual. Clarendon Press, Oxford

Spooner DM, Berg RG van den, Bamberg JB (1995) Examination of species boundaries of *Solanum* series *Demissa* and potentially related species in series *Acaulia* and series *Tuberosa* (sect. *Petota*). Sys Bot 20:295–314

Swaminathan MS, Hougas RW (1954) Cytogenetic studies in *Solanum verrucosum* variety *spectabilis*. Am J Bot 41:645–651

Watanabe KN, Orrillo M (1994) Disomic behaviour of polyploid tuber-bearing *Solanum* species. Jpn J Genet 69:637–643