

Resistance to root knot nematode, *Meloidogyne naasi* (Franklin) transferred from *Aegilops variabilis* Eig to bread wheat

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Summary — No genotype of bread wheat has been known to be resistant to cereal root knot nematode *Meloidogyne naasi*, although wheat relatives including *Aegilops variabilis* display complete resistance. In the progeny of the cross *Triticum aestivum* cv Chinese Spring x *Aegilops variabilis* no 1, lines as completely resistant as the *Aegilops* parent were selected. A dominant gene is involved in the resistance in this novel material, which did not present gall but sometimes root growth arrest when infested by *M naasi*. Cytogenetical analysis showed that the length of the alien segment is short or perhaps reduced to the gene itself. Either in the heterozygous state or in the homozygous state, alien information seems to have no negative effect either on the regularity of meiotic behaviour or on chromosomal stability. The value of the introgression into the wheat is discussed.

resistance to nematode / *Meloidogyne naasi* / wheat / *Aegilops variabilis* / introgression

Résumé — Transfert d'*Aegilops variabilis* dans le blé tendre, de la résistance au nématode à galle, *Meloidogyne naasi* (Franklin). Jusqu'à présent, on ne connaissait aucune lignée ou variété de blé tendre résistante à *Meloidogyne naasi*, nématode à galle des céréales. Par contre, des espèces voisines du blé dont *Aegilops variabilis* présentent une résistance totale. Dans la descendance en rétrocroisement de l'hybride interspécifique (*Triticum aestivum* L cv Chinese Spring x *Aegilops variabilis* n° 1), des lignées de blé à $2n = 42$ chromosomes possédant une résistance totale comme le parent *Aegilops* ont été sélectionnées (fig 1). Cette résistance est monogénique dominante (tableau I). Elle se caractérise par l'absence de galle et parfois l'induction d'arrêts de croissance des racines infestées (fig II). L'analyse cytogénétique de l'introgression a montré que la longueur du segment transféré est courte, voire réduite au seul gène de résistance. Que ce soit à l'état homozygote ou à l'état hétérozygote, l'information étrangère introduite ne semble avoir aucun effet négatif ni sur la régularité méiotique ni sur la stabilité chromosomique du matériel (tableau II). L'introgression pourrait être le résultat d'une recombinaison homéologue ou d'une translocation spontanée. L'originalité du matériel produit réside dans le fait qu'il constitue les seuls blés totalement résistants à *M naasi*. La sélection de variétés résistantes est envisageable.

résistance aux nématodes / *Meloidogyne naasi* / blé / *Aegilops variabilis* / introgression

INTRODUCTION

The cereal root knot nematode, *Meloidogyne naasi* can have a serious effect on the yield of wheat crops (Caubel *et al*, 1972; Kilpatrick *et al*, 1976; Gooris and d'Herbe, 1977; Person-Dedryver, 1986). No fully effective resistance has been found so far in bread wheat, *Triticum aestivum* L (Person-Dedryver, 1985). Therefore, improvement of its level of resistance was envisaged through exploitation of interspecific or intergeneric hybrids.

Searches for resistance in wheat relatives belonging to the tribe *Hordeae* were made. In the subtribe *Hordeinae*, Cook and York (1981) identified resistance to *M naasi* in *Hordeum vulgare* L, *H chilense* L and *H jubatum* L. In the subtribe *Triticinae* to which wheat belongs, Person-Dedryver and Jahier (1985) detected a complete resistance to the nematode in some lines of *Aegilops variabilis* ($2n = 28$, UUS^vS^v) and *Ae umbellulata* ($2n = 14$, UU) the donor of the *U* genome of *Ae variabilis*. Resistance of these *Aegilops* species is due to their ability to sup-

press both the development of the larvae into females and nematode reproduction. In other lines of these *Aegilops*, the root galls produced in response to invasion indicate susceptibility. Screening for resistant lines has involved selecting lines presenting few or no galls. The line number 1 of *Ae variabilis* belonging to the collection of the INRA Station in Le Rheu was found resistant to both *M naasi* and *Heterodera avenae*, the cereal cyst nematode which also has a severe incidence on wheat (Meagher, 1977; Esmenjaud *et al*, 1987; Rivoal and Sarr, 1987).

The introduction into bread wheat of its genes of resistance to both pests was undertaken. This paper reports on the first lines of wheat resistant to *M naasi* derived from a cross between this *Aegilops* and wheat.

MATERIAL AND METHODS

The wheat line Chinese Spring was crossed as female with *Ae variabilis* no 1. The F₁ hybrid was successive-

ly backcrossed twice as female to the varieties Rescler and Lutin. Progenies of 1 Back-Cross 2 plant were followed in self pollination as far as the F₅ generation and backcrossed to Lutin in F₃ (fig 1).

Evolution of the material was cytologically studied. Chromosome counting was carried out using a standard Feulgen technique. For meiotic analysis, anthers at first metaphase of meiosis were fixed in Carnoy's fixative (3:1) then squashed in 1% acetocarmine.

Seeds were disinfected in 0.2% HgCl₂ in a 95% alcoholic solution and grown individually on Agar (20 g/l) in Petri dishes using the technique described by Person-Dedryver (1984). The first root developed was taken for chromosome counting. The plants were infected with larvae of a *M naasi* population collected in Le Rheu, when the 3 following roots were between 1.5–3 cm long. About 70 juveniles were injected on to the agar of each Petri dish with a hypodermic syringe. The Petri dishes were placed in a growth chamber at 20–21 °C under a 16 h photoperiod. The observation of the plants and the counting of the galls developed per plant were carried out from 14–21 d after inoculation of the larvae. The plants found resistant with 0.1 or 2 galls per plant were grown in pots in a greenhouse.

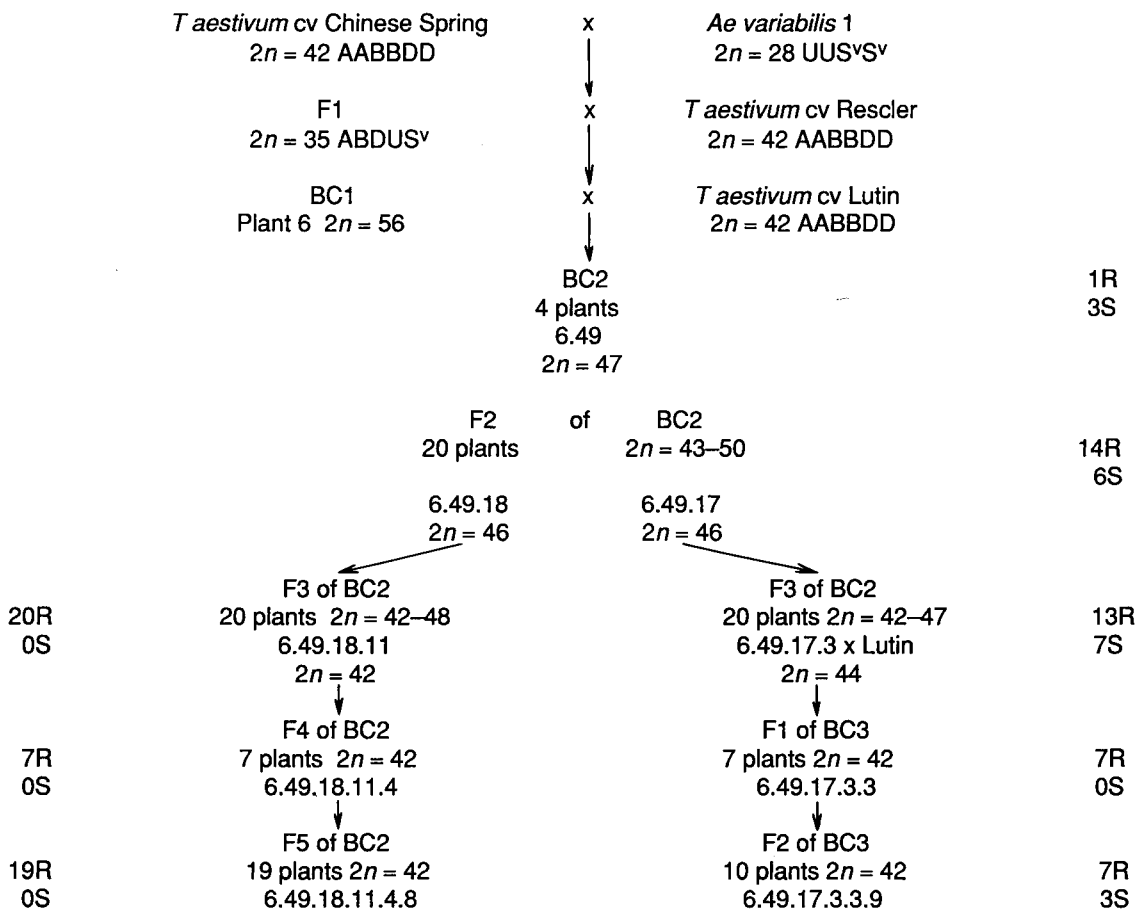


Fig 1. Steps in producing lines resistant to *Meloidogyne naasi*. The numbers of resistant (R); and susceptible (S) plants are given.

RESULTS

Selection of resistant lines

In each generation, the wheat lines Chinese Spring, Rescler and Lutin were good hosts for the nematode (fig 2C). Only the results concerning Lutin are recorded in table I. *Ae variabilis* no 1 (fig 2A) rarely formed 1 or 2 galls per plant. Plants selected in the backcross (BC) progenies of the interspecific hybrid were most often without galls or in some cases with 1 gall. In 2 cases plants with 2 galls were retained (F_2 and F_5 of BC_2) but their resistance could not be called into question since many more galls developed on the susceptible controls in the 2 corresponding tests and since their progenies were resistant. The genealogy of the material selected at each generation is presented in figure 1.

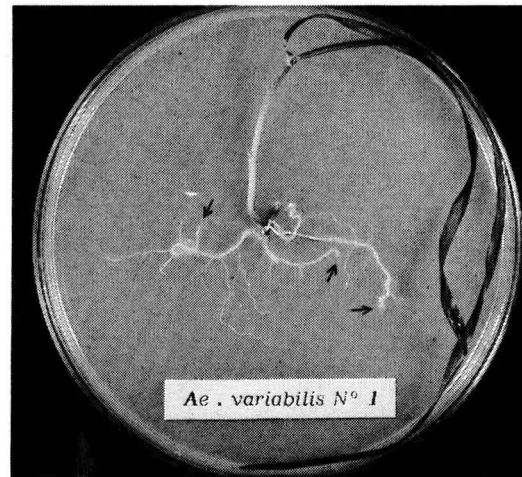
F_1 hybrid Chinese Spring x *Ae variabilis* no 1 displayed the same behaviour to the nematode as the resistant parent. BC_1 plant no 6 with 56 chromosomes was backcrossed to Lutin. From the 4 BC_2 plants obtained, only one (6.49) with 47 chromosomes was gall-free. Twenty plants from the self-pollination of this latter were analysed. A segregation for resistance showed the presence of a dominant gene ($\chi^2 = 0.267$) (table I). Among the 14 plants found to be resistant, 6.49.17 and 6.49.18 with 46 chromosomes were selfed.

Progenies of 6.49.18 were observed in F_3 , F_4 and F_5 of BC_2 . In each generation, all the plants were resistant and had 42 chromosomes.

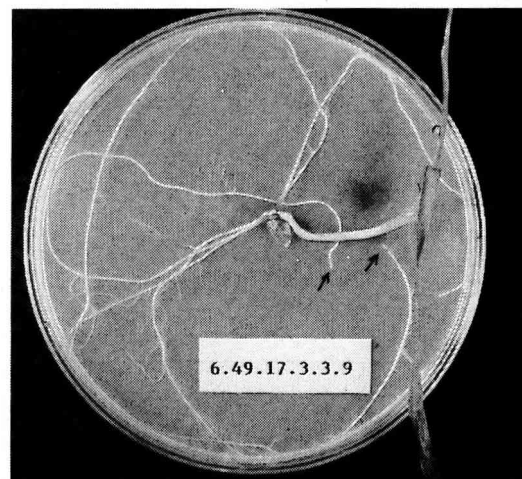
Twenty plants of the self progeny of 6.49.17 were tested for resistance. Thirteen of them were resistant including 6.49.17.3 with 44 chromosomes which were crossed with Lutin. The 7 plants in F_1 of BC_3 were all found resistant including 6.49.17.3.3 which had 42 chromosomes. The segregation for resistance in the self progeny of this plant was: 7 resistant, 3 susceptible and confirmed that only 1 gene is involved in the resistance.

The resistant material including *Ae variabilis* no 1 displayed 2 types of resistance. In the same line, no gall developed on roots in contact with the parasite and the plant showed: I – a growth stop (fig 2A,B), or II – a growth non altered. Sometimes the 2 resistant types were observed on the roots of the same plant. There was visible reaction of the plant when the growth of roots was stopped, probably if the sites of penetration of many larvae were close to each other. If the 2 types were induced by 2 different quantities of

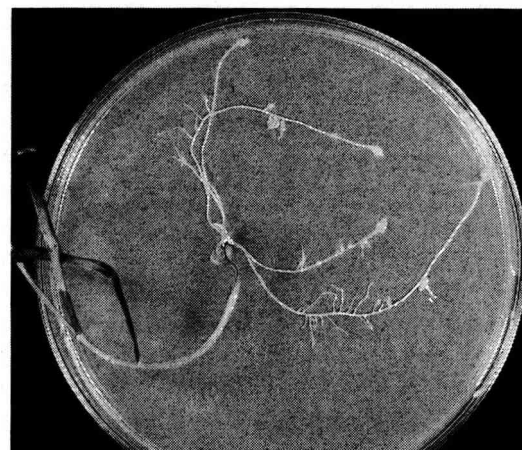
larvae penetrating in the roots of a plant, they probably do not express 2 different genetical determinisms of resistance.



a



b



c

Fig 2. Root systems 15 d after inoculation with *M naasi* of : A) *Ae variabilis* no 1 (without gall); B) a resistant plant derived from the cross CS x *Ae variabilis* no 1 (arrows indicate root growth arrests); C) the susceptible wheat Lutin (with galls).

Table I. Distribution of the plants susceptible or resistant to *Meloidogyne naasi* in the different generations issued from the cross Chinese Spring x *Ae variabilis* no 1.

Material with the same figure in brackets were tested at the same time with the same susceptible control. CS : Chinese Spring.

Material	No of plants	No of chromosomes	No of plants	Resistant Plants No of galls /plant		Susceptible plants No of plants /plant			Mode	χ^2	P
				\bar{X}	Range	\bar{X}	Range				
F ₁ C Sx <i>Ae var</i> 1	20	34–36	20	0.1	0–1						
Chinese Spring	20	42				20	21.6	9–45			
<i>Ae variabilis</i> 1	20	28	20	0.1	0–1						
BC ₂	4	47	1	0	0	3	6.3	3–12			
Lutin	11	42				11	4.5	1–11			
F ₂ of BC ₂	20	43–50	14	0.4	0–2	6	9.0	7–13	3R:1S	0.267	>0.60
Lutin	13	42				13	12.5	6–26			
F ₃ of BC ₂ (1)											
(6.49.18)	20	42–48	20	0.1	0–1						
Lutin	19	42				19	7.0	3–19			
F ₄ of BC ₂ (2)	7	42	7	0	0						
Lutin	7	42				7	32.0	23–42			
F ₅ of BC ₂ (3)	19	42	19	0.3	0–2						
Lutin	20	42				20	27.9	11–41			
F ₃ of BC ₂ (1)											
(6.49.17)	20	43–46	13	0	0	7	10.6	4–17	3R:1S	1.067	>0.30
F ₁ of BC ₃ (2)	7	41–42	7	0	0						
F ₂ of BC ₃ (3)	10	42	7	0.4	0–1	3	34.3	16–49	3R:1S	0.133	>0.70

Meiotic behaviour in the resistant plants

Chromosome pairing at metaphase I of meiosis was analysed in plants selected at each generation except in F₁ and F₂ of BC₂ (table II).

Most of the 35 chromosomes of the F₁ hybrid Chinese Spring x *Ae variabilis* no 1 did not pair (\bar{x} = 30.61 univalents). Plant BC₁ no 6 with 56 chromosomes is characterized by a relatively high level of asynapsis (\bar{x} = 19.50 univalents) and by a mean of 2.5 multivalents per cell. In F₃ of BC₂, plant 6.49.17.3 ($2n$ = 44) 2 chromosomes remained systematically unpaired and the plant 6.49.18.11 had a meiosis almost as regular as that of the wheat lines Chinese Spring and Lutin. The chromosome pairing measured by the mean number of paired arms per cell in the plants selected in the next 2 generations was comparable to that of the controls. More particularly, many pollen mother cells (PMC) with 21 ring bivalents were observed in each of the $2n$ = 42 chromosome selected plants.

DISCUSSION

The F₁ hybrid "Chinese Spring x *Ae variabilis* no 1" had the expected chromosome number of $2n$ = 35 (genomes ABDUS^v). The observed meiotic pairing in its PMCs was close to that reported by Driscoll and Quinn (1970). As the amount of pairing was very low and there was no evidence that rare bivalents observed were between chromosomes of wheat and *Aegilops* it appeared that the probability of recovering a recombination line resistant to the nematode was low and that resistant genotypes that could be selected in the progenies would be addition line(s).

In F₃, F₄ and F₅ of BC₂ at the origin of the homozygous resistant line (6.49.18.11.4) all the plants were found to be resistant. The plant F₂ of BC₂ 6.49.18 was therefore homozygous for resistance. In F₃, F₄ and F₅ meiotic chromosome pairing was analysed in 1 plant. It appears that meiotic regularity was comparable to that of Chinese Spring or Lutin indicating that homozygosity

Table II. Meiotic chromosome pairing in the parents and in the resistant plants selected in the progenies of the cross Chinese Spring x *Ae variabilis*. * Mean. ** Range, CS : Chinese Spring.

Material	Chromo- some, 2n	No of Cells	I	Chromosome association (mean no /cell)				Paired arms	
				II			III		IV
				Total	Rod	Ring			
F ₁ C Sx <i>Ae</i> var no 1	35	90	30.61	2.09	2.07	0.02	0.06	0.01	2.26
BC ₁ (no 6)	56	6	19.50	14.17	7.00	7.17	1.83	0.66	27.64
F ₂ of BC ₂ (6.49.17 and 6.49.18)	46								
F ₃ of BC ₂ (6.49.18.11)	42	74	0.30* (0-2)**	20.85 (20-21)	2.54 (0-7)	18.31 (14-21)			39.16
F ₄ of BC ₂ 6.49.18.11.4)	42	54	0.07 (0-2)	20.96 (20-21)	2.31 (0-5)	18.65 (16-21)			39.61
F ₅ of BC ₂ (6.49.18.11.4.8)	42	55	0.07 (0-2)	20.93 (20-21)	1.95 (0-5)	18.98 (16-21)			39.91
F ₃ of BC ₂ (6.49.17.3)	44	25	2.16 (2-4)	20.84 (19.21)	3.88 (1.8)	17.08 (13-20)		0.04 (0-1)	38.16
F ₁ of BC ₃ (6.49.17.3.3)	42	47	0.04 (0-2)	20.98 (20-21)	2.09 (0-5)	18.98 (16-21)			39.87
F ₂ of BC ₃ (6.49.17.3.3.9)	42	59	0.14 (0-2)	20.93 (20-21)	1.61 (0-7)	19.32 (14-21)			40.25
<i>Ae variabilis</i> no 1	28	57		14.00	1.79 (0-4)	12.21 (10-14)			26.21
CS	42	50	0.08 (0-2)	20.96 (20-21)	1.56 (0-5)	19.40 (16-21)			40.36
Lutin	42	64		21.00	0.92 (0-4)	20.08 (17-21)			41.08

for the alien information had no negative effect on pairing. However, this gave no further information on the nature of the alien introduction.

The resistant plants in F₁ of BC₃ from the cross between 6.49.17.3 (F₃ of BC₂) and Lutin were heterozygous for the gene(s) of resistance which is (are) dominant. The meiotic behaviour observed in the plants with 42 chromosomes including 6.4.17.3.3 was regular. More noticeably the chromosomes of 13% of the PMCs at metaphase I of meiosis formed 21 ring bivalents. This clearly showed that the transfer did not concern 1 entire chromosome or chromosomal arm of *Ae variabilis* no 1 carrying the resistance. A short alien segment was introduced into wheat so that

pairing between the chromosome carrying it and its homologue was not disturbed at all. This also resulted in chromosomal stability in the progenies of the resistant plant; indeed all the plants in F₄ and F₅ of BC₂ and in F₁ and F₂ of BC₃ were found to be euploid (2n = 42 chromosomes).

The nature of the alien introduction has not yet been defined. Did it originate spontaneously or through homoeologous recombination? Miller *et al* (1988) laid emphasis on the relatively high frequency of spontaneous non-Robertsonian translocations between *Ae comosa* 2M chromosome and its wheat homoeologous. As a low amount of meiotic pairing was found in F₁ hybrid

Chinese Spring x *Ae variabilis* no 1, the introgression might be the result of a spontaneous translocation.

At which generation did the transfer occur? The observed segregation for resistance in F₂ of BC₂ is 14 resistant: 6 susceptible. The probability that it fits with 3: 1 ratio is higher than 0.6 (table I). An event of recombination or of translocation occurred in F₁ or BC₁. It was probably in BC₁ since only 1 plant out of 4 was found to be resistant in BC₂. Moreover, the segregation in F₂ of BC₂ indicates that there is no distortion in the transmission of the gene(s) of resistance. In any case, further investigations will be made by analysing larger progenies of reciprocal crosses between Lutin and heterozygous plants.

The behaviour towards *M naasi* of the selected lines is the same as in *Ae variabilis* no 1. It is likely that all the resistance was transferred. We are checking this assumption by analysis of the segregation for resistance in the F₂ generation of a cross between a susceptible line of *Ae variabilis* and line no 1 used as the resistant parent in the programme.

A disomic addition line originating from another BC₁ plant has recently been obtained, the additional pair carrying the gene(s) of resistance (unpublished data). Biochemical marking will be performed initially to define its homeologous group and to characterize the alien information in the recombinant lines.

More backcrosses to Lutin are needed to produce a resistant isogenic line. This latter, and Lutin will be grown in a *M naasi* infested field to assess tolerance to the nematode and to check that the introgression has no negative effects on agronomical traits before using it in wheat breeding. Thus, we will be able to measure the incidence on yield due to the root knot nematode since up to now no cultivar has shown to be resistant.

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