Theor Appl Genet (1990) 79:331–336

THEORETICAL AND APPLIED GENETICS

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Resistance to *Heterodera avenae* in the rye genome of triticale

R. Asiedu*, J. M. Fisher and C. J. Driscoll*****

Waite Agricultural Research Institute, Glen Osmond, South Australia 5064

Received October 7, 1989; Accepted November 2, 1989
Communicated by J. Mac Key

Summary. The cereal cyst nematode, *Heterodera avenae* Wollenweber, is a serious pest of cereals in many countries. A high level of resistance to the unique Australian pathotype of the nematode has been demonstrated in a triticale line (T701-4-6), which was originally obtained from CIMMYT. The level of resistance is similar to that in rye cultivar, South Australian, but higher than that in the wheat line (AUS 10894), hitherto reported to have useful resistance to the Australian pathotype. The gene for resistance was located on rye chromosome 6 (*6R*) after backcrossing the T701-4-6 line to wheat and correlating the resistance with the presence of individual rye chromosomes identified by morphological, cytological, and isozyme markers. Preliminary evidence suggests that the gene is located on the long arm of *6R*. To transfer the resistance to wheat, double monosomics of *6R* and *6D* in a *ph1bph1b* homozygous background were selected from F_2 progeny from a cross of disomic *6R* substitution for *6D* to the *ph1b* mutant. Selfed seeds from these F_2 plants will be screened for wheat-rye chromosome recombinants.

Key words: *Heterodera avenae* – Resistance – Isozymes – C-banding – Recombination

Introduction

The cereal cyst nematode (*Heterodera avenae* Woll.) has been recorded in at least 31 countries (Meagher 1977) where it attacks mainly oats, wheat, barley, rye, triticale, and some wild grasses (Duggan 1961; Cook 1982). Of the ten or more different pathotypes differentiated world wide, a single unique pathotype, Ha13 (Andersen and Andersen 1982), has been recorded in Australia (McLeod 1976; O'Brien and Fisher 1979; Brown 1974, 1982), contrary to some earlier suggestions (O'Brien and Fisher 1974; Ellis and Brown 1976).

Plants infested with *H. avenae* suffer reduced tillering (Davies 1961), premature ear formation (Duggan 1961), and small or poorly filled heads. The assessment of direct economic injury by *H. avenae* is complicated by variations in tolerance of the cultivars, the level of soil nutrition, and crop rotation history. Nevertheless, losses of 20%–50% have been reported for different cereals in a number of countries (Fushtey and Johnson 1966; Cotten 1970; Gurner et al. 1980; Rovira and Simon 1982).

The available methods of control are crop rotation (Millikan 1938), host resistance (O'Brien and Fisher 1974), nematicides (Gurner et al. 1980), and host tolerance (Fisher et al. 1981). A combination of resistance and rotation should be sufficient for control, but only one gene for resistance has been identified in wheat (Nielsen 1966; O'Brien et al. 1980). This source of resistance may sustain a minimum of 3–5 eggs/gm of soil of the Australian pathotype, with a potential yield loss of 15%–30% in an intolerant wheat cultivar like Olympic (Fisher 1982a). Recently, a line of triticale that has a high level of resistance to *H. avenae* has been identified (Fisher 1982b), and the work reported here showed that the resistance was due to the rye chromosome *6R*. Joshi and Singh (1978) and Koebner and Shepherd (1985) have reported successful gene transfer from cereal rye to wheat through induced homoeologous recombination, and attempts are being made to transfer this resistance to wheat.

* Current address: CIMMYT, Lisboa 27, Mexico, D. F. Mexico
** To whom reprint requests should be sent
*** Current address: Sultan Qaboos University, P. O. Box 32484 Al-Khod, Muscat, Sultanate of Oman

Materials and methods

Seed stocks

Seeds of hexaploid triticale line T701-4-6 were originally obtained from CIMMYT, Mexico, and Chinese Spring lines and rye cv South Australian were obtained from Waite Institute stocks. Dr. A. J. Rathjen provided seeds of hexaploid wheat cvs Aroona and Halberd, which are susceptible to the nematode (O'Brien and Fisher 1974; J. M. Fisher, personal communication). Seeds of Spring Wheat (AUS 10894), resistant to *H. avenae* (O'Brien and Fisher 1974), were obtained from Waite Institute stocks, and Dr. K. W. Shepherd supplied seeds of *Aegilops variabilis*.

Cytology, embryo rescue, electrophoresis, and nematode assay

Examination of somatic chromosomes, C-banding, cytological analyses of meiosis, embryo rescue, and electrophoresis followed standard procedures with minor modifications. The growth medium described by Cooper and Driscoll (1985) was used for all embryo rescue. For electrophoreses of the isoenzyme alcohol dehydrogenase (ADH) (E.C. 1.1.1.1.), sample extracts were obtained from the brush end of the endosperm, leaving the embryo portion for germination. Cellulose acetate (cellogel from Chemetron, Italy) was used as the separating medium and staining followed Tanksley (1979). Seedling leaf extracts were electrophoresed in vertical polyacrylamide gel slabs for analyses of glutamate oxaloacetate transaminase (GOT) (E.X. 2.6.1.1.) (Hart 1975). The assay of Fisher (1982b) was used to screen for resistance to *H. avenae*, and each seedling was inoculated with 100 second-stage larvae per 1 ml of water on each of the five inoculation dates. When root tips were required for cytological preparations, one seminal root of each seedling was excised before planting.

For comparison of resistance, 20 seeds each of T701-4-6 and AUS 10894, 5 seeds of rye cv South Australian, and 6 of Halberd were assayed in a completely randomized design using the method of Fisher (1982b).

Plant maintenance

Plants selected from the resistance screening were transplanted into pots for tillering, seed production, and crossing. All crosses were made between plants growing in a glasshouse at approximately 20 °C and under natural light.

Chromosomal location of resistance gene

T701-4-6 was crossed in reciprocal fashion with Aroona and Chinese Spring wheats. When wheat was the maternal parent, embryo rescue of developing F_1 seeds onto artificial medium was necessary. The parents, F_1, and backcross progenies from crosses to both Aroona and Chinese Spring were subjected to cytological and the above electrophoretic analyses, in addition to screening for their reaction to *H. avenae* attack. A hairy peduncle on a mature plant was taken as evidence of the presence of rye chromosome *5R* (or at least its long arm) (Schlegel et al. 1986).

Induction of homoeologous recombination

Crosses were made between a disomic *6R(-6D)* substitution line (B83-9C-136-3-1), which was selected in this project and Sears' (1977) *ph1bph1b* mutant line, with the aim of inducing pairing and recombination of *6R* with *6D* or other wheat chromosomes. The resulting F_2 progeny were screened for the presence of *6R* and *6D* through cytology and electrophoresis, and were analyzed at meiosis for evidence of homoeologous pairing indicative of homozygosity for *ph1b*. The putative homozygotes were testcrossed to *Aegilops variabilis* to confirm their *ph1b* status by analysis of pairing behavior in their progeny.

Table 1. Comparison of T701-4-6 with standard resistance and susceptible lines

Cultivar/Line	No. of *H. avenae* females per plant: Mean ± SEM[a]	No. of seedlings tested
T701-4-6	0.40 ± 0.66	20
South Australian Rye	2.40 ± 1.98	5
AUS 10894	8.15 ± 3.96	20
Halberd	40.66 ± 9.86	6

Least significant difference (Lsd) (0.01 level) for comparing T701-4-6 with AUS 10894 = 3.78
Lsd (0.05 level) for comparing T701-4-6 with South Australian rye = 4.47
[a] SEM – Standard error of the mean

Results

Comparative resistance

Halberd, the standard susceptible cultivar, allowed the production of an average of 40.66 females per plant, while the three resistant lines restricted reproduction of the nematode (Table 1). T701-4-6 allowed the production of fewer females per plant than AUS 10894.

Chromosomal location of resistance gene

As shown in Fig. 1a and c, wheat cvs Aroona and Chinese Spring were susceptible and partially resistant, respectively, as compared to the high level of resistance in T701-4-6. All F_1 progenies were resistant even though the range of reactions of (T701-4-6 × Aroona) F_1 plants extended beyond that of T701-4-6 (Fig. 1b and d).

The zymogram phenotypes for ADH and GOT-2 in the wheat and triticale parents and for South Australian rye are shown in Fig. 2a and b. The presence of rye ADH and GOT-2 bands in backcross progenies was considered evidence for the presence of chromosomes *4R* and *6R*, respectively (Schlegel et al. 1986). Figure 2c shows a C-banded mitotic preparation of T701-4-6 with the rye chromosomes labelled, and it demonstrates that chromosome *2R* of rye has been substituted by *2D* of wheat.

Comparison of the results of rye chromosome identification with those of the nematode test showed that BC_1 plants possessing chromosome *6R* supported an average of only 1.45±0.18 females per plant (Table 2). In contrast, when *6R* was absent and the plants carried other rye chromosomes, more females developed, irrespective of the number and combination of these other chromosomes. For instance, a plant with rye chromosomes 1, 3, 4, and 7 carried 22 females, and another plant with rye

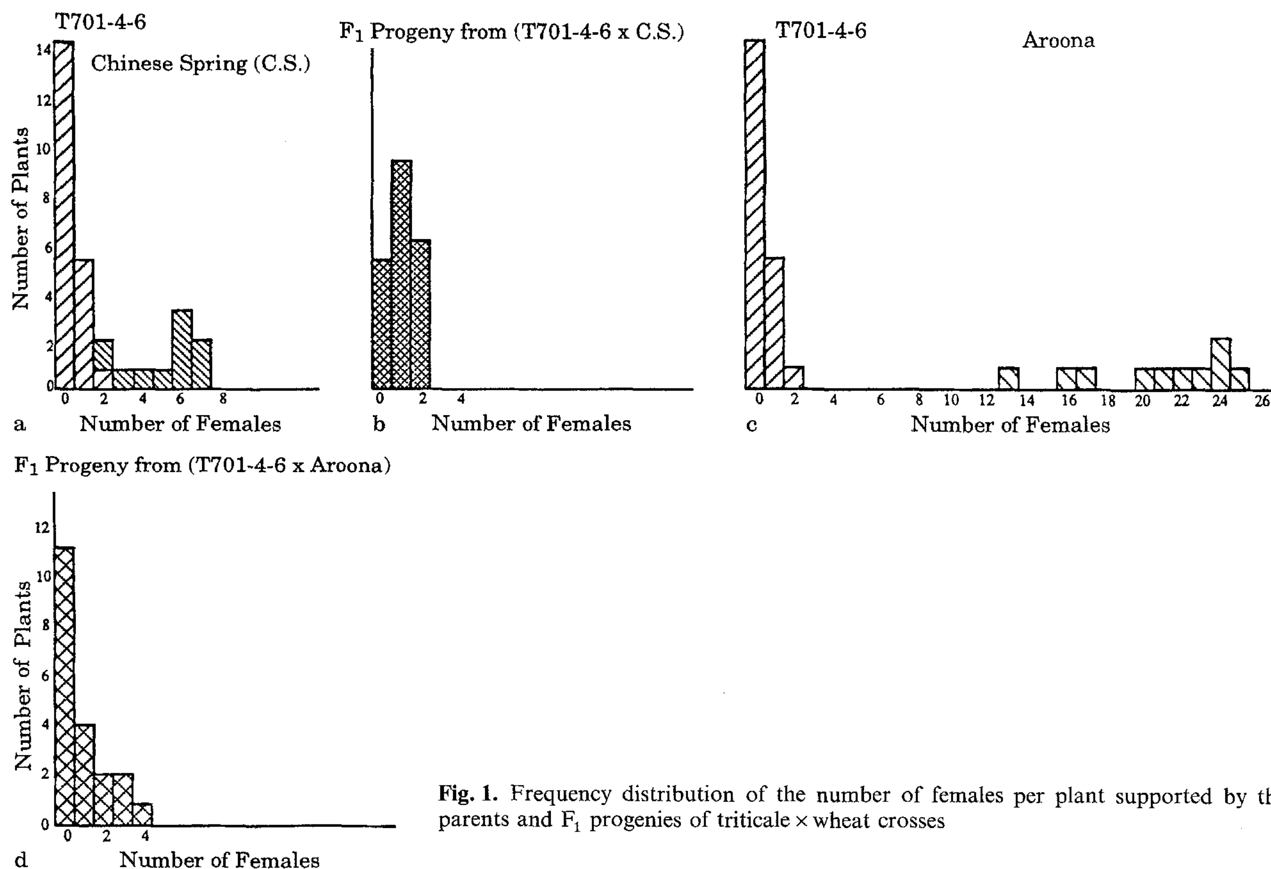

Fig. 1. Frequency distribution of the number of females per plant supported by the parents and F_1 progenies of triticale × wheat crosses

Table 2. Number of *H. avenae* females supported by parents and BC_1 plants in relation to the presence of rye chromosomes

Lines or rye chromosome present in BC_1 plants[a]	Mean	Standard error	Range	No. of plants
T701-4-6	0.50	0.22	0 – 3	16
Chinese Spring	10.68	1.03	3 – 18	16
Aroona	23.06	1.50	11 – 33	16
1R	16.74	2.30	3 – 44	23
3R	16.31	2.86	1 – 48	16
4R	19.44	2.26	1 – 44	27
5R	15.56	2.15	0 – 33	25
6R	1.45	0.18	0 – 4	53
7R	17.00	3.88	6 – 44	9

[a] Entries *1R*, *3R*, *4R*, *5R*, and *7R* refer to BC_1 plants with these respective rye chromosomes, either singly or with other rye chromosomes provided *6R* was absent. Entry *6R* refers to BC_1 plants with that rye chromosome alone or in combination with other rye chromosomes

chromosomes 1, 4, and 5 supported 31 females. However, seven BC_1 plants, five of them from crosses to Chinese Spring, which apparently did not possess chromosome *6R*, restricted female development to less than the upper limit of numbers observed in the F_1 progenies.

Eighty-nine BC_2 or BC_1F_2 plants in which *6R* was identified supported either one or no females, whereas plants without *6R* carried relatively high numbers. The influence of *6R* was also evident from progeny tests of BC_1 plants, which had only *6R* added to the complete or partial wheat chromosome complement. Progenies which carried *6R* proved resistant (mean of 0.5 females per plant), whereas those without it were susceptible (mean of 21.6 females per plant). Four BC_2 plants with the long arm of *6R* (i.e., *6RL*) and another with an isochromosome of that only carried 0–2 females per plant.

Introduction of homoeologous recombination

One resistant BC_1F_2 derivative (B83-9C-136-3-1) from crosses involving Chinese Spring showed a meiotic metaphase 1 configuration of mostly 20 bivalents of wheat and 1 of rye (from C-banding). Its GOT-2 isozyme pattern confirmed the presence of *6R* and the absence of *6D* of wheat (pattern identical to that of T701-4-6 parent). From cytological and/or isozyme (GOT-2) analyses of 100 F_2 progeny of crosses between this *6R(-6D)* substitution line and Sears' (1977) *ph1bph1b* mutant line, 24 were selected as having both *6R* and *6D*, out of which 19 had the required total chromosome number of 42. Most of these F_2 plants had some pollen mother cells (PMCs)

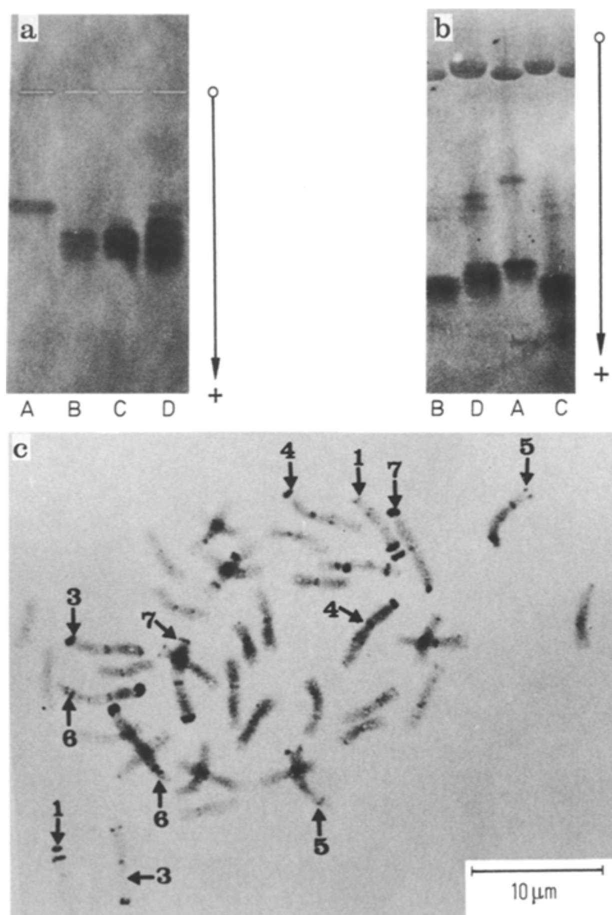

Fig. 2. **a** Alcohol dehydrogenase zymogram phenotypes. **b** Glutamate oxaloacetate transaminase zymogram phenotypes. *A* S.A. Rye; *B* Chinese Spring wheat; *C* Aroona wheat; *D* T701-4-6. The *arrows* indicate the direction of current flow. **c** A C-banded mitotic metaphase preparation of T701-4-6. The rye chromosomes present are labelled with their homoeologous group numbers

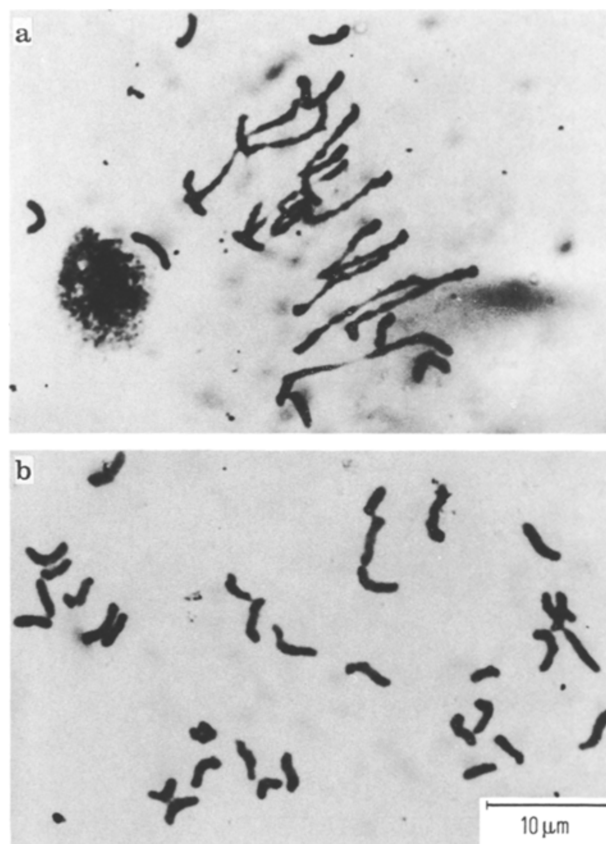

Fig. 3a and b. Meiotic configuration in wheat × *Aegilops variabilis* hybrids. **a** High pairing. **b** Low pairing

with multivalents at first meiotic metaphase, so it was difficult to use that criterion (Sears 1977) for the selection of *ph1b* homozygotes. The mean meiotic metaphase 1 configurations (based on 50 PMCs each), which were typical of most F_2 plants were:

$$1.85^{I*}\,(0-5)^{\#} + 2.20^{II}\,(0-6) + 17.00^{II**}\,(12-20) + 0.51^{III}\,(0-2) + 0.04^{IV}\,(0-1)$$

^{I*} = univalents; $^{\#}$ = range; ^{II} = open bivalents; ^{II**} = closed bivalents; ^{III} = trivalents; ^{IV} = quadrivalents.

However, there were four plants which, on the basis of 50 PMCs each, had the following mean configuration:

$$2.92^{I}\,(1-5) + 5.21^{II}\,(2-11) + 13.82^{II**}\,(8-17) + 0.14^{III}\,(0-1) + 0.07^{IV}\,(0-1).$$

Owing to the higher incidence of univalents and open bivalents in the PMCs, these four plants were classified as probable homozygous *ph1b* plants. Their genotype was confirmed by listing at least four progeny in each case from crosses to *Ae. variabilis*, and all progeny showed high levels of pairing at MI (Fig. 3a). In contrast, progeny from four F_2 plants showing normal meiotic chromosome pairing when crossed to *Ae. variabilis* gave progeny showing extremely low frequencies of pairing (Fig. 3b).

From the meiotic analyses of the four F_2 plants, one of them was found to be disomic for *6R* and most probably monosomic for *6D*, unless there had been a univalent shift, since the total number of chromosomes was 43. The other three plants were monosomic for both *6R* and *6D* and produced a total of 1,220 selfed seeds, all of which are available to be screened for recombination products.

Discussion

The resistance of T701-4-6 to CCN was superior to that of AUS 10894 and comparable to that of rye cv South Australian, although the small sample of rye necessitates caution in any close comparison. The degree of susceptibility of Halberd is similar to that reported by O'Brien

et al. (1980), but more females were produced on AUS 10894 than they obtained. Genetic differences between seed lots of AUS 10894, better nutritional status, and/or growing conditions of the test plants may explain the apparent inconsistency. The assay used in this experiment (Fisher 1982b) is a modification of that used by O'Brien et al. (1980) to improve the nutritional status of the plants and reduce the stress of the invading larvae on them.

The reaction of the F_1 plants suggests dominant action of the resistance gene in T701-4-6 (Fig. 1). Reactions of F_1 and backcross progenies from crosses of T701-4-6 to two susceptible triticale lines support this conclusion and further suggest monogenic inheritance (Asiedu 1986). The wider range of reaction of the (T701-4-6 × Aroona) F_1 plants giving progeny of 0–4 females per plant compared to 0–2 for the resistant parent (Fig. 1) may be attributed to the change in the genetic background of the resistance gene and/or hemizygosity of the gene (Ellis and Brown 1976).

The complete correlation between the presence of chromosome 6 of rye (*6R*) and resistance to the nematode (Table 2) suggests strongly that this chromosome carries the gene for resistance. Occasional BC_1 plants with chromosomes *1R*, *3R*, *4R*, or *5R* but lacking *6R* also produced low numbers of females (Table 2). However, considering the range of reaction of Chinese Spring (Table 2), it is not surprising that five out of the seven plants involved were derived from crosses to that line. These five plants could just be expressing the Chinese spring gene(s). It can only be speculated that the remaining two BC_1 plants either partially escaped infection or contained unidentified translocations of the critical segment of *6R*. It should be noted that the low numbers of females on those plants were not associated with any specific combinations of chromosomes. Different combinations of all the rye chromosomes, excluding *6R*, were found in very susceptible plants (i.e., plants carrying more than 20 females per plant). Thus, while some plants might have partially escaped infection and, therefore, produced only a few females, the presence of numerous females on a plant with a particular rye chromosome is good evidence against that chromosome being the critical one. Such results were obtained with all the rye chromosomes except *6R* (Table 2). The influence of *6RL* alone on resistance could not be conclusively established because a short arm telocentric for *6R* was not positively identified in any susceptible plants. Further tests are required to confirm the arm location of the resistance gene.

The occurrence of multivalents in PMCs of many of the F_2 plants at first meiotic metaphase could have resulted from translocations in the *ph1bph1b* mutant stock that was used. The choice of the four probable *ph1b* homozygotes on the basis of higher incidence of univalents and open bivalents at meiosis (Driscoll et al. 1979; Giorgi 1983; Yacobi and Feldman 1983; Koebner and Shepherd 1985) was verified by the meiotic configurations of F_1 hybrids from their testcrosses to *Ae. variabilis*.

Screening for recombinants in the progeny of these plants could be done by a combination of C-banding and isozyme analysis. Only glutamate oxaloacetate transaminase has been used in this work for the identification of *6R*, but aconitate hydratase, esterases, phosphogluconate dehydrogenase, and dipeptidase may be successfully used. The loci for all these isozymes are on the long arm of *6R* which, from the limited evidence available, may carry the gene for resistance to *H. avenae*. If however, the short arm is found to be critical, the locus of aminopeptidase and the heterochromatic telomere may be relied upon for selection of recombinants. Also, one of the isozymes on the long arm may be sufficiently close to the centromere to be useful for monitoring recombination in the short arm.

Acknowledgements. The senior author wishes to acknowledge the financial support of a University of Adelaide Scholarship for Postgraduate Research.

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