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A cereal cyst nematode (*Heterodera avenae* Woll.) resistance gene transferred from *Aegilops triuncialis* to hexaploid wheat

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Abstract The cereal cyst nematode (*Heterodera avenae*) is an important root parasite of common wheat. A high level of resistance was transferred to wheat from *Aegilops triuncialis* (TR lines) using the cross [(*T. turgidum* × *Ae. triuncialis*) × *T. aestivum*]. Low fertility (3–5 viable kernels per plant) was observed during the process but the surviving hybrid plants were highly vigorous. To obtain stable resistant lines further crosses to *T. aestivum* were performed. The resistance in TR lines seems to be transferred from the C genome of *Ae. triuncialis* (genomes CCUU). *Ae. triuncialis* was highly resistant to the two Spanish populations of *H. avenae* tested, as well as to four French races and two Swedish populations. The histological analysis showed a hypersensitive reaction in the roots of a resistant TR line inoculated with the Ha71 pathotype of *H. avenae*, whereas well-formed syncytia were observed in the roots of the susceptible control. Resistance to the *H. avenae* Ha71 pathotype seemed to be inherited as determined by a single dominant factor in the crosses between resistant TR lines and susceptible cultivars.

Key words *Aegilops triuncialis* · *Triticum aestivum* · *Heterodera avenae* · Cereal cyst nematode · Resistance gene

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Introduction

The transfer of genetic material from wild species to cultivated plants has been extensively exploited as a way to introduce interesting agronomic traits, such as resistance to different diseases, into highly productive cultivars. Alien gene transfer through hybridization with various grass genera has contributed significantly to the genetic improvement of hexaploid bread wheat (*Triticum aestivum* L., 2n = 6x = 42). The wheat grass genus *Aegilops* has been a particularly valuable source for wheat improvement.

The cereal cyst nematode *Heterodera avenae* is a severe pest of wheat causing a great loss in yield all over the world. To some extent, crop rotation and nematicides can reduce the soil-borne nematode population. However, both economically as well as for environmental reasons, the best way to avoid crop damage is to use resistant cultivars to the cereal cyst nematode (CCN). Because the nematode depends on wheat for its survival, resistant cultivars have generated a strong selection pressure on CCN populations to overcome resistance. This has resulted in the evolution of new virulent genes in the nematode and has continuously forced nematologists and wheat breeders to find new resistance genes. However, few sources of genetic resistance to CCN in the hexaploid wheat *T. aestivum* are available. To-date, only one resistance gene, *Cre1*, has been found in the cultivated wheat line Aus 10894/Loros (Slootmaker et al. 1974; O'Brien et al. 1980), and wild cereal species possessing resistance have been used for the introgression of this trait into wheat cultivars. The resistance gene *Cre2*, which could be the same to that of *CreX* (Jahier et al. 1996), has been transferred from *Aegilops ventricosa* to hexaploid wheat in the H-93-8 line (Delibes et al. 1993), and two different resistance factors (*Cre3* and *Cre4*) from *Triticum tauschii* have also been introduced into wheat (Eastwood et al. 1991). Other potential sources of

resistance to CCN have been recognized including: *Aegilops variabilis* (Rivoal et al. 1986; Brown 1974), *Aegilops triuncialis* (Brown 1973), *Aegilops umbellulata* (Rivoal et al. 1986) and cultivated rye (Asiedu et al. 1990).

Previous work from our laboratory has shown the effectiveness of a method to transfer genes from the wild grass *Ae. ventricosa* to the cultivated wheat *T. aestivum* (Doussinault et al. 1983; Mena et al. 1993). The strategy involves a cross of the donor species, *Ae. ventricosa* (genomes $D^vD^vM^vM^v$) with *T. turgidum* (AABB), which acts as a bridge, followed by the rescue of the sterile ABD^vM^v hybrid with pollen from the recipient species *T. aestivum* (AABBDD). Plants from this cross were fertile and stable lines with 42 chromosomes were derived from them after repeated selfing. Different studies have shown that genes from the donor species had been incorporated into the transfer lines both by chromosomal substitution and by recombination (Mena et al. 1993). Using this approach, resistance genes to the fungi *Pseudocercosporella herpotrichoides* and *Erysiphe graminis*, to the insect *Mayetiola destructor*, and to the cereal cyst nematode *Heterodera avenae* (CCN), have all been introduced into wheat chromosomes (Doussinault et al. 1983; Delibes et al. 1987, 1993, 1997).

We now report the application of the above described breeding scheme using *Ae. triuncialis* (genomes CCUU) as a donor species, with the same bridge and recipient species as before, to introduce resistance to the cereal cyst nematode into wheat. In this cross, enhanced homoeologous recombination should occur due to the known ability of the C genome to suppress the Ph diploidization mechanism of *T. aestivum* and *T. turgidum* (Kimber and Feldman 1987).

Materials and methods

Plant material

The breeding scheme used in the genetic transfer is represented in Fig. 1. *T. turgidum* H-1-1 (genomes AABB), used as the bridge species, was crossed with an accession of *Ae. triuncialis* A-1 (genomes CCUU) resistant to CCN, and the resulting hybrid (ABCU), which was male-sterile, was rescued with pollen from the recipient species (*T. aestivum* H-10-15, genomes AABBDD). After eight rounds of selfing, plants with 28–41 chromosomes were obtained (TR lines). One of them, (TR-353) with 41 chromosomes, was employed as a donor and different commercial wheats were used as alternative recurrent parents to obtain lines that are resistant to CCN in a hexaploid wheat background. All crosses were done in a greenhouse by standard manual procedures. Two generations per year were obtained and BC families were classified as resistant or susceptible to the Ha71 pathotype of *H. avenae*.

Cytological and histological observations

Somatic chromosomes were counted in root tips of germinating seeds, stained by the Feulgen reaction, after chromosome contraction in ice water for 48 h.

Histological studies were done as previously described (Delibes et al. 1993).

Tests of resistance to the nematode

Tests for resistance to CCN, under field conditions, were carried out in a naturally infested plot at the "La Higueruela" Experimental Station in Santa Olalla (Toledo, Spain) where the top layer of soil was previously homogenized before sowing.

Tests for resistance were also conducted in a greenhouse at a temperature of 16–18° C and a 90–95% relative humidity. Vernalization at 4–6 °C for 4 weeks under artificial light resulted in uniform plant size and seed production. Individual plants were grown in a greenhouse in pots filled with a mixture of sand and soil infested with cysts of the Ha71 pathotype from "La Higueruela". A comparison of the resistance of cultivars with different resistant genes was made by transplanting individual plants into pots filled with sterilized soil that were inoculated with cysts of a given pathotype.

The evaluation of the resistance was essentially as described previously (Delibes et al. 1993) and different wheat cultivars were used as susceptible controls.

For the analysis of the segregating populations in the field, individual seeds were space-planted and resistant to susceptible plant ratios were compared to appropriate expectations using chi-square tests.

Results and discussion

The transfer scheme

Three kernels of the intermediate hybrid between *T. turgidum* H-1-1 and *Ae. triuncialis* A-1 were obtained, and two of them gave plants that were viable and vigorous, but male-sterile. Seven spikes from these plants were pollinated with *T. aestivum* cv Almatense (H-10-15), yielding eight kernels (Fig. 1). These seeds were sown and selfed, and their progeny subjected to eight additional rounds of selfing. During all stages of this process 90% of the kernels germinated normally and, although 20–40% of the plants died in the greenhouse, the surviving plants were notably vigorous. These plants had low fertility, yielding only 3–5 kernels per viable plant, without any appreciable increase in fertility in the more advanced generations.

Somatic chromosomes were counted in mitotic roots of plants at different stages. Plants with 28–41 chromosomes were observed in early generations, whereas in the last two generations 95% of the plants had 40 chromosomes and only about 5% had 41 chromosomes.

The fertility observed in the progeny of this transfer experiment was significantly lower than in the cross involving *Ae. ventricosa* ($D^vD^vM^vM^v$). This could be due to the fact that the D^v genome of *Ae. ventricosa* is closer to the D genome of hexaploid wheat than either of the two different genomes (CCUU) present in *Ae. triuncialis*. This probably leads to a more regular meiosis in the former case and to a more extensive chromosomal rearrangement in the present case, due to

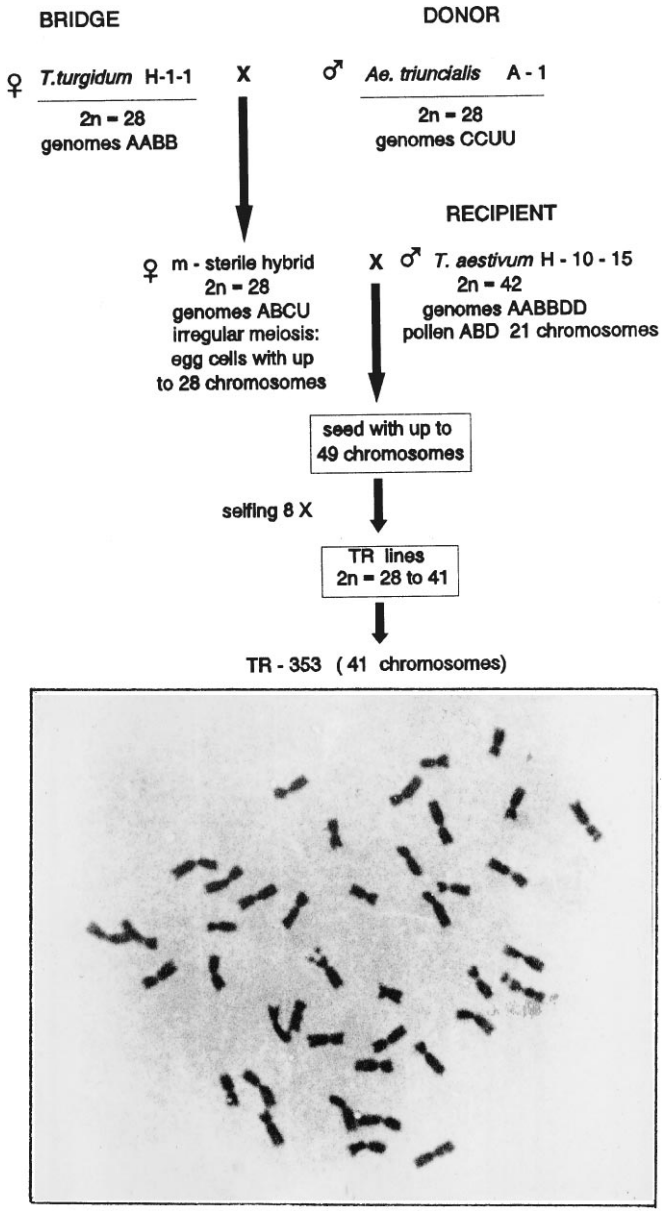

Fig. 1 Scheme followed for the genetic transfer from *Ae. triuncialis* (syn. *T. triunciale*) to hexaploid wheat. In the photograph the 41 chromosomes of TR-353 line obtained by this procedure are shown

the Ph-suppressing effect of the C genome (Kimber and Feldman 1987). Additionally, Endo and Gill (1996) have shown that when a particular chromosome from *Ae. cylindrica* or *Ae. triuncialis* was present in *T. aestivum* cv Chinese Spring in a monosomic condition, chromosomal breakages occurred in the gametes without the alien chromosome, generating various aberrations including deletions. This would explain why we found total sterility in the plants from a cross [(*T. turgidum* × *Ae. cylindrica*) × *T. aestivum*] that had been done simultaneously to that of *Ae. triuncialis.*

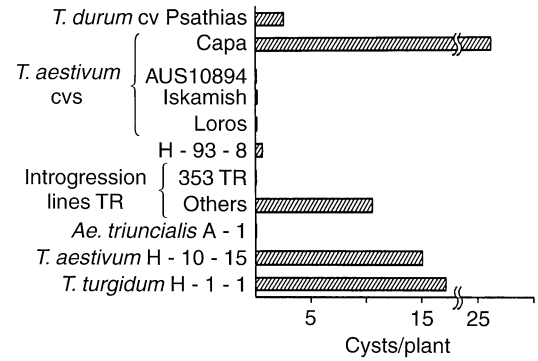

Fig. 2 Evaluation of the susceptibility to *H. avenae*, under field conditions, of introgression lines TR, their parentals, wheat cultivars with other known sources of resistance, with *T. aestivum* cv Capa as a susceptible control. Averages of 6–10 plants per stock are represented, except for the 20 TR lines with intermediate resistance, whose overall average is presented

CCN resistance to the Ha71 pathotype

Screening for CCN resistance among the 49 TR lines obtained from the cross [(*T.turgidum* H-1-1 × *Ae.triuncialis* A-1) × *T.aestivum* H-10-15] was carried out in a naturally infested field with pathotype Ha71 by counting the female nematodes on mature roots using stereoscopic microscopy. Beside TR lines and their progenitors, also included in the test were wheat cultivars with known sources of resistance such as the H-93-8 introgression line (*Cre2*), *T. aestivum* cvs Loros, Iskamish and Aus 10894 (*Cre1*), *T. durum* cv Psathias, and the susceptible wheat cultivar *T. aestivum* cv Capa used as a control. The results of this test are summarized in Fig. 2. Nine TR lines were not infested and one of them (line TR-353 with 41 chromosomes) was selected for further study because of its good performance in the tests for resistance in all the selfing rounds. The susceptibility of the other TR lines tested was in the same range as that of the H-10-15 wheat parent which, as previously described (Delibes et al. 1993), was a poor host for the nematode. A maximum of two or three cysts were developed on the roots of *Ae. triuncialis* and wheat cultivars carrying other resistant genes, except for *T. durum* cv Psathias which, although it was resistant, showed a higher level of infestation.

From these data it was not possible to decide whether the resistance had been transferred to TR lines from the C or from the U genome of *Ae. triuncialis.* Figure 3 shows the evaluation of susceptibility, under field conditions, of *Aegilops* belonging to the sections Cylindropyrum, Polylides, Comopyrum and of *Ae. ventricosa* from the Vertebrata section. All of them appeared with little or no infestation except for *Ae. umbellulata* (genome UU) which, although with a low number of cysts, presented the highest level of infestation. This result agrees with that of Rivoal et al.

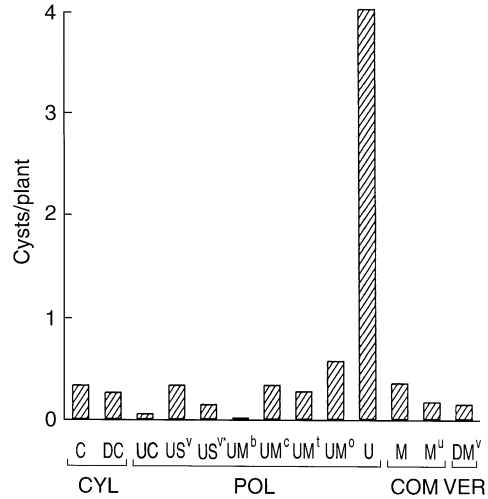

Fig. 3 Degree of infestation (cysts/plant), under field conditions, by *H. avenae* (pathotype Ha71) of different accessions of *Aegilops* belonging to the sections: Cylindropyrum [*Ae. caudata* (*C*), *Ae. cylindrica* (*DC*)]; Polylides [*Ae. umbellulata* (*U*), *Ae. ovata* (*UM*^{o}), *Ae. triaristata* (*UM*^{t}), *Ae. columnaris* (*UM*^{c}), *Ae. biuncialis* (*UM*^{b}), *Ae. variabilis* (*US*^{v}), *Ae. kotschyi* (*US*^{v*}), *Ae. triuncialis* (*UC*)]; Comopyrum [*Ae. comosa* (*M*), *Ae. uniaristata* (*M*^{u})] and Vertebrata [*Ae. ventricosa* (DM^{v})]. Averages of 10–20 plants per stock are represented. The *Aegilops* set was from the collection at I.N.I.A. (Madrid, Spain), kindly provided by Dr. E. Sánchez-Monge

(1986), who showed a lower level of resistance against Fr_{2-4} French races in three lines of *Ae. umbellulata* than in the hybrids between *T. aestivum* and *Ae. variabilis* (genomes UUS^{v}S^{v}). No significant differences were found between the levels of resistance in *Ae. comosa* (genome CC) and *Ae. triuncialis* (genomes CCUU). *Ae. comosa* had been earlier described as a poor host for the Fr1 and Fr4 races (Dosba and Rivoal 1982). These data and those of previous reports, where resistance genes in genomes M, D and S were described (Brown 1974; Delibes et al. 1993; Eastwood et al. 1991), suggest that the nine resistant TR lines carry, at the very least, a major resistance gene present in the C genome from *Ae. triuncialis.*

Histological examination was carried out on the roots of the TRrd-2 resistant line (derived from one BC_7, TR-353/7* *T. aestivum*), and of *T. aestivum* cv Anza used as a susceptible control, 7 days after inoculation with CCN in a growth chamber. Microscopic evidence shows that syncytia induced by the nematode infection were greatly developed in the vascular cylinder of roots of cv Anza (Fig. 4A). In the roots of the resistant line a hypersensitive reaction was observed, vacuolized and degraded syncytia were located outside the stele and affected the pericycle, the endodermis, and the inner layer of cortical cells. In the pericycle and endodermis some cells were necrotized as a consequence of the hypersensitive reaction (Fig. 4B). Differences in morphology and location between syncytia may be related to the degree of tolerance or resistance

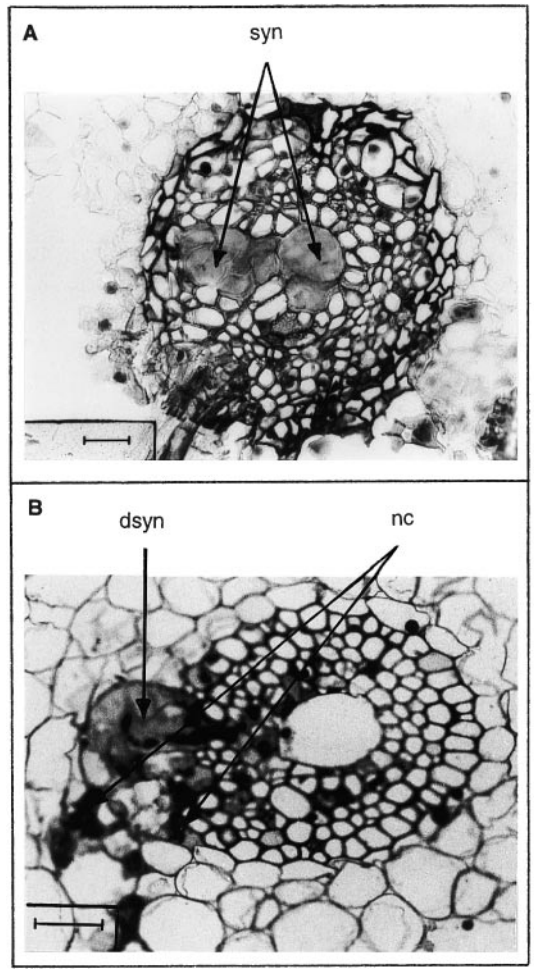

Fig. 4A, B Histological sections of parasited roots by CCN 7 days after inoculation. **A** Susceptible wheat (*T. aestivum* cv Anza); **B** resistant line (TRrd-2); *syn*, syncytia; *dsyn*, degraded syncytia; *nc*, necrosis

of the plants (Kim et al. 1986). The roots of the TRrd-2 line invaded by CCN showed the typical response of resistant plants, as described by Bleve-Zacheo et al. (1995).

Resistance to different pathotypes

Resistance of *Ae. triuncialis* to other Spanish and foreign populations was investigated by adding isolated cysts from different sources to the sterile soil in which the plants had been previously sown. The differential resistance coming from *Ae. triuncialis* and from other sources is summarized in Table 1. It shows that *Ae. triuncialis* is resistant to all populations of *H. avenae* tested, as well as to populations from Sweden (virulent to genes *Cre1* and *Cre2*) and to the Torralba de Calatrava Spanish population (virulent to gene *Cre1*). The results for the French race Fr1 during 2

Table 1 Resistance of *Ae. triuncialis* to different populations of *H. avenae* in comparison with other sources of resistance. P_1 from Santa Olalla, Spain (Sanchez and Zancada 1987), P_2 from Torralba de Calatrava, Spain (Valdeolivas and Romero 1990; Lopez-Braña et al. 1996; Romero et al. 1996), Fr1 to Fr4, France (Rivoal 1977) supplied by this author, N. Harene (Ireholm 1994) and Etelhem from Sweden supplied by A. Ireholm. *R*, resistant; (*R*) moderatly resistant; *S* susceptible

Source of resistance	Gene	Pathotype						
		FR			SP		SW	
		Ha11 Fr3	Ha41 Fr1	Ha12 Fr2–4	Ha71 P1	Torralba P2	N.Harene HgI	Etelhem HgIII
Ae. triuncialis A-1	*CreAet*	R	?	R	R	R	R	R
T. aestivum cv Loros	*Cre1*	R	R	R	R	S	(R)-S	(R)-S
Ae. ventricosa No. 11	*Cre2*	R	R	R	R	R	S	S

consecutive years were not conclusive and need to be confirmed.

Inheritance of CCN resistance

The number of genes transferred from *Ae. triuncialis* and their effectiveness in conferring resistance to *H. avenae* pathotype Ha71 was determined by studying the segregation of resistance in the $BC_{5-6}F_2$ families. These families were obtained by backcrossing line TR-353 as a donor parent with different cultivars of *T. aestivum* as alternative recurrent parents. In each backcross generation resistant plants (heterozygous for this trait) were selected, several BC_{5-6} plants were selfed and the seeds obtained were then used for segregation analysis. The degree of infestation of 261 individual plants, developed from these seeds, and of 49 plants of *T. aestivum* cv Anza used as a susceptible control was determined (Fig. 5). It is difficult to establish the demarcation point between resistance and susceptibility, but the most common criterion is to use 20% of the maximum infestation level. According to this criterion, F_2 plants were classified into resistant and susceptible using a threshold of infestation of 18 cysts/plant, equivalent to 20% of the maximum infestation level and very close to 50% of the infestation average of the control susceptible *T. aestivum* cv Anza. A 3:1 segregation of resistant versus susceptible plants was obtained [$\chi^2 = 3.587 < \chi^2 = 3.84$ ($df = 1$; $P = 0.05$)], which is compatible with the hypothesis of a single dominant resistance gene. This hypothesis was also suggested in the segregation analysis of 24 plants from F_2 progeny of one cross between the resistant line TRrd-5 (derived from one BC_7, TR-353/7* *T. aestivum*) and one susceptible line TRsd-1 of analogous origin (Fig. 6). F_2 plants were classified into resistant and susceptible using the same limit as before (18 cysts/plant) and a 3:1 segregation was also obtained [$\chi^2 = 0.88 < \chi^2 = 3.84$ ($df = 1$; $P = 0.05$)]. The infestation degree of the F_1 generation from this cross was determined in the greenhouse to take in advance one generation. A low level of inoculum was used in these conditions and the infestation in the hybrid was one cyst/plant, which is also consistent with the hypothesis of a dominant gene for resistance in this line. The infestation level in both resistant and susceptible plants was higher in the field than in the greenhouse. This result is similar to that of Eastwood (1995) who showed that there is an environmental component to the expression of resistance derived from *Ae. ventricosa* and that this may depend on inoculum density. We have also observed a lower expression of resistance in a wheat than in an *Aegilops* background, which agrees with previous reports (Delibes et al. 1993; Eastwood 1995).

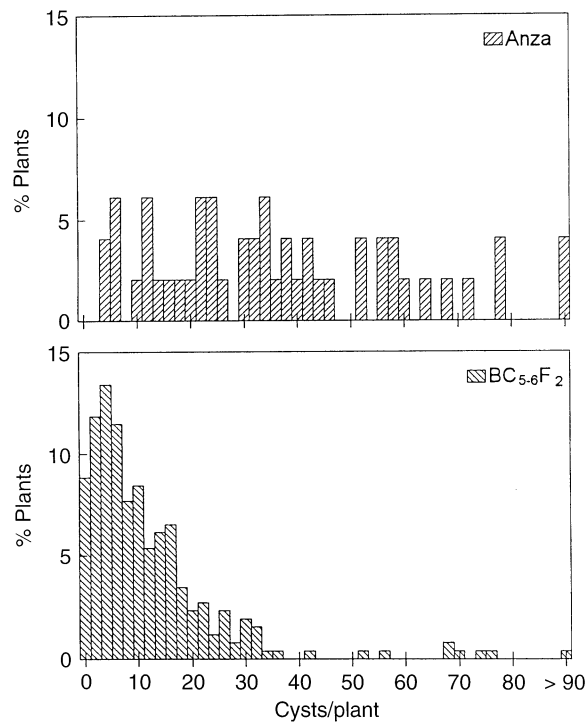

Fig. 5 Distribution of 261 plants obtained by selfing of heterozygous resistant plants (BC_{5-6}) with respect to CCN infestation. In the upper panel the degree of infestation of *Triticum aestivum* cv Anza (49 plants) as a susceptible control is shown

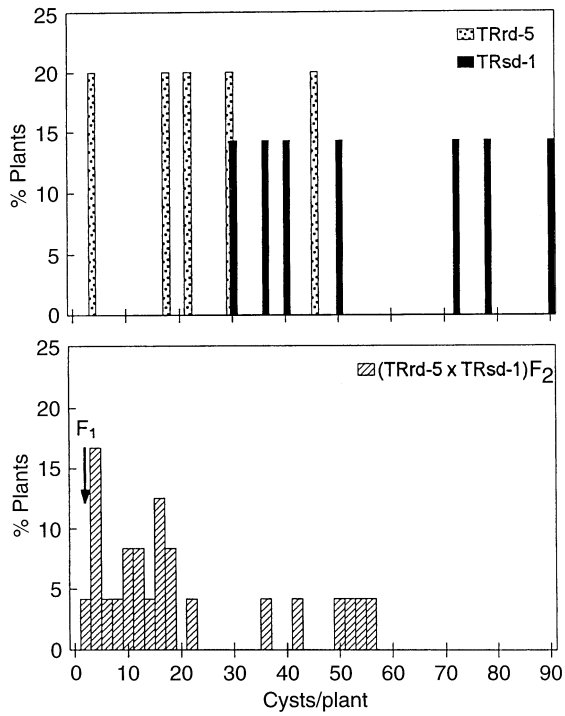

Fig. 6 Distribution of (TRrd-5 × TRsd-1) F_2 progeny and their parents (upper panel) with respect to CCN infestation. The *arrow* indicates the average number of cysts/plant of (TRrd-5 × TRsd-1) F_1

The crosses of line TR-353 with several wheats cultivars and breeding lines showed that it is possible to produce a sufficient number of viable and fertile progeny for efficient gene transfer.

In conclusion, the single resistance factor derived from *Ae. triuncialis*, which it is tentatively designated as *CreAet*, may represent a new source of genetic resistance to *H. avenae* available to wheat breeders.

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