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Resistance to the cereal cyst nematode (*Heterodera avenae* Woll.) transferred from the wild grass *Aegilops ventricosa* to hexaploid wheat by a "stepping-stone" procedure

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Abstract. Transfer of resistance to *Heterodera avenae*, the cereal cyst nematode (CCN), by a "stepping-stone" procedure from the wild grass *Aegilops ventricosa* to hexaploid wheat has been demonstrated. The number of nematodes per plant was lower, and reached a plateau much earlier, in the resistant introgression line H93-8 (1–2 nematodes per plant) than in the recipient H10-15 wheat (14–16 nematodes per plant). Necrosis (hypersensitive reaction) near the nematode, little cell fusion, and few, often degraded syncytia were observed in infested H93-8 roots, while abundant, well-formed syncytia were present in the susceptible H10-15 wheat. Line H93-8 was highly resistant to the two Spanish populations tested, as well as the four French races (Fr1–Fr4), and the British pathotype Ha11, but was susceptible to the Swedish pathotypes HgI and HgIII. Resistance was inherited as though determined by a single quasi-dominant factor in the F_2 generations resulting from crosses of H93-8 with H10-15 and with Loros, a resistant wheat carrying the gene *Cre1* (syn. *Ccn1*). The resistance gene in H93-8 (*Cre2* or *Ccn2*) is not allelic with respect to that in Loros. RFLPs and other markers, together with the cytogenetical evidence, indicate that the *Cre2* gene has been integrated into a wheat chromosome without affecting its meiotic pairing ability. Introduction of *Cre2* by backcrossing into a commercial wheat backgroud increases grain yield when under challenge by the nematode and is not detrimental in the absence of infestation.

Key words: Wheat – *Aegilops ventricosa* – *Heterodera avenae* – Cyst nematode – Resistance gene

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Introduction

The cereal cyst nematode (CCN), *Heterodera avenae* Woll., is a major economic constraint in many important wheat growing areas of the world. The use of nematocides to control CCN is not advisable because of health and environmental problems, as well as for economical reasons, and it is generally agreed that resistant cultivars are a key to effective methods for the reduction of nematode populations below damaging levels. There are few sources of genetic resistance to CCN in hexaploid wheat, *Triticum aestivum*. A dominant allele at a locus (*Cre1* or *Ccn1*) in chromosome 2B has been characterized in the line Aus 10894/Loros, which has been extensively used as a source of resistance in breeding programmes (Slootmaker et al. 1974; O'Brien et al. 1980). Wild grasses, such as *Aegilops ventricosa* (Dosba et al. 1978), *Ae. squarrosa* (Eastwood et al. 1991), and *Ae. triuncialis* (Brown 1973), as well as cultivated rye, *Secale cereale* (Asiedu et al. 1990), have been long recognized as potential sources of CCN resistance for wheat, although the genetic basis of their resistance has not been investigated and chromosomes of the alien species do not usually recombine with those of wheat.

In *Ae. ventricosa* (genomes $D^vD^vM^vM^v$), we have demonstrated that genes from the D^v genome appear with high frequency (30–60%), and those from the M^v genome at low frequency (< 4%), in wheat introgression lines obtained by a "stepping-stone" procedure which involved an intermediate hybrid between the donor and a bridge species (Delibes and García-Olmedo et al. 1984; Delibes et al. 1977; Doussinault et al. 1983; García-Olmedo et al. 1984). As shown in Fig. 1, certain egg cells from the non-self-fertile hybrid yield seeds when pollinated by the recipient species,

T. aestivum (AABBDD). Plants from these seeds were fertile and, after repeated selfing, stable lines with 42 chromosomes (H-93 lines) were derived from them. Using RFLPs and other makers, it has been shown that gene transfer between the D^v and D genomes occurred by recombination, while that from the M^v genome involved both the introgression of chromosomal segments and chromosome substitutions (García-Olmedo et al. 1984; Mena et al. 1993). The only previously reported direct hybrid between the donor and the recipient species was male sterile (Dosba and Cauderon 1972). This hybrid would probably have been a less adequate intermediate because it had all the genomes present in hexaploid wheat (ABD) and, when crossed with ABD pollen, would have had a greater tendency to eliminate the M^v genetic material while selecting for the euploid chromosome number (2n = 42). We now report the transfer of CCN resistance from *Ae. ventricosa* to hexaploid wheat and the inheritance of this resistance as a single Mendelian factor (*Cre2* or *Ccn2*) which is not an allele of the *Cre1* gene in Loros/Aus 10894.

Materials and methods

Biological materials

Lines H93-1 to H93-70 and their progenitors, *Triticum aestivum* cv Almatense H10-15, *T. turgidum* H1-1, and *Ae. ventricosa* AP-1 (see Fig. 1) were originally obtained from M. Alonso Peña (Cuenca, Spain), who carried out the initial crosses. Commercial wheats were from the collection at IRTA (Lerida, Spain). *T. aestivum* cv Loros was the gift of S. Andersen (Copenhagen, Denmark). Genetic crosses were carried out by standard manual procedures. Introduction of the resistance from line H93-8 into the commercial wheat cv Yécora rojo was carried out by backcrossing and selection in the greenhouse. Six seeds from each resistant plant were sown for the next backcross. One resistant plant from the third backcross was selfed and 12 plants from its progeny were sown on naturally-infested soil. Two of these, which were phenotypically close to Yécora rojo and resistant to the nematode, were selected and their progeny was used for the grain-yield test in the presence or absence of nematocide (see Fig. 9).

Cysts of *Heterodera avenae* were from the following origins: the Spanish pathotype Ha71 was from Santa Olalla (Toledo, Spain) and has been described by Sanchez and Zancada (1987); a second Spanish population was from Torralba de Calatrava (Ciudad Real, Spain) and has been described by Valdeolivas and Romero (1990). French races Fr1–Fr4, described by Rivoal (1977) and later classified into pathotypes Ha41 (Fr1), Ha12 (Fr2 and 4) and Hall (Fr3), were supplied by this author; Swedish pathotypes HgI and HgIII were obtained from A. Ireholm (Alnarp, Sweden), While the Hal1 pathotype was donated by R. Cook (Aberystwyth, UK).

Nematode tests

Infestation under field conditions was investigated in a naturally-infested plot, from the "La Higueruela" Experimental Station in Santa Olalla (Toledo, Spain), whose top soil was homogeneized with a bulldozer before sowing. The comparative resistance test of line H93-8 against different pathotypes was carried out by transplanting individual plants into buried plastic cylinders (6 cm diameter, 20 cm high) that had been filled with sterilized soil and inoculated with 25 cysts of a given pathotype. Infestation under laboratory conditions was achieved by growing individual plants in small pots (6 cm diameter) which had been filled with a mixture of an artificial substrate and infested soil (50% of each) and kept humid at 5 °C for 4 weeks before planting the pre-germinated seedlings. When nematocide treatment was carried out, the seeds were impregnated with 5 cm^3/kg of methyl N, N'-dimethyl-N-(mehtylcarbamoxyl-oxy)-1-thiooxaminate (Oxamyl; Du Pont).

Histological examinations

To observe and count the nematodes during the early stages of infestation, roots were washed, stained in a boiling solution of 0.05% acid fuchsin in lactophenol, and washed in lactophenol for 12 h. For a detailed microscopic examination, portions of infested roots were fixed in 3% glutaraldehyde in 0.05 M sodium cacodylate buffer (pH 7.2) for 4 h, rinsed in the same buffer, postfixed in 2% osmium tetroxide for 4 h at 4 °C, dehydrated in an ascending ethanol series, and embedded in Spurr's medium. Semi-thin sections were cut with an LKB ultratome III, stained with toluidine blue, and observed and photographed under the light microscope.

RFLPs and other markers

RFLPs and other markers were analyzed as described in Mena et al. (1993), except for aminopeptidase isozymes (AMP-1), which were separated by isoelectric focusing and stained according to Koebner and Martin (1989), using L-leucyl-β-naphtylamide as substrate.

Results

CCN resistance in the H93-8 wheat/Aegilops introgression line

A preliminary screening for CCN resistance among introgression lines H93-1 to H93-70, obtained as indicated in Fig. 1, was carried out in a naturally-infested field by visual inspection of the roots at maturity. Line H93-8 appeared with little or no infestation, while several other lines showed low numbers of cysts. These H93 lines, together with their progenitors and several commercial wheat cultivars, were then subjected to a more quantitative test in the same field, in which the top soil had been previously homogenized. The results of this test are summarized in Fig. 2. A high level of resistance was confirmed for line H93-8, while the susceptibility of the other H93 lines tested was in the same range as that of the H10-15 wheat parent, which was the most resistant of the *T. aestivum* cultivars.

The temporal course of infestation in H93-8 was compared to those of the H10-15 parent and of the susceptible cv Anza both under field and under greenhouse conditions (Fig. 3). The development of infestation was very similar in both environments, except that it developed faster in the greenhouse because the seeds

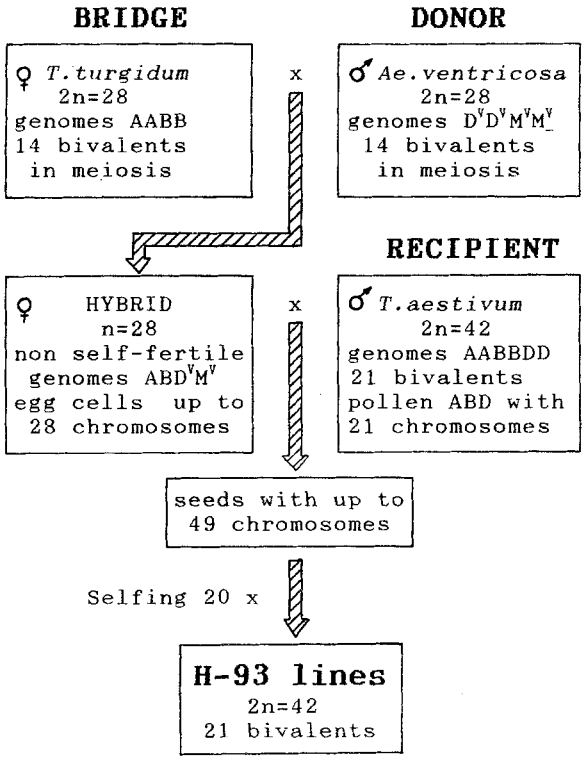

Fig. 1. Scheme of the genetic transfer from *Ae. ventricosa* to hexaploid wheat

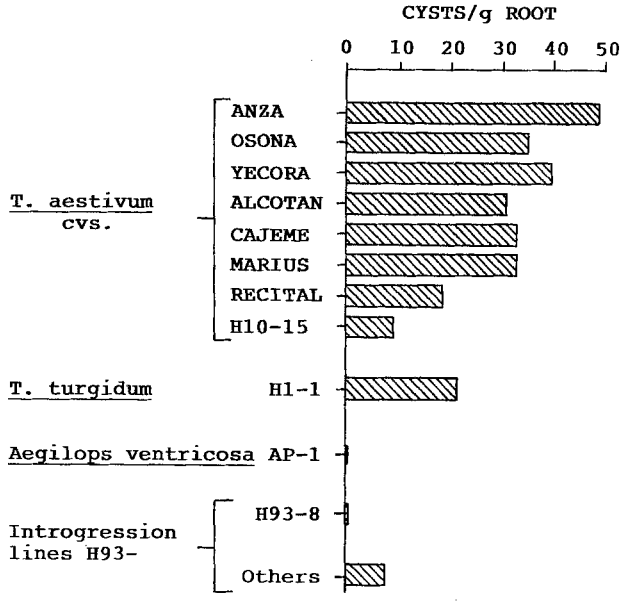

Fig. 2. Evaluation of susceptibility to the cyst nematode *Heterodera avenae* under field conditions. Averages of 5–12 plants per stock are represented, except for the 17 H93 lines with intermediate resistance, whose overall average is presented

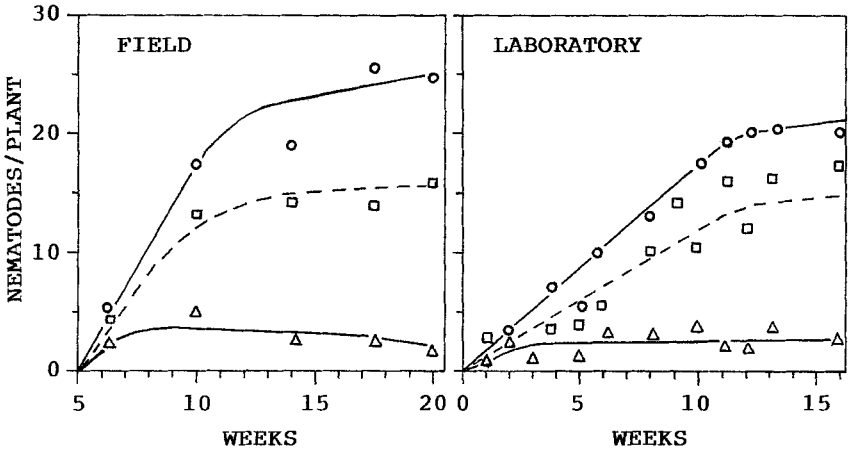

Fig. 3. Time course of infestation under field and under laboratory conditions in H93-8 (△), H10-15 (□), and cv Anza (○). Each point represents the average of five plants

had been pre-germinated and the soil had been kept humid at 4–5 °C for 5 weeks before sowing. In both experimental situations, the number of nematodes per plant was lower and reached a plateau much earlier in the resistant line, in comparison with the other two wheats.

A histological examination of the roots was carried out in greenhouse-infested H10-15 and H93-8 at 7 days and 15 days after sowing. As illustrated in Fig. 4, typical cell wall degradation and cell fusion, leading to well-formed syncytia without necrosis, were observed in the susceptible H10-15 wheat. By contrast, in the resistant H93-8 line, necrosis (hypersensitive reaction) occurred in the vicinity of the nematode and little cell fusion was observed, with few syncytia, which usually appeared as degraded and vacuolized.

The effect on grain yield of dressing the seeds with nematocide in the H93-8 and H10-15 wheats was determined as shown in Fig. 5. In two leading commercial cultivars used as controls, yield was markedly increased in response to the treatment, whereas no

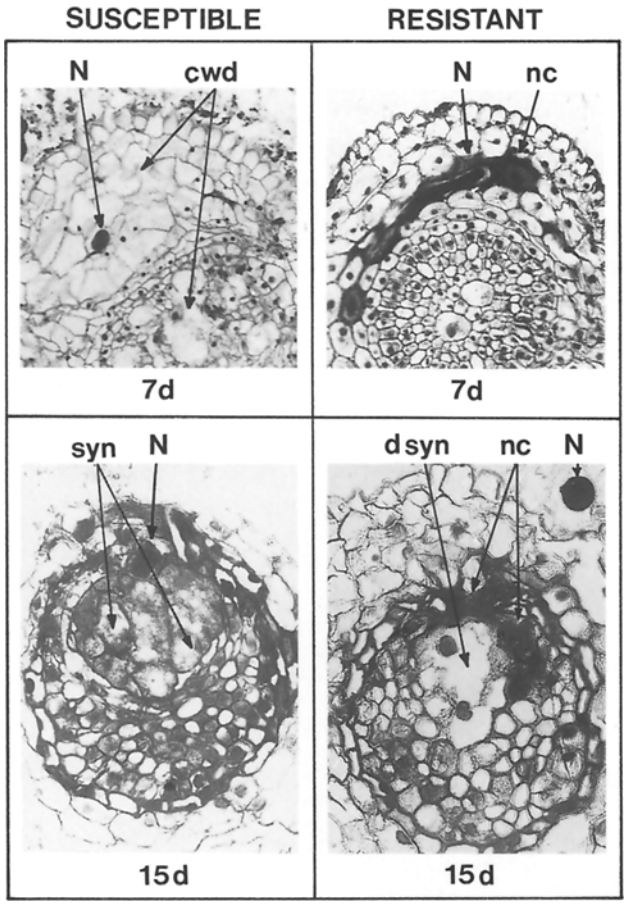

Fig. 4. Histological sections of roots from susceptible (H10-15) and resistant (H93-8) wheats infested with CCN under greenhouse conditions. *N*, nematode; *cwd*, cell wall degradation; *nc*, necrosis; *syn*, syncytia; *dsyn*, degraded syncytia

significant effect was observed in the H10-15 wheat and a significant decrease was observed in the resistant H93-8 line. These results, together with those in Fig. 2, implied that the H10-15 wheat used as receptor in the gene transfer was rather tolerant to the nematode and that the nematocide had a negative effect on yield, which in the susceptible wheats used as controls must have been much smaller than that caused by the nematodes.

All the above experiments were carried out with the Ha71 pathotype, which naturally infested the experimental field. Resistance of the H93-8 line to other pathotypes was investigated by adding isolated cysts from different sources to sterile soil in which the plants had been previously sown. As shown in Fig. 6, line H93-8 was resistant to all pathotypes tested, except against the two Swedish ones. Failure to infest the control cv Capa by pathotype HgI was unexpected as cv Capa had been previously reported as susceptible to it.

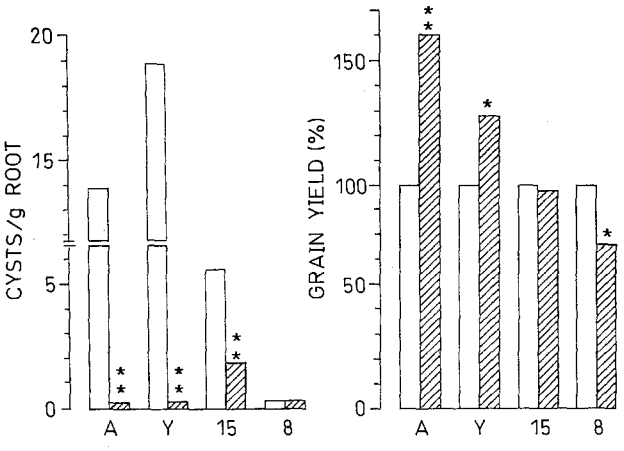

Fig. 5. Infestation and grain yield of H93-8 (*8*), H10-15 (*15*), cv Anza (*A*), and cv Yécora rojo (*Y*) with (*shaded*) or without (*open*) oxamyl seed dressing. Each bar represents the average of 20 replicas of 25 seeds in a random plot. The untreated samples were taken as 100% in each case. Significance of $P < 0.01$ (**), $P < 0.05$ (*)

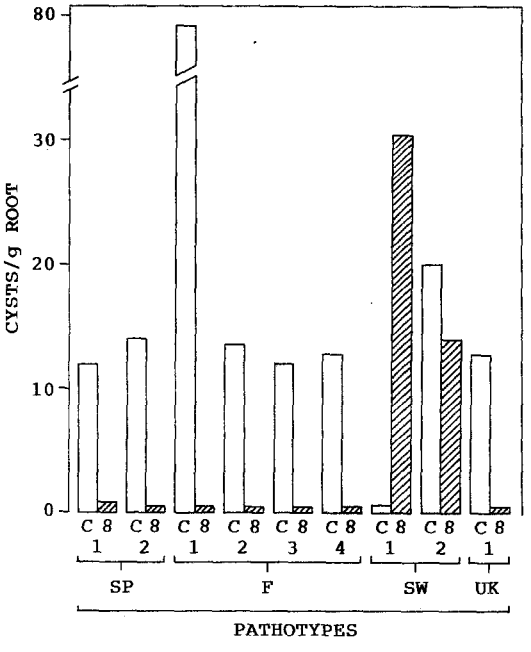

Fig. 6. Resistance of line H93-8 (*8*) to different CCN pathotypes from Spain (*Sp*): Ha71 (*1*) and the Torralba population (*2*); France (*Fr*): races 1 to 4; Sweden (*Sw*): HgI (*1*) and HgIII (*2*); and the United Kingdom (*UK*): Hal1 (*1*). Wheat cv Anza was used as a control (*C*) in all cases, except for the Swedish pathotypes, in which cv Capa was used. Each value is the average of five plants

Inheritance of CCN resistance

The resistant H93-8 line was crossed to the parental H10-15 wheat, as well as to the previously described resistant line Loros, and the degree of infestation of individual F_2 plants was determined (Fig. 7). If the (H10-15 × H93-8) F_2 plants are classified into resistant

and susceptible, using the lower limit of the confidence interval of the mean ($P = 95\%$) for the susceptible parent as a demarcation point, a 3:1 segregation of resistant versus susceptible plants is obtained [$\chi^2 = 1.98 \ll \chi^2(\text{gl} = 1; P = 0.05) = 3.84$]. However, there are plants more susceptible than the F_1 among those classified as resistant, and more susceptible than H10-15 among those classified as susceptible (Fig. 7). In the distribution of the (H93-8 × Loros) F_2 shown in Fig. 7, most of the plants fall into the resistant class, but a few clearly susceptible ones are also present.

Line H93-8 has been characterized both by cytogenetical methods and by RFLP/isozyme analysis as carrying chromosomes $5M^v$ and $7M^v$ from *Ae. ventricosa* (Mena et al. 1993). CCN resistance was not associated with the markers for these chromosomes in the H93 lines, as several of the lines carrying these chromosomes (Mena et al. 1993) were not resistant, and resistant plants which lacked both chromosomes were identified in the (H93-8 × H10-15) F_2, as judged

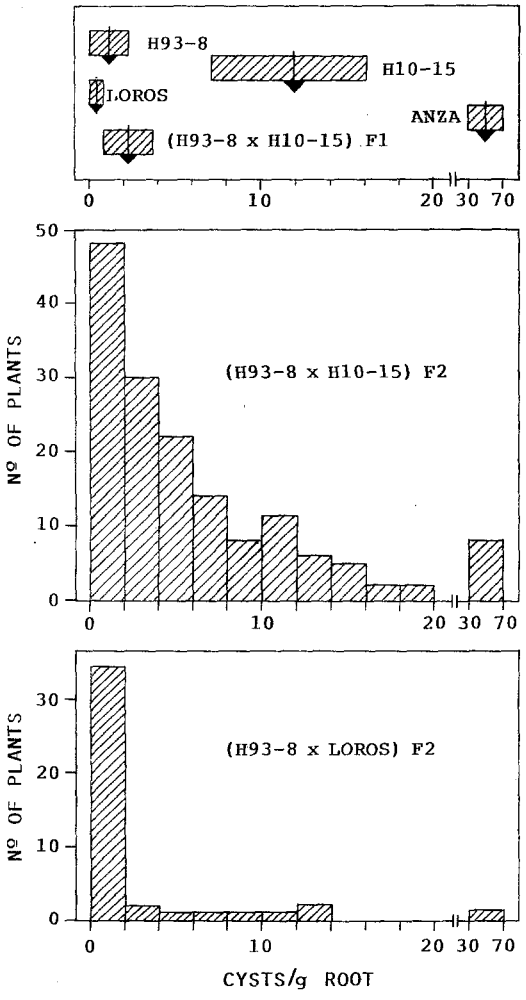

Fig. 7. Distributions of the (H93-8 × H10-15) F_2 and of the (H93-8 × Loros) F_2 with respect to CCN infestation. In the upper panel the average (*vertical arrow*) and the 95% confidence interval (*shaded*) are represented for H93-8 (15 plants), H10-15 (38 plants), Loros (5 plants), Anza (23 plants), and (H93-8 × H10-15) F_1 (22 plants)

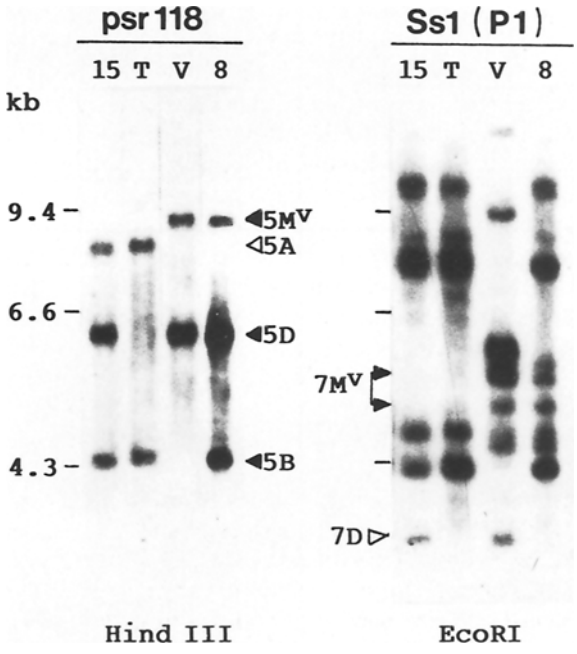

Fig. 8. RFLP markers of chromosomes $5M^v$ and $7M^v$ present in line H93-8. Probes psr118 and Ss1(P1) are from M. D. Gale and Ç. Maraña, respectively, as described in Mena et al. (1993). *T. aestivum* H10-15 (*15*); *T. turgidum* H1-1 (*T*); *Ae. ventricosa* AP-1 (*V*); and H93-8 (*8*). Relevant fragments present in H93-8 are indicated by *full horizontal arrowheads* with the corresponding chromosome designation. *Empty arrowheads* point to relevant missing fragments

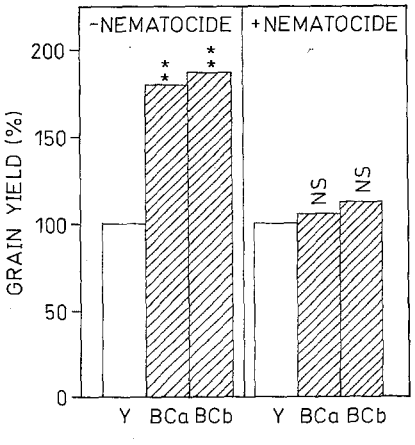

Fig. 9. Grain-yield test of two independent backcrosses (*BCa and BCb*) of the resistance of H93-8 into cv Yécora rojo (*Y*). Each value represents the average of four replicas of 20 seeds in a Hill-plot design, sown in a naturally-infested field. The yield of the recurrent parent, cv Yécora rojo, was taken as 100% in each case. Significance $P < 0.01$ (**), not significant (NS)

from the absence of the RFLP markers indicated in Fig. 8. It has been claimed that CCN resistance in *Ae. ventricosa* is associated with chromosome $6M^v$ (Dosba and Rivoal 1981; Rivoal et al. 1986). The following markers of this chromosome were absent from H93-8 (data not shown): $AcoM^v1$ (Mena et al. 1993) from the long arm, and $Xpsr167\text{-}E\text{-}6M^v$, $Xpup28\text{-}H\text{-}6M^v$ (Mena et al. 1993), and $AmpM^v1$ (unpublished), all from the short arm.

A yield test was carried out to investigate the effect of incorporation of the resistance into a commerical background (Fig. 9). Results from this test showed that when the resistance was backcrossed into cv Yecora rojo, a significant yield increase was associated with it in untreated naturally-infested soil, whereas no difference was observed between the original wheat and two independent backcrosses when the seeds were dressed with nematocide.

Discussion

Resistance to CCN was transferred from *Ae. ventricosa* ($D^vD^vM^vM^v$) to only one H93 introgression line out of 70 tested, a low transfer frequency which has been previously shown to be characteristic for genes from the M^v genome in the particular transfer procedure used (Delibes et al. 1977; Mena et al. 1993). The level of resistance in line H93-8 was high, as few nematodes were able to develop in its roots under heavy infestation conditions. The early stabilization of the number of nematodes per plant in this line indicates either that the initial infestation prevents infestation at later stages or that failure to induce well-formed syncytia by the nematodes leads to their death and eventual disintegration.

Differences in grain yield in the presence or absence of nematocide have several implications. The hexaploid wheat used as recipient in the genetic transfer (H10-15) was tolerant to CCN, as defined by Trudgill (1991) – "an atribute of the host genotype which is independent of resistance and relates to its ability to withstand or recover from damaging effects of nematode attack and yield well, even though differences in tolerance will affect nematode multiplication rates on susceptible and partially resistant plants". The nematocide oxamyl seemed to be deleterious to grain yield, but in the commercial cultivars tested yield was affected to a much greater extent by the nematode than by the nematocide. The results also indicated that the introduction of resistance into these wheats should be of practical interest.

Line H93-8 was resistant to a wide range of pathotypes which was similar to that found for the previously described resistance in Loros/Australia 10894 (Andersen and Andersen 1982; Ferris et al. 1989).

The shift towards susceptibility of the H93-8 × H10-15) F_2 distribution could be explained in terms of a quasi-dominant gene for resistance in H93-8 and a tolerance gene in H10-15, which would segregate independently of each other in the F_2, thus allowing for a fraction of highly susceptible plants that would lack both factors. The (H93-8 × Loros) F_2 distribution would also be consistent with the hypothesis of a quasi-dominant resistance gene in H93-8, if it is further postulated that the gene is non-allelic and in a different chromosomal location with respect to the gene *Cre1*, present in Loros/Australia 10894. This would explain both the predominance of resistant plants and the appearance of some susceptible ones. A different chromosomal location for the two genes is a plausible assumption, as it has been previously reported the *Cre1* is located on chromosome 2B (Slootmaker et al. 1974) while resistance in *Ae. ventricosa* has been associated with chromosome $6M^v$ (Rivoal et al. 1986). However, none of the genetic markers corresponding to chromosome $6M^v$ are present in line H93-8, which means that if the resistance gene was originally located in that chromosome, it has been transferred to chromosome 6D, or to some other location, in a segment not covered by the markers, and that most of that chromosome is absent in line H93-8. Chromosomes $5M^v$ and $7M^v$, which are present in line H93-8, do not recombine with their wheat homoeologues and resistance was not linked to markers associated with them, so it can be concluded that the resistance factor has been incorporated into a wheat chromosome that is able to regularly pair at meiosis in hybrids between line H93-8 and hexaploid wheats.

Resistance has been incorporated into the genetic background of commercial cultivars through backcrossing as predicted for a single quasi-dominant factor. Incorporation of the resistance also had the predicted positive effect on yield under infestation conditions, while it had no deleterious effect on yield in the absence of infestation. The designation of *Cre2* (or *Ccn2*) is proposed for the resistance gene reported here.

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References

Andersen S, Andersen K (1982) Suggestions for determination and terminology of pathotypes and genes for resistance in cyst-forming nematodes, especially *Heterodera avenae*. EPPO Bull 12:379–386

Asiedu R, Fisher JM, Driscoll CJ (1990) Resistance to *Heterodera avenae* in the rye genome of triticale. Theor Appl Genet 79:331–336

Brown JAM (1973) Cereal cyst nematode. Comparative resistance in wheat and progress towards alien-resistance transfer. In: Sears ER, Sears LMS (eds) Proc 4th Int Wheat Genet Symp, Columbia, pp 1–7

Delibes A, García-Olmedo F (1973) Biochemical evidence of gene transfer from the M^v genome of *Aegilops ventricosa* to hexaploid wheat. In: Sears ER, Sears LMS (eds) Proc 4th Int Wheat Genet Symp, Columbia, pp 161–165

Delibes A, Sanchez-Monge R, Garcia-Olmedo F (1977) Biochemical and cytological studies of genetic transfer from the M^v genome of *Ae. ventricosa* into hexaploid wheat. A progress report. In: Sanchez-Monge E, García-Olmedo F (eds) Proc 8th Congress of EUCARPIA "Interspecific hybridization in plant breeding", Madrid, pp 81–89

Dosba F, Cauderon Y (1972) A new interspecific hybrid *Triticum aestivum* ssp vulgare × *Aegilops ventricosa*. Wheat Inf Service 35:22–24

Dosba F, Rivoal R (1981) Les lignées d'addition blé-*Aegilops ventricosa*. II. Étude de leur comportement vis-á-vis d'*Heterodera avenae*. Agronomie 1:559–564

Dosba F, Doussinault G, Rivoal R (1978) Extraction, identification and ultilization of the addition lines *T. aestivum-Ae. ventricosa*. In: Ramanujan S (ed) Proc 5th Int Wheat Genet Symp, New Delhi, pp 332–337

Doussinault G, Delibes A, Sanchez-Monge R, García-Olmedo F (1983) Transfer of a dominant gene for resistance to eyespot disease from a wild grass to hexaploid wheat. Nature, 303:698–700

Eastwood RF, Lagudah ES, Appels R, Hannah M, Kollmorgen JF (1991) *Triticum tauschii*: a novel source of resistance to cereal cyst nematode (*Heterodera avenae*). Aust J Agri Res 42:69–77

Ferris VR, Faghihi J, Ireholm A, Ferris JM (1989) Two-dimensional protein patterns of cereal cyst nematodes. Phytopathology 79:927–933

García-Olmedo F, Delibes A, Sanchez-Monge R (1984) Transfer of resistance to eyespot disease from *Aegilops ventricosa* to wheat. Vortr Pflanzanzüchtg 6:156–158

Koebner RMD, Martin PK (1989) Chromosomal control of aminopeptidases of wheats and its close relatives. Theor Appl Genet 78:657–664

Mena M, Orellana J, Lopez-Braña I, García-Olmedo F, Delibes A (1993) Characterization of wheat/*Aegilops ventricosa* introgression and addition lines with respect to the M^v genome. Theor Appl Genet 86:197–204

O'Brien PC, Fisher JM, Rathjen AJ (1980) Inheritance of resistance in two wheat cultivars to an Australian population of *Heterodera avenae*. Nematologica 26:69–74

Rivoal R (1977) Identification des races biologiques du nematode a kystes des céreales, *Heterodera avenae* Woll., en France. Ann Zool Ecol Anim 9:261–272

Rivoal R, Dosba F, Jahier J, Pierre JS (1986) Les lignées d'addition blé-*Aegilops ventricosa* Tausch. IV. Etude de la localisation chromosomique de la résistance à l'égard d'*Heterodera avenae*. Agronomie 6:143–148

Sanchez A, Zancada MC (1987) Characterization of *Heterodera avenae* pathotypes from Spain. Nematologica 33:55–60

Slootmaker LAJ, Lange W, Jochemsem G, Schepers J (1974) Monosomic analysis in bread wheat of resistance to cereal root eelworm. Euphytica 23:497–503

Trudgill DL (1991) Resistance to and tolerance of plant parasitic nematodes in plants. Annu Rev Phytopathol 29: 167–192

Valdeolivas A, Romero MD (1990) Morphometric relationships of some members of the *Heterodera avenae* complex (*Nematoda:* Heteroderidae). Nematologica 36:292–303