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Molecular-genetic characterisation of a new nematode resistance gene in wheat

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Abstract Bread wheat lines introgressed with *Aegilops ventricosa* chromosomes were evaluated for their resistance to the Australian cereal cyst nematode (CCN, *Heterodera avenae*) pathotype Ha13. Higher levels of resistance relative to the phenotype of the *Cre1* CCN resistance gene in wheat were found in the donor *Ae. ventricosa* parental lines and chromosome-5N^v substitution or addition lines. The newly identified resistance to pathotype Ha13 on chromosome 5N^v, designated, *Cre6*, was shown to be independent of the *Ae. ventricosa*-derived *Cre2* gene, effective against several European pathotypes. Another *Ae. ventricosa* derived gene, *Cre5*, showed partial resistance to pathotype Ha13. Inhibition of Ha13 female nematode reproduction was ranked in the order *Cre6* >*Cre1* >*CreF* ≥ *Cre5*. *Cre6* was inherited as a single dominant locus. Gene sequences encoding nucleotide-binding sites and leucine-rich repeats (NBS-LRR) from the *Cre3* CCN-pathotype Ha13 resistance locus were used as probes to isolate related sequences from one of the donor *Ae. ventricosa* parents. Related sequences from *Ae. ventricosa* (71–73% similarity at the amino-acid level to the *Cre3*-derived sequences) of chromosome 5N^v origin were identified and served as diagnostic molecular markers for the presence of 5N^v. CCN-susceptible plants, found as variants in some of the purported chromosome 5N^v lines, were also found to be missing the diagnostic 5N^v RFLP markers assayed by the NBS-LRR probe. An alloplasmic chromosome-5N^v addition line with *Ae. ventricosa* cytoplasm in the wheat cultivar, Moisson, background was particularly variable, with 43% CCN-susceptible plants and a corresponding loss of the diagnostic chromosome-5 molecular markers.

Keywords Cereal cyst nematode · Resistance genes · Wheat introgressions · RFLP · Nucleotide binding site-leucine rich repeat · *Aegilops ventricosa*

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Introduction

The cereal cyst nematode (CCN) (*Heterodera avenae* Woll.) is an important disease in many wheat-growing regions of the world. In south-eastern Australia, this disease can cause significant losses in wheat production, where on average an 8% reduction in grain yield has been reported; although yield losses in individual fields can be more than 50%. In 1995 losses due to CCN were estimated at A$70 million, in spite of the widespread cultivation of resistant varieties (Rathjen et al. 1998).

In the last three decades, various *Triticum*, *Aegilops* and *Secale* species have been screened as potential sources of germplasm with resistance to CCN. Incorporating resistance into wheat cultivars and breeding lines is considered the most cost-effective control measure for reducing nematode populations. Consequently, resistance genes, which include *Cre1*, located on chromosome 2B from wheat (Slootmaker et al. 1974), have been used in Europe and Australia. One of the long arms of wheat chromosome 7 has been suggested to carry a gene found in other wheats resistant to the Australian CCN pathotype; this is derived from the cultivar Festiguay (CreF) (Paull et al. 1998). Other sources of resistance include *Cre3*, located on chromosome 2D, and *Cre4*, both of

which are from *Aegilops tauschii* (syn. *Triticum tauschii*) (Eastwood et al. 1991, 1994), *Cre2* and *Cre5* (previously designated *CreX*) from *Aegilops ventricosa* (Delibes et al. 1993; Jahier et al. 1996), *CreAet* from *Aegilops triuncialis* (Romero et al. 1998) and *CreR* from *Secale cereale* (Asiedu et al. 1990). The *Cre1* source confers resistance to the Australian CCN pathotype (Ha13) and several European pathotypes that include Ha11 and Ha12. *Cre3* confers a high level of resistance to the Australian pathotype, but is susceptible to both the Ha11 and Ha12 European pathotypes.

Amongst 70 *Ae. ventricosa* (genome designation D^vN^v) introgression lines into breadwheat, Delibes et al. (1993) identified, a line designated H-93–8 that carries the introgressed *Cre2* gene giving resistance to several European pathotypes. H-93–8 was also shown to contain a double-chromosome substitution involving $5N^v$(5A) and $7N^v$(7D). Delibes et al. (1993) examined isozyme and molecular markers diagnostic for the $5N^v$ and $7N^v$ chromosomes, but were unable to correlate the markers with the presence of the *Cre2* gene. Additionally, none of the introgressed lines with either a single $5N^v$ or $7N^v$ chromosome substitution was shown to be resistant to the European pathotypes tested. In a separate study, Jahier et al. (1996) reported partial resistance to some of the European pathotypes in wheat lines carrying *Cre5* located on a $2N^v$ segment present on chromosome $6N^v$ substitution or addition lines.

Colinearity of molecular markers across homoeologous chromosomes in wheat is highly conserved. Molecular markers and cytogenetic analysis have been used to further demonstrate conserved homoeologous relationships between *Ae. ventricosa* and wheat chromosomes (Mena et al. 1993; Bardsley et al. 1999). Molecular markers based on gene sequences encoding nucleotide-binding sites and leucine-rich repeats (NBS-LRR) at the *Cre3* locus (Lagudah et al. 1997) have been shown to cross-hybridise with related sequences at the *Cre1* locus and introgressed segments carrying *Cre5* (Lagudah et al. 1998; Seah et al. 2000b). Family members of the gene sequences from the *Cre3* locus present on group-2 chromosomes were also found on homoeologous group-5 and -7 chromosomes of wheat (Seah et al. 1998; Spielmeyer et al. 1998).

Resistance to the European pathotypes found in *Ae. ventricosa*, and some of its derivatives in bread wheat, has not been demonstrated against pathotype Ha13. The objectives of the present study were firstly to evaluate resistance expression to pathotype Ha13 using *Ae. ventricosa*, line H-93–8, and other single-chromosome substitutions and additions of *Ae. ventricosa* in wheat. Secondly, to determine the inheritance of a newly identified resistance phenotype to Ha13 found in H-93–8 and single-chromosome $5N^v$ substitution/addition lines. Thirdly, to ascertain the use of homologues of the NBS-LRR sequences from the *Cre3* locus isolated from *Ae. ventricosa* as probes diagnostic for chromosome $5N^v$ that carries the newly identified resistance to the CCN-pathotype Ha13.

Materials and methods

Plant materials

Ae. ventricosa accessions #10 and AP-1 were the parental sources used to introgress the alien chromosomes into the bread wheat cultivars Moisson, VPM1 and Almatense-H-10–15 (hereafter simply referred to as H-10–15). Line H-93–8, which carries, at least, a double-chromosome substitution, $5N^v$(5A) and $7N^v$(7D), and H-93—35, with a single substitution $5N^v$(5D) from *Ae. ventricosa* accession AP-1, were produced in the H-10–15 wheat background. Another *Ae. ventricosa* accession #11 used in the present study is related to AP-1. Lines VB and MB are chromosome $5N^v$ addition lines in a Moisson-cultivar background. The cytoplasm from VB is derived from *Ae. ventricosa*. Other genetic stocks in a Moisson background include a chromosome $6N^v$ addition, as well as a substitution line $6N^v$(6D). A translocated segment from $2N^v$ to wheat chromosome 2A is present in the wheat line VPM1. Lines VD and VC contain *Ae. ventricosa* chromosome-$4N^v$ and -$7N^v$ additions respectively. Reciprocal crosses from H-93–8 × H-10–15 and H-93–35 × H-10–15, F_1 and F_2 progeny of H-93–35 × H-93—8, were used in the genetic analysis of resistance to CCN. Other wheats used were: 'Meering', an Australian wheat cultivar used as a susceptible control to CCN-pathotype Ha13; Frame, an Australian variety with tolerance and partial resistance to CCN-pathotype Ha13; and AUS10894, a landrace and the source of *Cre1*.

Inoculum and CCN resistance tests

The inoculum used was CCN-infested sandy loam soil collected from a field plot in Rainbow, Victoria, with an initial CCN population of approximately 20 eggs per g. The soil was collected after harvest, before rain, and stored air-dry in a bin until used. Before use, the nematodes were hatched by moistening the soil and mixing thoroughly so that the soil was damp but friable. The soil was then stored in 45-l plastic bins for 6 weeks at 15°C. The soil was then potted (285–300 g per pot) and seeds planted as single seed per pot in a completely randomised design in the greenhouse with a temperature range of 15—25°C for a further 6 weeks. At least 12 plants per genotype were used in the CCN bioassay. Soil was carefully washed from the roots and resistance assessed by counting the number of white females on the roots of each plant using a stereomicroscope at 6.4 × magnification. The nematode reproduction values obtained were then used to classify plant resistance relative to the control: a mean value of less than 5 cysts (range 1–10) per plant root was resistant; a mean value greater than 30 cysts per plant root was susceptible, and 10–30 as moderately resistant.

DNA analysis

Genomic DNA was isolated and processed from leaves as described in Lagudah et al. (1991b). Modifications to the DNA extraction buffer made use of 1% sarkosyl, 100 mM Tris-HCL, 100 mM NaCl, 10 mM EDTA, 2% polyvinyl-pyrrolidone, and was adopted with some of the samples. A genomic DNA library of *Ae. ventricosa* #11 in lambda GEM11 (Promega) was as described in Seah et al. (2000a). Positive lambda clones (designated V1–V25), that hybridised to probes derived from the NBS-LRR sequences at the *Cre3* locus, were further subcloned into *Sac*I, *Sma*I and *Kpn*I /*Eco*RI sites in pBluescript (Stratagene). RFLP analyses using genomic DNA from wheat and *Ae. ventricosa* addition lines were performed using previously described procedures (Lagudah et al. 1991a). In addition to the *Ae. ventricosa* NBS-LRR homologues isolated from the lambda library two other cDNA clones that assay homoeologous group-5 chromosomes (psr118 and 128, kindly supplied by Dr. Mike Gale IPSR Norwich, UK) were used as probes in RFLP analyses.

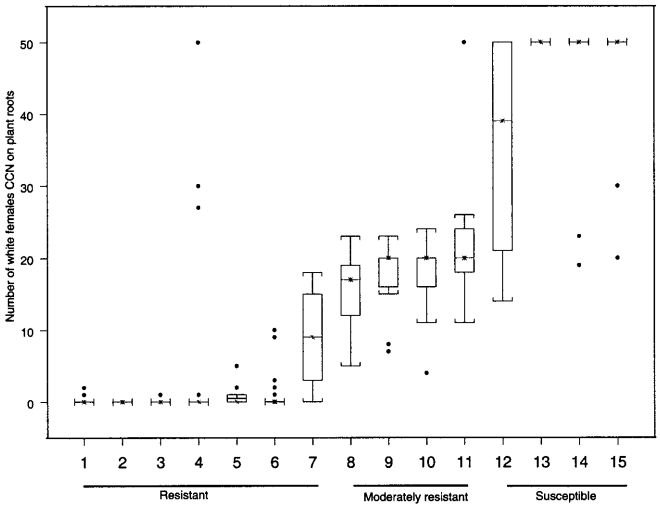

Fig. 1 Box plot of white female CCN counts on roots of wheat/*Ae. ventricosa* introgression lines, addition lines and susceptible wheat cultivar controls. *1* H-93–8, *2 Ae. ventricosa* #10, *3 Ae. ventricosa* #11, *4* H-93–35, *5* MB addition line 5N^v (R), *6* VB addition line 5N^v (resistant class), *7* Aus 10894 (source of *Cre1*), *8* Frame (a derivative of Festiguay, source of *CreF*), *9* Moisson 6N^v(6D), *10* Moisson 6N^v addition, *11* VPM1, *12* VB addition line 5N^v (susceptible class), *13* Moisson, *14* H-10–15, *15* Meering. Each line with an asterisk represents the median. The *edges* of the box indicate the upper and lower quartiles. The dots represent the complete range of the cyst counts per plant

Data analysis

To examine differences among the lines, an analysis of variance was employed. A box plot of the sample distribution of counts was used to graphically display the raw data. The F_2 data were analysed using a chi-square test to determine the goodness-of-fit to the expected simple Mendelian ratio.

Results

CCN-pathotype Ha13 reproduction on *Ae. ventricosa* and its derivatives in wheat

Absolute or near-complete inhibition of female nematode (cysts) reproduction with zero cysts, or a single cyst per plant was found in both parental *Ae. ventricosa* accessions (#10 and 11) (Fig. 1). This high level of resistance was also found in the introgressed lines, H-93–8, MB, and H-93–35. Approximately 57% of plants of the addition line VB were highly resistant, but 43% were susceptible. A common characteristic of these highly resistant, introgressed lines, was the presence of chromosome 5N^v derived from *Ae. ventricosa*. Further analysis on the non-resistant plants among this group of chromosome-5N^v addition or substitution lines using molecular markers diagnostic for 5N^v is described later. Differences in the cytoplasmic origins of the 5N^v chromosome addition lines MB and VB were compared for their effects on nematode resistance. Neither the cytoplasmic origin from *T. aestivum* found in line MB, nor the *Ae. ventricosa* source present in line VB, had an effect on the high level of resistance associated with chromosome-5N^v introgression. The resistance levels to pathotype Ha13 associated with chromosome 5N^v are higher than those observed for the *Cre1* resistance gene present in AUS10894 (Fig. 1).

A second group of *Ae. ventricosa* introgressions into wheat showed an intermediate level of nematode reproduction, with a mean value of 20 cysts per plant (range, 10–30) (Fig. 1). These resistance levels are comparable to those found in the partially resistant wheat cultivar, Frame (which carries the *CreF* gene). Introgressed lines in this category of resistance included chromosome substitution 6N^v(6D) and the addition line 6N^v present in a Moisson wheat background, as well as the line VPM1. Introgressed chromosome-6N^v in wheat has been shown to comprise a translocation of 6N^{v}S. 6N^{v}L-2N^{v}S (Jahier et al. 1996). VPM1 wheat contains only the 2N^{v}S introgressed segment.

The recurrent parents used in producing the *Ae. ventricosa* introgressions were shown to be highly susceptible, with cyst numbers per plant in excess of 50 (Fig. 1). The susceptibility of Moisson and H-10–15 wheat varieties to CCN-pathotype Ha13 was comparable to the susceptible wheat control, Meering, used in this study (Fig. 1).

Table 1 Resistance and susceptible phenotypes of F_1 and F_2 generations of wheat / *Ae. ventricosa* introgression lines to the Australian CCN pathotype (Ha13). Values for significance at $P = 0.05$, 1 *df*, 3.84 or $P = 0.01$, 1 *df*, 6.63

Line	Disease reaction		Expected ratio	χ^2
	Resistant	Susceptible		
H-93–35/H-10–15 F_1	11.00	0.00		
H-10–15/H-93–35 F_2	71.00	26.00	3:1	0.93
H-93–35/H-10–15 F_2	65.00	27.00	3:1	0.17
H-93–8/H-10–15 F_1	7.00	0.00		
H-93–8/H-10–15 F_2	45.00	46.00	3:1	31.68
H-93–35/H-93–8 F_2	61.00	0.00		

Inheritance of resistance to the CCN Ha13 pathotype in crosses between *Ae. ventricosa* introgression lines and *T. aestivum*

The F_1 plants from the cross between H-93–35/H-10–15 and H-93–8/H-10–15 showed high levels of resistance, similar to the resistant parent, indicating that inheritance to resistance conferred by H-93–35 and H-93–8 to the Australian CCN pathotype is dominant (Table 1). The F_2 family derived from H-93–35/H-10–15 segregated into 65 resistant and 27 susceptible plants, while its reciprocal cross, showed 71 resistant and 26 susceptible plants. Assuming a single locus for resistance, this observed ratio fits the theoretical 3:1 Mendelian phenotypic ratio for a single dominant gene (Table 1). However, the F_2 population derived from the H-93–8/H-10–15 cross deviated significantly from the expected 3:1 ratio ($P < 0.001$) (Table 1). This deviation was due to an

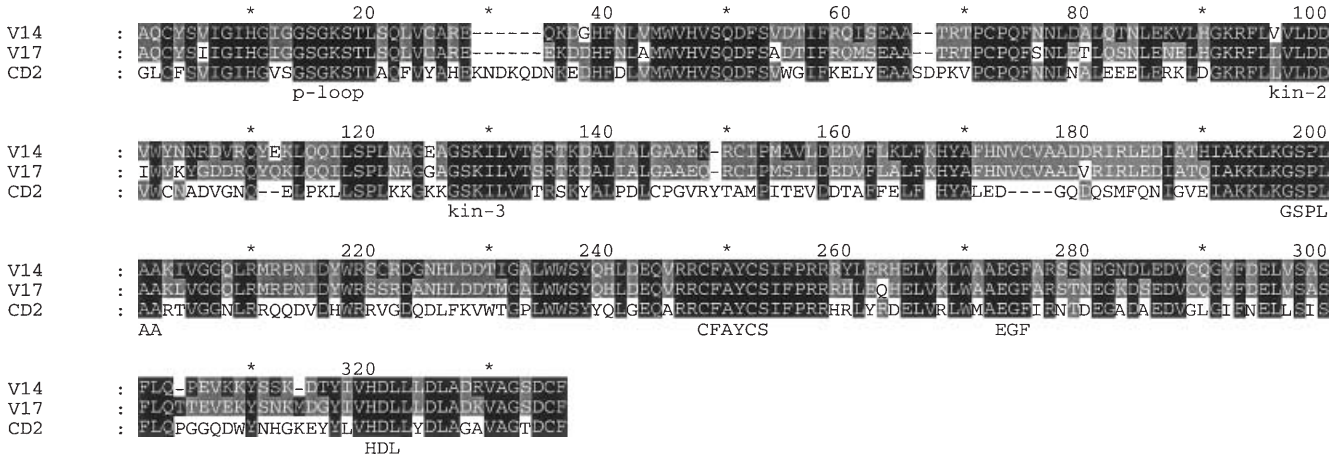

Fig. 2 Deduced amino-acid sequence alignment of clones V14NBS, V17NBS and CD2 in the region spanning nucleotide-binding sites and other conserved motifs found in plant disease resistance gene sequences. *CD2* is a cDNA derived from sequences at the *Cre3* locus in *Ae. tauschii*. The *regions* shown for V14 and V17 define the probes used as V14NBS and V17NBS respectively. In addition to the NBS region [P-loop, kinase 2 (kin2), kinase 3 (kin-3)] the other conserved motifs indicated are 'GSPLAA', 'CFAYCS', 'EGF' and 'HDL'

increase in the proportion of susceptible plants in the F_2 progeny from H-93–8/H-10–15, which may have resulted from poor transmission of the critical alien chromosome $5N^v$ in the F_1 hybrid H-93–8/H-10–15. Meiotic analysis showed that chromosome $5N^v$ substitution for 5D in line H-93–35 was transmitted more regularly, while a poorer transmission of $5N^v$ occurred in substitutions for 5A found in H-93–8.

To determine whether H-93–35 and H-93–8 shared a common gene for resistance to the Australian CCN pathotype, the F_2 family from H-93–35/H-93–8 was evaluated. All F_2 plants from the cross (H-93–35/H-93–8) resulted in highly resistant plants (Table 1). The absence of susceptible plants suggests that resistance to pathotype Ha13 in line H-93–35 is the same as the gene present in H-93–8.

In F_2 plants from crosses between the $6N^v$(6D) substitution line and H-93–8, susceptible phenotypes, as well as partially and highly resistant plants, were identified. This observation suggests that genes controlling the partial and high levels of resistance derived from $6N^v$(6D) and H-93–8, respectively, are non-allelic.

Molecular-genetic characterisation of introgressed lines

Several clones that hybridised to DNA probes derived from the *Cre3* CCN resistant locus were isolated from the *Ae*. *ventricosa* #11 genomic DNA library in bacteriophage lambda (Seah et al. 2001a). Subclones from two of the lambda clones, V14 and V17, contained gene sequences with open reading frames encoding for NBS-LRR-deduced proteins. Deduced amino-acid comparisons of the NBS regions of V14 and V17 relative to one of the *Cre3*-derived members (CD2) used in probing the library gave similarities of 73 and 71% respectively (Fig. 2). Corresponding regions between V14 and V17 were 92% similar and 87% identical. Specific probes from either V14 or V17 were used in RFLP analysis on all the available *Ae*. *ventricosa* addition lines that include chromosome-$4N^v$ and -$7N^v$ introgressions.

A diagnostic RFLP, unique to $5N^v$ chromosome addition or substitution lines, which could also be traced to the *Ae*. *ventricosa* parental source, was identified (data not shown). When subclones from either V14 or V17, which contain sequences from the coding region spanning the nucleotide-binding sites (designated V14NBS and V17NBS) to the start of the leucine-rich region, were used as probes (see Fig. 2), RFLPs diagnostic for chromosome $5N^v$ (Fig. 3: 18 kb and 2.0 kb) and additional fragments in line H-93–8 (Fig. 3: 2.6 kb and 1.6 kb, open arrows) were observed. The 2.6-kb fragment was also present in the *Ae. ventricosa* donor parents, but was absent in a chromosome-$7N^v$ addition line in Moisson (data not shown) as well as all the $5N^v$ substitution and addition lines. Conclusive location of the 1.6-kb fragment that was absent in *Ae. ventricosa* #10 but present in *Ae*. *ventricosa* #11, AP-1 and H-93–8 (Fig. 3), could not be established. Thus line H-93–8 carries homologues of V14/V17 NBS-LRR sequences that are neither present on $5N^v$ nor $7N^v$.

The diagnostic RFLP for chromosome $5N^v$ (detected on either DraI-, *Hin*dIII- or *Sac*I-restricted genomic DNA) was examined on all plants from H-93–35, MB and VB lines that were tested in the nematode bioassay. All CCN-susceptible plants, including among these expected chromosome-$5N^v$ introgressions, also lacked the diagnostic RFLP assayed by probes from V14NBS/V17NBS and the 3' end of the leucine-rich repeat region for the presence of the $5N^v$ segment (Fig. 4).

In line H-93–35, of the 18 plants tested in the CCN bioassay, three were susceptible and confirmed by RFLP analysis as lacking the diagnostic V14 or 17 NBS fragment. Similarly, with line VB, 22 out of 51 plants were susceptible to CCN and showed the corresponding absence of the $5N^v$ diagnostic RFLP. It is likely that transmission of the alien chromosome $5N^v$ in the allo-

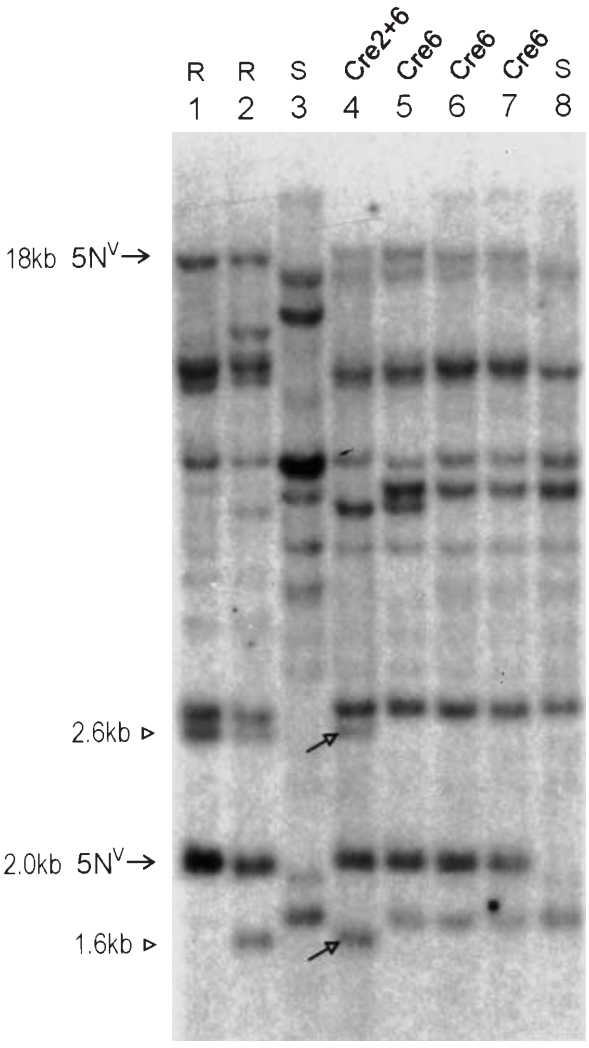

Fig. 3 RFLP analysis of *Sac*I-restricted genomic DNA from *Ae. ventricosa* and its derivatives probed with V17NBS. The *arrowed bands* (18 kb and 2.0 kb) are the diagnostic RFLPs unique to the chromosome-5N^{v} addition and substitution. Fragments designated with an *open arrow* (2.6 kb and 1.6 kb) are present in H-93–8 but are not of 5N^{v} origin. *Lanes: 1 Ae. ventricosa* #10, *2 Ae. ventricosa* #11 (the same set of RFLPs was found with AP-1, not shown), *3* H-10–15, *4* H-93–8, *5* H-93–35, *6* MB, *7* VB, *8* Moisson

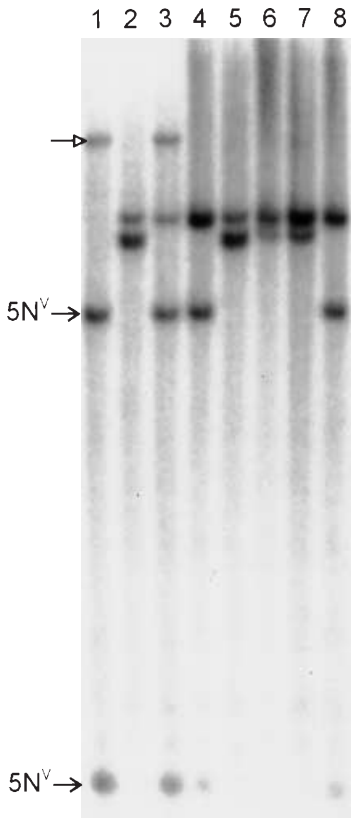

Fig. 4 RFLP analysis of *Hind*III-restricted genomic DNA from *Ae. ventricosa*, wheat derivatives and CCN-resistant and susceptible plants found in line H-93–35 probed with V14–3'. The probe V14–3' is from a 1-kb fragment taken from the leucine-rich repeat to the 3' untranslated region. *Arrowed* 5N^{v} bands show the diagnostic chromosome 5N^{v} RFLP. *Lanes: 1 Ae. ventricosa* #11, *2* H-10–15, *3* H-93–8, *4* H-93–35, *5* H-10–15, 6 H-93–35S1, CCN susceptible, *7* H-93–35S2, CCN susceptible, *8* H-93–35R1, CCN-resistant plant

plasmic VB line is poor by comparison with the stable euplasmic MB line. Alternatively, the VB plants may have been derived from a monosomic, rather than a disomic, 5N^{v} chromosome-addition parental seeds, and thus segregate with cytotypes of 2n = 42, 43 and 44.

Previous studies have shown that homologues of gene sequences derived from the *Cre3* locus present on homoeologous group-5 chromosomes were localised to the distal regions on the long arms (Seah et al. 1998; Spielmeyer et al. 1998). To investigate regions outside of the distal long-arm region, two probes, psr118 and 128 that map to the proximal region of the long and short arms of group-5 chromosomes (Gale et al. 1993) were used in RFLP analysis on lines H-93–35, VB and MB. Corresponding 5N^{v} marker loci for psr118 and 128 were only found in line MB and the CCN-resistant H-93–35 and VB plants, and were absent in the susceptible plants (data not shown). These results were identical to the observations made with the V14 or V17NBS probes (Fig. 4), and suggest the likelihood of the complete, or almost complete, loss of the alien chromosome 5N^{v} in the CCN-susceptible H-93–35 and VB plants.

Discussion

The H-93 series of bread wheat lines are derivatives of *Ae. ventricosa* AP-1 introgressions into bread wheat, whose resistance to the Australian CCN pathotype (Ha13) was unknown until the current study. H-93–8, which carries the *Cre2* gene, was reported to be highly resistant to several European CCN pathotypes (Delibes et al. 1993). In the current study, H-93—8 and MB authenticated chromosome-5N^{v} substitutions or additions in lines H-93–35 and VB, as well as the *Ae. ventricosa* parental accessions #10 and #11, were also found to be

completely resistant the to Australian CCN pathotype. Additionally, this result demonstrated that the same *Ae*. *ventricosa* introgression-line in bread wheat (H-93–8) with *Cre2*, which confers resistance to several European CCN pathotypes, also confers resistance to the Australian CCN pathotype. This, therefore, raises the question: are *Cre2* and the gene on 5N^v that confer resistance to the Australian CCN pathotype the same or different genes? Line H-93–35 carries 5N^v(5D) and has been shown to be susceptible to European CCN pathotypes for which the *Cre2* gene is effective. Thus, H-93–35 lacks *Cre2*, but contains another gene that is highly resistant to the Australian pathotype, as observed for H-93–8, MB and VB in the present study. The common link in all the newly identified high levels of resistance to pathotype Ha13 among the introgressed lines is the presence of chromosome 5N^v. Inheritance studies suggested that a major dominant gene confers resistance to Ha13. Delibes et al. (1993) ruled out chromosome 5N^v as the carrier of *Cre2*, based on their observation that neither line H-93–35 nor molecular markers diagnostic for the presence of 5N^v gave rise to resistant plants characteristic of *Cre2*. Contrary to the observations in the present study, the high level of resistance to pathotype Ha13 was present on chromosome-5N^v introgression lines, which thus carried a gene that is independent of *Cre2*. Since there is no previous report of a gene for CCN resistance on chromosome 5N^v transferred into wheat, we propose *Cre6* as the designated name for the newly identified resistance to Ha13. Furthermore, in the F_2 progeny from the cross H-93–35 × H-93–8 no susceptible plants were identified, as expected from parental lines sharing the same gene, Cre6.

In barley, the resistance gene, *Ha4*, located on the long arm of chromosome 5H, is known to confer resistance to *H. avenae* pathotype Ha13 (Barr et al. 1998). Although barley CCN resistance genes *Ha2/3* are on group-2 chromosomes, similar to the *Cre1* and *Cre3* genes, which parallels the observation between *Ha4* and *Cre6* on group-5 chromosomes, there is as yet no direct evidence that these barley and wheat CCN-resistant genes constitute orthologous genes.

The chromosome-5N^v substitution or addition lines in wheat analysed in the present study were independently derived; H-93–8 and H-93–35 carry 5N^v from the parental accession *Ae*. *ventricosa* #11 (AP-1), whereas MB and VB are derivatives of *Ae*. *ventricosa* #10. Because both *Ae*. *ventricosa* parental accessions showed the same level of resistance to pathotype Ha13 as their respective 5N^v introgression lines, the gene *Cre6* is most-likely to be present in both accessions.

Independently of *Cre6*, we also showed that partial resistance to *H. avenae* pathotype Ha13 is present on 6N^v(6D), which also contains *Cre5* (present on the translocated 2N^v segment in 6N^v). Partial resistance was also reported for the European CCN pathotypes, Ha41 and Ha12, in different wheat backgrounds carrying 6N^v(6D) (Rivoal et al. 1986; Jahier et al. 1996). In the present study, the wheat line VPM1 exhibited partial resistance levels against pathotype Ha13 similar to the Moisson 6N^v(6D) line. Both VPM1 and the 6N^v(6D) line contain the 2N^v segment with the rust resistance genes *Lr37*, *Yr17* and *Sr38* (Seah *et al*. 2000a). VPM1 has recently been shown to possess partial resistance to European CCN pathotypes and genetic analysis indicated that it also carried the *Cre5* gene in addition to the triple rust resistance genes (J. Jahier, P. Abélard, A.M. Tanguy, R. Rivoal, and H. Bariana, unpublished). Thus the observed partial resistance phenotype to the Australian pathotype for VPM1 and Moisson 6N^v(6D) may be due to the effect of *Cre5*. Resistance levels to pathotype Ha13 associated with *Cre6* found in the present study were equivalent to the levels for the *Cre3* (synonym *CcnD1*) phenotype (Eastwood et al. 1994) when compared to the *Cre1* gene. Different CCN resistance genes vary in their efficacy towards inhibiting female Ha13 nematode reproduction and, on the basis of the present study, can be ranked as: *Cre6* >*Cre1* >*CreF* ≥ *Cre5* (VPM1).

In the absence of homoeologous recombination between chromosome 5N^v and the wheat group-5 chromosomes, the precise location of *Cre6*, relative to diagnostic molecular markers for 5N^v, could not be determined. Homologous recombination expected between two *Ae. ventricosa* accessions will enable the precise localization of the *Cre6* gene. However, this approach will require the identification of a CCN-susceptible *Ae. ventricosa* accession to be used as one of the parents in the cross. Unfortunately, both parental accessions used in the current study are resistant and their respective chromosome 5N^v was inferred to also carry *Cre6*. Cytogenetic strategies based on induced homoeologous recombination could also be exploited to introduce *Cre6* on the smallest segment possible from chromosome 5N^v into wheat. Assuming colinearity of genes between wheat and the corresponding region of the *Ha4* gene on barley chromosome 5HL, the previous observation that gene sequences from the *Cre3* locus detected homologues diagnostic for *Cre1* and *Cre5* (Lagudah et al. 1998; Seah et al. 2000b), and the presence of other homologues on the long arm of group-5 chromosomes, make it likely that *Cre6* is located on the distal region of chromosome 5N^v. In addition to adopting cytogenetic strategies for allosyndesis, gene constructs based on the *Cre3*-related homologues in the V14 and V17 clones could be investigated as potential candidate genes in complementation experiments through wheat transformation.

Provided no deleterious agronomic traits are associated with the chromosome-5N^v substitutions, they can be readily crossed into appropriate wheat backgrounds in order to utilise *Cre6*. No deleterious phenotypes have as yet been reported for a derivative of the commercial cultivar, Fidel, developed in France and carrying the chromosome 5N^v(5D) (J. Jahier, unpublished). Resistance to powdery mildew caused by *Erysiphe graminis* was also transferred from *Ae. ventricosa* chromosome 5N^v and present in H-93–8 and H-93–35 (Delibes et al. 1987). In Australia, where *Cre6* will be more appropriate, the V14 or V17 NBS markers reported in this study, and other diagnostic markers for chromosome 5N^v, can be used to monitor the introgression of the new CCN resistance.

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