Mol Breeding (2016) 36:122
DOI 10.1007/s11032-016-0549-9

Cereal cyst nematode resistance gene *CreV* effective against *Heterodera filipjevi* transferred from chromosome 6VL of *Dasypyrum villosum* to bread wheat

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Received: 10 April 2016 / Accepted: 17 August 2016 / Published online: 24 August 2016

Abstract Cereal cyst nematodes (CCN) are a global economic problem for cereal production. *Heterodera filipjevi* is one of the most commonly identified and widespread CCN species found in many wheat production regions of the world. Transferring novel genes for resistance to *H. filipjevi* from wild relatives of wheat is a promising strategy for protection of wheat crops. A set of wheat–*Dasypyrum villosum* chromosome addition lines, T6V#4S·6AL translocation lines and their donor parental lines were tested for their response to the nematode. *D. villosum* and wheat–*D. villosum* disomic addition line DA6V#4 were resistant. As T6V#4S·6AL translocation lines were susceptible, resistance was presumed to be located on chromosome 6V#4L. The objective of this study was to produce and characterize wheat–6V#4L translocations and confirm the chromosome location of the resistance. Introgression lines T6V#4L·6AS, T6V#4L-4BL·4BS and DT6V#4L were developed and subjected to molecular cytogenetic analysis. These and four additional wheat–6V#4 introgression lines were tested for response to *H. filipjevi* in the greenhouse. The results indicated that introgression lines DA6V#4, T6V#4L·6AS, T6V#4L-4BL·4BS, T6V#4L·6V#4S-7BS and DT6VL#4 had higher levels of *H. filipjevi* resistance than their recurrent parent. However, Del6V#4L-1 and translocation line T6V#4S·6AL were equally susceptible to wheat cv. Chinese Spring. The CCN resistance gene, temporarily named *CreV*, was therefore physically mapped to chromosome arm 6V#4L FL 0.80–1.00. Translocation chromosomes T6V#4L·6AS transferred to a modern wheat cv. Aikang 58 with its co-dominant molecular markers could be utilized as a novel germplasm for CCN resistance breeding in wheat.

Keywords Wheat · *Dasypyrum villosum* · *Heterodera filipjevi* · *CreV*

Electronic supplementary material The online version of this article (doi:10.1007/s11032-016-0549-9) contains supplementary material, which is available to authorized users.

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Introduction

Cereal cyst nematodes (CCN) are major soilborne parasites that cause serious yield losses in cereal crops

in many parts of the world (Nicol and Rivoal 2008). CCN damages the roots of barley, oats and wheat through invasion of root cells and establishment of feeding sites. Symptoms include excessive root branching, reduced plant vigor, small spikes and reduced yield. The *Heterodera avenae* group parasitizing cereals consists of 12 valid species; the three economically most important in cereal production are *H. avenae*, *H. filipjevi* and *H. latipons* (Nicol and Rivoal 2008). Effective management of this parasite in Australia demonstrated that control can be achieved through host resistance and maintenance of nematode populations below economically damaging thresholds (Ogbonnaya et al. 2009).

Genetic stocks with stable resistance to CCN are necessary for breeding resistant cultivars, but resistance genes are uncommon in *Triticum aestivum*. Only two CCN resistance genes have been identified from hexaploid wheat, namely *Cre1* in AUS10894 (Williams et al. 1994) and *Cre8* in cv. Festiguay (Paull et al. 1998; Jayatilake et al. 2015). Otherwise, the wild relatives of wheat are important sources of CCN resistance that can be transferred to cultivated wheat (Barloy et al. 2007). To date, nine *Cre* genes have been transferred from wild wheat relatives, including *Cre2* (Delibes et al. 1993), *Cre5* (Jahier et al. 1996, 2001) and *Cre6* (Ogbonnaya et al. 2001) from *Aegilops ventricosa*, *Cre3* and *Cre4* from *Aegilops tauschii* (Eastwood et al. 1991, 1994), *Cre7* from *Aegilops triuncialis* (Romero et al. 1998) and *CreR* from *Secale cereale* (Taylor et al. 1998). *CreR* was located on the long arm of chromosome 6R and its transfer to wheat was attempted by crossing a disomic substitution line 6R(6D) to the *ph1bph1b* Chinese Spring mutant line (Asiedu et al. 1990). Among resistance genes, *Cre1* and *Cre8* have been the most useful sources used in Australian wheat breeding programs (Murray and Brennan 2009), although their effectiveness depends on the particular race and CCN species (Nicol et al. 2009). For example, the *Cre3* gene proved to be highly effective in Australia and moderately effective against *H. avenae* populations in Hebei province in China, but was not effective in Henan province (Nicol and Rivoal 2008; Peng et al. 2009). The known *Cre* genes are mostly *H. avenae* resistance genes, with only *CreR* and *Cre1* being effective against *H. avenae* pathotype Ha13 in Australia and local populations of *H. avenae* in Henan, as well as against *H. filipjevi* in Turkey (Nicol et al. 2009). *H. filipjevi* is currently the most common and widespread CCN species occurring in dry-land winter wheat production regions of China and Turkey. The distribution of *H. filipjevi* has been demonstrated to be wider than previously documented, with losses in wheat in Turkey averaging 40 %, and is especially damaging under drought conditions (Nicol et al. 2009). Recent field micro-plot studies in China have also indicated its economic importance (Peng et al. 2009). Global warming and increased retention of maize-straw in farming systems will exacerbate the impact of this nematode in intensive production systems of the middle-east region in China (Peng et al. 2009). Of the published *Cre* genes, only *CreR* was found to provide high levels of *H. filipjevi* resistance in China, while the other genes tested (*Cre1*, *Cre2*, *Cre3*, *Cre7* and *Cre8*) were found to be ineffective (Yuan et al. 2011). Recently, in a collection of 290 winter wheat accessions, 1 % of the wheat accessions were ranked as resistant and 16 % as moderately resistant to *H. filipjevi* (Pariyar et al. 2016). The restricted number of wheat germplasms with resistance to *H. filipjevi* therefore makes it necessary to find and transfer novel resistant genes from wild relatives.

Dasypyrum villosum (2n = 14, genomes VV), a cross-pollinated relative of bread wheat, has many agronomically important traits. Seven different *D. villosum* accessions have been introgressed into bread wheat (De Pace et al. 2011) and the beneficial alien genes located on chromosomes 1V, 2V, 4V, 5V and 6V were transferred into bread wheat by translocations (Chen et al. 1995; Zhang et al. 2005, 2010; Qi et al. 2011; Zhang et al. 2014, 2015). Zhang et al. (2012) tested a set of wheat–*D. villosum* addition lines for response to *H. filipjevi* and found that *D. villosum* and the chromosome 6V disomic addition line DA6V were both highly resistant. This indicated that *D. villosum* may be a valuable source of resistance to CCN in wheat. The present study describes the production of wheat–*D. villosum* introgression lines involving chromosome 6V and localization of a CCN resistance gene to the long arm of a T6V#4L·6AS chromosome.

Plant materials

The plant materials used in this study included *T. aestivum* cv. Chinese Spring and *D. villosum* accession GP005 (2n = 14, VV, the donor of the V chromatin in the present study). In order to distinguish this

accession from others, the chromosomes of GP005 were numbered #4 by De Pace et al. 2011). Details of other lines, including *T. durum* cv. ZY1286–*D. villosum* amphiploid (2n = 42, AABBVV), *T. aestivum–D. villosum* disomic substitution line DS6V#4 (6A), translocation line T6V#4S·6AL, and other wheat–*D. villosum* genetic stocks, are given in Table 1. These materials were all developed and are maintained at the Cytogenetic Institute, Nanjing Agricultural University (CINAU). Seventy-six *T. aestivum–D. villosum* translocations involving chromosomes 1V#4 to 7V#4 developed by Bie et al. (2007) and Cao et al. (2009) were used in a search for those translocation lines carrying molecular markers on chromosome 6VL. Wheat cultivar Aikang 58 was used as a recurrent backcross parent for translocated chromosomes and cv. Wenmai 19 was used as a *H. filipjevi*-susceptible control.

In order to produce compensating Robertsonian translocations (RobTs) involving chromosomes 6A of wheat and 6V#4 of *D. villosum*, crosses were made between DS6V#4 (6A) and a homozygous *ph1bph1b* Chinese Spring mutant line. F1 plants with 42 chromosomes that were double monosomic for chromosomes 6A/6V were identified cytologically and self-pollinated. The F2 progeny were genotyped by molecular markers for putative RobTs.

Cytological procedures

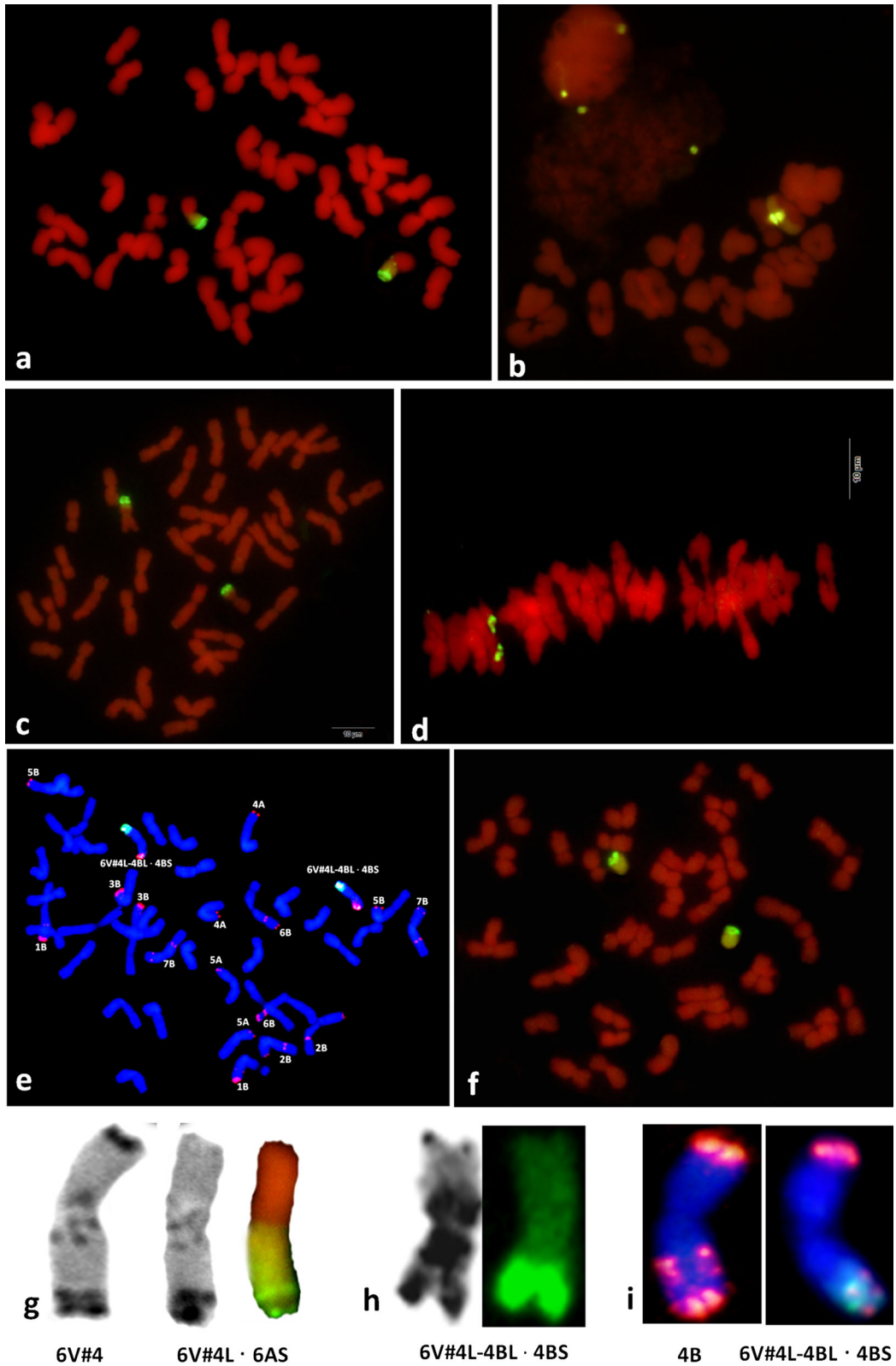
Chromosome C-banding was conducted according to Gill et al. (1991). Genomic in situ hybridization (GISH) and fluorescence in situ hybridization (FISH) followed Zhang et al. (2004). The total genomic DNA of *D. villosum* labeled with fluorescein-12-dUTP was used to detect segments of chromosome 6V. Clone *pSc119.2*, a 120-bp tandem-repeat sequence from rye inserted in the plasmid pBR322 (Mukai et al. 1993) and labeled with digoxigenin-11-dUTP, was used for identification of the wheat B-genome chromosomes. Images of chromosomes were captured with an Olympus BX60 fluorescence microscope fitted with a SPOT Cooled Color Digital Camera (Diagnostic Instruments; http://www.diaginc.com).

PCR sequence-tagged site (STS) marker analyses

Three previously reported 6V#4 arm-specific expressed sequence tag (EST)-PCR markers, CINAU15 for 6V#4S and 6L-4 and WMC256 for

Table 1 Wheat–*Dasypyrum villosum* 6V#4 genetic stocks used in this study

Line designation	Genetic stock	Description/background	Reference
11R274	DA6V#4	Chinese spring–*D. villosum* disomic addition 6V	Zhang et al. (2013)
92R137	T6V#4S·6AL	Yangmai 5	Chen et al. (1995)
92R112	Del 6V#4L-1	Chinese spring–*D. villosum* chromosome 6V deletion line. The breakpoint was located at fraction length (FL) 0.66 in the long arm	Qi et al. (1998)
11R082	T6V#4L·6VS–7BS	Chinese spring	Bie et al. (2015)
NAU423	T6V#4L·6AS	Chinese spring	This study
NAU424	T6V#4L–4BL·4BS	Chinese spring	This study
NAU426	DT6V#4L	Chinese spring ditelosomic 6V#4L	This study
14zrq7-29-1	T6V#4L·6AS	(Aikang 58 × NAU423)/Aikang 58 BC2F2	This study
14zrq7-29-2			
14zrq7-29-3			
14zrq7-29-4			
14zrq7-29-5			
14zrq202-1	T6V#4L-4BL·4BS	(Aikang 58 × NAU424)/Aikang 58 BC2F2	This study
14zrq202-2			
14zrq202-3			
14zrq202-4			
14zrq202-5			


a
b
c
d
e
f
g
h
i
5B
4A
6V#4L-4BL · 4BS
3B
3B
4A
6B
1B
7B
5A
5A
6B
2B
2B
1B
6V#4L-4BL · 4BS
5B
7B
6V#4
6V#4L · 6AS
6V#4L-4BL · 4BS
4B
6V#4L-4BL · 4BS

◀**Fig. 1** Cytogenetic analyses of wheat–6V#4L introgression lines. GISH using *D. villosum* genomic DNA labeled with digoxigenin-11-dUTP as probe. *D. villosum* chromatin fluoresced with a *yellowish–green color*. **a** and **b** Mitotic and meiotic GISH patterns of NAU423 containing a pair of T6V#4L·6AS Robertsonian translocation chromosomes; **c** and **d** mitotic and meiotic GISH patterns of NAU424 containing a pair of T6V#4L-4BL·4BS translocation chromosomes. **e** Dual-color GISH/FISH patterns of NAU424. GISH used *D. villosum* DNA as probe (*green*) and B-genome chromosomes painted by the *pSc119.2* probe (*red*) are marked. **f** Mitotic GISH patterns of NAU426 containing a pair of 6V#4L chromosome arms. **g** From *left* to *right*: C-banding pattern of 6V#4; sequential C-banding and GISH patterns of T6V#4L·6AS; **h** sequential C-banding and GISH patterns of T6V#4L-4BL·4BS translocated chromosome; **i** dual-color *pSc119.2* FISH patterns of chromosome 4B and T6V#4L-4BL·4BS translocated chromosome. (Color figure online)

6V#4L, were used to identify translocations involving chromosome 6V (Table 2). GWM570 for chromosome 6A long arm was used to select homozygous translocations (Qi et al. 2011). A further seven 6VL-specific markers were selected from 53 EST-based primers to amplify different regions of wheat homeologous group-6L chromosomes. The primers were designed using Conserved Primers 2.0 software (Frank et al. 2009). Genomic DNA was isolated from young leaves using a DNA Secure Plant Kit (Tiangen Biotech Co., Ltd, Nanjing, China). The procedures for PCR amplification followed those of Zhang et al. (2015). The PCR products were separated on 8 % polyacrylamide gels in 1× TBE buffer.

Table 2 Sequences of 6V#4-specific STS primers used in this study

Primer code	EST	Location	Tm (°C)	Primer sequence 5′–3′
CINAU692	Ta.12477.1.S1_at	6VL	56	Forward: TGGAGTGGAACGCAGTG
				Reverse: TCAACCAGCTAGACAAGACA
CINAU796	Ta#S32636387	6VL	55	Forward: GGCTCCAATATCTTCGGTCA
				Reverse: AAGCCCTGCTCCCCTATATC
CINAU860	Ta#S32733555	6VL	57	Forward: CTCCAGAACAGTCCGCCTTA
				Reverse: AGCCTGCAAGTGCTCAAACT
CINAU871	Ta#S13146969	6VL	55	Forward: TGGTGGCCAGCAAGTTAAG
				Reverse: TGCTGTTCTTCATTGGGTTG
CINAU933	Ta#S17884703	6VL, 6AL	56	Forward: GCCTGGGTGTGACAAATACC
				Reverse: GGCGTCCAAAATCTAATCCA
CINAU954	Ta#S16222288	6VL, 6AL	55	Forward: GCAGCTCATGGATCAGACAA
				Reverse: GGCCAAGTTCCAGATAAGCA
CINAU250	–	6VL	55	Forward: TGGAGTGGAACGCAGTG
				Reverse: ATCAACCAGCTAGACAAGACA
6L-4[a]	BE471191	6VL, 6AL	55	Forward: TGGCTGATGATTCTGCTTCA
				Reverse: CCACAAGGTTCAGCCAAGTT
CINAU15[b]	Hv-S/TPK	6VS, 6AS, 6BS, 6DS	55	Forward: AGATCCAACACCAGTTCAAG
				Reverse: ATGTTATGGAGGCTTGTGTC
WMC256[c]	–	6VL, 6AL	53	Forward: CCAAATCTTCGAACAAGAACCC
				Reverse: ACCGATCGATGGTGTATACTGA
GWM570[d]	–	6AL	60	Forward: TCGCCTTTTACAGTCGGC
				Reverse: ATGGGTAGCTGAGAGCCAAA

[a] 6L-4 is a 6VL-specific marker developed by Bie et al. (2015)

[b] CINAU15 is a 6VS-specific marker based on the sequence of a serine/threonine kinase (*Hv-S/TPK*) (Cao et al. 2006)

[c] WMC256 is also 6VL-specific (Zhang et al. 2006)

[d] GWM570 is 6AL-specific (Qi et al. 2011)

CCN response assessment

Mass culture of CCN in a controlled greenhouse was used to screen the plant material over 3 years in order to determine response to a *H. filipjevi* Xuchang population. The procedures followed those of Zhang et al. (2012). Ten seedlings per line were planted individually in tubes (30 mm × 130 mm) filled with sterile soil and inoculated four times. After 75 days the number of white females of *H. filipjevi* per tube was counted. Relative resistance index (RRI) was used as the evaluation indicator (Zhang et al. 2012): RRI = [1 – (the mean number of white females per plant on a tested line/the mean number of white females per Wenmai 19 check plant)]. The scale of resistance was classified based on the RRI as: RRI = 1, immune (I); $0.9 \leq$ RRI < 1.0, highly resistant (HR); $0.7 \leq$ RRI < 0.9, resistant (R); $0.5 \leq$ RRI < 0.7, moderately susceptible (MS); $0.3 \leq$ RRI < 0.5, susceptible (S); RRI < 0.3, highly susceptible (HS).

Results

Development of chromosome 6VL-specific STS markers

Seven of the 53 STS primer pairs specifically identified the 6V#4 chromosome and polymorphism was detected among cv. Chinese Spring, *T. durum* cv. ZY1286 (AABB), *T. durum* cv. ZY1286–*D. villosum* amphiploid (AABBVV), and 6V chromosome disomic addition line DA6V#4. Further analysis revealed that all seven molecular markers were absent in T6V#4S·6AL; they were therefore assigned to chromosome arm 6V#4L (Table 1). Including two previously reported 6VL-specific markers, a total of nine loci on the long arm of chromosome 6V, including three located in the distal region, were used to detect 6V#4L segments in the 76 *T. aestivum–D. villosum* translocation lines.

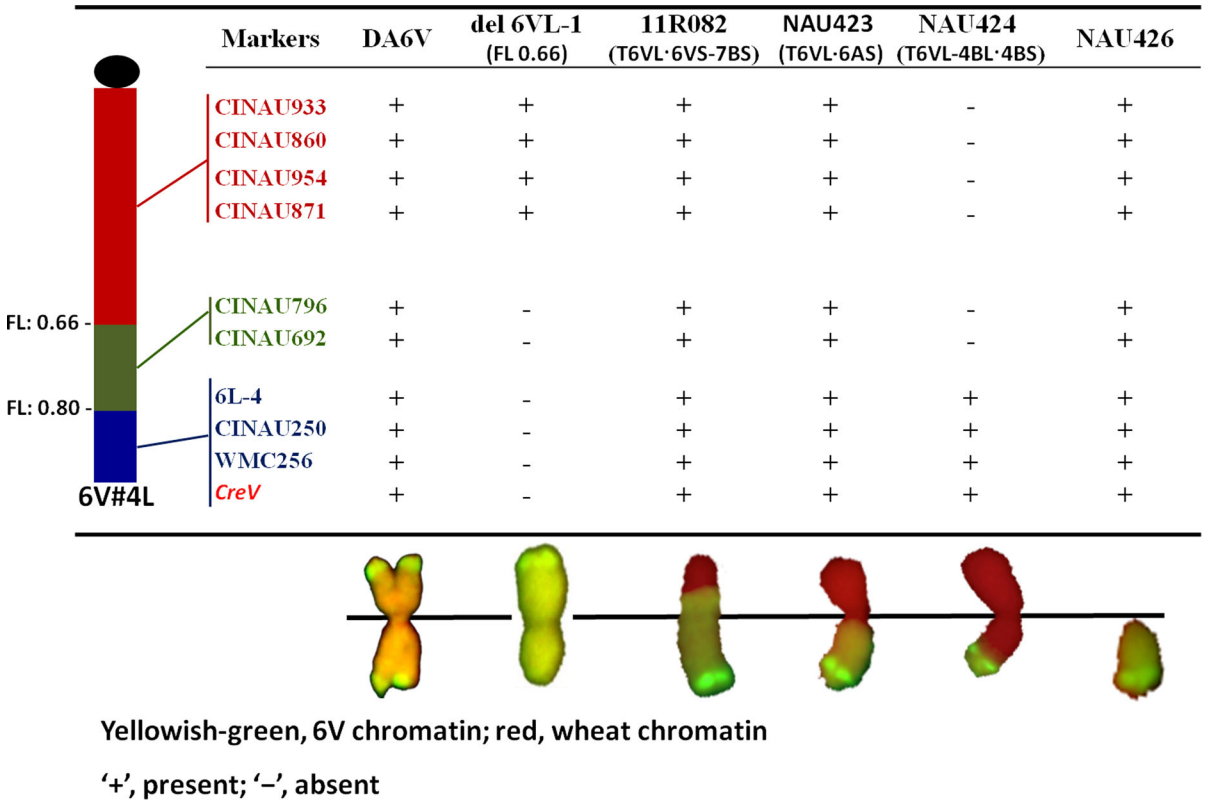


Fig. 2 A rough physical map of 6VL including nine molecular markers in three bins. The cereal cyst nematode (CCN) resistance gene *CreV* was physically mapped to the FL 0.80–1.00 region of 6V#4L

Table 3 Responses of genetic stocks to *H. filipjevi* in the greenhouse

Material number	Genetic stock	No. of seedlings	Mean number of females	Relative resistance index (RRI)	Rating
Evaluation in 2012					
11R274	DA6V#4	10	16.1 ± 4.6	0.72	R
92R137	6V#4S·6AL	10	32.8 ± 6.2	0.43	S
92R112	Del 6V#4L-1	10	41.0 ± 15.5	0.40	S
11R082	T6V#4L·6VS-7BS	10	16.0 ± 9.2	0.72	R
NAU423	T6V#4L·6AS	10	16.9 ± 4.4	0.71	R
NAU426	DT6V#4L	10	9.8 ± 2.5	0.83	R
Chinese spring		10	34.3 ± 7.9	0.43	S
Wenmai 19		10	57.4 ± 4.2	0.00	HS
Evaluation in 2013					
11R274	DA6V#4	10	10.1 ± 3.2	0.81	R
92R137	6V#4S·6AL	10	28.3 ± 9.1	0.51	MS
92R112	Del 6V#4L-1	10	22.4 ± 4.8	0.57	MS
11R082	T6V#4L·6VS-7BS	10	14.4 ± 3.5	0.73	R
NAU423	T6V#4L·6AS	10	16.0 ± 3.8	0.70	R
NAU424	T6V#4L-4BL·4BS	10	1.25 ± 1.3	0.98	HR
NAU426	DT6V#4L	10	12.2 ± 6.1	0.79	R
Chinese spring		10	25.4 ± 8.2	0.52	MS
Wenmai 19		10	52.9 ± 6.2	0.00	HS
Evaluation in 2014					
14zrq7-29-1	T6V#4L·6AS	10	1.6 ± 2.3	0.96	HR
14zrq7-29-2	T6V#4L·6AS	10	4.3 ± 2.7	0.90	HR
14zrq7-29-3	T6V#4L·6AS	10	6.5 ± 9.2	0.85	R
14zrq7-29-4	T6V#4L·6AS	10	7.9 ± 5.9	0.82	R
14zrq7-29-5	T6V#4L·6AS	10	6.1 ± 4.4	0.86	R
14zrq202-1	T6V#4L-4BL·4BS	10	3.5 ± 4.1	0.92	HR
14zrq202-2	T6V#4L-4BL·4BS	10	3.4 ± 2.3	0.92	HR
14zrq202-3	T6V#4L-4BL·4BS	10	3.3 ± 2.9	0.93	HR
14zrq202-4	T6V#4L-4BL·4BS	10	2.0 ± 3.3	0.96	HR
14zrq202-5	T6V#4L-4BL·4BS	10	3.0 ± 1.4	0.93	HR
Aikang 58		10	28.9 ± 2.0	0.35	S
Wenmai 19		10	44.5 ± 11.5	0.00	HS

Development and characterization of translocation lines

A total of 153 F2 plants derived from F1 plants double monosomic for 6V and 6A were screened with markers CINAU15 for 6V#4S and 6L-4 for 6V#4L. 6VL- or 6VS-specific polymorphic markers were observed in seven plants. Of them, four plants were positive for the 6VS marker and were highly resistant to powdery mildew, indicating that they were wheat-6VS Robertsonian translocations. The other three plants had only the 6V#4L marker and were susceptible to powdery mildew, suggesting that they involved misdivision products for 6V#4L. Homozygote line NAU423 was identified in F3 plants by GISH (GISH using *Dasypyrum villosum* genomic DNA labeled with digoxigenin-11-dUTP as probe and the alien chromatin in colour Fig. 1 is yellowish-green color). Mitotic and meiotic GISH patterns showed that NAU423 was a RobT line with $2n = 42$, pairing as 21 bivalents at meiotic metaphase I (Fig. 1a, b). Sequential C-banding and GISH

revealed that the opposite arm of the translocated chromosome in NAU423 did not have recognizable C-banding patterns, which indicated that the chromosome arm could belong to wheat A genome (Fig. 1g). Molecular markers analysis showed that the diagnostic band of 6V#4S-specific marker CINAU15 was absent in NAU423 (Fig. S1d), but all the diagnostic bands of nine 6V#4L-specific markers were present with simultaneous absence of 6AL-specific bands, indicating that this translocation chromosome could be T6V#4L·6AS (Fig. S1a, b and c).

NAU424 (Fig. 1c) and NAU426 (Fig. 1f) were identified among the 76 introgression lines by molecular markers and GISH analysis. NAU424 was a small alien terminal translocation segment with its breakpoint at FL 0.80. The alien segment was the terminal part of 6VL and the diagnostic bands of three 6VL-specific molecular markers were present whereas the other six 6VL-specific molecular markers were not (Fig. 2). The standard FISH patterns for *pSc119.2* established for the wheat cv. Chinese Spring by Mukai et al. (1993) were compared with the *pSc119.2* pattern of NAU424 (Fig. 1e, i). The combined sequential C-banding and GISH patterns (Fig. 1h) showed that the translocation involved wheat chromosome arm 4BS and segment of chromosome arm 4BL. Chromosome at meiotic meiosis I (MI) configurations in pollen mother cells (PMCs) showed 21 ring or rod bivalents (Fig. 1d), among which one ring bivalent was formed by two wheat–*D. villosum* translocation chromosomes, indicating that this line was homozygous. Hence, NAU424 was a homozygous 6V#4L-4BL·4BS translocation line. NAU426 was a disomic telosomic addition line because all nine 6VL-specific markers were present whereas the 6VS-specific marker CINAU15 was absent (Fig. 2).

Characterization of resistance in homozygous translocation lines

Previous screening of a set of *D. villosum* disomic addition stocks from cv. Chinese Spring revealed that the disomic addition line DA6V#4 was resistant to *H. filipjevi* (Zhang et al. 2012). Homozygous lines T6V#4S·6AL (92R137), T6V#4L·6AS (NAU423), Del6V#4L-1 (92R112), T6V#4L·6VS-7BS (11R082) and DT6V#4L (NAU426) along with resistant DA6V#4 (11R274), susceptible recurrent parent cv. Chinese Spring and the highly susceptible cv. Wenmai 19 were tested for reaction to *H. filipjevi* (Table 3). T6V#4S·6AL and Del6V#4L-1 exhibited low RRIs similar to the susceptible parent, Chinese Spring. The other lines, including T6V#4L·6AS, DT6V#4L, T6V#4L·6VS-7BS and DA6V#4, showed significantly higher RRIs than their background parent in both years. All of these lines had the same alien whole arm, 6V#4L, suggesting that a CCN resistance gene was located on this chromosome arm. T6V#4L-4BL·4BS (NAU424) also exhibited a higher RRI (0.98) than cv. Chinese Spring (0.52) in the greenhouse experiment carried out in 2013. For further confirmation, the RRI was determined in 2014 for five homozygous T6V#4L·6AS lines and five homozygous T6V#4L-4BL·4BS lines derived from BC2F2 populations of crosses Aikang 58 × NAU423 or Aikang 58 × NAU424. All the translocation selections were resistant whereas Aikang 58 was susceptible. We therefore presumed that a resistance gene, temporarily named *CreV* was located in the alien segment, which represented FL 0.80–1.00 of 6V#4L. Roots of cv. Aikang 58 (Fig. S2d) infested by CCN showed more CCN eggs and abnormal branching than those of 14zrq7-29-1 (T6V#4L·6AS) (Fig. S2e), confirming the resistance attributable to *CreV*.

Discussion

Employing alien genes from the wheat secondary gene pool, such as *D. villosum*, is an effective way of increasing genetic diversity and improving cultivated wheat (Li et al. 2007). *CreV* is the first nematode resistance gene, discovered in *D. villosum*, that is effective against *H. filipjevi*. The integrated use of cytogenetic stocks, molecular markers, GISH, and FISH analyses, allowed the introgression of gene *CreV* from *D. villosum* into bread wheat by developing translocated chromosomes T6V#4L·6AS and T6V#4L-4BL·4BS, and this gene was mapped to 6VL-0.80-1.00. Qi et al. (2011) developed a compensating wheat–*D. villosum* RobT T6V#3L·6AS with a temperature-sensitive gene *Sr52* resistant to stem rust race Ug99. In the present study, two wheat–*D. villosum* translocation lines, T6V#4L·6AS (NAU423) and T6V#4L-4BL·4BS (NAU424) with the *H. filipjevi* resistance gene, *CreV,* were developed and transferred into cv. Aikang 58. The chromosome arm 6VL in different accessions of *D. villosum* may carry different

beneficial genes and the presence of *Sr52* gene on 6V#3L and *CreV* on 6V#4L opens up the possibility of pyramiding the two genes into the same line by the crossing T6V#4L·6AS, *CreV* × T6V#3L·6AS, *Sr52*.

Host-plant resistance is the most effective method of controlling *H. avenae* and *H. filipjevi*. The compensating translocation 6V#4L·6AS present in the stock NAU423 has a *H. filipjevi* resistance gene, *CreV*, which may be useful in wheat improvement. After backcrossing twice, the T6V#4L·6AS translocation line and its recurrent parent Aikang 58 are very similar in their major agronomic characteristics, including plant height, spike length, seed weight, flowering and maturity stages (Fig. S2a, b and c). However, the detailed effects of T6V#4L·6AS translocated chromosomes on yield- and quality-related traits are currently unknown. Further research is also needed to confirm the value of this translocated chromosome in different wheat backgrounds against both *H. filipjevi* and *H. avenae* under field conditions.

Ionizing radiation has proved to be an efficient method of inducing chromosome translocation (Sears 1993). Seventy-six translocation lines involving 1V–7V chromosomes were developed by irradiating *T. durum–D. villosum* amphiploid (AABBV) pollen using a ^{60}Co source (Bie et al. 2007; Cao et al. 2009), but most of them were non-compensating translocations because the chromosome breakages and rejoins produced by radiation are random. Two molecular markers (6L-4 and WMC256) allowed for the identification of a 6VL-translocation line NAU424 from the 76 lines of the translocation pool. Sequential C-banding analysis was used to identify the wheat chromosome arms involved in NAU424 translocations. The C-banding patterns preliminarily determined that the translocated chromosomes in NAU424 could belong to the B genome (Fig. 1h). Dual-color *pSc119.2* FISH patterns can not only differentiate the wheat and *D. villosum* chromatin, but also determine the identities of particular chromosomes of the B genome. Referring to the standard FISH pattern for *pSc119.2* (Mukai et al. 1993) established for wheat variety Chinese Spring, it could be concluded that the wheat chromosome involved in the translocation with 6V#4L in NAU424 was chromosome 4B (Fig. 1e, i). The terminal and internal *pSc119.2* FISH patterns of the long arm of chromosome 4B were absent in the T6V#4L-4BL·4BS translocated chromosome, indicating that approximately 60 % of the long arm of chromosome 4B was substituted by approximately a 20 % segment of the long arm of chromosome 6V#4L in this translocated chromosome (Fig. 1i). The genes located on the missing segment of the long arm of chromosome 4B in NAU424 cannot be compensated by the alien chromatin of *D. villosum*. Thus, T6V#4L-4BL·4BS translocated chromosomes could have a negative effect on agronomic characteristics and fertility. After transferring T6V#4L-4BL·4BS translocated chromosomes into a modern wheat cultivar Aikang 58, ten lines involving this translocated chromosome were all vigorous but less fertile. The spikelet fertility of nine lines was approximately 70 %, while one line was less than 50 %. Therefore, the T6V#4L-4BL·4BS translocation line may not be used in wheat breeding except for physical mapping of the alien genes.

Due to the high cost and time involved in phenotypic evaluation of CCN resistance, molecular markers will be of great benefit for selection. Nine informative molecular markers which are sufficient to recover the alien chromosome arm 6 V#4L could be used for molecular-assisted selection. When using 6V#4L·6AS lines as resistant donors, co-dominant 6VL-specific EST-PCR markers that distinguish 6VL and 6AL can be used in agarose gel electrophoresis, as a convenient marker-assisted breeding assay. Of the nine 6VL-specific markers evaluated in this study. 6L-4, WMC256, CINAU933 and CINAU954 are easily used and co-dominant. In addition, the molecular markers developed in three bins of chromosome arm 6V#4L in the present study will not only facilitate the selection of T6V#4L·6AS translocated chromosomes, but also will be useful for screening the shortening 6V#4L segment retaining the *CreV* gene by *ph1b*-induced recombinants or ionizing radiation on T6V#4L·6AS translocated chromosomes.

In conclusion, the novel *H. filipjevi* resistance gene *CreV* was identified and physically mapped to chromosome bin 6VL-0.80–1.00. *CreV* is present in the translocation lines 6V#4L·6AS and 6V#4L-4BL·4BS, which were transferred to an adapted cv. Aikang 58 line. The co-dominant molecular markers developed are available for marker-assisted selection in breeding programs.

Acknowledgments This study was supported by the National Natural Science Foundation of China (Project no. 30871519) and The 863 High-tech Program (Project no. 2012AA101105). The authors would like to thank Dr. Ian Riley, Australia and Prof. Bob McIntosh, the University of Sydney Plant Breeding

Institute Cobbitty, NSW, Australia, for their comments on the manuscript.

Author contributions Conceived and designed the experiments: Ruiqi Zhang; Developed translocation lines NAU424 and NAU426: Yigao Feng; Developed the translocation line NAU423: Haifeng Li; Identified the CCN resistance: Hongxia Yuan, Junli Dai and Honglian Li; Wrote the paper: Ruiqi Zhang, Aizhong Cao and Liping Xing.

Compliance with ethical standards

Conflict of interest All the authors have no conflicts of interest and agree with publication.

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