J. Wheat Res. 5 (1) : 35-40

Tracking of cereal cyst nematode resistance genes in wheat using diagnostic markers

Pradeep Sharma*, Manoj Saini, Om Prakash Gupta, Nupur Gupta, Arun Kumar Singh[1], Rajan Selvakumar, Vinod Tiwari and Indu Sharma

Directorate of Wheat Research
Karnal-132 001, India

Abstract

Cereal cyst nematode (CCN), *Heterodera avenae*, has been reported throughout the world causing reduction in yield of wheat. The major focus of this study was to screen wheat accessions using diagnostic markers for resistance genes to identify the genes for resistance against CCN infestation and aid in the development of the resistant cultivars of bread wheat. Two PCR based co-dominant markers tightly linked to *Cre3* and *Cre5* resistance genes against CCN were used in this study to screen advanced lines from India and two international nurseries. In this study, only RAJ 1 was found positive for presence of *Cre3* and *Cre5* genes.

Key words: *Cre*3, *Cre*5, CCN, wheat

Received: 23 May 2013/Accepted: 05 June 2013

Introduction

The Cereal Cyst Nematode (CCN) complex is a group of 12 valid species, with *Heterodera avenae*, *H. filipjevi* and *H. latipons* considered the most economically important species in West Asia, North Africa and Mediterranean countries. The sedentary *Heterodera spp.* has a global distribution which leads to significant yield losses in many countries of the world, particularly where rainfed cereal predominated (Nicol *et al.,* 2003). *H. avenae* is the most studied CCN species with several biotypes found mostly in temperate cereals (Nicol and Rivoal, 2008) which causes yield loss in wheat ranging from 15-20 per cent in Pakistan, 40-92 per cent in Saudi Arabia and 23-50 per cent in Australia. However, in barley losses were ranged from 17-77 per cent in Saudi Arabia follwoed by 20 per cent in Australia (Nicol, 2002). In India, *H. avenae*, was identified from Sikar area of Rajasthan by Vasudeva (1958). Yield loss upto 47 per cent due to CCN in sandy soils of Rajasthan during late 1960's and early 1970's has been reported in Kalyansona variety of wheat (Mathur *et al*., 1980). Five biotypes of *H. avenae* have been reported from Rajasthan and Haryana, out of which two biotypes were authenticated (Mathur *et al*., 1974). Subsequently, Bishnoi and Bajaj (2004) reported existence of two species namely, *H. avenae* in Rajasthan, southern Haryana and Madhya Pradesh and *H. filipjevi* in northern Haryana, Punjab and Himachal Pradesh. In some areas of India, infestation has resulted in complete crop failure (Van Berkum and Seshadri, 1970).

Eight genes for resistance to CCN have been identified in hexaploid wheat and few in barley also. *Cre1* has come from *Triticum aestivum* chromosome 2B (Williams *et al.,* 1994), *Cre2* transferred to wheat from *Aegilops ventricosa* (Delibes *et al.,* 1993), *Cre3* on chromosome 2D transferred from *A. tauschii* (Eastwood *et al.,* 1994), *Cre4* in *A. tauschii* (Eastwood *et al.*,1991), *Cre5* on VPM1 segment of chromosome 2A from *A. ventricosa* (Jahier *et al.,* 2001), *Cre6* in *A. ventricosa* 5NV (Ogbonnaya *et al.,* 2001) , *Cre7* in *A. truincialis* (Romero *et al.,* 1998) and *Cre8* on chromosome 6B in *T. aestivum* (Williams *et al.,* 2003). CCN resistance genes have also been mapped in rye (6R) (Taylor *et al.,* 1998) and barley (2H) (Kretschmer *et al.,* 1997). A linkage disequilibrium study (Paull *et al.,* 1998) found that an RFLP locus, *Xcd0347*, that was associated with the Festiguay derived CCN tolerance in wheat *cvs* Molineux, Frame and Barunga. *Cre1* confers resistance to several European pathotypes of *H. avenae* as well as the Australian biotype, although with varying degree of nematode reproduction in different genetic backgrounds. Comparison of *Cre1* with the nematode resistance gene, *Cre3* derived from the diploid D genome progenitor of wheat *A. tauschii*, showed that both provide resistance to the Australian biotype but differ in their specificity to European and Middle-East biotypes (Ogbonnaya *et al.,* 2001). Some of these genes have been mapped and molecular markers either linked or co-segregating with resistance genes have been reported for *Cre1* (Williams *et al.,* 1994), *Cre3* (Eastwood *et al.,* 1994; Lagudah *et al.,* 1997) and *Cre6* (Ogbonnaya *et al.,* 2001).

Molecular marker based diagnostics has emerged as a potential tool to screen wheat germplasm for use in incorporating resistance against CCN in wheat cultivars. The aim of this study was to screen wheat genotypes/elite lines for the presence of *Cre3* and *Cre5* resistance genes to help in the development of CCN resistant wheat cultivars.

[1] Current address: Division of Nematology,IARI, New Delhi
* Correspondence author email: *neprads@gmail.com*

Materials and methods

CCN response studies under artificial condition: Present study was carried out at Directorate of Wheat Research, Karnal, during 2010-11 and 2011-12. Twenty accessions each from Cereal Cyst Nematode Host Differential (CCNHD 0910) and Soil-Borne Pathogen Spring Wheat (SBPSW INT 0910) nurseries obtained through CIMMYT-Turkey (Table 1 & 2) were screened for identifying their response against Karnal CCN biotype under artificial conditions. One month before harvesting, plant samples were collected as per standard procedure. Response scoring was done as per scale (Kaur *et al.*, 2008): R – upto 4 cysts/plant; MR– 4.1 to 9 cysts/plant; S – 9.1 to 20 cysts/plant; HS – > 20 cysts/plant.

Table 1. Screening for presence of CCN resistance genes and host plant response data under artificial conditions in SBPSW nursery

S.No.	Acc. No.	Varieties/Germplasm	Cre3 (bp)	Cre5 (bp)	CCN population (cysts/ plant)	Host response
1	030798	AUS GS50AT34/SUNCO// CUNNINGHAM	-	250	15	S
2	031036	CHIRYA.3	-	250	16	S
3	020615	CROC_1/AE.SQUARROSA (224)//OPATA	-	250	11	S
4	20617	CROC_1/AE.SQUARROSA (224)//OPATA	-	250	14	S
5	20616	CROC_1/AE.SQUARROSA (224)//OPATA	-	250	14	S
6	30800	SUNCO/2*PASTOR	-	250	19	S
7	030947	SUNR23 (GALA 2-49/ (CN#133/ SUNSTATE*4) //SUNSTATE)	-	250	12	S
8	30898	VL411R	-	-	21	HS
9	20632	SABUF/7/ALTAR84/AE.SQ.(224)/YACO/6/ CROC_1/AE.SQ.(205)/5/ BR12*3/4/IAS55*/ CI14123/3/IAS55*4/EG.AUS//IAS55*4/ALD	-	250	19	S
10	31038	SABUF/3/BCN//CETA/ AE.SQUARROSA(859)	-	-	58	HS
11		VENTURA(SUNT76G)	-	250	13	S
12	20591	FRAME	-	250	15	S
13	20596	GS50A	-	250	12	S
14	20626	ID-2150	-	250	13	S
15	990659	MILAN	-	250	17	S
16	050903	AUS4930.7/2*PASTOR	-	250	16	S
17	30883	6R(6D)	-	250	11	S
18	030901	VP1620	-	250	11	S
19		2-49	-	-	25	HS
20	951027	SERI	-	250	18	S

Note: R: resistant; MR: moderately resistant; S: susceptible; HS: highly susceptible

Isolation of genomic DNA: Total genomic DNA extraction was carried out as per the procedure described by Sharma *et al.* (2013) for screening of resistance genes *Cre3* and *Cre5,* co-dominant markers, in selected wheat accessions/ advanced lines.

PCR amplification: Microsatellite markers linked to *Cre* genes such as *Cre3* (F- 5'GAG GAGTAAGACACATGCCC-3' & R- 5' GTGGCTGGAGATTCAGGTTC-3) and *Cre5* (F-5' ATGGAGATATTTGGCCTACAAC-3 & F-5 CTTGACTTCAAGGCGTGAC-3) were designed to amplify CCN resistance gene. Oligonucleotides were custom synthesized from SIGMA Aldrich Pvt Ltd, USA. PCR for the amplification of template DNA was done using Gradient Thermo cycler S 1000™ (BioRad, USA). Total volume of PCR reaction mixture was kept 25 µl, containing 10x PCR buffer, 10 µM dNTPs, 10 µM of each primers, 1 U *Taq* DNA polymerase and 1 µl template DNA (200ng). PCR amplification was performed with initial denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 30 sec and extension at 72°C for 1 min. Final extension was done at 72°C for 7 min followed by cooling at 4°C

Table 2. Screening for presence of CCN resistance genes and host plant response data under artificial conditions in CCNHD nursery

S.No.	Acc. No.	Varieties/Germplasm	Cre3 (bp)	Cre5 (bp)	CCN population (cysts/ plant)	Host response
1	30883	6R(6D)	-	250	17	S
2	20591	FRAME	-	250	14	S
3		SILVERSTAR	-	250	10	S
4	30903	VP5053	-	250	28	S
5	20628	T-2003	176	250	9	MR
6		RAJ 1	176	250	2	R
7	020626	ID-2150	-	250	12	S
8	990659	MILAN	-	250	13	S
9	030857	AUS 4930.7/2*PASTOR	-	250	13	S
10	030798	AUS GS50AT34/SUNCO// CUNNINGHAM	-	250	13	S
11	30898	VL411R	-	-	14	S
12	020615	CROC_1/AE.SQUARROSA (224)// OPATA	-	-	13	S
13	20616	CROC_1/AE.SQUARROSA (224)// OPATA	-	-	12	S
14	030901	VP1620	-	250	16	S
15	980872	F130L1.12/ATTILA		-	25	HS
16		SONMEZ	-	-	38	HS
17		CPI133859	-	-	25	HS
18		CPI133872 [two plots in WNT05]	-	-	21	HS
19	950590	KATE A-1	-	250	18	S
20		PRINS	-	-	21	HS

Note: MR: Moderately Resistant; R: Resistant; S: Susceptible; HS: Highly Susceptible

PCR based screening: For PCR analysis, a total of 114 wheat accessions, including 40 international nursery lines and 74 Indian wheat varieties and advance varietal trial genotypes (Table 3) were screened for the presence of *Cre*3 and *Cre*5 genes.

Results and discussion

Evaluation of SBPSW and CCNHD nurseries against CCN: The forty genotypes from each SBPSW and CCNHD (Table 1 & 2) were screened for resistance to CCN (Karnal population) showed that the response of entries varied from susceptible (S) to highly susceptible (HS) response to Karnal CCN population.

Seventeen accessions of SBPSW nursery (Table 1) exhibited susceptible response, while three entries showed highly susceptible response under artificial screening. While comparing these results with PCR data, the entries showing HS response lacked the presence of *Cre5* gene (Table 1). The presence of *Cre3* could not be detected in any of the SBPSW entries.

In CCND nursery, genotype Raj-1 exhibited resistant (R) and T-2003 showed moderately resistant (MR) response to the CCN population screened (Table 2). It is noteworthy to mention here that both the genotypes possessed *Cre3* and *Cre5* genes. However, 12 out of the 20 accessions in this nursery showed presence of *Cre5* gene and S response; whereas in five accessions which did not have either of the CCN resistance genes e.g., accessions 980872, SONMEZ, CP133859, CP133872 and PRINS, exhibited HS response. This observation is similar to that of HS response by genotypes from SBPSW nursery.

The artificial response study showed that for incorporating resistance against CCN, the combination of *Cre3* and *Cre5* genes is essential. However, further studies are also required to explore possibilities of other CCN resistance genes as reported in literature (Ogbonnaya *et al.* 1998; Williams *et al.,* 1994). Genotype RAJ 1 could be exlpoited in Indian wheat breeding programme for incorporating resistance against CCN infestation.

Allelic variation in advanced Indian wheat varieties and advance varietal genotypes: In another study involving 74 lines comprising varieties and advance varietal trial (AVT) genotypes from the Indian wheat breeding programme (Table 3) were tested for the presence of *Cre*3 and *Cre*5 genes using diagnostic markers. In this study, *Cre*3 marker gave ~176 bp allele (Fig 1A) in 28 genotypes. This marker gave additional bands ~220 bp in 27 genotypes and ~280 bp in 7 genotypes while its presence could be detected as many as in 12 genotypes (Table 3). Similarly, for *Cre*5 marker expected ~250 bp allele (Fig 1B) was detected in 27 genotypes. In addition to this allele two more alleles were also observed *viz.* ~280 bp allele in 15 lines and ~350 bp allele in 5 lines, while no alleles could be identified in 21 lines.

Table 3. Allelic variation for *Cre3* and *Cre5* genes in Indian wheat varieties and advance lines

S.No	Varieties/ Genotypes	*Cre3**	*Cre5**	S.No	Varieties/ Genotypes	*Cre3**	*Cre5**
1	HD 2643	-	-	44	WHD 943	176	-
2	NW 1014	-	-	45	DH 2967	280	280
3	NW 2036	-	-	46	WH 1081	220	250
4	UP 2565	-	-	47	UP 2744	220	280
5	HUW 510	176	250	48	PBW 343	280	250
6	HD 2236	176	250	49	PBW 590	220	280
7	HD 2307	176	280	50	PDW317	176	-
8	HD 2009	280	280	51	PDW 175	-	-
9	HD 2851	220	280	52	WH 1021	220	280
10	HD 4502	176	300	53	DBW 50	176	250
11	HD 2380	176	280	54	PBW 55	176	280
12	NP 839	220	-	55	PDW 315	176	-
13	NP 818	220	-	56	DBW 60	-	250
14	NP 846	-	250	57	UAS 325	176	250
15	NP 852	176	280	58	PBW 639	220	250
16	GW 1139	176	-	59	WH 1094	-	280
17	GW 10	280	350	60	PBW 636	220	250
18	GW 503	176	300	61	PBW 645	220	-
19	K 9162	220	280	62	HD 4722	176	-
20	K 9351	176	250	63	HI 1571	220	280
21	K 9533	-	-	64	UAS 324	220	250
22	K 816	176	-	65	DBW 59	220	-
23	K 8962	220	-	66	DBW 58	176	250
24	UP 1109	220	250	67	HD 3035	176	300
25	UP 262	220	250	68	PBW 631	220	250
26	PBW 222	176	280	69	HUW 638	176	-
27	PBW 138	176	250	70	GW 1255	176	-
28	PBW 34	176	350	71	HUW 636	220	-
29	PBW 154	176	250	72	HD 3024	220	-
30	PDW 274	176	350	73	HD 3043	-	250
31	PDW 233	220	350	74	HUW 635	-	250
32	PDW 291	220	350				
33	HI 1500	220	300				
34	HI 1077	-	250				
35	HI 784	280	250				
36	RAJ 1114	176	250				
37	RAJ 3077	176	250				
38	PBW 629	220	250				
39	DBW17	280	250				
40	PBW 373	280	280				
41	C 306	220	250				
42	PBW 621	220	250				
43	PBW 396	220	300				

***** – no amplification

The presence of additional alleles as observed in this study might be due to InDels during selection process. The presence of additional allelic bands besides the diagnostic *Cre3* and *Cre5* shows that there is much more allelic variation in Indian wheat genotypes which requires further studies and identification of the *Cre* genes present in them.

The information presented herewith will be quite helpful in utilization of these identified genotypes for further enhancement of genetic resistance in the respective zones and at the breeding centers.

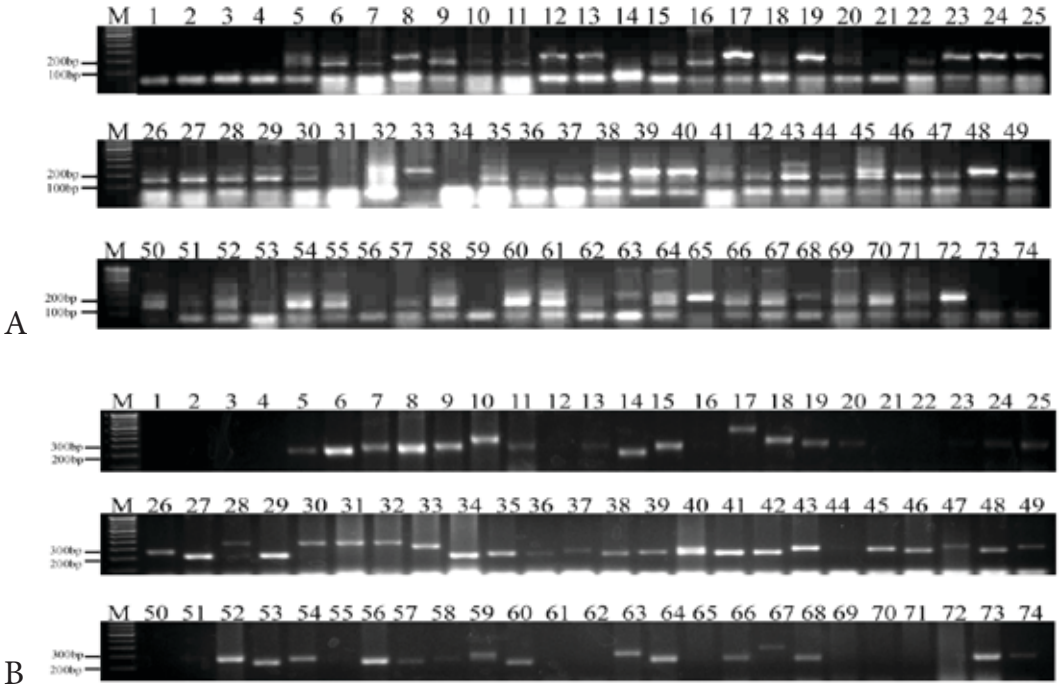

Fig. 1. Allelic variation observed in 74 advance lines. A: using *Cre3* and B: *Cre5* markers. M lane: 100bp ladder DNA; Numbering of the gel lanes are as per table 3 genotypes.

Acknowledgement

The authors are grateful to the CIMMYT-Turkey and Germplasm Unit, Directorate of Wheat Research, Karnal for supplying the seeds.

References

1. Bishnoi SP, Bajaj HK (2004). On the species and pathotypes of *Heterodera avenae* complex of India. *Indian Journal of Nematology* **34**: 174-152.
2. Delibes A, Romero MD, Aguaded S, Duce A, Mena M, Lopez-Brana I, Andres MF (1993). Resistance to the cereal cyst nematode (*Heterodera avenae*) transferred from the wild grass *Aegilops ventricosa* to hexaploid wheat by a "stepping stone" procedure. *Theoretical and Applied Genetics* **87**:402-408.
3. Eastwood RF, Lagudah ES, Appels R (1994). A directed search for DNA sequences tightly linked to cereal cyst nematode resistance genes in *Triticum tauschii. Genome* **37**:311-319.
4. Eastwood RF, Lagudah ES, Appels R, Hannah M, Kollmorgan JF (1991). *Triticum tauschii*, a novel source of resistance to cereal cyst nematode (*H. avenae*). *Australian Journal of Agricultural Research* **42**: 69-77.
5. Jahier J, Abelard P, Tanguy AM, Dedryver F, Rivoal R, Khatkar S, Bariana HS (2001). The *Aegilops ventricosa* segment on chromosome 2AS of the wheat cultivar 'VPM1' carries the cereal cyst nematode resistance gene *Cre5. Plant Breeding* **120**:125-128.
6. Kaur DJ, Sharma VS, Sohu VS, Bains NS (2008). Reaction of wheat genotypes to a population of *Heterodera avenae* from Punjab, India. *Nematologia Mediterranea* 36: 157-160 157
7. Kretschmer JM, Chalmers KJ, Manning S, Karakousis A, Barr AR, Islam AKMR, Logue SJ, Choe YW, Barker SG, Lance RCM, Langridge P (1997). RFLP mapping of the Ha2 cereal cyst nematode resistance gene in barley. *Theoretical and Applied Genetics* **94**: 1060-1064.
8. Lagudah ES, Moullet O, Appels R (1997). Map based cloning of a gene sequence encoding a nucleotide binding domain and leucine rich region at the Cre3 nematode resistance locus of wheat. *Genome* **40**: 659–665.
9. Mathur BN, Arya HC, Mathur RL, Handa DK (1974). The occurrence of biotypes ofcereal cyst nematode (*Heterodera avenae*) in the light soils of Rajasthan and Haryana, India. *Nematologica* **20**: 19-26.
10. Mathur BN, Handa DK, Swaroop S, Sethi CL, Sharma GL, Yadav BD (1980). On the loss estimation and chemical control of molya disease of wheat caused by *Heterodera avenae* in India. *Indian Journal Nematology* **16**: 152-159.
11. Ogbonnaya F, Moullet O, Kollmorgan J, Eastwood RF, Eagles HA, Lagudah ES (1998). The use of molecular markers to pyramid cereal cyst nematode resistance in wheat. In: Slinkard, A.E. (Ed.),

Proceedings of the 9th International Wheat Genetics Symposium, Volume 3. Saskatoon, Saskatchewan, Canada, August 1998 pp. 138–139.

12. Ogbonnaya FC, Subrahmanyam NC, Moullet O, de Majnik J, Eagles HA, Brown JS, Eastwood RF, Kollmorgen J, Appels R, Lagudah ES (2001). Diagnostic DNA markers for cereal cyst nematode resistance in bread wheat. *Australian Journal of Agricultural Research* **52:** 1367–1374
13. Paull JG, Chalmers KJ, Karakousis A, Kretschmer JM, Manning S, Langridge P (1998). Genetic diversity in Australian wheat varieties and breeding material based on RFLP data. *Theoretical and Applied Genetics* **96:** 435–446.
14. Romero MD, Montes MJ, Sin E, Lopez-Brana I, Duce A, Martin-Sanchez JA, Andres MF and Delibeset A (1998). A cereal cyst nematode (*Heterodera avenae* Woll.) resistance gene transferred from *Aegilops triuncalis* to hexaploid wheat. *Theoretical and Applied Genetics* **96**: 1135-1140.
15. Sharma P, Saini M, and Sharma I (2013). Molecular analysis of dimeric α-amylase inhibitor genes from Indian wheat genotypes. *Indian Journal of Biotechnology* **12**: 153-160
16. Singh AK, Sharma AK, Shoran J (2009). Heterodera avenae and its management on wheat in India. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 17-22. (CIMMYT: Ankara, Turkey)
17. Taylor C, Shepherd KW, Langridge P (1998) A molecular genetic map of the long arm of chromosome 6R of rye incorporating the cereal cyst nematode resistance gene, CreR. *Theoretical and Applied Genetics* **97**: 1000–1012.
18. Van Berkum JA, Seshadri AR (1970). Some important nematode problems in India. In 'Proceedings of the tenth international nematology symposium of the European Society of Nematologists, 8-13 September 1970, Pescara, Italy.' pp. 136-137.
19. Williams KJ, Fisher JM, Langridge P (1994). Identification of RFLP markers linked to the cereal cyst nematode resistance gene (Cre) in wheat. *Theoretical and Applied Genetics* **83:** 919–924.
20. Williams KJ, Lewis JG, Bogacki P, Pallotta MA, Willsmore KL, Kuchel H, Wallwork H (2003). Mapping of a QTL contributing to cereal cyst nematode tolerance and resistance in wheat. *Australian Journal of Agricultural Research* **54**: 731-737.