

## GENETICS OF RESISTANCE IN WHEAT AGAINST THE CEREAL CYST NEMATODE, *HETERODERA AVENAE* WOLL.

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**Summary.** The genetics of resistance of wheat to *Heterodera avenae* was studied with respect to F<sub>1</sub>, F<sub>2</sub>, and backcross progenies of cross combinations Raj 1482 × CCNRV 4, Raj 1482 × Raj MR 1, and Raj 1482 × AUS 15854. All F<sub>1</sub> were found resistant to cereal cyst nematode and the F<sub>2</sub> population segregated in a 3 resistant : 1 susceptible ratio. Thus, the resistance gene showed monogenic dominance over susceptibility. The back cross (test cross) segregated in a 1 resistant : 1 susceptible ratio, once again confirming the monogenic dominant nature of the resistance gene.

**Key words:** Breeding, inheritance, nematode development, *Triticum aestivum*.

There are about 80 species of plant parasitic nematodes associated with wheat (*Triticum aestivum* L.) but in India only two are considered to be key pests viz., the cereal cyst nematode, *Heterodera avenae* Woll., the causal organism of 'molya disease', and *Anguina tritici* (Steinbuch) Chitwood. The cereal cyst nematode (CCN), *H. avenae*, is one of the major nematode pest of oats (*Avena sativa* L.), wheat (*Triticum* spp.) and barley (*Hordeum vulgare* L.). In India, it is confined mainly to wheat growing areas of the states Haryana, Punjab, Jammu and Kashmir, Rajasthan, western Uttar Pradesh, Bihar and Madhya Pradesh (Swarup, 1986). In certain areas, yield losses caused by the nematode of up to 50-90% have been reported, with an annual loss estimate of 80 million rupees in the state of Rajasthan alone (Mathur *et al.*, 1980). Crop rotation and nematicides are effective at controlling this nematode. However, nematicides may leave residual toxicity and cause health hazards and are very expensive if used on a large scale in wheat cultivation, particularly in developing countries. Instead, the use of resistant cultivars is considered the best and most economical option for managing *H. avenae*. Concerted efforts have been made to study the genetics of resistance in wheat in other countries (Burrows, 1992; Cook and Rivoal, 1998; Cook, 2004; Eastwood *et al.*, 1994). However, no study had been conducted on the resistance of wheat cultivars to Indian populations of the cereal cyst nematode. Therefore, investigations were undertaken to obtain insights on the genetic nature of resistance in three Indian wheat (*Triticum aestivum* L.) lines/varieties to *H. avenae* pathotype I.

### MATERIALS AND METHODS

**Selection of parents.** Four resistant lines/varieties (Raj MR 1, CCNRV 2, CCNRV 4 and AUS 15854), two moderately resistant (BK 3102 and BK 3105) and two highly susceptible (Raj 1482 and Raj 3077) were selected from the already screened wheat germplasm (Pankaj *et al.*, 2006). All of them were supplied by the Agricultural Research Service (ARS), Durgapura, Jaipur, India. Of them, three resistant (Raj MR 1, CCNRV 4 and AUS 15854), and one susceptible (Raj 1482) were used in the studies on the development of the nematode and for the crossing programme (Table I). The selection of the parents used in the crossing programme was made on the basis of similarity in anthesis so that crosses could be made easily at the appropriate time. The two moderately resistant lines (BK 3102 and BK 3105) and line CCNRV 2 were discarded because mortality was observed in F<sub>1</sub> plants under field conditions.

**Study on development of the nematode.** A pot experiment was conducted to study the development of *H. avenae* in the above-mentioned three resistant and one susceptible wheat lines/varieties. Two seeds of each line/variety were sown in 10-cm-diameter earthen pots containing 500 g sterilized alluvial soil sand mixture (1:1) and thinned to one plant per pot after germination. Pots were irrigated regularly with distilled water to avoid any contamination. When 10 days old, the plants were inoculated with 4 J2s per cm<sup>3</sup> soil (2,000 J2s per pot). Two sets of pots were maintained (with ten pots of each line/variety), one for uprooting 35 days and the other 75 days after inoculation. The roots of plants uprooted 35 days after inoculation were washed with tap water and stained in acid fuchsin to facilitate counting the juvenile stages in the roots under a stereoscopic

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binocular microscope (Byrd *et al.*, 1983). The set up-rooted 75 days after inoculation was used for counting the number of white females per plant.

*Crossing programme.* The three resistant (Raj MR1, CCNRV 4, AUS 15854) and the highly susceptible (Raj 1482) parents were sown in microplots of 1 m<sup>2</sup> each consisting of three rows with 8-10 plants per row. All cross combinations were made at the Division of Genetics, I.A.R.I., New Delhi (India) during the winter season (November-April) of 2004-2005. The F<sub>1</sub> seeds were sown separately to raise the F<sub>1</sub> generation at Dalang Maidan, Lahaul valley (Himachal Pradesh, India) during the summer (May-September) season of 2005. F<sub>1</sub> plants were used as female parents, and were pollinated with their respective parents to obtain back crosses (BC<sub>1</sub> and test cross). The remaining uncrossed spikes of F<sub>1</sub> plants were collected as F<sub>2</sub> seeds. The F<sub>2</sub> seeds thus obtained were grown at ARS, Durgapura (Rajasthan) during November-April 2005-2006. Backcross, BC<sub>1</sub> and test cross populations of the three cross combinations were tested along with F<sub>1</sub> and F<sub>2</sub> plants for their reaction to the cereal cyst nematode *H. avenae* at ARS, Durgapura, Jaipur as described hereafter.

*Screening of filial and backcross generations.* Two surface sterilized seeds of wheat were sown in each of total 636 (15-cm-diameter) earthen pots containing 1 kg sterilized alluvial soil sand mixture (1:1). Pots were thinned to one seedling two weeks after germination. Each pot was then inoculated with 4-6 J2s per cm<sup>3</sup> (4,000-6,000 J2s per pot) of soil. The final cyst population was ascertained 90 days after nematode inoculation. For this, the soil was removed from each pot, suspended in 4 l water and sieved through nested 20 (840 µm) and 60 (250 µm) mesh sieves. The residue collected on the 60 (250 µm) mesh sieve was examined under a binocular microscope to count the number of cysts per plant. Based on the number of cysts formed, the plants were categorized as resistant (0-4 cysts/plant), moderately resistant (5-9 cysts/plant) and susceptible (10 and above cysts/plant), according to Pankaj *et al.* (2006).

*Nematode population used for inoculation.* Naturally infested field soil from ARS, Durgapura, Jaipur was collected during the first week of November 2004 and 2005 and cysts were extracted from about 6 buckets (each 15 l

capacity) of soil. For this, 1 kg lots of soil were suspended in 4 l water and sieved through nested 20 (840 µm) and 60 (250 µm) mesh sieves. The residue on the 60 (250 µm) mesh sieve was collected in a 500 ml beaker along with water. The water suspension with debris was observed under a binocular microscope and the brown cysts were picked out by hand. The average number of eggs per cysts was then determined by crushing five cysts in a counting dish containing distilled water, under a binocular microscope. The water suspension was then transferred to a graduated cylinder and the volume made up to 25 ml. The egg suspension was agitated by bubbling thoroughly and the eggs in three 1 ml aliquots were counted. To obtain second stage juveniles (J2s), the cysts were placed on wire (2 mm) mesh in Petri plates containing water and incubated at room temperature (20 ± 3°C) (Sethi and Dhawan, 1986). The J2s were collected twice at intervals of 2 days after hatching (i.e. beginning 48 h after start of incubation). Thus, 4-day-old J2s were used for inoculation. The plants were inoculated with 4 J2s/cm<sup>3</sup> soil, i.e. 4,000 J2s/15 cm diameter pots.

*Yield estimation of parent lines/varieties.* All the four parent lines/varieties were sown in naturally infested (initial population of 6-8 cysts/200 cm<sup>3</sup> = 325 g) soil, each cyst averaging 160-200 eggs and J2s) and uninfested fields during the winter season of 2004-2005. Each line/variety was sown in plots of 50 m<sup>2</sup>, replicated five times. The grain yield/plot was recorded after the harvest of the crop (5 months after sowing).

*Statistical Analysis.* A Chi-Square ( $\chi^2$ ) test was performed to test whether the observed plants in different generations (F<sub>1</sub>, F<sub>2</sub>, BC<sub>1</sub> and BC<sub>2</sub>) of the aforementioned cross combinations were in agreement with the theoretical or the expected frequencies based on the laws of inheritance. The following formula was used for calculations:

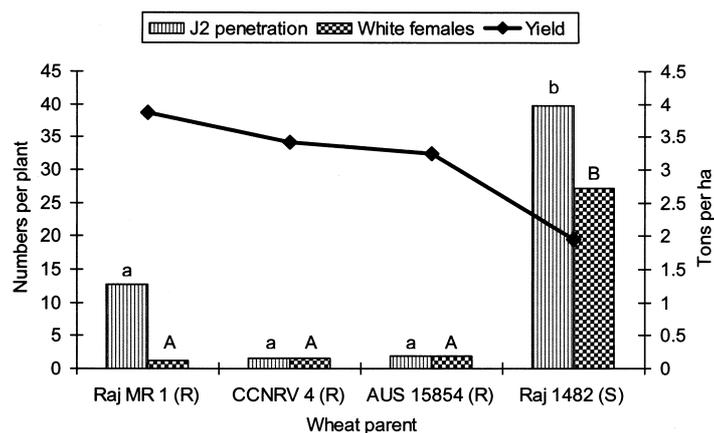
$$\chi^2 = \sum_{i=1}^k \frac{(O_i - E_i)^2}{E_i}$$

where, O<sub>i</sub> = Observed frequency in i<sup>th</sup> cell  
E<sub>i</sub> = Expected frequency in i<sup>th</sup> cell  
D.F. = K-1

An analysis of variance was carried out to examine the significance of variation between the parental varieties/lines by least significant difference (LSD) (Panse and Sukhatme, 1967).

**Table I.** Characteristics of the parent lines/cultivars used in the study.

Name	Pedigree	Reaction to <i>H. avenae</i>	Source
Raj MR 1	J 24 × AUS 15854	Resistant	ARS, Durgapura, Jaipur (Rajasthan), India
CCNRV 4	Indigenous Line	Resistant	ARS, Durgapura, Jaipur (Rajasthan), India
AUS 15854	Turkish Line	Resistant	Australia
Raj 1482	Indigenous variety	Susceptible	ARS, Durgapura, Jaipur (Rajasthan), India



**Fig. 1.** Comparison in terms of juvenile (J2) penetration (No./plant), white females (No./plant) and yield (Tons/ha) of resistant (R) and susceptible (S) wheat parents to *Heterodera avenae*. Small letters for comparing J2 penetration and capital letters for white females at  $P = 0.05$ , according to LSD.

## RESULTS AND DISCUSSION

Three resistant (Raj MR 1, CCNRV 4 and AUS 15854), and one susceptible (Raj 1482) wheat lines/varieties were used for the studies on the development of CCN and for the crossing programme. The new wheat variety resistant to CCN, Raj MR 1, developed from two genetically diverse cultivars in a single cross (J 24/AUS 15854), was kept as one of the parents in the breeding programme for the resistance to *H. avenae*. This variety exhibited a higher level of productivity in both CCN-infested (initial population of 6-8 cysts/200 cm<sup>3</sup> soil) and normal soils, with an increase in grain yield of 78.7% over Raj 1482 (susceptible) in infested soils. It also gave 19.0% higher yield than local varieties under timely-sown irrigated conditions in normal soils (Yadav *et al.*, 2002).

The number of J2 that had penetrated in the roots of resistant wheat cultivars was in the range 1.5-12.7 per root system compared with the susceptible check, Raj 1482 (40.0 per root system) (Fig. 1). The number of white females per plant was almost negligible (maximum 2) in the roots of resistant cultivars (Raj MR 1, CCNRV 4, and AUS 15854). Resistance is determined by a reduced number of females per plant (Mathur *et al.*, 1998). The penetration of the nematode into a resistant variety is significantly less and the inability of penetrated juveniles to develop into mature females in all the resistant varieties (Raj MR 1, CCNRV 4 and AUS 15854) confirmed their resistance reaction. Our results agree with findings of Pankaj *et al.*, 2006.

The yield under field conditions was also significantly higher in the resistant lines/varieties compared to the susceptible cv. Raj 1482 (Fig. 1). The ability of a wheat line to grow and yield well in the presence of the nematode is considered to be tolerance (Dixon and Harrison, 1994; Rao *et al.*, 1998; Pankaj *et al.*, 2006). Tolerance

traits are considered to be independent of resistance and susceptibility (Roberts, 1982). Incorporation of sources of resistance into highly tolerant lines is a sound approach for nematode control (Sharma and Sharma, 2000). Because of the differences in the effects of resistance and tolerance on nematode population densities, tolerance and resistance will have different effects on the productivity of cropping systems involving multiple crops with a range of degrees of tolerance and levels of resistance.

The  $F_1$  plants of the crosses Raj 1482  $\times$  CCNRV 4, Raj 1482  $\times$  Raj MR 1 and Raj-1482  $\times$  AUS 15854 were completely resistant to *H. avenae* (Tables II-IV), indicating the dominant nature of the resistance. The source of resistance in the resistant lines/varieties Raj MR 1 and CCNRV 4 was AUS 15854. The  $F_2$  plants segregated in a 3R:1S ratio, indicating the monogenic dominant nature of the resistance. The  $\chi^2$  calculated on the observed segregation ratio of the resistant : susceptible plants of the  $F_2$  generation of all three crosses, viz. Raj-1482  $\times$  CCNRV 4, Raj 1482  $\times$  Raj MR 1 and Raj 1482  $\times$  AUS 15854, was not significantly different from that of the 3:1 ratio characterizing the inheritance governed by a single dominant gene. Similarly the observed  $\chi^2$  value for test cross data was also found to be non-significant when compared with theoretical ratio 1:1. The back cross of  $F_1$  plants with resistant parents gave all resistant progeny (Tables II-IV). Therefore, the results of our study agree with Yadav *et al.* (1987) and Pankaj *et al.* (1995), who reported a single dominant gene in barley controlling resistance to pathotype I of *H. avenae*. These studies may help in selection and development of varieties of cereals resistant to nematode pests in India.

Single dominant resistance genes in pure line crops are likely to provide durable control only in unusual situations (Burrows, 1992; Cook, 2004; Eastwood *et al.*, 1994; Mathur *et al.*, 1994). The variety used in the present study (Raj MR 1) has already been released for cultivation in the *H. avenae* infested areas of Rajasthan in India. It has been grown mainly in wheat growing areas for the last 5-6 years. Continuous growing of this variety may be a cause of concern in coming years because of the virulence in *H. avenae* pathotype I (Swarup *et al.*, 1979), as demonstrated in Europe for another pathotype (Ha11) of this nematode (Lasserre *et al.*, 1996). Through the manipulation of cereal varieties, human impacts have caused genetic variation in plants during the relatively recent domestication of crops, and their current use in industrialized farming has also affected nematode variation (Atkinson, 1995). Moreover, the timeframe, spatial scales and likely nature of plant-nematode co-evolution in the longer period preceding domestication further emphasizes the significance and extent of the genetic complexity of the interactions (Atkinson 1995; Cook, 2004). It is important to take these factors into account during the identification of resistance sources and their exploitation through plant breeding (Cook and Rivoal, 1998).

**Table II.** Mode of segregation for resistance to *Heterodera avenae* in the wheat parents in different generations of the cross Raj 1482 × CCNRV 4.

Source Parents/cross	Generation	No. of Plants/families		Total plant observed	$\chi^2$	Mode of segregation
		R	S			
Raj 1482	P <sub>1</sub>	-	10	10		
CCNRV 4	P <sub>2</sub>	10	-	10		
Raj 1482 × CCNRV 4	F <sub>1</sub>	12	-	12		
	F <sub>2</sub>	43	17	60	0.356 <sup>ns</sup>	3:1
(Raj 1482 × CCNRV 4) × Raj 1482	BC <sub>1</sub> (Test cross)	35	25	60	2.5 <sup>ns</sup>	1:1
(Raj 1482 × CCNRV 4) × CCNRV 4	BC <sub>1</sub>	60	-	60		

ns = not significant

**Table III.** Mode of segregation for resistance to *H. avenae* in the wheat parents in different generations of the cross Raj 1482 × Raj MR 1.

Source Parents/cross	Generation	No. of Plants/families		Total plant observed	$\chi^2$	Mode of segregation
		R	S			
Raj 1482	P <sub>1</sub>	-	10	10		
Raj MR 1	P <sub>2</sub>	10	-	10		
Raj 1482 × Raj MR 1	F <sub>1</sub>	12	-	12		
	F <sub>2</sub>	46	14	60	0.089 <sup>ns</sup>	3:1
(Raj 1482 × Raj MR 1) × Raj 1482	BC <sub>1</sub> (Test cross)	29	31	60	0.05 <sup>ns</sup>	1:1
(Raj 1482 × Raj MR 1) × Raj MR 1	BC <sub>1</sub>	60	-	60		

ns = not significant

**Table IV.** Mode of segregation for resistance to *H. avenae* in the wheat parents in different generations of the cross Raj 1482 × AUS 15854.

Source Parents/cross	Generation	No. of Plants/families		Total plant observed	$\chi^2$	Mode of segregation
		R	S			
Raj 1482	P <sub>1</sub>	-	10	10		
AUS 15854	P <sub>2</sub>	10	-	10		
Raj 1482 × AUS 15854	F <sub>1</sub>	12	-	12		
	F <sub>2</sub>	44	16	60	0.089 <sup>ns</sup>	3:1
(Raj 1482 × AUS 15854) × Raj 1482	BC <sub>1</sub> (Test cross)	33	27	60	0.9 <sup>ns</sup>	1:1
(Raj 1482 × AUS 15854) × AUS 15854	BC <sub>1</sub>	60	-	60		

ns = not significant

The results of the study on the inheritance of the resistance in wheat to *H. avenae* pathotype I, would be of use in breeding nematode resistant wheat varieties for other agro-climatic zones of the country and for other pathotypes of *H. avenae*. Moreover, it will further be utilized for molecular characterization of resistance in wheat cultivars bred in India against *H. avenae* pathotype I.

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