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Research Article

Characterization of cereal cyst nematode (*Heterodera avenae*) infecting wheat in Punjab

Jaspreet Singh and Daman Jeet Kaur*

Department of Plant Breeding and Genetics
Punjab Agricultural University Ludhiana-141 004, India

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****Corresponding author***

Email: *daman_wheat2007@yahoo.com*
Tel.: *9417087367*

Abstract

Three populations of *Heterodera avenae* collected from Wheat – Maize, Wheat – Cotton and Wheat – Rice rotation fields were characterized using two sets of host differentials i.e., CCNHD 1982 and CCNHD Modified received from CIMMYT, Maxico. Reaction of all the host differentials was mostly the same to all three populations. Host differentials Ansi, Ortalan, Morocco and Bajo Aragon (CCNHD 1982) and VP1620 (CCNHD Modified) were resistant. The cultivars viz., Capa, Zita, Silva KVL 191, Siri, 1.376; CC4658, Iskamish K-2-Light, Martin 403-2, MK H. 72-646, Psathias and AUS 10894 from CCNHD 1982, while Raj 1, Croc_1/*Ae.Squarrosa*(224)// Opata//020615 and F372 from CCNHD Modified were moderately resistant to all three populations. Rest of the host differentials were categorized into moderately resistant to highly susceptible to one or other population. On the basis of the results, it was concluded that all the three populations belonged to same pathotype i.e., Ha41 pathotype of Ha1 group.

Keywords: Cereal cyst nematode, *Heterodera avenae,* Pathotypes, Wheat, Resistance

1. Introduction

Wheat (*Triticum aestivum* L.) is the most important human food grain and ranks second in total production as a cereal crop behind maize. India is the second largest producer of wheat next to China and achieved all time high production of 93.9 million tonnes during 2011-12 from 29.90 million hectare area (Paroda *et al.,* 2012), while Punjab known as "Wheat bowl of India" produced 19.21 million tonnes of wheat from 35.13 lakh hectare area with productivity of 4893 kg per ha (Anonymous, 2012). Globally, a loss of 10% of world crop production has been estimated as a result of plant nematode damage (Whitehead, 1998). About 90 species of plant parasitic nematodes have been reported to be associated with wheat crop. Those of economic importance include: cereal cyst nematode, root lesion nematode, root knot nematode, seed gall nematode and stem nematode (McDonald and Nicol, 2005. Out of these cereal cyst nematode, *Heterodera avenae* Woll. (CCN) is the most important and the most studied plant-parasitic nematode on wheat (Toktay *et al.,* 2013). Its worldwide distribution, predominance in areas where cereals are grown and devastating negative impact on yields make them major pests affecting the world's food supply (Cook and Noel, 2002). It can cause about 40-50 per cent yield loss that can reach up to 60-75 per cent in case of severe infection (Mathur *et al.,* 1980). In isolated areas losses in wheat up to 100 per cent have been reported in India (Van Berkum and Seshadri, 1970). In 1960s, CCN caused losses worth Rs. 40 million and Rs. 30 million in wheat and barley, respectively in Rajasthan. The annual loss caused in wheat has been estimated to the tune of Rs. 66 crores in Haryana alone (Kanwar *et al.,* 2007). In Punjab, *H. avenae* population was adversely affected with the cropping sequence shift from maize-wheat and groundnut-wheat to rice-wheat. During 1990s under the rice-wheat crop rotation it remained below damaging threshold level. Since 2003, infestation of CCN was recorded in rice-wheat rotation fields (Kaur *et al.,* 2009).

The nematode can be managed by cultural practices, chemicals, using CCN resistant cultivars or by integrating these approaches. However, resistance is considered to be most economically effective method of managing CCN. The use of resistant cultivars requires a sound knowledge of the virulence spectrum of the targeted species, including pathotypes. Knowledge of genetic variability within nematode populations of agricultural

interest is important for selection of appropriate control strategies (Ganguly and UmaRao, 2003). To date, the pathotypes of *H. avenae* have been recognized with the test developed by Andersen and Andersen (1982) designated as, "The International Cereal Test Assortment" for Defining Cereal Cyst Nematode Pathotypes, which has been modified by Rivoal and Cook (1993). Earlier work in India to determine variability in *H. avenae* using international differentials including clones of a few grasses, revealed the occurrence of 5 biotypes in the state of Rajasthan alone (Mathur *et al.,* 1974) and two biotypes have been reported in another study by Swarup *et al.,* 1979. Biotype I comprised populations from the state of Rajasthan (Jaipur and Udaipur) and Haryana (Narnaul). On the other hand, Biotype II included populations from Punjab (Hoshiarpur and Ludhaina). Based on host differential reaction along with isozyme profile and molecular characterization of biotype II revealed that it is *H. filipjevi* which is a subspecies in the *H. avenae* species complex (Bishnoi *et al.,* 2004 and Umarao and Sashi, 2008). The cultivars resistant to CCN from other states have proven to be susceptible to the Ludhiana population (Kaur *et al.,* 2009). In order to get effective and durable cultivar resistance, a sufficient understanding of the number of species and pathotypes within species is essential. Keeping this in view, the present investigation was under taken with objective to characterize the pathotypes of CCN populations occurring in Punjab.

2. Materials and methods

2.1 Identification and maintenance of pure cultures of different populations of Heterodera avenae: Pure cultures of *Heterodera* populations collected from Wheat – Maize, Wheat – Cotton and Wheat – Rice rotation fields during 2011-12 and 2012-13 were procured from wheat section, Department of Plant Breeding and Genetics (already collected and maintained). Identification of *Heterodera* was done on the bases of cyst cone. Cysts were cut at the posterior end with sharp blade and mounted in Canada balsam.

2.2 Seed multiplication of Host differentials: Seeds of host differentials i.e., Two sets namely CCNHD 1982 (27 host differentials) and CCNHD Modified (25 host differentials) received from CIMMYT, Maxico were multiplied in experimental area of the Department of Plant Breeding & Genetics and at PAU off-season research Station, Keylong, HP days 2010-11 and 2011-12.

2.3 Reaction of host differentials against H. avenae populations: The experiment was conducted under pot conditions. Number of cysts were estimated in soil collected from pure cultures and were standardized for 2 cysts/100g of soil of each population by mixing the steam sterilized soil. One kg of nematode infested soil was filled in earthen pots of 6 inch diameter in size. Sowing of host differentials along with 3 local checks (PBW 343, PBW 550 and DBW 17) was carried out. Four seeds were sown in each pot and each treatment was replicated four times and the pots were arranged on the bench in Completely Randomized Block Design. Irrigations were applied as and when required.

Observations were recorded in the month of March on number of white cysts per 250cc soil using the Cobb's Decanting and Sieving technique (Cobb, 1918). Reaction was categorized from highly resistant to highly susceptible following the scale suggested by the All India Coordinated Wheat and Barley Improvement Project (Table 1).

Table 1. Grading scale for categorization of differential reaction

Rating index	Cysts per plant	Host response
1	0 cysts	Highly Resistant
2	Up to 4 cysts	Resistant
3	4.1 to 9.0	Moderately Resistant
4	9.1 to 20	Susceptible
5	More than 20	Highly Susceptible

3. Results and discussion

H. avenae was identified on the basis of short vulval slit, bifenestrate and strongly developed bullae, while underbridge was absent (Mulvey, 1972).

3.1 Reaction of host differentials against H. avenae populations: Nematode reproduction and host reaction of the International Test Assortment to three *H. avenae* populations confirmed that the inoculum sources were virulent. Reaction of all the host differentials was mostly same in to all the three populations. Host differentials namely Ansi, Ortalan, Morocco and Bajo Aragon from CCNHD (1982) and VP1620 from CCNHD (Modified) were found resistant against all three *H. avenae* populations (Tables 2 and 3). However, Croc_1/Ae.Squarrosa (224)//Opata//020616 from CCNHD (Modified) has given a moderately resistant to Wheat-Maize population as compared to resistant in Wheat-Cotton and Wheat-Rice populations. The cultivars Capa, Zita, Silva, KVL 191, Siri, 1.376; CC4658, Iskamish K-2-Light, Martin 403-2, MK H. 72-646, Psathias and AUS 10894 from CCNHD (1982), while Raj 1, Croc_1/Ae.Squ(224)// Opata// 020615 and F372 CCNHD from CCNHD (Modified) were moderately resistant to all three populations. Salka was resistant to Wheat-Cotton population but moderately resistant to other two populations. Dalmatische, Marocaine (CCNHD 1982) and Taikong (CCNHD Modified) were resistant to Wheat-Cotton

population but were susceptible to Wheat-Maize and Wheat-Rice populations. While Silverstar and Kate A-1 were moderately resistant to Wheat-Cotton and Wheat-Rice populations and susceptible to Wheat-Maize population. There was not much difference in the reaction of rest of the host differential to all the three populations, they were categorized in to susceptible to highly susceptible.

The results obtained for host differentials Ortalan, Morocco and Bajo Aragon (CCNHD 1982) and for VP1620 (CCNHD Modified) against all three populations of *H. avenae* were similar as also demonstrated by Andersen and Andersen, (1982) and Rivoal and Cook, (1993) against *H. avenae* group Ha1 pathotype (Table 4) but the reaction of Martin 403-2 and AUS 10894 was different as these two cultivars were found moderately

Table 2. Reaction of host differentials (CCNHD 1982) to three populations of *H. avenae*

Sr. No	Name of host differential (CCNHD 1982)	Wheat-Maize Population		Wheat-Cotton Population		Wheat- Rice Population	
		Average (cysts/ 250 cc soil)	Reaction	Average (cysts/ 250 cc soil)	Reaction	Average (cysts/ 250 cc soil)	Reaction
1	VARDE/2081	21.50	HS	17.25	S	15.75	S
2	CAPA	6.75	MR	6.00	MR	6.00	MR
3	HERTA	22.25	HS	21.25	HS	16.00	S
4	SALKA	5.50	MR	3.75	R	4.75	MR
5	ANSI	3.50	R	3.25	R	3.50	R
6	EMIR	13.75	S	11.00	S	12.50	S
7	ZITA	6.25	MR	5.50	MR	5.75	MR
8	SILVA	6.75	MR	5.00	MR	4.75	MR
9	KVL 191	5.50	MR	6.50	MR	6.00	MR
10	VARDE/8861	37.50	HS	24.00	HS	25.75	HS
11	SIRI	5.75	MR	6.25	MR	4.75	MR
12	ORTOLAN	3.50	R	3.25	R	3.75	R
13	MOROCCO	2.75	R	3.25	R	3.75	R
14	SUN II	14.75	S	11.25	S	12.50	S
15	PUSA HYBRID BS1	27.50	HS	17.00	S	21.00	HS
16	1.376, CC4658	5.75	MR	5.00	MR	5.75	MR
17	LOROS X KOGA	22.00	HS	16.00	S	20.25	HS
18	ISKAMISH K-2-LIGHT	4.75	MR	4.50	MR	5.50	MR
19	BAJO ARAGON	2.75	R	1.75	R	3.00	R
20	MARTIN 403-2	7.00	MR	7.25	MR	6.75	MR
21	LA ESTUANZUELA	15.25	S	15.25	S	16.50	S
22	HARLAN 43	11.00	S	10.25	S	17.75	HS
23	DALMATISCHE	11.50	S	8.50	MR	11.50	S
24	MK H. 72-646	4.25	MR	4.50	MR	5.00	MR
25	PSATHIAS	7.00	MR	6.25	MR	6.75	MR
26	AUS 10894	7.50	MR	6.50	MR	6.75	MR
27	MAROCAINE	11.75	S	7.75	MR	9.50	S
28	PBW 550	15.00	S	16.50	S	15.50	S
29	PBW 343	13.50	S	16.00	S	14.50	S
30	DBW 17	10.25	S	10.00	S	11.00	S
	CD at 5%	2.71	-	2.56		3.04	-

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

Table 3. Reaction of host differentials (CCNHD Modified) to three populations of *H. avenae*

S r . No.	Name of host differentials (CCNHD Modified)	Wheat- Maize Population		Wheat- Cotton Population		Wheat- Rice Population	
		Average (cysts/ 250 cc soil)	Reaction	Average (cysts/ 250 cc soil)	Reaction	Average (cysts/ 250 cc soil)	Reaction
1	6R(6D)	11.75	S	10.00	S	10.25	S
2	FRAME	11.00	S	14.25	S	13.50	S
3	SILVERSTAR	10.75	S	7.25	MR	7.25	MR
4	VP5053	13.75	S	20.25	HS	13.75	S
5	T-2003	8.25	MR	12.75	S	12.00	S
6	RAJ 1	5.50	MR	5.00	MR	6.00	MR
7	ID-2150	7.25	MR	11.75	S	9.75	S
8	MILAN	11.75	S	12.25	S	11.50	S
9	AUS 4930.7/2*PASTOR	10.25	S	13.75	S	12.25	S
10	AUS GS50AT34/SUNCO// CUNNINGHAM	14.25	S	17.00	S	14.75	S
11	VL411R	12.25	S	14.75	S	17.00	S
12	CROC_1/*AE.SQUARROSA* (224)//OPATA//020615	5.50	MR	6.50	MR	6.50	MR
13	CROC_1/*AE.SQUARROSA* (224) //OPATA//20616	6.50	MR	3.25	R	3.75	R
14	VP1620	3.25	R	2.75	R	3.75	R
15	F130L 1.12/ATTILA	11.75	S	15.75	S	13.25	S
16	SONMEZ	16.25	S	21.50	HS	16.00	S
17	CPI133859	24.50	HS	18.25	S	20.50	HS
18	CPI133872	23.25	HS	20.75	HS	21.00	HS
19	KATE A-1	10.50	S	5.50	MR	6.50	MR
20	PRINS	14.50	S	12.25	S	12.75	S
21	MIRZABEY2000	28.25	HS	22.50	HS	16.50	S
22	AU/O652337//28*CA8-155/3/ F474S1-1.1	12.75	S	10.00	S	9.50	S
23	F372	6.25	MR	7.00	MR	10.25	S
24	TAIKONG	10.00	S	7.25	MR	9.75	S
25	ZHONGYU	13.50	S	11.00	S	12.75	S
26	PBW 550	14.50	S	15.50	S	14.75	S
27	PBW 343	11.75	S	13.75	S	14.50	S
28	DBW 17	12.25	S	10.50	S	11.50	S
	CD at 5%	2.62	-	2.84	-	2.95	-

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

Table 4. Comparative reaction of host differentials (CCNHD 1982) to "The International Cereal Test Assortment" for defining pathotypes and three populations of *H. avenae*

Pathotype	*Heterodera* avenae group Ha1 pathotypes							Ha2		Ha3		Present Study (*H. avenae* Population)		
	Ha 11	Ha 21	Ha 31	Ha 41	Ha 51	Ha 61	Ha 71	Ha 12	Ha 13	Ha23	Ha 33	W-M	W-C	W-R
	Barley													
Emir	S	S		S	-	R	S	S	S	S	S	S	S	S
Ortolan	R	R	R	R	R	R	R	S	S	S	S	R	R	R
Siri	R	R	R	S	S	S	R	R	S	S	S	MR	MR	MR
Morocco	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Varde/2081	S	-	-	S	-	S	S	S	S	S	S	HS	S	S
KVL191	R	R	R	-	S	S	S	R	-	-	-	MR	MR	MR
Bajo Aragon	R	-	-	R	-	R	R	R	S	S	R	R	R	R
Herta	S	S	R	-	R	-	R	S	S	-	-	HS	HS	S
Martin 403-2	R	-	-	R	-	R	R	R	R	S	S	MR	MR	MR
Dalmastische	(R)	-	-	S	-	R	(S)	S	S	(R)	S	S	MR	S
La Estanzuela	-	-	-	-	-	-	S	-	-	(R)	-	S	S	S
Harlan 43	R	-	-	-	-	-	R	R	-	R	S	S	S	HS
Salka	-	-	-	-	-	-	-	-	-	-	-	MR	R	MR
Zita	-	-	-	-	-	-	-	-	-	-	-	MR	MR	MR
Varde/2081	-	-	-	-	-	-	-	-	-	-	-	HS	HS	HS
Marocaine	-	-	-	-	-	-	-	-	-	-	-	S	MR	S
Oats														
Sunll	S	R	R	R	R	S	R	S	S	S	S	-	-	-
Nidar	S	-	-	S	-	S	R	S	S	S	S	-	-	-
Pusa Hybrid BS1	R	R	-	R	R	R	R	R	S	R	S	HS	S	HS
Silva	(R)	-	-	R	-	(R)	R	(R)	(R)	(R)	S	MR	MR	MR
Avena sterilis	R	R	-	R	R	R	R	R	R	R	R	-	-	-
IGV.H 76-46	R	-	-	R	-	R	R	R	S	S	S	-	-	-
Ansi	-	-	-	-	-	-	-	-	-	-	-	R	R	R
SUN II	-	-	-	-	-	-	-	-	-	-	-	S	S	S
1.376;CC465	-	-	-	-	-	-	-	-	-	-	-	MR	MR	MR
Wheat														
Capa	S	S	-	S	-	S	S	S	S	S	S	MR	MR	MR
Loros	R	R	-	R	-	(R)	R	R	(R)	S	S	HS	S	HS
Iskamish K-2-light	S	-	-	R	-	(R)		S	S	S	S	MR	MR	MR
AUS 10894	R	-	-	R	-	R	S	R	(R)	S	S	MR	MR	MR
Psathias	-	-	-	S	-			S	S	S	R	MR	MR	MR

Note: W-M = Wheat-Maize Population, W-C =Wheat-Cotton Population, W-R=Wheat-Rice Population, R = Resistant, MR = Moderately Resistant, S = Susceptible, HS = Highly Susceptible

resistant in present study. Recently studies conducted on the pathotype identification of three populations of CCN from Turkey according to scheme of Subbotin *et al* (2010) have demonstrated three primary groups of pathotype (Ha1, Ha2 and Ha3) distinguished by the reactions of barley differentials (Toktay *et al.,* 2013). Ortolan and Bajo Aragon which were found resistant in present study have been shown susceptible to *H. filipzevi* population (Subbotin *et al.,* 2010) and Morocco and Martin 403-2 were susceptible as against resistant and moderately resistant respectively. Whilst Silva, Psathias, MKH. 72-646, Aus 10894, Iskamish K-2 gave moderately resistant reaction to all the three population. It has been demonstrated earlier also that oats are resistant to *H. avenae* in India (Bajaj *et al.,* 1996) and in Australia (Cook and York, 1988).

The partial susceptibility of the wheat varieties and the new combination of virulence in the studied populations supports the proposition that populations of *H. avenae* may be highly heterozygous. For wheat Capa, Aus 10894 and Iskamish K-2 Light were also indicative of a divergent response (McDonald and Nicol, 2005; Turner and Rowe 2006, and Subbotin *et al.,* 2010), in this study, these differentials gave moderately resistant response.

Wheat cultivar VP1620 has also been shown resistant to Haryana population of *H. avenae* (pathotype Ha21) by Kanwar (2012) and mixed population of *H. avenae* of Punjab by Kaur and Sharma (2012). They have also reported Taikong, Seri and SUN 434G resistant. However, Raj MR1 was moderately resistant in present studies to all the three populations but has been shown resistant to pathotype Ha21. Croc_1/*Ae.Squarrosa* (224)//Opata which has been shown moderately resistant to pathotype Ha21 was also found moderately resistant in present study, while Croc_1/*Ae.Squarrosa* (224)//OPATA//020616 was resistant to two populations (Wheat-Cotton and Wheat-Rice). Line Croc_1/*Ae.Squarrosa* (224)//Opata from CIMMYT has also been reported resistant against *H. avenae* populations from Australia (Ha13) and Rajasthan (Nicol *et al.,* 2001). The susceptible check Milan has also shown susceptible reaction against all three populations evaluated in the present studies, while the resistant check, Silverstar has shown susceptible reaction to Wheat-Maize population but moderately resistant to Wheat-Cotton and Wheat-Rice populations of *H. avenae.*

Wheat line 6R(6D) was susceptible to all the three population tested as against highly resistant and ID-2150 was moderately resistant to Wheat-Maize population but susceptible to Wheat-Cotton and Wheat-Rice population has been shown moderately resistant in China against *H. filipjevi* (Yuan *et al.,* 2010; Toktey *et al.,* 2012). Taikong and SUN434G have also shown resistance to *H. avenae* in Punjab and ID-2150, Croc_1/*Ae. squarrosa* (224)//Opata, Iskamishk-2-Light, Croc_1/*Ae.squarrosa*(224)//Opata/ Janz, AUS 4930.6.5/Pastor were moderately resistant (Kaur and Sharma, 2012). As against resistant reaction of winter bread wheat Kate A-moderately resistant reaction has been noted against Wheat-Cotton and Wheat-Rice populations but susceptible to Wheat-Maize population. Saglam *et al.* (2009) also demonstrated moderate resistance in wheat cultivars (Katea, Sönmez, Milan and Silverstar) to the Turkish isolate TK1 (Haymana) of *Heterodera filipjevi.* Frame, a wheat cultivar which was categorized from Resistant to Moderately Resistant by Nicol *et al.* (2008) was indicated susceptible to all the three populations.

In conclusion, on the basis of these observations it can be said that all three populations of *H. avenae* belonged to the same pathotype i.e.,Ha 1 group of pathotypes. In Ha 1 group, there are seven pathotypes described as Ha11, Ha21, Ha31, Ha41, Ha51, Ha61 and Ha71. The characters of these three populations under study have been found to be close to Ha 41 pathotype. Similar results have also been reported by Swarup *et al.* (1979) for the Punjab population of *H. avenae.*

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