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Life Cycle of Cereal Cyst Nematode, *Heterodera avenae* on Resistant and Susceptible Barley Cultivars

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A B S T R A C T

The development of *Heterodera avenae* was studied on four resistant cultivars (BH 393, BH959, RD 2035 and DWRB 91) and one susceptible cultivar (BH902). After 20 days of germination inoculation was done with second stage juveniles @ 250 J_2 / pot. Plants of each cultivar were uprooted at 15 days interval after the inoculation and observations were taken till adults were formed. Roots of uprooted plants were stained with 0.1% acid fuchs in lactophenol and observations on the development stages were taken by mounting the nematode dissected out of root in lactophenol. Penetration of the J_2 of *H. avenae* was occurred insusceptible as well as in resistant cultivars but penetration was more in susceptible cultivars than resistant cultivars. In all the varieties, almost all the J_2 could reach the fourth stage. Infected roots exhibited only slight swelling at the site of infection. *H. avenae* completed life cycle from J_2 to female in 75 days on all varieties except, on RD 2035. Maximum number of cysts and cyst content was found in susceptible variety than resistant varieties but on RD 2035 no cysts were formed.

Introduction

Plant parasitic nematodes are among the most important groups of organisms affecting crop production. The cereal cyst nematode (CCN, *Heterodera avenae*) is a destructive nematode pest on cereal crops worldwide and cause significant losses in many countries (Cook and Noel, 2002; Smiley and Nicol 2009; Dababat *et al.*, 2015). In India, Vasudeva (1958) first reported it for the first time on wheat roots from Sikar district of Rajasthan and the disease caused by this nematode is commonly known as "Molya". In Haryana, *Heterodera avenae* has been reported from Mahendergard, Sirsa, Ambala, Gurgaon, Rohtak, Bhiwani, Faridabad and Hisar districts of Haryana (Kanwar *et al.*, 2011). Bird and Kaloshian (2003) estimated that these obligate parasites causing over 100 billion dollars annual crop losses. Abad *et al.*, (2008) estimated worldwide crop losses caused by nematodes to be $ 157 billion per annum.

Barley (*Hordeum vulgare*) is a main grain crop which is consumed in various forms. In Haryana, the production of barley is 0.14 million tons on 40,000 hectares (Anonymous, 2017). Van Berkum and Seshadri (1970) calculate losses worth Rs. 60 million in wheat

and barley in Rajasthan state only and also estimated losses of Rs 255 lakh for barley in three districts of Rajasthan alone. 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). Resistance in cereals against *Heterodera avenae* may operate at different levels. With the findings resistance in barley many workers traced the nature of resistance. Andersen (1961) observed that the larvae, which invaded the roots of resistant plants, did not mature into females. The adult females of *H. avenae* remained small sized and failed to extrude from the roots and, were, thus unable to copulate and reproduce on barley cultivar C-164 (Bajaj *et al.*,1996). Barley varieties BP 262, BP 264 and C-164 were found resistant to *H. avenae* and ratio of male to female was 106:1, 86:1, and 109:1, respectively (Bhatti *et al.,* 1976). Some resistant cultivars of barley like Rajkiran, RD 2052, RD 2035, RD 2508, BH-331, BH-338 and C-164 have been developed and released for cultivation in Molya prone areas of these states (Anon., 1979; Singh *et al.,* 2000).Therefore further investigations were planned on some aspects of the life cycle and development of *Heterodera avenae* on some barley varieties.

Materials and Methods

Seeds of five barley cultivars, susceptible (BH 902) and resistant (BH 393, BH959, RD 2035, DWRB 91) were obtained from the stock maintained in the Department of Plant breeding & genetics, CCS HAU, Hisar and sown in 15 cm diameter earthen pots filled with steam sterilized soil having pH 8.5 and EC 0.05. After germination one plant per pot was retained. Each treatment was replicated thrice. The juveniles were obtained by incubating the cysts in distilled water at 10º C for 30 days and collecting J_2 every third day and after 20 days of germination inoculation of larvae was done @ 250 J2 / pot. Inoculation was done by carefully removing the soil around the roots of plants in each pot to ensure direct and easy approach of juveniles to root system. The larval suspension was bubbled continuously for 10-15 seconds for required quantity and poured on exposed root system with pipette. Plants of each cultivar were uprooted at 15 days interval after the inoculation and observations were taken till adults were formed. Roots of uprooted plants were stained in 0.1% acid fuchs in lactophenol (McBerth *et al.,* 1941) and observations on the development stages were taken by mounting the nematode dissected out of root in lactophenol. The soil of each pot was processed through a 60 mesh sieve placed over a 300 mesh sieve by Cobb's decanting and sieving technique. White females and cysts remaining on the 60 mesh sieves were further processed by Baermann's funnel technique for the recovery of males.

Results and Discussion

The Penetration of second stage juveniles of *Heterodera avenae* occurred in susceptible as well as resistant cultivars of barley (Table 1). However, the penetration was more in susceptible cultivar than resistant cultivars (Fig. 1). The development of *Heterodera avenae* differed in the susceptible and resistant cultivars (Fig. 4 C and D). In case of susceptible variety (BH 902), third stage was observed on 30th day of inoculation. Infected roots exhibited slight swelling and lateral branching and at the site of infection (Fig. 4 E). Fourth stage females were recorded from 45th day onwards. Adults (males and females) were observed after 60 days of inoculation from soil and roots, respectively. Root tissues near the head of juveniles exhibited necrosis in the form of black spots (Fig. 4 F).

Table.1 Penetration and Developmental stages of *Heterodera avenae* in roots and soil of resistant and susceptible varieties of barley Days after inoculation

Days after inoculation	Resistant varieties								Susceptible variety	
	BH 393		**BH 959**		**RD 2035**		**DWRB 91**		**BH 902**	
	Root system	**Soil**	**Root system**	**Soil**	**Root system**	**Soil**	**Root system**	**Soil**	**Root system**	**Soil**
15	22 J_2	-	14 J_2	-	10 J_2	-	17 J_2	-	43 J_2	-
30	2 J_2, 19 J_3	-	12 J_3, 1 J_2	-	7 J_3, 2 J_2	-	14 J_3, 2 J_2	-	3 J_2, 36 J_3	-
45	1 J_3, 9 J_4♂, 8 J_4 ♀	-	1 J_3, 6 J_4♂, 5 J_4 ♀	-	1 J_3, 4 J_4♂, 2 J_4♀	-	1 J_3, 9 J_4♂, 4 J_4 ♀	-	2 J_3, 28 J_4♀, 7 J_4♂	-
60	6 J_4♀	7 ♂	3 J_4♀	3 ♂	1 J_4♀	2 ♂	3 J_4♀	5 ♂	25 J_4♀, 2 ♀	4 ♂
75	2 ♀	2 ♀	-	2 ♀	0 ♀	-	0	1 ♀	s11 ♀	8 ♀

J_2 = Second stage juvenile, J_3 = Third stage juvenile, J_4♀ = Fourth stage female juvenile, J_4 ♂ = Fourth stage male juvenile, ♀ = Adult female, ♂ = Adult male

Fig.1 Total number of nematode penetration after 15 days of inoculation in resistant and susceptible

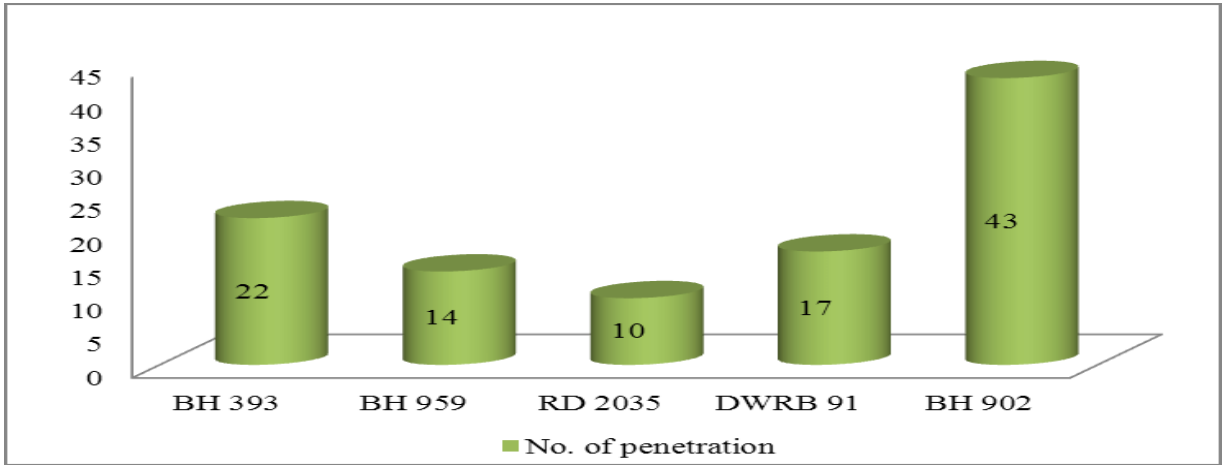


Fig.2 Total number of cyst formed per plant on different barley varieties

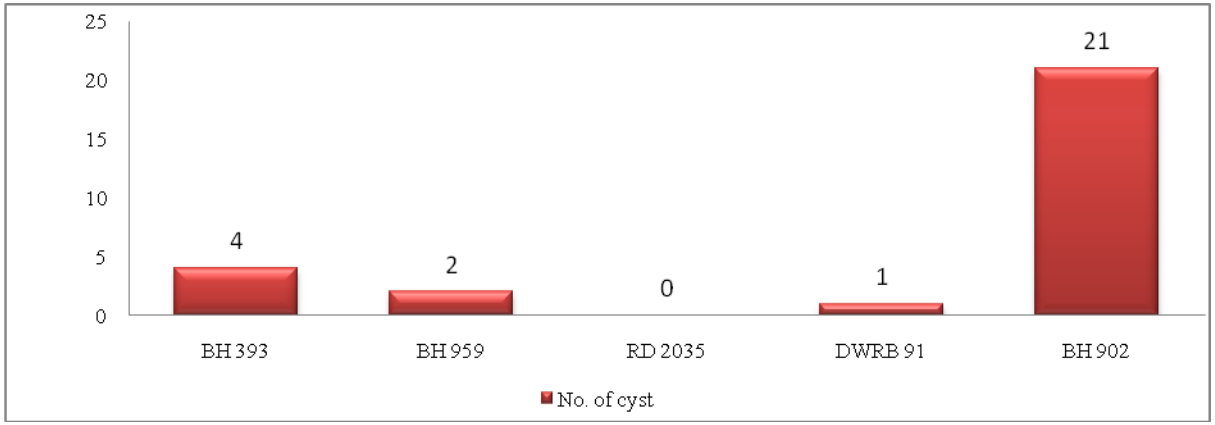


Fig.3 Average cyst content in different varieties of barley varieties

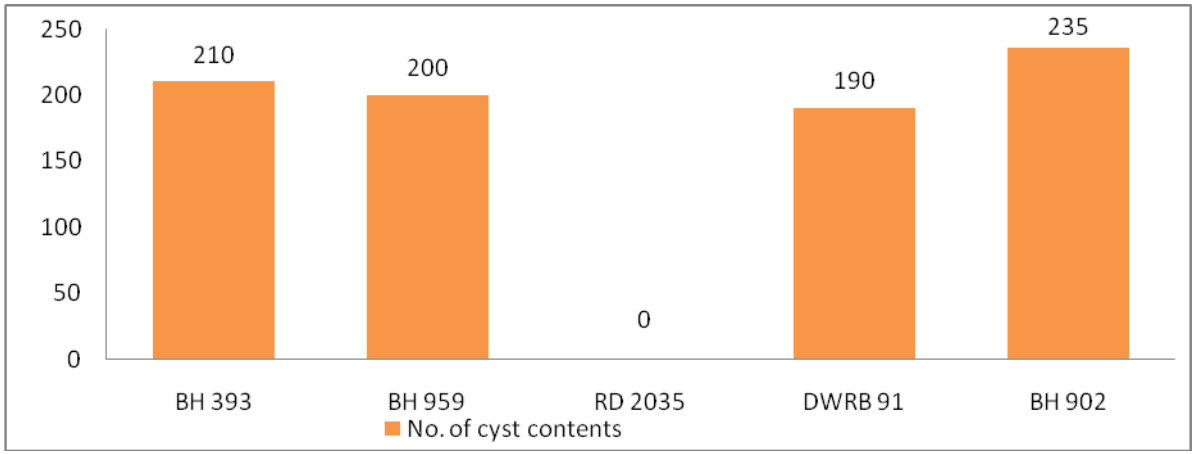
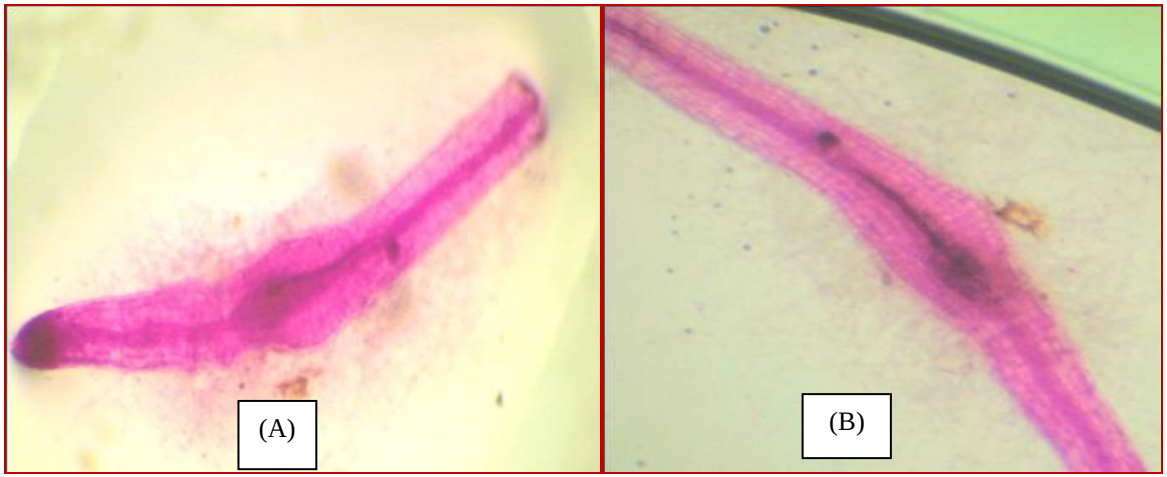
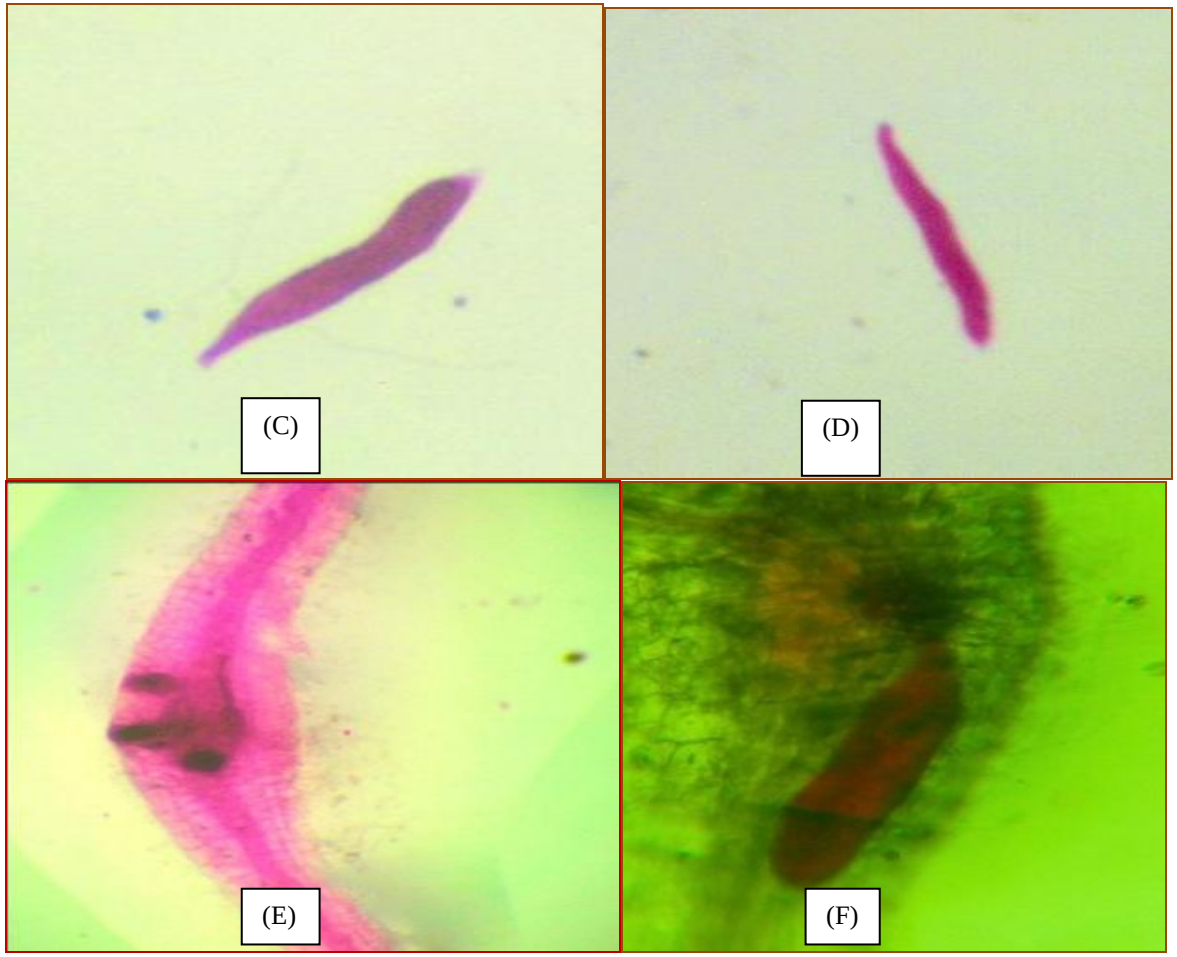


Fig.4 Penetration of J2 after 30 days of inoculation in susceptible variety (A) and resistant variety (B), Developmental stages of *Heterodera avenae* in susceptible variety (C) and resistant variety (D), Swelling of root (E) and syncytium formation at the feeding site of nematode penetration(F).




(C)
(D)
(E)
(F)

In all the varieties, almost all second stage juveniles could reach the fourth stage. Infected roots exhibited only slight swelling at the site of infection. However, males were able to reach adulthood but the development was comparatively slow. In RD 2035, no female or cyst was produced while in other varieties less number of cysts, as compared to the susceptible one, was formed (Table 1). In RD 2035, the fourth stage juveniles of female failed to develop further in the roots and got disintegrated at this stage itself. Comparatively, cyst formed earlier in susceptible variety than the resistant varieties. A good number of males were recorded in resistant varieties indicating that male development was not much affected which led to change in male: female ratio in favour of males as compared to susceptible check. *Heterodera avenae* completed life cycle from J_2 to female in 75 days on all varieties except, on RD 2035 although less females developed on resistant varieties.

Maximum number of cysts (21) was found on BH 902 than other resistant varieties (Fig. 2). The average cyst content was estimated by dilution method. The cyst content was found 210, 200, 190 and 235 on varieties BH 393, BH 959, DWRB 91 and BH 902 respectively. No cyst content could be recorded in variety RD 2035 as no cyst was formed on it. The highest cyst content was found in the susceptible variety (Fig. 3). Fewer numbers of juveniles penetrated the resistant cultivars than susceptible cultivar. There are several studies which reported less nematode penetration in resistant varieties (Bishnoi *et al.,* 2008; Price *et al.,* 1983). This is chiefly due to the difference in chemicals exuded from roots of resistant and susceptible plants. Less penetration of endoparasites or exodus from roots is due to pre-inflectional resistance in the host plant. Further development of *Heterodera* spp. depends upon successful establishment of feeding sites (syncytia) in the host in absence of which nematode development is seized or adversely affected. Among resistant cultivars, in RD 2035 lesser number of juveniles that had penetrated the roots reached to J_3 and J_4 stages but no further development was observed. In this variety, no female or cyst was produced while in other resistant varieties very less number of cysts as compared to the susceptible one were formed. RD 2052 had a different type of resistant mechanism than reported for C-164 (Bajaj *et al.,* 1996) where development was normal up to adult female stage. However, such females remained small sized and failed to protrude out of roots and copulate.

In resistant varieties, penetration was less as compared to the susceptible one. Similar observation was made by Bishnoi *et al.,* (2008) who indicated that maximum number of J_2 penetrated in susceptible barley variety RD 103 while lesser in resistant variety RD 2052 and RD 2035. They recorded good number of males in RD 2052 indicating that males development was not much affected which led to change in male: female ratio in favour of males as compared to susceptible check.

Maximum numbers of cysts (21) were found on susceptible barley BH 902. On resistant varieties, less number of cysts (1-4 per plant) developed except on RD 2035, where no cyst was formed. The size of cyst formed in BH 902 variety was larger as compared to the resistant varieties. Bishnoi *et al.,* (2008) reported small size white cysts without eggs on RD 2052 and RD 2035 barley varieties. Production of small-sized cysts with few eggs by cereal cyst nematodes on resistant hosts is a common phenomenon (Cook and Mizen, 1991; Bajaj *et al.,* 1996; Kanwar *et al.,* 2004).

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