J. Phytol. Res. **28** (1 & 2) : 7-13, 2015 **ISSN 0970-5767**

INTERACTION STUDIES BETWEEN CEREAL CYST-NEMATODE AND VESICULAR-ARBUSCULAR MYCORRHIZAL (VAM) FUNGI ON BARLEY IN JAIPUR, DISTRICT, RAJASTHAN

INDU RANI SHARMA*
Department of Botany, Govt. College, Kota, Rajasthan, India
* Corresponding author : E-mail: moonindu30@gmail.com

Cereal-cyst nematode (*Heterodera avenae*) is one of the most serious pathogen of wheat and barley crops in the state of Rajasthan, limiting agricultural productivity. In the present interaction study the potential of VAM species *viz*. *Glomus fasciculatum and Glomus aggregatum* singly and in combination was evaluated against *H. avenae* infestation on barley roots. Analysis of covariance of yield and plant growth data indicated better growth and yield of barely when its roots were associated with VAM species. A significant reduction in *H. avenae* cyst population and egg content of the cysts to minimum level was evident in VAM associated plants. Host's roots and VAM species interaction was found to be beneficial symbiotic association which resulted in improved plant growth and yield of the host plant.

Keywords: Barley; *Heterodera avenae; Glomus fasciculatum* and *Glomus aggregatum*; Symbiotic association.

Introduction

In India, *Heterodera avenae* was first reported from Sikar district of Rajasthan state[1,2]. The nematode was found to be a serious pathogen of wheat and barley causing "Molya disease" characterized by deformed root system. Survey studies revealed that due to congenial climatic conditions and sandy soil, the nematode established and distributed in different areas of Rajasthan including Jaipur district[3]. Barely is an important food and forage crop, extensively grown in the state of Rajasthan. It was found to be severely infested with the nema-pathogen that results in an atrocious state of the host and causes great loss to the crop production every year. Amongst the various kinds of organisms engaged in the biocontrol of nematodes, VAM fungi are the most promising group. The role of VAM as biocontrol agents in limiting yield losses due to nematodes infestations was investigated by different workers[4,5]. Their beneficial symbiotic association with host roots increases the plants ability to absorb Phosphorus (P), minor elements and water[6]. During survey of Jaipur district areas, plant samples collected from *H.avenae* infested local fields, when observed showed intimate association of *Glomus* species with the roots of barley and the cysts of *H. avenae*. Therefore, it was logical to further explore the nature of interaction between the VAM fungi, Cereal-cyst nematode and the host plant barley. In the present study interaction between the nematode and *Glomus* species

viz. *Glomus fasciculatum* and *Glomus aggregatum* individually and their combined effect on the growth and yield of barley were evaluated.

Material and Methods

Isolation, Identification and Culture of VAM fungi: Spores of VAM spp. were isolated from the soil samples collected from different localities by using standard wet sieving and decanting technique given by Gerdemann and Nicolson[7]. For identification of VAM spp. the root pieces retained on 710 µm sieve were examined under a dissecting microscope to assure the presence of hyphae with spores and sporocarps of VAM fungi. The organic matter retained on 250 µm sieve was examined under stereomicroscope for the presence of large spores and sporocarps. Similarly matter retained on 106 µm and 53 µm sievs were also observed for the presence of clusters of small spores and detached spores respectively. For counting and identification of spores Doncaster nematode counting dish was used.

For examining VAM hyphae and spores associated with host's roots, roots pieces were boiled in 10% KOH solution for 5 minutes, washed in tap water stained in 0.1% Trypan blue in lactoglycerol for 12 hrs and then transferred to lactoglycerol.

From the VAM infested cysts spores and hyphae were isolated simply by crushing the cysts in watch glasses containing. D.W. and were examined for the identification of VAM species under stereomicroscope. For identification purpose standard key given by Trappe[8] was used. Two *Glomus* species *viz., G. fasciculatum* and *G. aggregatum* were identified.

Above said identified two species were maintained separately on wheat for getting pure inoculums. For pure cultures, sterilized wheat grain were sown in pots containing steam sterilized soil + sand (3:1) mixture. The mycorrhizal inoculum consisted of chlamydospores of each *Glomus* species obtained from the local field soil and infested *H. avenae* cysts were placed just near the roots of 1 week old wheat seedling. After 90 days plants were uprooted and roots were washed, processed and examined for the presence of each *Glomus* species.

Experimental study: For the interaction study pot trials were set. Pure inoculum of each *Glomus* species was added separately as well as in combination to the 15 cm diameter pots containing autoclaved soil + sand (3:1) mixture and mixed thoroughly. Inoculum of both the species consisted on an average 280 chlamydospores per 10g hyphae plus infected roots. Surface sterilized seeds of barley variety RD103 were sown in VAM inoculated pots. One week old seedlings were nematized with one thousand freshly hatched active juveniles of *H.avenae* around the roots for infestation. Following treatments were comprised for experimental set up:

1. G. fasiculatum [G.F.(10g] + G. aggregatum [G.A.(10gm)]
2. G.F.(10g)
3. G.A. (10g)
4. G.F. (10g)+G.A. (10g)+Nematode (N)
5. G.F. (20g) + G.A. (20g) + N
6. G.F. (10g) + N
7. G.F. (20g) + N
8. G.A. (10g) + N
9. G.A. (20g) + N
10. N

All the treatments were replicated four times. Data on plant growth characters, nematode reproduction and spore count with percent mycorrhizal root infection were recorded after 90 days of nematization. Number of cysts per root system plus per

100g soil and average cyst content (number of eggs) were also recorded. VAM root association levels were assessed from randomly collected root material after cutting the entire root system into 1 cm pieces. Roots were stained and preserved as described before. The percent VAM colonization was calculated from the frequency distribution using following formula given by Giovanneti & Mosse[9].

$$\% \text{ Colonizaton} = \frac{\text{Number of VAM positive segments}}{\text{Total Number of segments scored}} \times 100$$

Spores count in soil was also determined by the technique described earlier.

Observations and Results

Analysis of soil samples collected from H. avenae infested fields showed presence of spores, sporocarps and hyphae of VAM fungi. Barley roots examined revealed presence of extra as well as intrametrical hyphae bearing spherical vesicles at their tips and in intercalary positions containing oil globules. Arbuscules developed from intracellular hyphae were also evident in host cells. Hyphae were abundantly observed in the cortical region of roots, but they were absent in endodermal and stellar region. Young roots were found to be containing plenty of arbuscules, but in older roots vesicles with oil globules, chlamydospores and sporocarps were abundant.

Similarly crushed cysts also showed presence of both *Glomus fasciculatum* and *Glomus aggregatum* species. In some cysts, cyst content (eggs) was totally replaced by VAM hyphae and spores. (Fig.1, 2). The most commonly identified species; *G. fasiculatum* and *G. aggregatum* were characterized as follows:

*G. fasciculatum***:** Spores globose to subglobose, ovate, ellipsoid or irregular; dimensions at maturity 50-125 µm; surface smooth to roughened; double walled; yellow sporogenous hyphae thickened at the point of attachment with a diameter 5 to 20 µm (Fig-1a)

G. aggregatum: spores subglobose, ovate, ellipsoid or irregular; dimensions at maturity 225-250 µm; surface coarse-roughened, double walled; sporogenous hyphae white to hyaline, non perceptible, cylindric or flared towards point of attachment, pore closed by septum or inner wall (Fig-1b).

The mycorrhizal culture multiplied on wheat for experimental purpose also consisted of chlamydospores, along with hyphae and infected roots.

Experimental Study: Data depicted in Table-1, showed effect of *G. fasciculatum* and *G. aggregatum* singly and in combination on plant growth and yield of barley and multiplication of *H. avenae.* A significant reduction in disease incidence, better plant growth and yield in terms of seed weight were obtained by the treatment of *Glomus* species as compared to untreated control and only *H. avenae* infested plants.

Regarding plant growth parameters, like root-shoot lengths were recorded to be maximum in G.F. (10g) + G.A. (10g) treatment and were minimum in 'N' treated plants. Intermediate shoot and root lengths were evident in the treatments, where single dose (10g) of each species and higher dose in combinations were used.

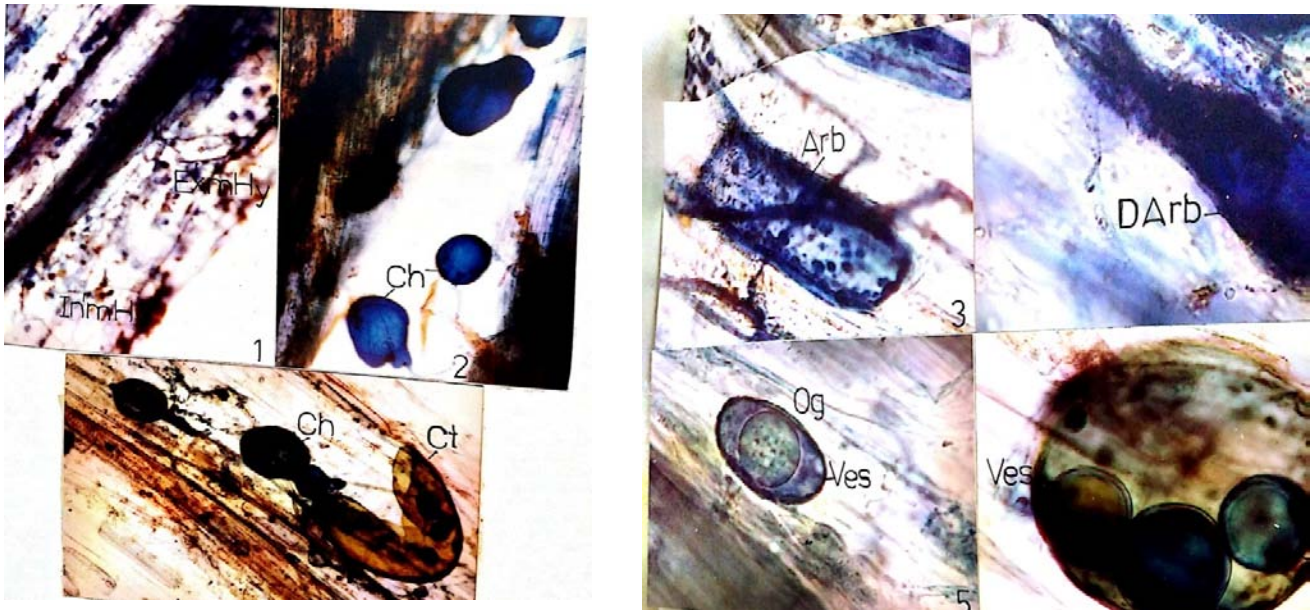
Generally, both fresh and dry weights of shoot were higher in VAM treated plants as compared to 'N' alone treated plants. Shoot fresh weight ranged between 2.852g to 14.898 g, being maximum in G.F. (10g) + G.A. (10g) treatment and minimum in 'N' treated plant. Similar trend was evident regarding shoot dry weights and seed weight per plant.

In the case of roots also fresh and dry weights increased with increase in VAM

Table: 1. Interaction of VAM fungi with *H. avenae* infecting Barley (Mean of 4 replicates)

S. No.	Treatments	Length (CM)		Fresh Wt. (g)		Dry Wt. (g)		Seed Wt. (g)	Cysts per root system + per 100 g soil	No. of Egg/ Cyst	% Mycorrhizal infection	VAM Spore Count
		Shoot	Root	Shoot	Root	Shoot	Root					
1	2	3	4	5	6	7	8	9	10	11	12	13
1	GF (10g) +GA (10g)	74.5	40.00	14.898	4.875	1.655	0.609	1.325	0 (1)	0 (1)	99.16	3334.3
2	GF (10g)	67.6	38.8	13.754	3.495	13.84	0.439	1.221	0	0	85.30	1991.0
3	GA (10g)	61.4	37.1	6.735	1.948	0.846	0.256	1.002	0	0	80.3	2422.0
4	GF (10g)+GA(10g) + Nematode	54.1	31.4	4.751	0.753	0.590	0.115	0.500	67.8	260.60	91.3	2578.0
5	GF (20g) + GA (20g) + Nematode	61.1	34.6	6.252	1.748	0.782	0.246	0.630	26.3	211.00	100.0	2825.0
6	GF (10g) + N	46.3	20.8	4.032	0.602	0.502	0.075	0.227	100.0	231.3	64.0	4387.0
7	GF (20g)+N	48.7	30.8	4.195	0.602	0.533	0.094	0.481	72.6	165.3	60.1	2447.0
8	GA(10g)+N	45.2	12.6	3.201	0.153	0.413	0.057	0.075	126.3	300.6	87.4	4911.00
9	GA (20g)+N	45.9	20.3	3.555	0.500	0.444	0.073	0.125	101.0	245.0	98.9	1620.0
10	Nematode	36.4	9.2	2.852	0.152	0.353	0.017	0.027	162.6	338.3	0 (1)	0 O(1)
11	Control	60.2	34.4	5.224	0.904	0.654	0.217	0.601	0 (1)	0 (1)	0 (1)	0 (1)
	SEM±	0.293	0.388	0.029	0.015	0.008	0.011	0.008	0.512	0.516	0.308	0.171
	CD at 5%	0.611	0.810	0.061	0.032	0.018	0.024	0.017	1.070	1.079	0.644	0.357
	CD at 1%	0.833	1.103	0.081	0.042	0.024	0.130	0.022	1.456	1.467	0.875	0.485

GF = *Glomus fasciculatum*; GA = *Glomus aggregatum*; N= Nematode



(a) **(b)**

Fig. 1 (a) *G. fasiculatum* (Hyphae, arbuscules, vesicles, chlamydospores and sporcarps), **(b)** *G. aggregatum* (Hyphae, arbuscules, vesicles, chlamydospores)

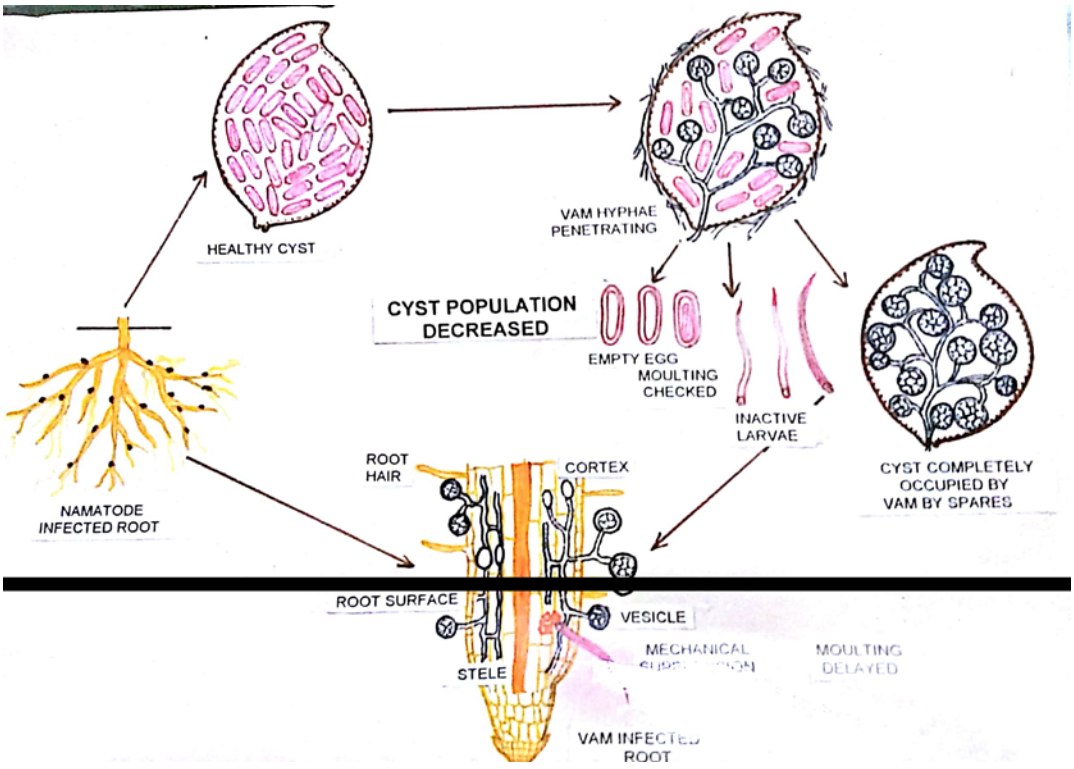


Fig. 2 Control of *Heterodera avenae* by VAM Fungi

inoculations. Root fresh weight (40g) was maximum in the G.F. (10g) + G.A.(10g) treatment, whereas it was minimum (9.2g) in nematode('N') treatment. Root dry weights also exhibited similar trend.

A marked decrease in disease manifestation was evident by the application of *Glomus* species. Maximum cyst number (162.6 per root system + 100g soil) was recorded in the 'N' treated plants. It reduced to minimum (26.3 per root system + 100 g soil) in the treatment, where 20g dosages of both the species were used. As compared to *G. aggregatum*, *G. fasiculatum* was more efficient in reducing cyst number.

The Cyst content (Eggs + larvae) did not show any definite trend. In the case of concomitant application of *Glomus* species most of the cysts were found to be empty containing hyphae with spores (Fig-2). Data revealed a negative correlation between the nematode and mycorrhizal association with the host plant.

Percent mycorrhizal infection was more in G.F. (10g) + G.A. (10g) treatment, which gradually increased with higher dosages (20g) of VAM, however it was recorded to be minimum in the G.A. (10g)+N treatment. Spore counts also showed similar trend. Data were found to be statistically significant.

Discussion

Survey of study area revealed that *H. avenae* and *Glomus* species are indigenous to local fields of barley. Results of present investigation indicated better establishment of *Glomus* species on the root of barley naturally as well as in pot trial. Their association was found to be mutually beneficial symbiosis that caused better growth and yield of barley as compared to non mycorrhizal plants. Barley was proved to be the good host for establishment of VAM species. Hayman also reported a considerable infection on barley host[10].The genus *Chloris gayana* with 98.5% root colonization by *G. fasciculatum* was considered to be the best host by Srinivasan and Bagyaraj[11]. In the present investigation on an average percent root colonization by VAM ranged between 80.3-85.3 percent, so it can also serve as an efficient host.

The results obtained during pot trial indicated that barley and VAM fungi interation mitigated the deleterious effect of the nematode on barley variety RD103 and increased host tolerance level against the pathogen.

References

1. Vasudeva RS 1958, Scientific Report of the Indian Agri. Res. Inst. for the year ending 30th June, 1957. IARI, New Delhi, India.
2. Prasad N, Mathur RL and Sehgal SP 1959, Molya disease of wheat and barley in Rajasthan *Curr. Sci.* **28** 453.
3. Mathur BN, Arya HC, Nanda DK And Mathur RL 1975, On the symptoms and distribution of molya disease of wheat and barley caused by *Heterodera avenae* in light soils of Rajasthan. *Indian J. Mycol. and Pl. Pathol.* **5** 91-94.
4. Schonbeck F 1987, Mycorrhizas and Plant health a contribution to biological protection of plants. *Angewandte Botanik* **61** 9-13.
5. Smith GS 1987, Interaction of mycorrhizal fungi with nematodes, In: Veech JA and Dickens DW (eds.), Vistas on Nematology, Society of Nematologists 292-300.
6. Harley JL and Smith SE 1983, Mycorrhizal Symbiosis, London Academic Press.
7. Gerdemann JW and Nicolson TH 1963, Spores of mycorrhizal Endogone

species extracted from soil by wet sieving and decanting. *Trans. Br. Mycol. Soc.* **46** 235-244.

8. Trappe JM 1982, Synoptic Key to the genera and species of Zygomycetes mycorrhizal fungi. *Phytopathology* **72** 1102-1108.
9. Giovannetti M and Mosse B 1980, An evaluation of technique for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytol.* **84** 489-500.
10. Hayman DS 1982, Practical aspects of vescicular- arbuscular mycorrhiza. In : Subba Rao NS (eds.), Advances in Agricultural Microbiology, Oxford and IBH Publishing Co., Oxonian Press Ltd. Faridabad 325-373.
11. Srinivasan MN and Bagyaraj DJ 1988, *Chloris gayana* a better host for mass production of *Glomus fasciculatum* inoculums. *Pl. & Soil* **106** 289-290.