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Novel approaches in managing wheat and barley nematodes - an overview

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ABSTRACT

Among several biotic factors involved, nematodes have been playing substantial deleterious role in various parts of the country (like Rajasthan, Haryana, U.P, Bihar and M.P,) in reducing the yields of wheat. The tragedy is that by the time we notice them damaging, they already have much damaged the crop. Majority of farmers is not aware of their presence (nematodes), nor are very satisfactory means available at the farm level to create awareness about them or to contain them. There are reports that more than 25 species of nematode associated with wheat rhizosphere and plants six of them are endoparasites, but out of these, two species have got to be the major hurdles in realizing the full potential of wheat. These two are, *Heterodera avenae* (the molya disease causing agent) and *Anguina tritici* (the ear cockle disease causing agent / wheat seed gall nematode). Though the yield loss due to *Heterodera avenae* has been recorded to be around 50% or even total loss of the crop, but in case of *Anguina tritici*, in some cases and in some years, it has gone up to 80% or more as it happened in year 1997 in Bihar and in year 1999 in several districts of M P.

Keywords: *Heterodera avenae, Anguina tritici*, cereal cyst nematode, Ear cockle nematode

Wheat, the most important cereal crop occupies prominent position in Indian agriculture after rice as it provides surplus food for our teeming population and extends job opportunities for agricultural workers. The wheat production has been hovering between 68 and 75 million tonnes hailing from approximately 27 million-hectare area in the country. Though, we are complacent but to any one's surprise lot of produce is being waste on account of several factors. There occurs several biotic and abiotic factors per *se*, which if managed efficiently, our production level can go even quite up.

CEREAL CYST NEMATODE (CCN), HETERODERA AVENAE, THE INCITER OF MOLYA DISEASE

This nematode was reported for the first time from Sikar district of Rajasthan by Vasudeva in 1958. Since then, it has also been reported from Sikar, Jhunjhunu, Churu, Chomu, Bharatpur, Tonk, Ajmer, Dausa, Jaipur, Hanumangarh, Sawai Madhopur, and Udaipur. In Haryana, it is found in Ambala, Bhiwani, Faridabad, Fatehabad, Gurgaon, Hissar, Mahendragarh, Rewari, Rohtak, and Sirsa. The disease is characterized by typical patchy growth and stunted plants in the field .The patches tend to coalesce if the wheat growing is continued in the same field year after year. Infested plants exhibit yellowing, less tillering, reduced leaf area, fewer leaf and roots, delayed emergence of ears, and reduced number of spikelets (resemble with nutrient deficiency symptoms). Also the affected plants have stiff, thin, and narrow leaf blade in comparison to healthy ones. Under our condition, main root gets elongated, with a bunch of rootlets at the extreme end. If the plants of wheat are uprooted after 75-80 days of sowing, white cyst bodies (females) are seen attached with the roots and roots show knotty appearance and also they just look like pearl.

The infested roots are short, with multiple branches giving a bunchy appearance (called coral roots) and often bearing small gall like formations. The disease intensity mostly depends upon the environmental and ecological factors which influence both the host and the parasite. Damage to crop is governed by soil moisture, soil texture, inoculum load, and age of host plant. A positive correlation exists between degree of infestation of CCN and crop damage. This way it can be established that malady is due to *Heterodera avenae*. Further, there are two biotypes available in *Heterodera avenae*. Rajasthan and Haryana population constitutes one biotype and Punjab (Ludhiana & Hoshiarpur) other Swarup *et al.* 1979. This was proved by employing host differentials like Siri and Martin which were susceptible to Punjab population but resistant to Rajasthan and Haryana population whereas La-estanzuella was found to give reverse reaction with these population. By resorting to host differential, populations of these two pathotypes have been diagnosed but inconsistency in reaction is observed year after year. Bisnoi and Bajaj (2004) studied eight population of CCN from India and found that population from Kangra (H.P) Ludhiana (Punjab) and Ambala (Haryana) belong to *H. filipjevi*. Unfortunately, there is no complete International Test Assortment that allows clear differentiation of current cereal cyst nematode complex. For more authentic results on biotype variability, the population collected from different places may be resorted to molecular tools so that this pervasive confusion is resolved.

I) LATEST APPROACHES IN CONTAINING CCN:

Resistance is one of the major control measures to reduce the population of nematodes below damaging thresholds level and, through extensive screening, several sources of plant resistance against CCN have been identified in different alien sources, land races and in important cultivars. Though resistance to CCN has been identified in hexaploid wheat, *Triticum ventricosum*, Rye, Barley, Oats, and in *Triticum tauschii*. Two classes of resistance have been observed in *T. tauschii* and these confer either a high (CcnD1) or a moderate (CcnD2) level of resistance in allohexaploids. Several cultivars of Wheat, Barley, Oats, and Rye have been produced incorporating resistance, but a significant limitation to breeding CCN resistant cultivars has been now that many breeding programmes are not breeding for resistance to this nematode, due to limited technical and financial resources and underestimation of the impact of the nematodes. Besides, the time required, high labour input cost, and lack of consistency of the biological assays used to select resistant genotypes are also responsible. Prioritizing nematode problems for resistance, would have considerable impact on grain production in regions where the nematodes can be implicated with yield loss. This will pave the way for resistance as a control method to reduce nematode population below economic thresholds. This method is environmentally sound, cost effective, sustainable, accessible, and achievable with combined research between nematologist and plant breeders. A better understanding of the evolution of nematode pathotypes and species is still required, however, to assist control measures. Improved identification of species and individuals in mixed population, associated with the molecular characterization of pathotypes or markers linked to the virulence features, will allow more rapid integration of resistance in to cereals. An alternative strategy in breeding CCN resistant cultivars is to use molecular markers linked to the resistance genes. Such an assay can identify genetic loci independently, allowing two or more genes to be combined in a single genotype. Techniques frequently used in identifying molecular markers include DNA hybridization, and PCR. Restriction Fragment Length Polymorphism (RFLP) arising from sequence variation is revealed in DNA hybridization experiments as changes in the length of DNA fragments after cleavage by restriction enzymes take place. Molecular technologies have been applied to identify markers for various CCN plant resistant genes using techniques such as RAPD and RFLP in both barley (Kreetschmer *et al.* 1997; Barr *et al.* 1998) and wheat (Eagles *et al.* 2001a, b). In some Australian cereal breeding programmes, markers for both wheat and barley are being implemented using marker-assisted selection to pyramid resistance genes against *H. avenae*. RFLPs are the most commonly used and informative molecular markers in cereal genome mapping. Again unlike RFLP analysis which is time consuming and expensive, molecular markers based on the Polymerase Chain Reaction (PCR) allow high throughput of material and have low start-up and running costs. The technique, PCR based allele-specific amplification assay from RFLP clone Tag 605 for use as a PCR based wheat chromosome 2B genetic marker has been employed. This region of 2B is of importance to wheat breeding programmes since it maps close to *Cre1*, a gene conferring resistance to cereal cyst nematode.

The conversion of the existing RFLP marker to a PCR based assay system represents a major goal in developing a cheap and rapid assay for screening for *Cre1* gene. In an experiment, the RFLP locus Xglk 605 identified by probe Tag 605 mapped to a proximal position on the long arm of wheat chromosome 2B about 7c M away from a gene conditioning resistance to cereal cyst nematode in the wheat line AUS 10894.

The development of CCN resistant cultivars may be accelerated through the use of molecular markers. A number of resistance genes against this pest are well known; one of them, the single dominant *Ha2* resistance gene, has been shown to be active against the Australian pathotype and maps to chromosome 2 of Barley. Segregation analysis identified two RFLP markers flanking the resistance gene in two double haploid populations of Barley. The *Ha2* gene confers resistance to *H. avenae*, arresting female development at about 15 days by causing the degradation of feeding sites in the root steele. The recent mechanization and computerization of the bioassay have reduced the cost but the test still takes 14 weeks and is confined to the winter month. The trend is going to identify molecular markers linked to the *Ha2* gene for resistance to *H. avenae* that can be used for marker assisted selection in barley. The objective has been to determine if additional loci could be identified from alternative sources. Such loci would provide an opportunity to pyramid genes for resistance to CCN. RFLP markers can provide powerful tools for the indirect selection of agronomically important genes in breeding programme.

Molecular markers can offer several important advantages over the bioassay for CCN resistance. RFLP markers described here can effectively replace the bioassay in all early generation screens. Of the particular significance will be availability of CCN resistant malting quality varieties permitting an increase in the cultivated area of malt barley and leading to higher growth returns (the area under Rajasthan, Haryana, eastern as well as southern part of UP can go up). Additionally, cultivation of CCN resistant wheat and barley will allow growers to effectively reduce both the cost of cultivation and levels of nematodes in soil. This will provide growers with more flexibility in rotations by being able to grow CCN susceptible, but otherwise, excellent, varieties of wheat, barley and oats.

A high level of resistance was also transferred to wheat from *Aegilops triuncialis* (TR lines) using the cross (*T. turgidum and Ae triuncilialis*) and *T. aestivum*). Low fertility (3-5 viable kernels per plant) was observed during the process but the surviving hybrid plants were highly vigorous. To obtain stable resistant, further crosses to *T aestivum* were performed. The resistance in TR lines seems to be transferred from the C genome of *Ae triuncilialis* (genome CCUU). *Ae triuncilialis* was highly resistant to the two Spanish populations of *H. avenae* tested, as well as to four French races and two Swedish populations. The histological studies showed that hypersensitive reaction in the roots of a resistant TR line inoculated with the *Ha*71 pathotype of *H. avenae*, whereas well formed syncitia were observed in the roots of the susceptible control. As a result, resistance to the *H. avenae* *Ha*71 pathotype seemed to be inherited as determined by a single dominant factor in the crosses between resistant TR lines and susceptible cultivars.

To target the *H. avenae* (cereal cyst nematode) resistance locus, a D genome amplified sequence (Dgas44), present in all chromosomes of the D genome, is being exploited to detect new sources of polymorphism. Another approach will apply analysis to bulk DNA pools from homozygous lines, resistant (CcnD1) and susceptible to CCN and generate RAPDs using 10 mer random and semi random primers.

Modest approach like *Cre* gene of the wheat line AUS 10894 that confers resistance to the cereal cyst nematode can be applied. Using a pair of near isogenic lines that differed only in a small chromosome segment containing the Cre locus, 58 group -2 probes were screened; two (Tag 605 and CD0 588) were found that detected polymorphism between the NILs.

II) INNOVATIVE NEMATODE ASSAY:

Wheat, Barley, Oat and Rye seedlings are planted in 3 cms tubes filled in with sterile soil and inoculated five times at 3 day intervals @ 100 second stage juveniles per tube. After 12 weeks at 15 0C the number of cysts formed on roots of each plant are counted. Then after cyst grading, the reaction is determined. This has lessened the drudgery of present day process and procedure for screening the wheat and barley against CCN.

EAR COCKLE DISEASE

This disease is diagnosed once the symptoms have already appeared in the field, but at early stage, plants can be observed for having basal swelling (just after 20-25 days of sowing) of stem at the ground level with a whitish tinge. Twisting and crinkling of leaves and of stem is quite evident. Dwarfing of plants and less tillering are also observed. Affected plants show early emergence of earheads with spreading spikelets giving a broader look to the earheads in comparison to healthy ones. Again if cool weather prevails, tundu appears and ears get yellow slime mass. Few grains or whole grains in affected earheads get converted in to galls. Upon examining the seed after threshing or before sowing, if some small roundish, dark brown or black coloured cockled are found in grain or seed lot, it means disease may appear in ensuing crop. The appearance of the disease is highly dependent on weather factors. Thorough picking of cockled grains from the seed lot may help in checking the appearance of disease in the next crop. To manage this disease, the farmers are advised not to sow cockled grains, take healthy truthfully labelled or certified seeds from reliable sources in the country. The seed agencies must ensure that they are selling seeds only of pure nature, which do not have any contamination with seedgall. The extension agencies must popularize the simple technique available for checking the disease. The techniques, available are water floatation, sieving, salt sedimentation etc for mixing seed free of nematode galls.

OTHER NEMATODES

There are certain other nematodes like *Helicotylenchus, Tylenchorynchus, Hoplolaimus, Meloidogyne graminicola, Merlinius brevidens, Pratylenchus thornei* which are commonly found in the wheat fields. Though, there are no apparent damage caused by these nematodes but certainly they weaken the plants during the growth period. Sometimes, these nematodes predispose the plants to secondary infection by fungal, bacterial and viral pathogen. These nematodes have become more like root health issues in the wheat crop than the real inciter of the disease. If they are not managed efficiently, they may pose second generation problems in wheat. At some point of time, they may act like vectors, for many viral, bacterial disease of wheat, which are not found in India. These nematodes are diagnosed by isolating them from soil and observing on root mass. Barring *Meloidogyne graminicola* and *Pratylenchus thornei,* all are seen in soil for which soil slurry is made in water and by using different sieves, the nematodes are isolated from soil and finally through tissue papers straining, the suspension is collected. Thus collected suspension is seen under stereobinocular microscope, and identity of nematode is established. For *M graminicola,* root knots having eggs and other stages of nematode are observed on fresh root under Stereobinocular Microscope. Roots are observed in the same way for *P. thornei* also. The AICWIP (Wheat Nematology of All India Coordinated Wheat Improvement Project) stationed at DWR, Karnal is closely monitoring the behaviour of these nematodes under wheat and wheat based cropping system in different wheat growing zones of the country since inception of Wheat Nematology programme at DWR. This can be termed as preparedness on the AICW&BIP part and also doing base work for monitoring, evaluating and taking appropriate actions in tackling the dynamism/damage caused by these micro entities.

EVALUATION OF NEMATODE PROBLEMS

A) *HETERODERA AVENAE*

The problem of molya disease is serious in Rajasthan and southern parts of Haryana. Though reports of occurrence are there from various parts of NWPZ, but seeing the soil type, weather and other related parameters, there is nothing alarming. Molya disease causes more damage to barley compared to wheat. Its ETL has been found to be as low as 5 juveniles per cubic centimetres of soil to cause significant damage to Wheat, Barley and Triticale. Kanwar and Paruthi (2000) reported that 20% increase in wheat yield due to the management of CCN in Infested field by using carbofuran. The problem of CCN used to exist in Punjab earlier, but now again raising its head in rice-wheat cropping system. But in Rajasthan and southern Haryana, because of sandy nature of soil, same cropping system and also due to arid climate prevailing there, the molya problem is still glaring and deciding factor in realizing the actual output. To contain these diseases, a thorough management strategy basing on sound host resistance system, is desired. Falling on the line, AICWBIP (DWR, Karnal) is thoroughly screening the entries against CCN every year at multilocations and under hot spot conditions. As a result, few varieties have been realized for the state of Rajasthan. Farmers in the states of Rajasthan and Haryana are not in position to grow wheat and barley crops efficiently rather they seem to be shifting for other crops (Mustard, Pulses, Fenugreek etc) which are not affected by this nematode and if they grow these crop for one or two years, nematode population reduces. Here comes the question of raising staple food crops. The wheat production has not found favour with the farmers because of Molya disease in the state. Crores of rupees go waste as farmers remain unable to harvest more of harvesteable yield. The development and release of CCN resistant cultivars may act as a boon to resource starved, marginal farmers residing in harsh climatic locations in arid zone.

B) *ANGUINA TRITICI*

In the year 1997, some farmers belonging to districts of Darbhanga and Madhubani in Bihar state could not harvest wheat because of ear cockle problem. Some fields were discarded because of misconception/superstition that eating such cockled grain/bhusa (toori) might poison the eaters, be it human beings or cattle. In the 1999, ear cockle problem emerged in the state of MP also where it entrapped Panna, Satna, Chhatarpur, and Tikamgarh districts. In few fields, the infection ranged up to 100% as a consequence there was hue and cry among the populace and at the spotting of the disease, no body could believe that it was due to nematode. The severity of the disease depends upon the presense of number of cockles in seed lot. The farmers remain unaware of the impending situation and take lightly the sowing of the seed without having it properly cleaned. Now this aspect has to be thoroughly stressed so that no farmer could sow infested seed. The state government agencies, seed producing and distributing agencies, agricultural research institutes / stations, NGOs, DD, AIR, private TV channels etc. located in the endemic areas should spread the message of clean cultivation to the farmers so that no yield is waste.

C) OTHER NEMATODES

These are *Helicotylenchus, Tylenchorynchus, Meloidogyne graminicola, Hoplolaimus and Pratylenchus.* Though there are no apparent damage caused by these nematodes, but certainly they weaken the plant system during the growth period. Continuous monitoring of these nematodes is need of hour and DWR (AICWBIP) is alive to it.

MANAGEMENT OF WHEAT NEMATODE:

MOLYA DISEASE

Deep summer ploughing (2-3 times) of the infested field during May-June to expose cysts to harsh weather factors including sunlight that in turn kills the cysts, thereby reduces the population by 40% in each season.

Follow crop rotation if possible with coriander fenugreek, onion, mustard, gram, carrot and garlic for bringing down the cyst population to 47-55% level. The cultivation of wheat/barley has increased the CCN population to the level of 160-180%. The thing is that whether these crops find favour with farmers or not.

Host resistance – Raj MR 1 of wheat can be put to use against CCN. Cereal Cyst Nematode (CCN) resistant barley varieties like, RD-2035 and RD-2052 and BH 393 can be grown in infested areas to raise bumper crop of barley.

Apply, if required, carbofuran 3G @ 1.5 Kg ai /ha or sebufos 10G @ 2 kg a.i./ha at the time of sowing in soil.

EAR COCKLE DISEASE

The only solution lies in sowing clean, healthy, uncontaminated seed in the field. For this, extension, developmental machinery and publicity wing of state/central government required to be proactive.

Once the symptoms have appeared, no need to go for any chemical application as this will be mere wastage of chemical and do not get frightened as eating of cockled grain will not poison any more.

If farmers are to sow their own saved seed and if, infestation is observed in the seed lot, they are advised to go for through sieving or brine solution treatment.

OTHER NEMATODES

Insecticides applied for insect pest control (termite, white grub, cut worms, etc) do take care of these entities.

Continuous watch on dynamics of the population is desired to monitor the population.

INTEGRATED NEMATODE MANAGEMENT

MOLYA (CCN):

Summer ploughing in May/ June to expose cyst to weather factors. Apply carbofuran or sebuphos, if the field is having past history of CCN infestation. Farmer may opt non-graminaceous crops in the affected fields. The point to be remembered here is that if the farmers are going for crop rotation, they ought not to apply nematicide as it is a costly item. If possible, they may grow some genotypes like Raj MR 1 of Wheat, RD-2035, RD-2052 and BH 393 of Barley that possess resistance/tolerance against CCN.

EAR COCKLE (ECN):

Farmers should use cockle free seed only. In case they are to use their own seed, they should ensure that it is free from galls. If galls are found in seed lot, they should resort to sieving or water floatation or brine solution treatment as the case demands. For brine solution (2 – 5 % ordinary salt solutions) they can use at the rate of 200 – 250 g salt per 10 lit. of water. Once seed lot is put in the brine solution, galls will float on the surface and they can be easily skimmed off. The settled seed at bottom be washed 2 – 3 times in ordinary water and shade dried. Then they can be used as seed. In spite of dry or wet cleaning, some galls are bound to be with treated seed and they will certainly cause some symptoms to appear in the field, for that selective rouguing is advised at early stages. Above all, the seed agencies are to play crucial role in checking this menace.

FUTURE STUDIES REQUIRED:

- The riddle of existence of biotypes/pathotypes needs to be thoroughly addressed through use of molecular tools and breeding for resistant varieties of wheat and barley should be initiated.
- There are dire needs of developing resistant varieties for which breeders and nematologist have to put their efforts. The issue of pathotypes and resistant material development go hand in hand.
- In addition to host resistance, other management strategies like crop rotation, agronomic practices and biological control, need to be implemented in larger interest as an integrated option with resistance.
- Sound and effective publicity for preventing and containing ear cockle disease are required.

REFERENCES

Anonymous. 1985 – 86 to 2000 – 01. All India Coordinated Wheat Improvement Project Reports (Nematology)

Barloy D J, Lemonie F, Dredryver and Jahier J. 2000. Molecular markers linked to the *Aegilops variabilis* derived root knot nematode resistance gene *Rkn-mn1* in wheat. *Plant Breeding* **118**: 169-172.

Barr A R, Chalmers K J, Karakousis A, Kretschmmer J M, Manning S, Lance R C M, Lewis J, Jefferies S P and Langridge P. 1998. RFLP mapping of a new cereal cyst nematode resistance locus in barley. *Plant Breeding* **117**: 185-187

Bishoi S P and Bajaj H K. 2004. On the species and pathotypes of *Heterodera avenae* complex of India. *Indian Journal of Nematology* **34**: 147-52

Eagles H A, Bariana H S, Ogbonnaya F C, Rebetzke G J, Hollamby G L, Henry R J, Henschke P H and Carter M. 2001. Implementation of markers in australian wheat breeding. *Australian Journal of Agricultural Research* **52**: 1349-1356

Kanwaar R S and Paruthi I J. 2000. Wheat yield influenced by nematicites application in *Heterodera avenae* infested field. National Nematology symposium on integrated nematode management for sustainable agriculture in changing agro-ecological secnario in the new millenium, OUAT, Bhuvnashwar, Nov, 23-24, p. 58.

Kretschmer J M, Chalmers K J, Manning S, Karakousis A, Barr A R, IslamA K M R, Logue S J, Choe Y W, Barker S J, lance R C M, Langridge P. 1997. RFLP mapping of the *Ha2* cereal cyst nematode resistance gene in barley. *Theoretical and Applied Genetics* **94**: 1060-1064.

Lagudah E S and Apples R. 1993. A directed search for DNA sequences tightly linked to cereal cyst nematode resistance genes in *Triticum tauschii*. *Genome* **37**: 311-319.

Ogbonnaya F C, Seah S, Delibes A, Jahier J, Lopez-Brana I, Eastwood R F, and Lagudah E S. 2001 Molecular genetic characterization of a new nematode resistance gene in wheat. *Theoretical and Applied Genetics* **102 (4)**: 623-629.

Ogbonnaya F C, Subrahmanyam N C, Moullet O D E, Majnik J, Eagles H A, Brown J S, Eastwood RF, Kollmorgen J, Appel R and Lagudah E S. 2001 Diagnostic DNA markers for cereal cyst nematode resistance in bread wheat. *Australian Journal of Agricultural Research* **52**: 1367-1374.

Swarup G, Sethi CL, Seshadri AR and Kaushal K K. 1979. On the biotypes of *H. avenae*, the casual organism of molya disease of wheat and barley in India. *Indian Journal of Nematology* **9** : 164-68

Twenty Years of Coordinated Wheat Research.1961-1986. (Ed) Tandon J P and Sethi A P. Wheat Project Directorate, AICW&BIP, IARI, New Delhi-12 : 189-206.

Vasudeva R S, 1958. Scientific report of Indian Agricultural Research Institute for the year ending 30th June 1958.

Williams K J, John M Fisher and Peter Langridge. 1996. Development of a PCR based allele-specific assay from an RFLP probe linked to resistance to cereal cyst nematode in wheat. *Genome* **39**: 798-801.