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EFFECT OF CRUCIFEROUS TISSUE CULTURES AND MEDIA ON THE PENETRATION OF *HETERODERA AVENAE* IN WHEAT *IN VITRO*

by
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Summary. Excised roots and callus cultures of wheat and crucifers were raised on synthetic media. Differences in nematode behaviour were observed with combinations of wheat and crucifers. MS solidified agar medium was better for propagation and maintenance of excised roots. *Eruca sativa* suppressed penetration of roots by juveniles of *Heterodera avenae* significantly. Use of gnotobiotic technique for the establishment of antagonism of crucifers to cereal cyst nematode on wheat is described.

Gnotobiology has proved to be a useful research tool in the study of many stylet-bearing nematodes. Examples of research with cyst and root-knot nematodes include the influence of host nutrition on the development and sex ratios of *Meloidogyne incognita* (McClure and Viglierchio, 1966; Tanda, 1983; Tanda and Atwal, 1988; Tanda *et al.*, 1988) and the use of excised roots to evaluate resistance to *Meloidogyne* spp. (Dropkin and Boone, 1966). More recently, excised roots have been used in monoxenic studies of various aspects of the biology and host parasite relationships of the cyst nematode *Heterodera glycines* (Lauritis *et al.*, 1983). However, it is not known if root (exudates of crucifers are inhibitory *in vitro* and are suitable media for penetration and maintenance studies. My objective was to determine the effect of crucifer tissue cultures and of different media on the penetration of the cereal cyst nematode, *Heterodera avenae* Woll., in wheat using gnotobiotic techniques.

Materials and methods

Seeds of wheat and crucifers were surface sterilized with 0.2% and 0.1% mercuric chloride solutions respectively, for 12-15 minutes. The seeds were then rinsed in sterile distilled water, transferred to culture tubes (25 x 150 mm) containing water agar (15 ml per test tube) and placed in the dark for germination.

Root-tips were excised from 5-7 day old, aseptically grown seedlings of wheat (*Triticum aestivum* L. cv. WL-711), brown mustard (*Brassica campestris* L. cv. Dichotoma), raya (*B. nigra* L. cv. RLM-619), toria (*B. juncea* L. cv. TIC-1) and taramira (*Eruca sativa* L. cv. ITSA) and were cultured on Murashige and Skoog's medium (1962), supplemented with IAA (0.1 mg/l) + Kinetin (0.1 mg/l). The media were gelled with 0.7% agar, except liquid media, and pH was adjusted to 5.8 (Tanda, 1983). Treatment combinations were as listed in Table II. The excised root-tips elongated and thickened considerably, and showed lateral branches. The cultures of excised roots were inoculated with surface-sterilized (0.1% Hg Cl_2) cysts of *H. avenae* at the rate of 2 cysts per test tube (containing approx. 400 juveniles). The cysts were placed near the tips of the excised roots. Callus cultures were raised from root segments grown on B-5 (Gamborg *et al.* 1968) + 2, 4-D (2 mg/l) + Kinetin (0.1 mg/l). The root segments proliferated at both the cut ends, and within 3 weeks the whole segment was covered with whitish to brown callus.

The cultures used for different media and tissues were inoculated with 100 juveniles (Table I). Each treatment was replicated four times. The cultures were incubated at 15 ± 2 °C. Root penetration by the juveniles was observed one and two weeks and on media studies one week after inoculation, respectively. The data were analysed statistically.

Results and discussion

The juveniles started to emerge from cysts within 2-3 days in the tissue cultures. They aggregated near the root-tips of the wheat. However, the behavioural response of the nematode differed when exposed to different tissue culture combinations of wheat and crucifers.

Juvenile (J2) penetration was more when the excised roots were cultured on MS solidified agar medium (8-12%) compared with the MS liquid medium (0-4%). MS agar was

an efficient medium for culturing as well as propagation of excised roots of wheat (Table I). Further, it was found that excised root system is a better system for maintenance and penetration studies of *H. avenae*, as there was more than 8 to 12% J2 penetration and the adult formation was earlier in excised roots than on callus. Callus tissues were found to be an inefficient system for maintenance of *H. avenae* because there was poor penetration (Tanda, *et al.*, 1980). The difference might be due to the roots possessing a well-developed vascular system and the nematodes, being sedentary endoparasites, feed better (Zuckerman, 1971; Tanda, 1984).

In vitro studies to determine the antagonistic effect of crucifers on the penetration of *H. avenae* in wheat excised roots revealed that *E. sativa* suppressed J2 penetration up to the maximum (5.6 mean number of J2s penetrated) (Table II). Root penetration was significantly highest in wheat two weeks after inoculation (Table II) being the major host of *H. avenae*. There was no significant difference among the treatments except with wheat cultured with wheat. Until now, antagonism of cruciferous plants against phytonematodes has not been studied with gnotobiotic techniques except sesame against *M. incognita* in okra (Tanda, 1983; Tanda, *et al.*, 1988).

To conclude, media and tissue cultures standardization and the antagonism of crucifers in wheat against the cereal cyst nematode, under gnotobiotic conditions in tissue cultures emphasizes its utility as an effective tool not only for studying the life cycle or other biological aspects in germ free conditions in excised root cultures, but also for the evaluation of available nematicidal compounds and various organic and inorganic sterilants under a strictly controlled environment. There is also a scope for using biotechniques for selecting nematode-resistant germplasm cell lines or restructuring plants for nematode-resistance in crop improvement programmes.

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TABLE I - *In vitro comparison of penetration of* Heterodera avenae *in excised roots/callus cultures of* Triticum aestivum *on different media one week after inoculation*

Media/culture	J2 penetration	(Mean ± S.E.)	Remark
MS liquid + IAA (0.1 mg/l) + Kinetin (0.1 mg/l)	+	(3.2 ± 1.1)	Nematodes do not penetrate well; good for culturing excised roots/propagation
MS agar + IAA (0.1 mg/l) + Kinetin (0.1 mg/l)	+++	(10.6 ± 1.3)	Nematodes penetrate well; efficient means for propagation and maintenance
Excised roots	+++	(10.7 ± 1.2)	Nematodes penetrate well; efficient system of maintenance and penetration studies
Callus tissue	+	(3.4 ± 1.0)	Nematodes do not penetrate well; inefficient system for maintenance

+ = Poor (0-4% J2 penetration; ++ = Good (4-8% J2 penetration); +++ = Very good (8-12% J2 penetration); ++++ = Best (12-16% J2 penetration).

TABLE II - *Effect of crucifers on the penetration of* H. avenae *in* in vitro *grown 3 week-old-wheat excised roots cultured on MS agar media*

Excised Roots	Number of J2 penetrated in excised roots (Weeks after inoculation)		Mean
	1	2	
Wheat + Toria	4.8 ± 1.5*	7.8 ± 1.7	6.3
Wheat + Taramira	4.1 ± 1.2	7.2 ± 1.4	5.6
Wheat + Raya	5.0 ± 1.7	8.1 ± 1.9	6.5
Wheat + Brown mustard	5.2 ± 2.0	8.5 ± 2.1	6.8
Wheat + Wheat	9.8 ± 3.1	19.2 ± 3.4	14.5
Mean	4.2	10.2	

* Average number ± S.E. (Average of 4 repeats); C.D. (P = 0.05) for treatments: 1.7; for weeks: 1.5.

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