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EXPRESSION OF RESISTANCE TO KARNAL BUNT (*NEOVOSSIA INDICA*) IN EMBRYO CULTURES OF WHEAT

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ABSTRACT

Joint culturing of *Neovossia indica* and embryos of resistant (HD 29 and WH 283) and susceptible (HD 2009, HD 2329 and WH 147) wheat genotypes was done on modified MS medium. Growth of *N. indica* did not affect the callusing frequency of the embryos but embryos formed a circular inhibition zone around themselves in which pathogen growth was absent. The diameter of this zone was little more in resistant genotypes and varied from 2.42 cm in WH 283 to 2.76 cm in HD 29. In susceptible lines it ranged from 1.74 to 2.12 cm. The ability to form this inhibition zone was lost when just initiated callus or 15 days old callus was cultured in presence of the pathogen. The callus growth was not affected by the pathogen except in initial stage.

Key Words : *Neovossia indica*, Joint culturing, Inhibition zone

Screening against Karnal bunt is carried out in the field by creating artificial epiphytotic conditions at boot leaf stage and disease development is dependent upon climatic conditions [1]. The present investigations were planned to explore the possibility of *in vitro* screening of wheat varieties against *Neovossia indica.*

MATERIAL AND METHODS

N. indica (Mitra) Mundkur isolated from the intact bunted grains was cultured on Potato Dextrose Agar (PDA) slants and kept at 20 ± 1°C for ten to fifteen days in an incubator. The 0.1 ml of spore suspension (about 10,000 secondary sporidia/ml) prepared from the actively growing pathogen was spread in petriplates containing Murashige and Skoog [2] medium supplemented with 2, 4-Dicholorophenoxy acetic acid (2 mg/l), Naphthalene acetic acid (0.5 mg/l) and Casein hydrolysate (200 mg/l). Four to five wheat embryos excised from sterilized seeds of each of the five wheat genotypes (HD 2009, HD 2329, WH 147, HD 29 and WH 283) were placed in each

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of the pathogen inoculated petriplates at 25 ± 1°C in dark. This joint culturing of wheat embroys and *N. indica* was done in at least ten petriplates per genotype.

In two other sets of experiment, just initiated calli and fifteen days old calli from embryos of all the five genotypes were cultured in petriplates in presence and absence of *N. indica*. Inoculation with *N. indica* was carried out in green house at boot leaf stage [3] and coefficient of infection (CI) was worked out [4]. The genotypes showing CI less than 5 per cent were graded as resistant and others as susceptible.

RESULTS AND DISCUSSION

Both *N. indica* and wheat embroys grown on modified MS medium showed fairly good growth individually. In joint culturing, a circular inhibition zone was formed (Fig. 1) around the wheat embryos, both in resistant (WH 283, HD 29) as

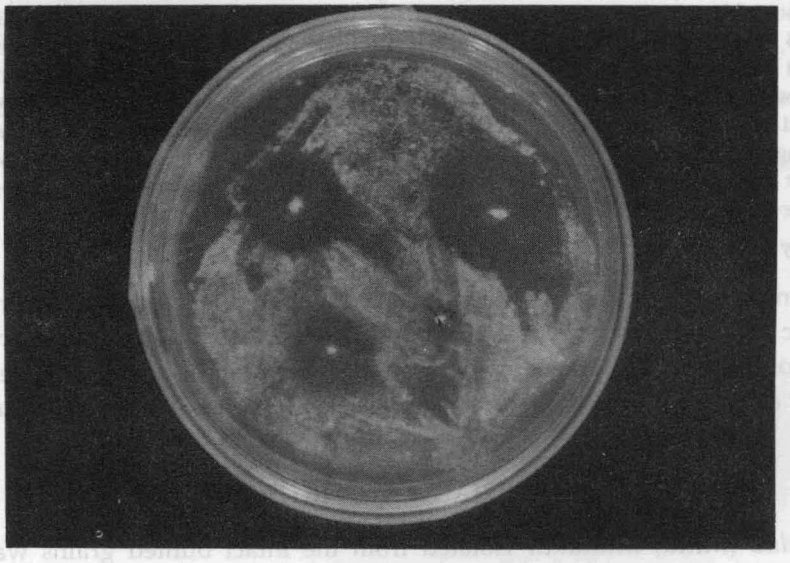

Fig. 1. Joint culturing of wheat embroys and *N. indica* showing inhibition zones

well as susceptible (HD 2009, WH 147, HD 2329) genotypes. This showed that something was secreted by the embryos which did not allow the growth of the pathogen. However, the nature of the chemical could not be ascertained. The inhibition zone was well defined and the diameter of the circular zone could be measured.

Significant variation existed among the genotypes for diameter of inhibition zone (Table 1). It was slightly more in resistant genotypes (2.76 and 2.42 cms in HD 29 and WH 283 with CI 0.43 and 3.46, respectively) as compared to that in susceptible genotypes (1.74, 2.12, 2.12 cm in WH 147, HD 2009 and HD 2329 with CI 35.00, 31.43, 29.06, respectively). Thus, there might be some quantitative differences for the chemical produced among the genotypes.

Table 1. Karnal bunt incidence and inhibition zone in five wheat genotypes

Genotypes	Coefficient of infection	Diameter of inhibition zone (cm)
HD 29	0.43 ± 0.09	2.76
WH 283	3.46 ± 0.95	2.42
WH 147	35.00 ± 1.23	1.74
HD 2009	31.43 ± 1.50	2.12
HD 2329	29.06 ± 0.38	2.12
CD	—	0.17

Time taken for callus induction did not show much variation (3 to 5 days) both in presence and absence of *N. indica*. Even the frequency of callus induction in these two conditions did not differ significantly (Table 2) in all the five genotypes. This reflected that embryos were fully protected against the pathogen in the inhibition zone. Microscopic observations indicated absence of pathogen in the inhibition zone.

Table 2. Callus induction from embryos of five wheat genotypes

Genotypes	Per cent embryos showing callus induction	
	In absence of pathogen	In presence of pathogen
HD 29	52.22 (46.26)	54.30 (47.46)
WH 283	64.06 (53.17)	62.50 (52.67)
WH 147	89.73 (71.56)	87.50 (69.03)
HD 2009	85.89 (67.98)	83.30 (65.92)
HD 2329	48.40 (44.08)	45.83 (42.20)
CD	3.96	2.01

Values in parentheses are arc sine transformations.

In other sets of experiments instead of embryos just initiated calli or fifteen days old calli were cultured in plates already inoculated with *N. indica*. In these

Fig. 2. Joint culturing of wheat calli and *N. indica* showing no inhibition zone

cases inhibition zone was completely absent (Fig. 2) showing thereby chemical in the inhibition zone was secreted by the embryo only and capacity to produce it was lost as the callusing started in the embryo. After ten days of culturing of just initiated calli visual observations indicated that both in presence or absence of *N. indica* there was better callus growth in genotypes HD 2009 and WH 147 (Table 3). However, in the presence of *N. indica*, the callus growth was slightly poorer as compared to that in control (in absence of *N. indica*) in all the genotypes. Visual observations on culturing of fifteen days old callus showed almost the same growth both in presence or absence of *N. indica*. But, callus color in the presence of *N. indica* was slightly darker in resistant genotypes as compared to that in susceptible genotypes. It has also been reported earlier [5, 6] that fungus grew well over the calli of resistant as well as susceptible genotypes.

Microscopic observations on susceptible genotypes indicated more mycelium, an increase in cell size of the calli and cells were loosely placed. These changes, probably, made calli more fragile. However, in the resistant genotypes the cells of

Table 3. Callus growth from embryos of five wheat genotypes in presence and absence of *N. indica*

Genotypes	Growth of just initiated calli		Growth of 15* days old calli
	In absence of *N. indica*	In presence of *N. indica*	
HD 29	+++	+	+++
WH 283	+++	+	+++
WH 147	++++	+++	++++
HD 2009	++++	+++	++++
HD 2329	+++	+	+++

++, +++, ++++ denote low, moderate and good growth of callus, respectively. *In the presence and absence of *N. indica*.

the calli were small, densely packed and placed in clumps. The compactness of the callus decreased in fifteen days old callus cultures as compared to that in preceding stage in all the five genotypes. Significantly more mycelium on one month old callus of Sonalika (wheat cultivar susceptible to *N. indica*) as compared to that of Dolma (Barley cultivar resistant to *N. indica*) has been reported earlier (7). More fragile nature of calli of susceptible genotypes has also been reported by Gill *et al.* (4).

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