Genetic analysis of Karnal bunt (*Neovossia indica*) resistance in wheat

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Embryos excised from seeds of six generations (P_1, P_2, F_1, BC_1, BC_2 and F_2) of a cross WH 283 × WH 533 were cultured on modified MS medium already inoculated with secondary sporidia of *Neovossia indica*. Significant variations for callusing response (CR) (54·55–75·55%) were observed among generations but the presence or absence of *N. indicia* did not affect callusing response. A clear inhibition zone (IZ) was formed around each embryo showing callusing. The diameter of IZ varied significantly among generations and was maximum in the resistant genotype, WH 283 (3·60 cm). Fresh weight and dry weight of calli, initiated from embryo cultured and inoculated with *N. indica*, varied significantly among generations. Coefficient of infection as well as percentage of infection reflected the overdominance of susceptibility. Generation mean analysis showed that the three parameter model was adequate for diameter of IZ only. Six-parameter model showed that additive (in presence of *N. indica*), additive and additive × dominance (in absence of *N. indica*) effects were also significant. Complementary type of epistasis for fresh weight of calli and dominance, and dominance × dominance effects for dry weight of calli were observed in the presence of *N. indica*. Magnitude of additive effects was higher for diameter of IZ in three parameter model. Therefore, selection might assist in improving this trait and thus indirectly help in attaining the resistance towards *N. indica*.

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1. Introduction

Karnal bunt of wheat caused by *Neovossia indica* (Mitra) Mundkar has become a disease of serious concern in some parts of the world like northwest India, Pakistan and northwest Mexico. Consequently, stringent quarantine measures have been adopted in several countries, which may affect not only wheat grain trade but also germplasm exchange as well. Since the pathogen is seed, soil and air borne, a limited control is achieved through the application of fungicides (Singh *et al* 1985). One method of controlling the disease is to develop resistant cultivars through screening against *N. indica*. Screening is carried out by creating artificial epiphytotic conditions at boot leaf stage which is time and labour consuming operation (Chona *et al* 1961; Aujla *et al* 1980; Singh and Krishna 1982). Screening and multiplication of different wheat varieties under laboratory conditions using *in vitro* culture techniques may speed up the resistance breeding programmes. Hence, the present investigations were planned to study the nature and magnitude of gene effects of inhibition zone formed by the wheat embryos, callusing response both in presence and absence of *N. indica*, effect of *N. indica* on callus growth and to study the Karnal bunt resistance of adult plant stage in parents and F_1s.

Keywords. Callusing response; coefficient of infection; fresh and dry weight of calli; *Neovossia indica*

Abbreviations used: CR, Callusing response; IZ, inhibition zone; MS, Murashige and Skoog medium; PDA, potato dextrose agar .

2. Material and methods

Neovossia indica, causal organism of Karnal bunt of wheat, was isolated from the bunted grains. The teliospores were dusted directly over potato dextrose agar (PDA) slants in test tubes and kept at 18–20 ± 1°C for 10–15 days in a BOD incubator. The tubes were kept in an upright position so that allantoid sporidia got showered on the slants of culture medium. Subculturing of the pathogen was done after every 10–12 days on the fresh medium to maintain the pathogen in a sporulating condition. Embryos excised from mature seeds of six generations, P_1, P_2, F_1, BC_1, BC_2 and F_2, of a cross involving a resistant and susceptible parent, viz. WH 283 × WH 533, were cultured on MS medium supplemented with 200 mg/l casein hydrolysate, 2 mg/l 2, 4-dichlorophenoxyacetic acid and 0·5 mg/l of *a*-naphthalene acetic acid in petriplates already inoculated with 0·1 ml of spore suspension (10^3 secondary sporidia) of *N. indica*. The pedigree of these genotypes is given below:

Genotype	Pedigree
WH 283	HD 1981/Raj 821
WH 533	Veery-5 (s)

At least 20 embryos from each other parents, F_1s, backcrosses, and 50 embryos from F_2 were cultured in three replications. The same number of embryos were cultured in petriplates, as control, i.e. in the absence of *N. indica*. Callusing response (in terms of percent embryos showing callusing) and diameter of inhibition zone were recorded. In another experiment at least 20 embryos from each of the parents, F_1's, backcross and 100 embryos from F_2 were cultured with one embryo per tube for callus initiation, followed by inoculation with *N. indica* after callus initiation. The same number of tubes were also maintained as control, and the cultures were incubated at 25 ± 1°C in dark. Fresh weight and dry weight of callus were recorded after 30 days of culturing. Data were analysed by factorial completely randomized design. Joint scaling test (Cavalli 1952) and generation mean analysis (Hayman 1958) were carried out for all the traits under investigations. Five plants from each of the parents (WH 283 and WH 533) and F_1's were raised in the earthen pots in three replications and were inoculated with the sporidial suspension prepared from freshly sporulating 10–12 days old culture. About 2 ml spore suspension was injected in each tiller at boot-leaf stage during the evening hours (Aujla *et al* 1983). After maturity, the inoculated ear heads were harvested and grains were removed carefully by hand. Percent infected grains and coefficient of infection were worked out as suggested by Aujla *et al* (1989). The grains obtained from inoculated earheads were separated into different grades as 0, 1, 2, 3 and 4; and data were analysed using completely randomized design.

3. Results and discussion

Significant variation was observed for callusing response of embryos among different generations and varied from 54·44% to 75·55% – overall, the callusing response was low. Agarwal and Tiwari (1995), Kintzois *et al* (1996),

Table 1. Callusing response, fresh weight and dry weight shown by embryos in different generations of the cross WH 283 × WH 533/WH 283.

	Callusing response		Fresh weight (mg)			Dry weight (mg)		
Generations	In the presence of *N. indica*	In the absence of *N. indica*	In the presence of *N. indica*	In the absence of *N. indica*	Relative weight[+] (%)	In the presence of *N. indica*	In the absence of *N. indica*	Relative weight[†] (%)
WH 283	72·22	72·22	110·49*	158·58*	69·67	7·16*	11·41*	62·75
WH 533	54·44*	54·44*	60·40*	113·77*	56·60	4·91*	8·63	56·89
WH 283 × WH 533 (F_1)	63·33*	63·32*	100·84	154·98	65·06	7·08	10·58	66·91
WH 283 × WH533 (F_2)	74·44*	74·44*	98·73	162·66*	60·84	6·57	10·96	59·94
WH 283 × WH533/WH 283 (BC_1)	75·55*	73·33*	106·21*	140·76	75·45	6·94	9·54	72·74
WH 283 × WH533/WH 533 (BC_2)	71·11	72·22	82·33*	138·66	59·37	5·87*	9·37	62·64
Mean	68·51	68·32	93·83	144·83		6·42	10·08	
CD for genotype	4·29		10·56			0·73		
CD for treatments	NS		6·09			0·42		
CD for genotype × treatment	NS		NS			NS		

*Significant at 5%.

[†]Weight of callus in the presence of *N. indica* to that in the absence of *N. indica* $\frac{\text{weight in the presence of } N.\ indica}{\text{weight in the absence of } N.\ indica} \times 100.$

NS, Non significant.

Ozgen *et al* (1998) and Tandon *et al* (2000) also reported significant differences among genotypes for callusing ability. However, in the present study there were no significant differences in callusing ability of different generations due to the presence or absence of *N. indica*. This reflected that pathogen did not affect callus initiation from embryos of these generations. Low callusing ability of embryo excised from mature grains were also reported by Bartok and Sagi (1990), Chauhan and Singh (1995), Ozgen *et al* (1996) and Arya (1999). Maximum callusing response (75·55%) in the presence of *N. indica* was observed in backcross WH 283 × WH 533/WH 283 (table 1). Arya (1999) and Tandon *et al* (2000) also reported that *N. indica* had no effect on callus initiation in resistant and susceptible genotypes. Significant variations were observed due to generations, treatment and generation × treatment interaction for fresh weight and dry weight of callus. This indicated that fresh and dry weight of calli are affected significantly by the pathogens. The observations on fresh weight and dry weight results indicated that among generations, the minimum effect of *N. indica* was in WH 283 (P_1) and maximum in WH 533 (P_2). A clear inhibition zone was formed around each of the embryos showing callusing including those from susceptible genotypes, in all the six generations of cross. Generations differed significantly for the diameter of inhibition zone (figure 1) and this was maximum (3·60 cm) in WH 283, the resistant genotype. Percent infected grains and coefficient of infection showed significant variations among all the generations. Coefficient of infection and percentage of infected grains was maximum in F_1 of WH 283 × WH 533 and minimum in WH 283 (resistant genotype) (table 2), which suggests that susceptibility is dominant over resistance.

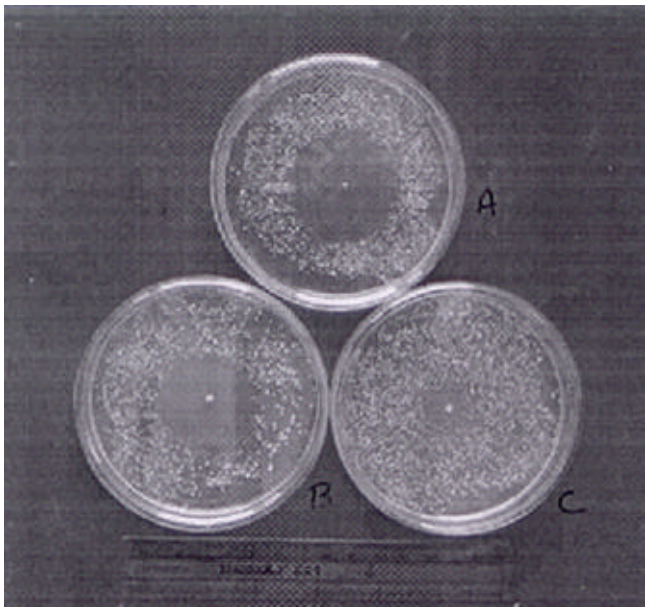

Figure 1. Inhibition zone shown by embryos of highly resistant (**A**), resistant (**B**) and susceptible (**C**) generations.

Three parameter model indicated that both additive and dominance effects were significant for callusing response (in the absence of *N. indica*) and for fresh and dry weight (both in the presence and absence of *N. indica*). None of the estimates of gene effects for callusing frequency in the presence of *N. indica* were found significant. This may be due to cancelling effects of contribution of individual loci in the net effect or due to high standard error of the estimates (table 3). The additive–dominance model was found to be inadequate for callusing response, fresh and dry weight, as indicated by c^2, showing the presence of epistatic interactions. Six-parameter model (table 4) showed that in presence of *N. indica*, only additive (4·440) effects were significant for callusing response. In the absence of *N. indica*, additive (1·110) and dominance × dominance (31·128) were significant. In the presence of *N. indica* additive (24·030), dominance (3·810) and dominance × dominance (16·060) were significant

Table 2. Diameter of inhibition zone, coefficient of infection and percentage of infection shown by embryos in different generations of the cross WH 283 × WH 533.

Generations	Diameter of inhibition zone (cm)	Coefficient of infection (%)	Percentage of infection (%)
WH 283	3·60*	3·96*	9·73*
WH 533	2·33*	14·87*	32·56*
WH 283 × WH533 (F_1)	2·56*	21·90*	48·57*
WH 283 × WH533 (F_2)	2·72		
WH 283 × WH533/WH 283 (BC_1)	3·07*		
WH 283 × WH533/WH 533 (BC_2)	2·71		
Mean	2·83	17·24	26·95
CD	0·28	0·25	0·05

*Significant at 5%.

Table 3. Estimation of genetic parameter for different characters of cross WH 283 × WH 533 in wheat using three parameter model.

Characters	m	(d)	(h)	c^2
Callusing frequency				
In the presence of *N. indica*	71·211 ± 1·92	2·870 ± 2·69	0·996 ± 10·59	15·889**
In the absence of *N. indica*	63·900 ± 3·84	7·359** ± 2·84	16·203** ± 5·26	14·713**
Fresh weight				
In the presence of *N. indica*	92·846 ± 1·37	29·121** ± 7·15	8·147** ± 2·86	48·121**
In the absence of *N. indica*	136·322 ± 4·26	11·388** ± 4·18	18·897** ± 1·86	23·672**
Dry weight				
In the presence of *N. indica*	6·053 ± 0·09	1·126** ± 0·22	1·027** ± 0·25	1·075**
In the absence of *N. indica*	9·017 ± 0·33	0·556* ± 0·17	1·450** ± 0·50	22·102**
Diameter of inhibition zone	2·987 ± 0·22	0·607** ± 0·14	– 0·258 ± 0·25	4·444 (NS)

*Significant at 5%.
**Significant at 1%.

Table 4. Estimation of genetic parameter for different characters of cross WH 283 × WH533 in wheat using six parameter model.

Characters	m	(d)	(h)	(i)	(j)	(l)	Types of epistasis
Callusing frequency							
In the presence of *N. indica*	74·440 ± 1·92	4·440* ± 1·69	– 4·440 ± 10·59	– 4·440 ± 9·38	– 8·900 ± 8·99	– 35·560 ± 16·47	–
In the absence of *N. indica*	74·440 ± 3·84	1·110* ± 0·54	– 6·664 ± 18·26	– 6·660 ± 17·19	– 15·560 ± 8·81	31·128** ± 4·98	–
Fresh weight							
In the presence of *N. indica*	98·730 ± 1·37	24·030* ± 7·15	3·810** ± 0·86	– 17·540 ± 15·32	1·300 ± 16·39	16·060** ± 4·27	Complementary
In the absence of *N. indica*	162·260 ± 4·26	2·180 ± 4·78	– 71·555 ± 21·86	– 90·360 ± 19·56	– 40·450 ± 19·01	113·990* ± 32·23	–
Dry weight							
In the presence of *N. indica*	6·573 ± 0·09	1·070 ± 1·22	8·379** ± 2·50	– 0·600 ± 2·47	– 0·110 ± 2·55	1·270* ± 0·57	–
In the absence of *N. indica*	10·963 ± 0·33	0·227 ± 0·27	– 5·590 ± 1·57	– 6·146 ± 1·46	– 2·323 ± 1·22	9·640** ± 2·10	–

*Significant at 5%.
**Significant at 1%.

for fresh weight of calli. Same sign of (h) and (l) indicated that complementary type of epistasis was present. Only dominance × dominance (113·990) gene effects were found to be significant in the absence of *N. indica*. In case of dry weight, dominance (8·379) and dominance × dominance (1·270) effects were significant in the presence of *N. indica* while in the absence of *N. indica*, all type of gene effects were non-significant except dominance × dominance (9·640) (table 4). c^2 value indicated the adequacy of additive–dominance model for diameter of inhibition zone. As additive effects were significant in present investigations, selection *in vitro* might be partially helpful for improving wheat for inhibition zone and thus indirectly providing the resistance against the Karnal bunt.

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