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Effect of *Neovossia indica* on embryo culture of wheat [*Triticum aestivum* (L.) em Thell] and its reflections on *in vivo* resistance

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Abstract

In an effort to study the effect of *Neovossia indica* on embryo culture of wheat [*Triticum aestivum* (L.) em Thell], 17, 20 and 23 days old embryos were excised from immature grains of different wheat genotypes. These were cultured on modified MS medium in presence and absence of the pathogen. The pathogen had no effect on callus initiation and callusing frequency. Some of the 23 days old embryos from the wheat genotypes formed a circular inhibition zone (IZ) around themselves where no growth of pathogen could be seen. The formation of IZ was observed around every embryo excised from mature grains and cultured in presence of the pathogen. The mean diameter of IZ was found to be 3.19 cm in resistant, 2.26 cm in susceptible and 1.62 cm in highly susceptible genotypes showing coefficient of infection less than 5%, 5 to 20 % and more than 20 %, respectively at adult plant stage.

Key words: Wheat, *Neovossia indica*, embryo culture, resistance.

Introduction

Sustainability of wheat production has been greatly interfered by disease epidemics. Some of the diseases, which were considered to be of minor importance earlier, have now assumed serious proportions. Karnal bunt of wheat caused by *Neovossia indica* (Mitra) Mundukar is one of them. Infected kernels are infested with masses of dark, powdery, fishy smelling fungal teliospores, resulting in poor consumer quality of wheat products. The pathogen being quite resistant to physical and chemical treatment, easily spreads to new areas calling for stringent quarantine measures locally and globally [1, 2, 3]. It has been reported earlier from our laboratory that a circular inhibition zone is formed around the mature wheat embryos co-cultured with *N. indica* on a defined medium [4]. The present investigation was carried out to study the effect of *N. indica* on the cultured wheat embryos and to establish any link of inhibition zone formation (in different time regimes) with disease resistance/susceptibility in wheat using some resistant, susceptible genotypes and hybrid genotypes.

Materials and methods

Fourteen wheat genotypes (Table 2) were sown in green house in earthen pots in three replications along with PBW 343 and WH 542.

Joint culturing of wheat embryos/callus and N. indica: *N. indica* was isolated from the intact bunted grains by dusting the teliospores directly on Potato Dextrose Agar (PDA) medium. A spore suspension containing 10^4 secondary sporidia per ml in water was prepared from actively growing culture, 0.1 ml of this (1000 sporidia) was plated in Petri plates containing Murashige and Skoog (MS) [5] medium supplemented with 200 mg/1 of casein hydrolysate, 2 mg/1 of 2,4-dichloro phenoxy acetic acid (2,4-D) and 0.5 ml/1 of naphthalene acetic acid (NAA). Embryos were excised from immature grains collected at 17, 20 and 23 days after anthesis (stage I, II & III respectively) and also from overnight presoaked, mature grains. These were placed in Petri plates already plated with *N. indica*. Twenty embryos from each replication in each genotype at each stage were cultured at 25±1°C under dark with scutellum of the embryos facing away from the surface of the medium. The same numbers of embryos were also cultured as control in absence of *N. indica*. Observations were recorded for days to callus induction, per cent embryos showing callus induction and diameter of inhibition zone.

Twenty embryos from each genotype per replication were also cultured on modified MS medium with one embryo per culture tube. When callus formation was initiated, each culture tube was inoculated with 0.05 ml of sporidial suspension of *N. indica*. Observations were recorded for IZ formation.

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Table 1. Analysis of variance for days to callusing in wheat embryos (excised from immature seeds after different days of anthesis) cultured in presence and absence of *N. indica* on modified MS medium

Source of variation	df	Mean sum of squares
Genotypes	13	9.435**
Treatments	1	0.862
Stages	2	11.233**
Genotypes × treatment	13	0.961
Genotypes × stage	26	12.223**
Treatments × stage	2	1.327
Genotypes × Treatments × Stages	26	8.437**
Error	168	1.648

Treatments presence or absence of *N. indica*, stages = 17, 20 and 23 days after anthesis, **Significant at 1%

excised from immature grains in presence and in absence of *N. indica* showed significant variation among the genotypes. However, non-significant differences for days to callus initiation were observed between treatments (i.e. between embryos cultured in presence and cultured in absence of *N. indica*). This indicated that *N. indica* did not promote or delay the callusing tendency of wheat embryos. Significant differences could be seen due to stages and interactions due to genotypes x stages or genotypes × treatments × stages (Table 1.). This reflected that embryos of a genotype showing early callusing at one stage may not show callusing at the same time at other stage in presence or in absence of *N. indica*. In general seventeen days old embryos took more time to callus initiation than 20 or 23 days old embryos (Table 2.). Thus appropriate stage

Table 2. Days to callus induction in wheat embryos (excised from mature seeds) at different days of anthesis on modified MS medium.

Genotypes	Stage-I (17 days)		Stage-II (20 days)		Stage - III (23 days)	
	In presence of *N. indica*	In absence of *N. indica*	In presence of *N. indica*	In absence of *N. indica*	In presence of *N. indica*	In absence of *N. indica*
WH 533	5.17	5.08	2.10	2.02	2.15	2.11
WH 283	4.26	4.31	2.17	2.13	2.36	2.24
HD 2329	2.11	2.17	2.20	2.12	2.00	2.07
WH 147	2.13	2.25	2.11	2.04	2.09	2.09
HD 29	2.01	2.00	2.13	2.12	2.20	2.22
HD 2009	3.32	3.21	2.00	2.13	2.22	2.18
HD 29 × WH 147(F_1)	5.26	4.98	2.11	2.07	2.01	2.10
WH 533 × WH 147(F_1)	2.09	2.25	2.09	2.00	2.19	2.13
HD 2329 × WH 283 (F_1)	3.17	3.01	2.12	2.00	2.29	2.22
HD 29 × WH 283 (F_1)	3.03	3.01	2.23	2.13	2.16	2.19
SC* 16	3.21	3.17	2.09	2.02	2.10	2.11
SC* 18	3.03	3.02	2.19	2.11	2.32	2.17
SC* 19	3.33	3.31	2.12	2.02	2.17	2.11
UP 2338	2.26	2.14	2.12	2.06	2.23	2.27
Mean	3.17	3.15	2.10	2.07	2.18	2.03
CD for genotypes		0.83	CD for genotype × stages			1.45
CD for treatments		0.31	CD for treatment × stage			0.54
CD for stages		0.38	CD for genotype × treatment x stage			2.05
CD for genotype × treatment		1.18	SC* = Soma clones of HD 2009			

In vivo studies: At boot leaf stage, individual ears of five plants sown in earthen pots in green house were inoculated with 2 ml of sporidial suspension using hypodermic syringe during evening hours [6]. For successful infections and disease development, optimum temperature (18-22°C) and relative humidity (80-100 %) were maintained in the green house. At maturity infected seeds were separated into different grades as suggested by Aujla *et al.*, [7] and coefficient of infection was calculated as given by Gill *et al.*, [1].

Results and discussion

Joint culturing of wheat embryos and N. indica: Analysis of variance for days to callus initiation from embryos of embryos for early callusing could be achieved after 20 days of anthesis. Karadimova *et al.* [8] also reported that embryos excised and cultured within 19 to 22 days of anthesis were more responsive to callus initiation. Zhang and Scilleur [9] observed that optimum stage for callus initiation was 16-22 days after anthesis, depending upon the genotypes. Khatri [10] reported that embryos excised and cultured from grains collected at 16-20 days of anthesis were the best material for callus induction.

Callus initiation was delayed in embryos excised from mature grains as compared to that from immature grains. On an average 4 days were taken for callus initiation from mature embryos and there were no

Table 3. Percentage of embryos (excised from immature and mature grains) showing callus induction and morphology of calli in presence and in absence of *N. indica* on modified MS Medium.

Genotypes	From immature grains				From mature grains			
	In presence of *N. indica*		In absence of *N. indica*		In presence of *N. indica*		In absence of *N. indica*	
	% callus induction	Callus morphology	% callus induction	Callus morphology	% callus induction	Callus morphology	% callus induction	Callus morphology
WH 533	88.66	+	88.33	+++	44.44*	+	47.88*	+
WH 283	88.66	++++	88.00	++++	67.77*	+	68.88*	+
HD 2329	87.00	+++	86.00	++++	46.77*	+	47.77*	+
PBW 343	-	-	-	-	64.55	+	66.66	+
WH 147	87.00	+	87.33	+++	69.93*	+	70.10*	+
HD 29	87.00	++++	85.33	++++	52.32	+	51.11	+
WH 542	-	-	-	-	52.44	+	51.10	+
HD 2009	87.33	+	86.33	+++	74.44*	+	75.55*	+
HD 29 × WH 147 (F_1)	87.33	+	85.33	+++	63.44	+	64.44	+
WH 533 × WH 147 (F_1)	85.00	+	86.66	+	55.66	+	55.66	+
HD 2329 × WH 283 (F_1)	88.66	+	87.00	+++	69.99*	+	71.71*	+
HD 29 × WH 283 (F_1)	86.00	++++	87.33	++++	45.66*	+	46.66*	+
SC* 16	88.66	++++	88.33	++++	65.55	+	65.66	+
SC* 18	86.66	++++	85.33	++++	55.77	+	56.77	+
SC* 19	87.70	+	88.00	+++	43.33*	+	44.44*	+
UP 2338	88.66	+++	87.00	+++	57.77	+	57.77	+
Mean	87.45		86.87		58.12		58.82	
CD for genotypes			5.90		7.25			
CD for treatments			2.23		2.56			
CD for genotypes × treatments			8.35		10.25			

*Significantly different from mean, - Not tested, ++++ Very good callus, +++good callus, ++ poor callus, + very poor callus.

significant differences among the genotypes. Chauhan and Singh [11] also reported that embryos excised from immature grains started callusing in 2-3 days whereas embryos excised from mature grains started callusing in 4-5 days. On the contrary Agarwal and Tiwari [12] observed that embryos excised from immature grains started callusing in 4-6 days and mostly independent of genotypes. Analysis of variance for per cent embryos (excised from immature grains) showing callus induction indicated no significant differences among the genotypes in presence and also in absence of *N. indica*. In all the genotypes, 85-90 per cent immature embryos showed callus initiation. However, the percentage was fairly lower for mature embryos showing callus induction. It varied between 43.33 (44.44) to 74.94 (75.55) in presence (absence) of *N. indica*. There were significant differences for it among the genotypes. The overall growth and morphology of the calli was better from immature embryos than from mature embryos (Table 3). High frequency of callus induction from embryos excised from immature grains was also reported by Dahiya *et al.* [13] and Ozgen *et al.* [14] whereas low frequency of callus initiation from embryos excised from mature grains was reported by Bartok and Sagi [15] and Ozgen *et al.* [16]. Presence of pathogen did not affect the percentage of embryos showing callusing. Tandon *et al.* [4] and Kumar *et al.* [17] also reported that *N. indica* had no effect on callus initiation in resistant and susceptible genotypes. Both *N. indica* and wheat embryos grown on modified MS medium showed good growth individually. In joint culturing a circular inhibition zone was formed around the embryos excised from mature grains of all the genotypes. This showed some biochemical product was secreted by the embryos which did not allow growth of the pathogen. Inhibition zone was not formed in joint culturing of *N. indica* and 17 or 20 days old embryos. However, it could be seen in joint culturing of *N. indica* and embryos (of 3 genotypes) excised after 23 days of anthesis. As inhibition zone formation coincided with the stage when grain development had taken place, it could be said that inhibition zone formation might be a characteristic of embryos excised from maturing/mature grains. Further research is needed to ascertain the exact nature of the chemical responsible for inhibition zone formation.

The diameter of inhibition zone formed by embryos excised from mature grains varied significantly among the genotypes (Table 4). This may be due to the quantitative differences among the genotypes for the production of the chemical responsible for inhibition zone formation. Visual observations showed that there was no growth of *N. indica* inside the inhibition zone. Moreover, inhibition zone was formed by every mature embryo showing callusing reflecting that chemical produced by the embryo did not interfere with its callusing ability but hindered the growth of the pathogen.

Correlation of disease severity with inhibition zone: *In vivo* studies for disease severity indicated that out of 16 genotypes, five were resistant (coefficient of infection, CI < 5%) seven were susceptible (CI = 5-20%) and four highly susceptible (CI > 20%) (Table 4). Significant rank correlation (–0.87) was observed between disease severity and diameter of inhibition

Table 4. Inhibition zone formed by mature embryos of resistant, susceptible and highly susceptible genotypes of wheat against *N. indica* on modified MS medium

Genotypes	Diameter of inhibition zone (cm)	Co-efficient of infection (< 5%)	Genotypes	Diameter of inhibition zone (cm)	Co-efficient of infection (5-20%)	Genotypes	Diameter of inhibition zone (cm)	Co-efficient of infection (>20%)
WH 283	3.49	3.83	WH 533	2.41	13.33	HD 2329	2.03	22.69
HD 29	3.64	2.05	WH 542	2.45	19.86	WH 147	1.86	26.50
HD 29 × WH 283	3.51	3.33	HD 29 × WH 147	2.16	19.07	HD 2009	1.42	34.57
SC* 18	2.14	4.93	HD 2329 × WH 283	2.30	16.55	WH 533 × WH 147	1.19	39.07
SC* 19	3.21	4.25	SC* 16	1.21	11.26			
			UP 2338	2.58	11.45			
			PBW 343	2.72	9.65			
Mean	3.19			2.26			1.62	

zone. More diameter of inhibition zone was formed by genotypes where CI was lower and *vice-versa*. The mean diameter of inhibition zone was 3.19 cm in resistant genotypes, 2.26 cm in susceptible genotypes and 1.62 cm in highly susceptible genotypes.

Joint culturing of wheat callus and N. indica: No inhibition zone formation could be observed when just initiated calli were cultured in presence of *N. indica*. This loss of ability to form inhibition zone in joint culturing after callus initiation was also reported by Tandon *et al.* [4]. It is because of the fact that developing wheat embryo and initiated callus are totally dissimilar tissues morphologically and biochemically.

Thus from the above studies, it could be concluded that the presence of *N. indica* in culture does not affect the callus initiation or callusing frequency of the embryos but it affects the callus growth. The inhibition zone formation is a characteristic of mature embryo or more precisely development specific since even 17 and 20 DAP embryos were unable to elicit this response. Further investigation into the chemical nature of substance secreted and inhibition zone formation may prove useful to measure resistance to karnal bunt in wheat improvement programmes.

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