Indian J. Genet., 69(1): 62-65 (2009)

Short Communucation

Karnal bunt resistance in wheat embryos on different time regimes of grain development in bixenic cultures

Veena Chawla, Subhash Kaushik, Minakshi Jattan and Neelam R. Yadav*

Deptt. of Genetics, *Deptt. of Biotechnol. & Molecular Biology, CCS Haryana Agricultural University, Hisar 125 004

(Received: May 2008; Revised: December 2008; Accepted: February 2009)

www.IndianJournals.com
Members Copy, Not for Commercial Sale
Downloaded From IP - 61.247.228.217 on dated 27-Jun-2017

Karnal bunt (KB) [(*Neovossia indica* (Mitra) Mundkur)], an important disease of wheat causes partial conversion of individual kernels into sori, filled with teliospores and sterile cells resulting in losses in both yield and quality [1]. The infected grains emit an unpleasant fishy odour due to trimethylamine. The disease has a great significance in global wheat trade because of stringent quarantine measures. The development of KB resistant varieties is difficult as the disease expression is highly influenced by the environment. So finding some easy way to screen against the disease will help to develop resistant genotypes using conventional as well as molecular techniques.

It has already been reported by Tandon *et al.* [2] that in joint culturing of wheat embryo and Karnal bunt pathogen in the petri plates, a circular zone (referred as inhibition zone) is formed around the wheat embryo due to host pathogen interactions. This tendency is lost if the pathogen comes in contact after initiation of callusing of the embryo. In the present investigation, the effect of *N. indica* was studied on callusing response (per cent embryos showing callus initiation), callusing state (the very good callus was given rank 4, good as 3, poor as 2 and very poor as 1), per cent embryos showing inhibition zone (IZ) and diameter of IZ at different days of embryo development.

Three susceptible (WH 542, WH 147, HD 2329) and two resistant (HD 29 and WH 283) wheat genotypes were sown in the farm area of Department of Genetics. Bixenic culturing of wheat embryos (20 embryos for each of the three replications) excised after 15, 18, 21, 24, 27, 30, 33, and 36 days of pollination (DAP) (designated as stage 1-8 in figures 1-4) and *N. indica* was done in petri plates. Spore suspension of *N. indica* (1000 sporidia/0.1 ml) was spread in petri plates containing Murashige and Skoog (MS) [3] medium supplemented with 200 mg/l of Casein hydrolysate, 2 mg/l of 2, 4-Dichlorophenoxy acetic acid (2, 4-D) and 0.5 ml/l of Naphthalene acetic acid (NAA). Embryos were excised from immature grains; placed in petri plates already plated with *N. indica* and incubated at 25 ± 1 °C under dark.

At 15 DAP stage, callusing response was very low in all the 5 genotypes i.e. varying between 21.66% (HD 29) to 46.66% (WH 542 and WH 147) (Table 1). Callusing state was also very poor. Karadimova *et al.* [4] also reported that embryos excised and cultured within 19 to 22 days of anthesis were more responsive to callus initiation. Zhang and Scilleur [5] observed that optimum stage for callus initiation was 16-22 days after anthesis, depending upon the genotypes.

At 18 DAP stage, callusing response and callusing state were better in all the genotypes varying from 51.66% (HD 29) to 75% (HD 2329) and 1.54 (WH 283) to 2.84 (WH 147), respectively. Up to 21 DAP stage, callusing response further improved significantly in all the genotypes, maximum being in HD 2329 (95%). Best callusing response (Fig. 1) and callusing state (Fig. 2) were observed at 24 DAP stage. The best callus was observed in WH 147 genotype. Callusing response and callusing state declined with further increase of DAP, minimum being at 36 days of pollination when grains were almost mature. High frequency of callus induction from embryos excised from immature grains was also reported by Özgen *et al.* [6] and Arya *et al.* [7] whereas low frequency of callus initiation from embryos excised

www.IndianJournals.com
Members Copy, Not for Commercial Sale
Downloaded From IP - 61.247.228.217 on dated 27-Jun-2017

Table 1. Callusing response and inhibition zone formation in bixenic culture of *N. indica* and wheat embryos at different days of pollination

DAP	WH542				WH147				HD2329				HD29				WH283			
	CR (%)	C.St	IZ No. (%)	Dia-meter of IZ	CR (%)	C.St	IZ No. (%)	Dia-meter of IZ	CR (%)	C.St	IZ No. (%)	Dia-meter of IZ	CR (%)	C.St	IZ No. (%)	Dia-meter of IZ	CR (%)	C.St	IZ No. (%)	Dia-meter of IZ
15DAP	46.66	1.10	00.0 (00.0)	0.00	46.66	2.30	00.0 (00.0)	0.00	26.66	1.56	00.0 (00.0)	0.00	21.66	1.15	00.0 (00.0)	0.00	30.00	1.00	00.0 (00.0)	0.00
18DAP	61.66	1.67	00.0 (00.0)	0.00	63.33	2.84	00.0 (00.0)	0.00	75.00	2.62	00.0 (00.0)	0.00	51.66	1.76	6.7 (12.5)	0.46	66.66	1.54	00.0 (00.0)	0.00
21DAP	73.33	2.63	31.4 (33.9)	0.76	73.33	3.71	08.6 (14.0)	0.11	95.00	3.61	24.6 (29.6)	0.83	85.00	2.94	27.4 (31.5)	0.91	88.33	2.91	03.7 (09.0)	0.40
24DAP	73.33	2.75	47.7 (43.6)	1.01	81.66	3.64	34.3 (35.7)	1.17	93.33	3.44	37.2 (37.3)	0.93	91.66	3.04	50.9 (45.5)	1.38	88.33	3.03	52.8 (46.5)	1.03
27DAP	75.00	2.73	57.7 (49.1)	1.56	76.66	3.48	56.4 (48.6)	1.44	88.33	3.03	54.7 (47.7)	1.08	86.66	3.25	57.7 (49.4)	1.86	76.66	2.93	69.5 (56.4)	1.90
30DAP	78.33	2.57	59.6 (50.5)	1.56	75.00	3.50	75.7 (60.5)	2.37	78.33	2.71	68.2 (55.6)	1.43	85.00	2.66	69.9 (56.7)	2.19	80.00	2.85	73.2 (58.9)	2.50
33DAP	70.00	2.02	78.5 (62.5)	2.10	66.66	3.01	79.7 (63.3)	2.35	73.33	2.60	75.3 (60.2)	1.93	76.66	2.46	80.5 (63.9)	2.69	81.66	2.81	79.6 (63.1)	2.93
36DAP	73.33	1.95	75.1 (60.0)	2.40	63.33	2.80	82.0 (65.2)	2.36	75.00	2.40	82.3 (65.1)	2.07	73.33	2.25	84.2 (66.5)	3.07	78.33	2.75	78.5 (62.4)	3.03
CD for DAP	4.63	0.11	3.02	0.10																
CD for varieties	3.66	0.09	2.39	0.08																
CD for interactions	10.35	0.26	6.76	0.24																

CR= Callusing Response; C.St = Callusing State; IZ = Inhibition Zone; ( ) = Values in parentheses are transformed values

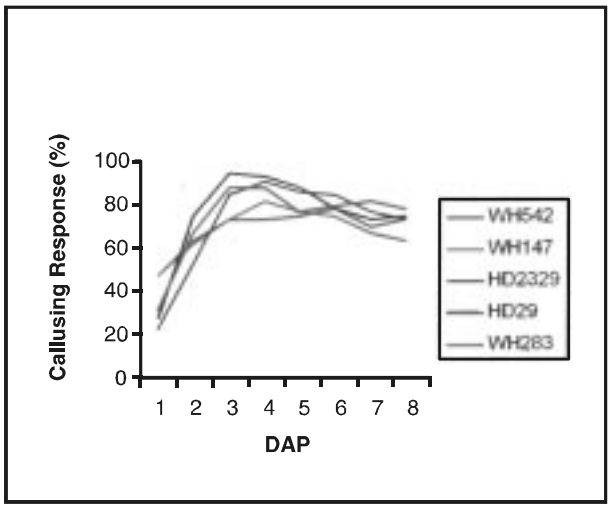

Fig. 1. Callusing response of wheat embryos at different days of pollination

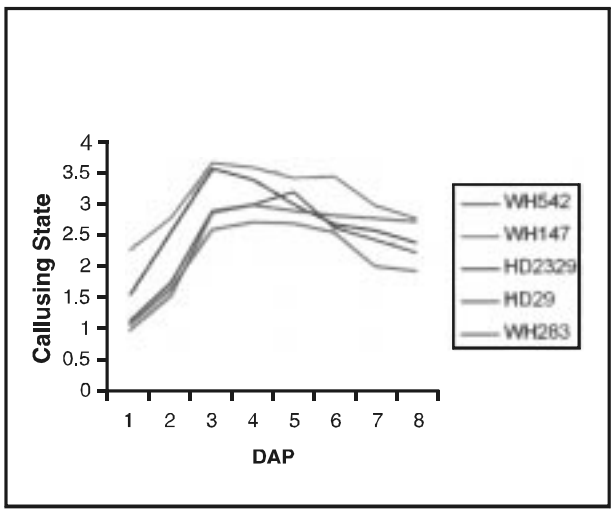

Fig. 2. Callusing state of wheat embryos at different days of pollination

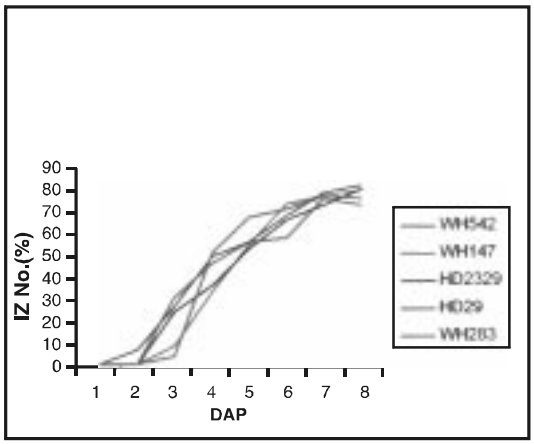

Fig. 3. Inhibition zone formation in bixenic culture of wheat embryos and *N. indica*

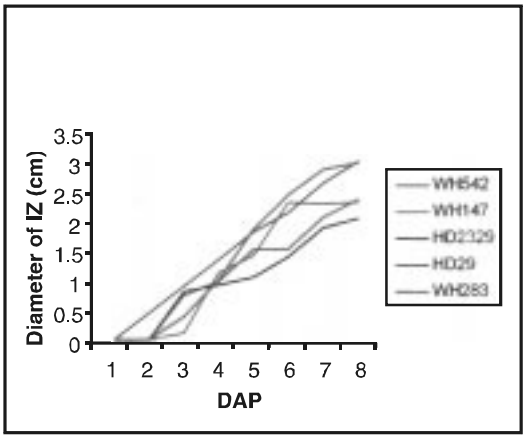

Fig. 4. Diameter of IZ in bixenic culture of wheat embryos and *N. indica*

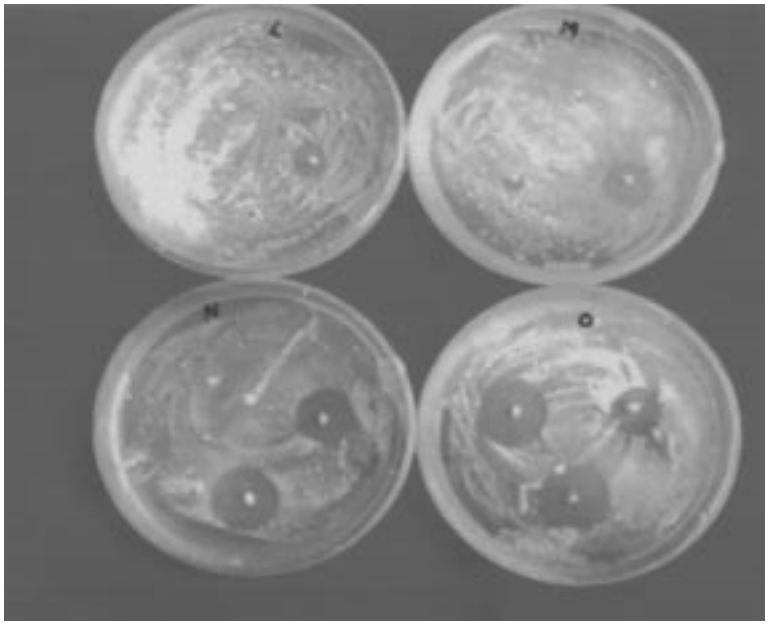

Fig. 5. Inhibition zone formation at 21(L), 24(M), 27(N) and 30(O) days of pollination

www.IndianJournals.com
Members Copy, Not for Commercial Sale
Downloaded From IP - 61.247.228.217 on dated 27-Jun-2017

from mature grains was reported by Bartok and Sagi [8] and Özgen *et al.* [9].

No inhibition zone was formed in bixenic culturing in any of the 5 genotypes at 15 DAP. At 18 DAP stage, inhibition zone was observed in 6.7 % embryos of only HD 29 genotype. At 21 DAP stage, IZ formation took place in all the 5 genotypes and per cent embryos forming IZ varied between 3.7% (WH 283) to 31.4% (WH 542) but the diameter of IZ was very small in all the genotypes (varied from 0.11 cm in WH 147 to 0.91 cm in HD 29). The per cent embryos forming IZ and also the diameter of IZ increased with the increasing age of the embryos in all the genotypes (Figs. 3 and 4). This showed that the genes responsible for secretion of a biochemical (which did not allow the pathogen growth and were responsible for IZ formation) were induced when the embryos were about 21 days old and their activity increased when the embryos proceeded towards maturity (Fig. 5). Maximum IZ was observed in resistant genotypes, HD 29 (3.07 cm) and WH 283 (3.03 cm) at 36 DAP stage. Tandon *et al.* [2] also reported bigger size of IZ at maturity in resistant genotypes than susceptibles. Extraction, identification and quantification of this biochemical may provide a solution for the control of KB pathogen.

Acknowledgement

Financial assistance through NATP (ICAR) project is greatfully acknowledged.

References

1. **Carris L. M., Castlebury L. A. and Goates B. J.** 2006. Nonsystemic bunt fungi—*Tilletia indica* and *T. horrida*: A Review of History, Systematics, and Biology. Ann. Rev. Phytopath., **44**: 113-133.
2. **Tandon J., Chawla V., Luthra O. P., Uppal S. and Beniwal M. S.** 2000. Expression of resistance to Karnal bunt (*Neovossia indica*) in embryo culture of wheat. Indian J. Genet., **60**: 281-285.
3. **Murashige T. and Skoog F.** 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant, **15**: 475-497.
4. **Karadimova M., Dorosiev L., Ganeva G. and Bochev B.** 1985. Regeneration of plants from callus tissue of *Triticum aestivum* L. Genetika i. Selektsiya, **18**: 157-162.
5. **Zhang L. J. and Scilleur P.** 1987. A simple and fast method to obtain high frequency of plant regeneration from mature and immature wheat embryos. Bull. Rech. Gembloux, **22**: 187-197.
6. **Özgen M., Turet M., Ozgen S. and Sancak C.** 1996. Callus induction and plant regeneration from immature and mature embryos of winter durum wheat genotypes. Plant Breeding, **115**: 455-458.
7. **Arya A., Yadav N. R., Yadav R. C. and Chawla V.** 2002. Comparison of immature and mature seeds for plant regeneration and *Agrobacterium* – mediated transformation in wheat (*Triticum aestivum* L.) In 2nd International Group Meeting on "Wheat Technologies for Warmer Areas" from 23-26 Sep. 2002 at Agharkar Research Institute, Pune (India).
8. **Bartok T. and Sagi F.** 1990. A new endosperm supported callus induction method of wheat (*Triticum aestivum* L.). Plant Cell Tissue Organ Cult., **22**: 37-41.
9. **Özgen M., Turet M., Altmok S. and Sancak C.** 1998. Efficient callus induction and plant regeneration from mature embryo culture of winter wheat (*Triticum aestivum* L.). Plant Cell Rep., **18**: 331-335.

www.IndianJournals.com
Members Copy, Not for Commercial Sale
Downloaded From IP - 61.247.228.217 on dated 27-Jun-2017