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BRIEF COMMUNICATION

Identification of RAPD markers linked to the karnal bunt resistance genes in wheat

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Abstract

A set of 104 wheat recombinant inbred lines (RILs) obtained from a cross between parents resistant (HD 29) and susceptible (WH 542) to karnal bunt (KB) (caused by *Neovossia indica*) were screened and used to identify random amplified polymorphic DNA (RAPD) markers linked with resistance to karnal bunt as these would allow indirect marker assisted selection of KB resistant genotypes. The two parents were analysed with 92 RAPD primers. A total of 65 primers proved functional by giving scorable polymerase chain reaction (PCR) products. Of these, 21 (32 %) primers detected polymorphism between the two parental genotypes. Using these primers, bulked segregant analysis was carried out on two bulk DNAs, one obtained by pooling DNA from 10 KB resistant RILs and the other similarly derived by pooling 10 KB susceptible RILs. One marker, OPM-20 showed apparent association with resistance to KB. This was confirmed following selective genotyping of individual RILs included in the bulks.

Additional key words: *Neovossia indica*, PCR, recombinant inbred lines, *Triticum aestivum*.

Wheat (*Triticum aestivum* L.) occupies a premier place among cereals in the world. However, the widespread occurrence of karnal bunt (KB) of wheat caused by *Neovossia indica*, affects the quality of wheat grain. KB was first detected in 1931 at Karnal (Haryana), hence the name Karnal bunt (Mitra 1931). The disease is distributed to all over north-west India in an endemic form and occurs in traces over a large part of south Asia (Warham 1986). Beside India and Pakistan, KB is reported from Syria (Williams 1983), Afghanistan, Mexico (Joshi *et al.* 1983), Nepal (Singh *et al.* 1989), USA (Ykema *et al.* 1996), Iran (Torabi *et al.* 1996) and South Africa (Crous *et al*. 2001).

Conventional approaches for controlling KB include cultural practices such as crop rotation, sowing of disease free seeds, *etc*. Chemical control of disease is very difficult and is not cost-effective. The most effective and economical method of disease management is through host plant resistance. The wheat cultivars with sufficient resistance to KB have been observed (Fuentes-Davila and Rajaram 1994). Developing KB resistant wheat cultivars with conventional methods is time-consuming and labour-intensive. An alternative to direct selection for KB resistance is the indirect selection for DNA markers linked to genes conferring KB resistance. The application of random amplified polymorphic DNA (RAPD) analysis has proven useful in plant genotype fingerprinting (Williams *et al.* 1990, Yang and Quiros 1993), pedigree analysis and detection of DNA polymorphism levels appropriate for the development of DNA markers in a number of self pollinating crops. The purpose of this study is to take advantage of RAPD to identify DNA markers closely linked to KB resistance in wheat.

Two wheat (*Triticum aestivum* L.) genotypes differing in KB resistance, HD 29 (resistant) and WH 542 (susceptible), and a set of 104 recombinant inbred lines (RILs) (F_8) derived from these parents were used in the presented study. The RIL population was developed following single-seed descent (SSD) method. DNA was isolated from leaf tissues using modified CTAB

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Abbreviations: CI - coefficient of infection; CTAB - cetyltrimethylammonium bromide; KB - karnal bunt; PCR - polymerase chain reaction; RAPD - random amplified polymorphic DNA; RIL - recombinant inbred line.
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procedure of Saghai-Maroof *et al.* (1984).

The screening of both the parent genotypes and their RILs against *N. indica* under artificial epiphytotic conditions in greenhouse was carried out. Five plants of both the genotypes and their RILs were cultivated in earthen pots in three replications. These were inoculated with the sporidial suspension of concentration 10 000 sporidia per cm^3 (Aujla *et al.* 1983). After maturity, the inoculated ear heads were harvested. Grains were removed carefully by hand and were separated into different grades. The percentage of infected grains and a coefficient of infection (CI) was calculated as described by Aujla *et al.* (1989).

The 10-base oligonucleotide random primers for the PCR were obtained from *Operon Technologies*, Alameda, USA; 92 random primers from different kits were used in this study. PCRs were carried out in 0.05 cm^3 reaction volumes each containing 50 ng of genomic template DNA, 0.2 μM of the particular primer, 100 μM of each dNTP, 0.002 cm^3 of Taq DNA polymerase 10X buffer, 1 unit Taq polymerase (*Perkin Elmer*, CA, USA) and 2.5 mM $MgCl_2$. PCR amplification was performed on a *PTC 100 Thermal Cycler* (*MJ Research Inc.*, Watertown, MA, USA) under the following conditions: Initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturing at 95 °C for 1 min, annealing at 35.1 °C for 1 min, extension at 72 °C for 2 min and final extension at 72 °C for 10 min. The amplification products were resolved on 1.5 % agarose gel and visualized under UV light following staining with ethidium bromide.

The frequency of RAPD polymorphism was calculated based on presence (taken as 1) or absence (taken as 0) of common bands (Ghosh *et al.* 1997). The binary data were used to compute Pair-wise similarity coefficient (Jaccard 1908) on NTSYS-PC.

The two parents differed sharply for mean incidence of the disease. The range of disease on the RILs was 0 - 81.25 % (Table 1). Analysis of variance for CI and percentage of infected grains showed significant variations among all RILs (Table 2). The distribution of KB of the RILs was resembled that of the resistant parent type. The distribution of KB disease severity on the RILs was skewed towards that of the resistant parent, suggesting the segregation of multiple genes with dominant or complementary gene action in wheat line HD 29. The inheritance of KB resistance has also been reported to be controlled by two recessive genes (Singh *et al.* 1993, Fuentes-Davila *et al.* 1995). Identification of RILs with lower and higher disease incidence than HD 29 and WH 542, respectively, suggests that WH 542 probably has minor genes for KB resistance. Singh *et al.* (1996) reported that resistance was controlled by a major gene along with some minor genes. Significant variations among all the recombinant inbred lines were observed for CI and percentage of infected grains (Table 2).

A total of 92 RAPD primers were used on two parental genotypes, HD 29 (resistant to KB) and WH 542 (susceptible to KB), to detect polymorphism. Of the

Table 1. Coefficient of infection (CI) and percentage of infected grains (Inf.) from different RILs derived from cross HD29 × WH 542. CD = 1.09 - 1.42.

RIL No.	CI [%]	Inf. [%]	RIL No.	CI [%]	Inf. [%]	RIL No.	CI [%]	Inf. [%]
1	33.30	75.0	36	00.00	00.0	71	00.00	00.0
2	00.00	00.0	37	00.00	00.0	72	00.00	00.0
3	00.00	00.0	38	00.00	00.0	73	12.50	29.0
4	00.00	00.0	39	00.00	00.0	74	00.00	00.0
5	20.00	45.6	40	00.00	00.0	75	25.00	46.0
6	00.00	00.0	41	00.00	00.0	76	00.00	00.0
7	00.00	00.0	42	00.00	00.0	77	00.00	00.0
8	00.00	00.0	43	00.00	00.0	78	00.00	00.0
9	00.00	00.0	44	00.00	00.0	79	10.52	23.6
10	06.25	25.6	45	60.00	72.3	80	00.00	00.0
11	11.11	29.9	46	00.00	00.0	81	03.22	06.9
12	20.00	35.6	47	00.00	00.0	82	00.00	00.0
13	00.00	00.0	48	00.00	00.0	83	00.00	00.0
14	00.00	00.0	49	00.00	00.0	84	00.00	00.0
15	00.00	00.0	50	10.53	25.6	85	00.00	00.0
16	00.00	00.0	51	00.00	00.0	86	00.00	00.0
17	00.00	00.0	52	04.54	13.5	87	00.00	00.0
18	00.00	00.0	53	00.00	00.0	88	18.75	33.0
19	10.52	25.6	54	00.00	00.0	89	00.00	00.0
20	00.00	00.0	55	00.00	00.0	90	07.14	15.9
21	81.25	98.8	56	15.00	25.0	91	00.00	00.0
22	00.00	00.0	57	00.00	00.0	92	00.00	00.0
23	00.00	00.0	58	00.00	00.0	93	00.00	00.0
24	25.00	46.6	59	00.00	00.0	94	00.00	00.0
25	00.00	00.0	60	00.00	00.0	95	47.05	78.9
26	00.00	00.0	61	00.00	00.0	96	08.00	12.5
27	00.00	00.0	62	00.00	00.0	97	00.00	00.0
28	00.00	00.0	63	00.00	00.0	98	00.00	00.0
29	18.75	39.5	64	00.00	00.0	99	00.00	00.0
30	30.76	48.5	65	00.00	00.0	100	05.25	12.5
31	00.00	00.0	66	00.00	00.0	101	00.00	00.0
32	00.00	00.0	67	00.00	00.0	102	00.00	00.0
33	09.52	19.8	68	00.00	00.0	103	00.00	00.0
34	00.00	00.0	69	00.00	00.0	104	00.00	00.0
35	09.52	15.5	70	00.00	00.0	HD29	00.00	00.0
						WH542	56.78	70.3

Table 2. Analysis of variance for coefficient of infection (CI) and percentage of wheat grains infected with *N. indica* (Inf.).

Source of variation	Degree of freedom	Mean sum of squares CI	Inf.
Genotypes	105	536.77	653.56
Error	212	15.86	0.51

Table 3. Similarity matrix among parents as obtained using the allelic diversity at 65 RAPD loci.

	HD 29	WH 542
HD 29	1.000	
WH 542	0.836	1.000

above primers, 65 primers proved to be functional primers giving scorable amplification products. Of these 65 functional primers, 21 (32 %) primers detected polymorphism between the two parental genotypes. The two parents had a similarity coefficient of 0.83 (Table 3.). The low genetic diversity in our study is due to the fact that only the genome regions conferring karnal bunt resistance were tagged, since the primers generate polymorphism between resistant and susceptible parents. The level of RAPD polymorphism within wheat itself has been reported to be low (Devos and Gale 1992). Joshi and Nguyen (1993) in a study of common bread wheat for 109 RAPD markers found that similarity among cultivars ranges from 0.60 to 0.90. Sun *et al.* (2003) also reported genetic similarity ranging from 0.64 to 0.98, while observing different levels of *Fusarium* resistance in wheat using RAPD.

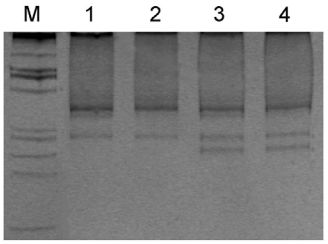

Fig. 1. Bulked segregant analysis of RILs (representing extreme groups) with OPM-20 primer. *Lane M*: 100 bp ladder marker; *2,4*: parents WH 542 and HD 29; *1,3*: bulk segregants for susceptible and resistant to karnal bunt.

Twenty-one RAPD primers that have detected polymorphism between the parental genotypes were used for conducting bulked segregant analysis (Michelmore *et al*. 1991) using two bulked DNAs, one obtained by pooling DNA from 10 KB resistant RILs and the other similarly obtained from 10 KB susceptible RILs. With 20 of the 21 primers used, no apparent association between the markers and KB resistance was observed. The solitary remaining OPM-20 primer exhibited apparent association with resistance to KB (Fig. 1). To further confirm this association, we carried out selective genotyping (Lander and Botstein 1989) of individual RILs belonging to the two bulks (Fig. 2). The results revealed that 4 out of 5 RILs from each of the two bulks showed amplification profiles characteristic of the corresponding parents. This suggested that OPM-20 might be associated with karnal bunt resistance.

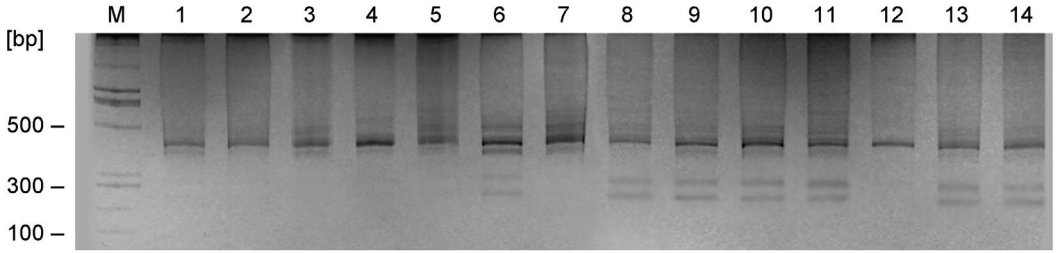

Fig. 2. Selective genotyping of RILs (representing extreme groups) with OPM-20 primer. *Lane M*: 100 bp ladder marker, *1,8*: parents WH 542 and HD 29; *2,9*: susceptible and resistant bulks; *3-7*: RILs susceptible to karnal bunt; *10-14*: RILs resistant to karnal bunt.

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