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Full Length Research Paper

Identification of micro satellite markers on chromosomes of bread wheat showing an association with karnal bunt resistance

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A set of 104 wheat recombinant inbred lines developed from a cross between parents resistant (HD 29) and susceptible (WH 542) to karnal bunt (caused by *Neovossia indica*) were screened and used to identify SSR markers linked with resistance to karnal bunt as these would allow indirect marker assisted selection of karnal bunt resistant genotypes. The two parents were analysed with 46 SSR primer pairs. Of these, 15 (32%) were found polymorphic between the two parental genotypes. Using these primer pairs, we carried out bulked segregate analysis on two bulked DNAs, one obtained by pooling DNA from 10 karnal bunt resistant recombinant inbred lines and the other similarly derived by pooling DNA from 10 karnal bunt susceptible recombinant inbred lines. Two molecular markers, Xgwm 337-1D and Xgwm 637-4A showed apparent linkage with resistance to karnal bunt. This was confirmed following selective genotyping of individual recombinant inbred lines included in the bulks. These markers may be useful in marker assisted selection for karnal bunt resistance in wheat.

Key words: Karnal bunt, *Neovossia indica*, Resistance, SSR and Wheat.

INTRODUCTION

Wheat (*Triticum aestivum* L. em. thell) belongs to the sub-tribe Triticinae and the tribe triticeae (= Hordeae) of the family Poaceae. India is the second largest wheat growing country of the world (DWR, 1996). More than half of its population depends on wheat, as it provides a major source of energy, protein and dietary fibre in human nutrition. Some diseases of wheat which were considered to be of minor importance have assumed serious proportions now. One such disease is karnal bunt caused by the fungus *Neovossia indica* (Mitra) Mundkur, which was first recorded in India in the 1930's (Mitra, 1931). It infects the plant at boot leaf stage and then pathogen penetrates individual florets, entering the embryo end of the kernel and proceeds along the ventral crease. The Infected parts of kernels are replaced by masses of dark, powdery, fishy smelling fungal teliospores (Joshi et al., 1980). In India, it has been reported that the disease is prevalent to varying extents in the northern states such as Punjab, Haryana, Delhi, Utter Pradesh, Bihar, Jammu and Kashmir, lower Himachal Pradesh, Madhya Pradesh and Rajasthan. It has also been reported from southern Nepal, Pakistan, Lebanon, Sweden, Syria, Turkey, Afghanistan, Iran, Mexico and USA (Ykema et al., 1996; Torabi et al., 1996; Crous et al., 2001; Haq et al., 2002).

Breeding for karnal bunt resistant cultivars requires a reliable method of selecting plants containing a resistance

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gene. Greenhouse screening is carried out by creating artificial epiphytotic conditions at the boot leaf stage, but this is both time consuming and labour intensive. An average 10% error rate is typical for the greenhouse screening method. So it is highly desirable to employ a screening technique that is based on molecular markers linked to the resistance genes (Ma et al., 1996). Both RFLP markers (Singh et al., 1994; Nelson et al., 1998) and PCR based markers, RAPDs (Dhaliwal and Haarjit Singh, 1997) and AFLPs (Singh et al., 1999) have been used for mapping karnal bunt resistance genes. Microsatellite or simple sequence repeats (SSR) markers have been successfully used to generate genome specific markers for mapping studies in hexaploid wheat (Roder et al., 1995) and to produce a micro satellite map of wheat (Roder et al., 1998). Identification of PCR-based markers linked to karnal bunt resistance offers the prospect of using marker-assisted selection schemes in developing resistant wheat cultivars. The purpose of present study is to take advantage of SSR to identify DNA markers closely linked to karnal bunt resistance in wheat.

MATERIALS AND METHODS

Plant material

Two bread wheat genotypes differing for karnal bunt resistance, namely HD 29 (resistant) and WH 542 (susceptible), and a set of 104 recombinant inbred lines (F_8) derived from these parents were used in the present study. The recombinant inbred line population was developed using singleseed descent (SSD). DNA was isolated from leaf tissues using modified CTAB procedure (Saghai-Maroof et al., 1984).

Evaluation of karnal bunt resistance

The screening of both the genotypes and their recombinant inbred lines against *N. indica* under artificial epiphytotic conditions was carried out in the green-house in 2001 and 2002. Five plants of both the genotypes and their recombinant inbred lines were grown in pots in three replications. These were inoculated with the sporidial suspension of concentration 10,000 sporidia per ml (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 the coefficient of infection were worked out as described by Aujla et al. (1989).

Polymerase chain reaction (PCR) amplification

DNA amplification were carried out in 25 µl reaction mixture, each containing 50 ng of template DNA, 2 µM micro satellite primers, 100 µM each of dNTPs, 2 µl of 10X buffer, 1 unit Taq polymerase (Perkin Elmer) and 2.5 mM $MgCl_2$. PCR amplification were performed on a PTC-100 Thermal Cycler (MJ Research, Inc. Watertown, MA, USA) under the following conditions: Initial denature-tion at 95°C for 5 min, followed by 40 cycles of denaturing at 95°C for 1 min, annealing at 50°C / 55°C / 60°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 6% polyacrylamide denaturing gels (PAGE) followed by silver staining (Tegelstrom, 1992).

Similarity coefficient

The frequency of micro satellite 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) and the similarity matrix thus obtained was subjected to cluster analysis using the UPGMA (Unweighted Pair-group method with Arithmetic average) algorithm on NTSYS-PC.

RESULTS

Disease reaction of the recombinant inbred lines

Two parent *viz*. HD29 and WH542 and their 104 recombinant inbred lines were screened against *N. ndica* in green-house. Ears at boot leaf stage were inoculated with suspension of secondary sporidia (10,000 sporidia/ml) of *N. indica* and reactions in terms of percentage of infected grains and coefficient of infection were worked out. The range of coefficient of infection (CI) on the recombinant inbred lines was 0 – 81.25 and 0 – 80.80 in years 2001 and 2002, respectively. Among the 104 recombinant inbred lines, there was a wide variation in the response to karnal bunt resistance, as shown in Figure 1. The distribution of karnal bunt disease on the recombinant inbred lines was towards the resistant parent type in both the years, thereby suggesting the segregation of some major additive effects from the parental line HD 29. Identification of recombinant inbred lines with lower and higher disease incidence than HD 29 and WH 542 respectively, suggested that WH 542 probably has minor genes for karnal bunt resistance. Significant variations among all the recombinant inbred lines were observed for coefficient of infection and per cent infected grains (Table 1.).

SSR markers based polymorphism among the parents

A micro satellite fingerprinting database was generated using 46 SSR primer pairs for both the parents differing in karnal bunt resistance. A total of 179 alleles were detected at 46 SSR loci. The number of alleles per locus ranged from 1 to 8 with an average of 3.9 alleles per locus. Since micro satellite primers are locus specific, only one loci should be amplified by each primer pair and so it was unexpected to find primers which amplified more than one locus. The detection of multiple alleles per locus is probably due to heterogeneity of the accessions rather than genetic heterozygosity. Of these 46, 15 (32 per cent) detected reproducible polymorphism between the two parental genotypes (Figure 2). The two parents had a similarity coefficient of 0.86 (Table 2). The low genetic diversity in our study might be due to the reason that only the genome regions conferring karnal bunt resistance were tagged, since the primers generate polymorphism between resistant and susceptible parents.

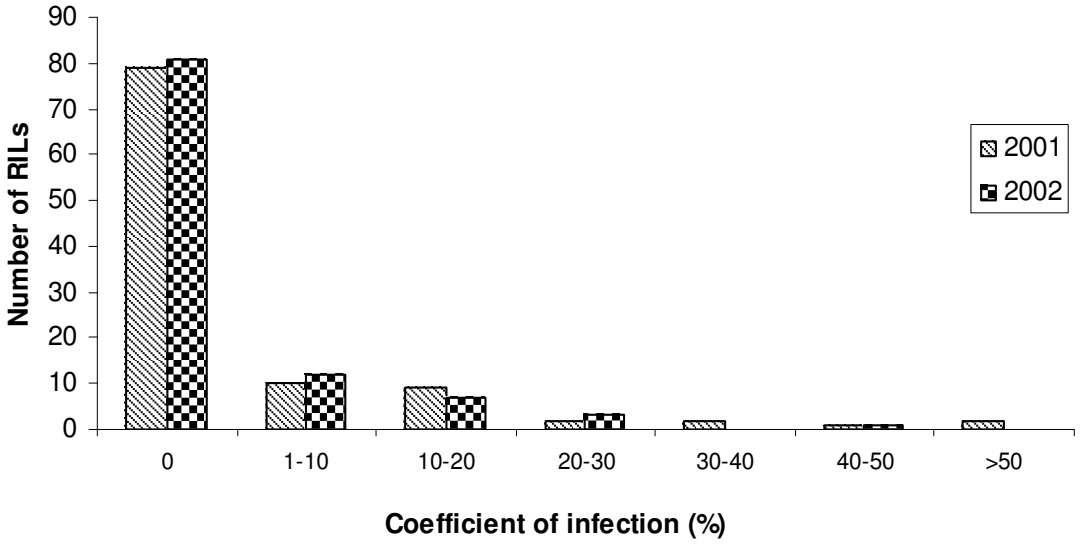

Figure 1. Histogram showing coefficient of infection on recombinant inbred lines during the year 2001 and 2002.

Table 1. Analysis of variance for coefficient of infection and percentage of wheat grains infected with *N. indica*.

Source of variation	Degree of freedom	Mean sum of squares	
		Coefficient of infection	Percentage of infection
Genotypes (2001)	105	536.77 **	653.56 **
Error	212	15.86	0.51
Genotypes (2002)	105	573.27 **	539.64 **
Error	212	0.55	0.86

** Significant at 1 per cent.

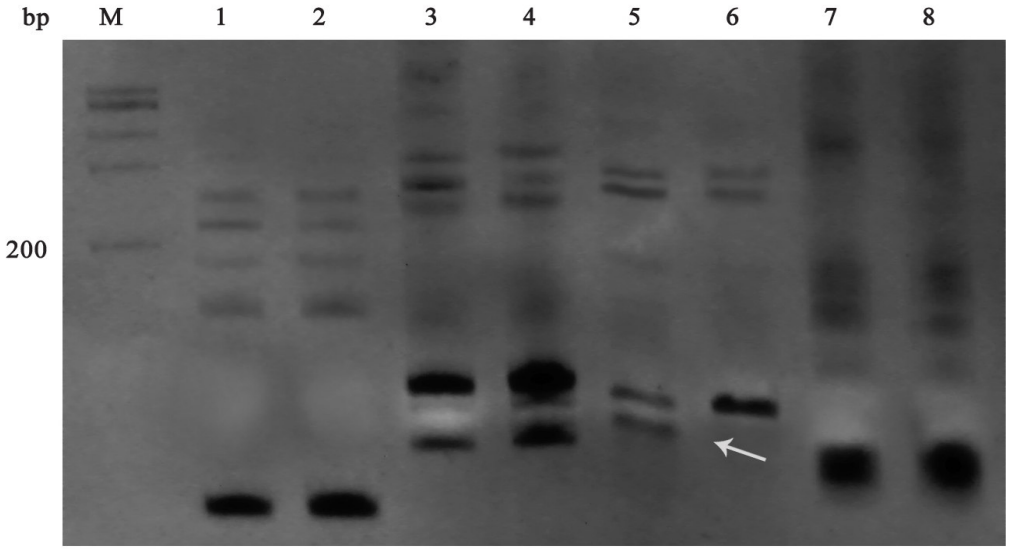

Figure 2. A representative sample of SSR polymorphism between parents HD 29 (Lanes 1, 3, 5, 7) and WH542 (Lanes 2, 4, 6, 8) using four primers: gwm 425-2A (Lanes 1, 2), gwm 469-6D (Lanes 3, 4), gwm 637-4A (Lanes 5, 6) and gwm 666-1A (Lanes 7, 8).

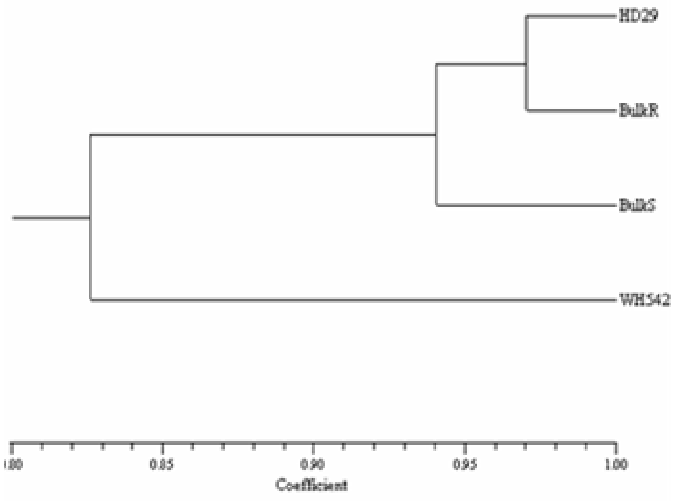

Figure 3. Dendrogram of parents and both the bulks based on SSR diversity data at 15 loci.

Table 2. Similarity matrix among parents as obtained using the allelic diversity at 46 SSR loci.

	HD 29	WH 542
HD 29	1.000	
WH 542	0.860	1.000

Table 3. Similarity matrix among parents, resistant and susceptible bulks obtained using the allelic diversity at 15 SSR loci.

	HD 29	Bulk R	Bulk S	WH 542
HD 29	1.0000			
Bulk R	0.9701	1.0000		
Bulk S	0.9253	0.9552	1.0000	
WH 542	0.7910	0.8208	0.8656	1.0000

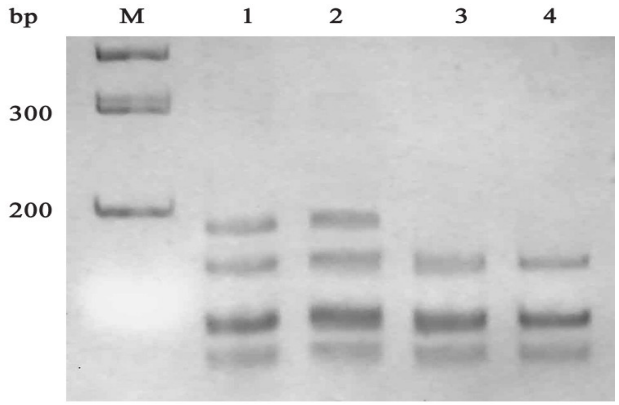

Figure 4. Bulked segregant analysis of recombinant inbred lines (representing extreme groups) with gwm 337-1D. Lane M: 100 bp ladder marker, 2, 3: parents HD 29 and WH 542, 1, 4: bulk segregates for resistant and susceptible to karnal bunt.

Specific SSR markers probably linked to karnal bunt resistance

Fifteen SSR primers, which detected polymorphism between the parent genotypes, were used for conducting bulked segregate analysis (BSA; Michelmore et al., 1991). A total of 10 resistant and 10 susceptible recombinant inbred lines were selected from HD29 X WH542 (F_8) segregating population based on their mean coefficient of infection. Extreme recombinant inbred lines with a CI of-0 (resistant) and a CI of- >12.0 (susceptible) were selected for preparing the bulks for SSR marker analysis. The similarity coefficient between the two parental genotypes was 0.791 using only the polymorphic markers (Table 3). The resistant bulk showed similarity of 0.970 with HD 29 which was higher than the similarity (0.820) with WH 542. However, the susceptible bulk also showed more or less equal similarity with HD 29 (0.925) and WH 542 (0.865). The cluster analysis split the bulks and parental genotypes into two groups. Group 1 was further divided into two subgroups. Subgroup-I contained HD 29 and the resistant bulk. Subgroup-II contained the susceptible bulk and the two subgroups merged at similarity coefficient of 0.94 (Figure 3). Group 2 comprised only WH 542, which merged with group 1 at similarity coefficient of 0.82. Two SSRs, Xgwm 337-1D and Xgwm 637-4A, exhibited amplification profiles that were characteristic of resistant and susceptible parents in the corresponding bulks following BSA. This indicated an association of these markers with karnal bunt resistance (Figure 4). To further confirm this association, selective genotyp-

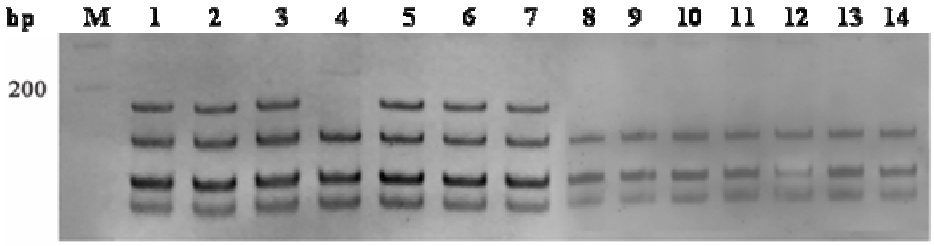

Figure 5. Selective genotyping of recombinant inbred lines (representing extreme groups) with gwm 337-ID primer. Lane M: 100-bp ladder marker; 1, 8: parents HD29 and WH542; 2, 9: resistant and susceptible bulks; 3 – 7: recombinant inbred lines resistant to karnal bunt; 10 – 14: recombinant inbred lines susceptible to karnal bunt.

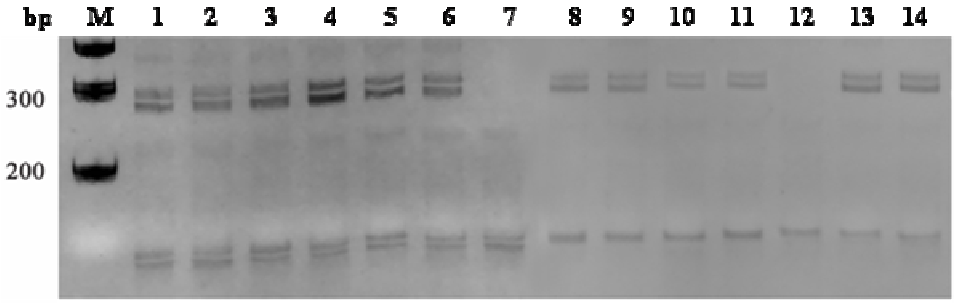

Figure 6. Selective genotyping of recombinant inbred lines (representing extreme groups) with gwm 637-4A primer. Lane M: 100-bp ladder marker; 1, 8: parents HD29 and WH542; 2, 9: resistant and susceptible bulks; 3 – 7: recombinant inbred lines resistant to karnal bunt; 10 – 14: recombinant inbred lines susceptible to karnal bunt.

ing of 5 individual recombinant inbred lines belonging to extreme resistant and 5 individual recombinant inbred lines belonging to extreme susceptible bulk was carried out with these two primers (Lander and Botstein., 1989). Using Xgwm 337-1D primer pair, the results revealed that four out of five recombinant inbred lines from resistant and all five recombinant inbred lines from susceptible bulk showed amplification profiles characteristic of the resistant and susceptible parents, respectively (Figure 5). Similarly, in case of Xgwm 637-4A, four out of five recombinant inbred lines belonging to each of the two bulks showed amplification profiles characteristic of the corresponding parents (Figure 6). This suggested an association of these two markers Xgwm 337-1D and Xgwm 637-4A, with karnal bunt resistance.

DISCUSSION

Green-house studies were conducted on the parents HD 29 and WH 542 and their recombinant inbred lines progenies generated via SSD. The percentage of seeds infected with karnal bunt was calculated and analysis of variance indicated that there were significant differences between the recombinant inbred lines. Singh et al. (1996) reported that karnal bunt resistance in wheat was controlled by both major and minor genes. The DNA polymorphisms between both the parental genotypes obtained by micro satellite marker amplification, is a useful tool for the assessment of genetic diversity and polymorphism (Lang et al., 2001). In our study multiple alleles were amplified at each locus. In two earlier studies, more than one locus per micro satellite primer pair was detected and mapped in bread wheat (Roder et al., 1998; Stephenson et al., 1998; Vasu et al., 2000). Prasad et al. (2000) also reported relatively higher number of alleles per locus. Struss and Plieske (1998) reported 8.6 alleles per locus in barley. Ahmed (2002) detected a total of 156 allelic variants at 43 SSR loci, ranging from 2 to 8 alleles per locus with an average of 3.6 and genetic similarities ranged from 30.1 ('Era' and 'Klasic') to 90.1 ('Neepawa and 'Thatcher') between genotypes.

Our study found that two markers Xgwm 337-1D and Xgwm 637-4A were associated with karnal bunt resistance in wheat. Vasu et al. (2000) found the following micro satellite loci that were associated with karnal bunt resistance gene(s), Xgwm382, Xgwm369, Xgwm637, Xgwm156 and Xgwm617 which mapped to 2AS, 3AS, 4AL, 5AL, and 6AL. Singh et al. (2003) also reported that

the SSR locus Xgwm538 could be used, marker-assisted selection for karnal bunt resistance in wheat. Our results, together with the above reports, suggest that several genes-QTL for karnal bunt resistance are present in different genetic backgrounds.

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