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Development and molecular marker analysis of Karnal bunt resistant near isogenic lines in bread wheat variety PBW 343

Sunish K. Sehgal[1], Gurvinder Kaur, Indu Sharma and Navtej S. Bains[2]

Department of Plant Breeding, Genetics and Biotechnology, Punjab Agricultural University, Ludhiana 141 001

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Abstract

Karnal bunt (KB) resistant near isogenic lines (NILs) were developed using the stock (KBRL 22) with high KB resistance and the widely grown wheat cultivar (PBW 343) as recipient. Genetic analysis in BC_5F_2 and BC_5F_3 populations revealed involvement of up to three additive genes for KB resistance. Single plants from lines which were uniform for resistance and susceptibility in BC_5F_3, were advanced to BC_5F_4 and screened further for establishment of extreme NILs. The KB resistant NILs were screened for presence of donor alleles at 93 polymorphic SSR loci using the recipient parent (PBW 343) as control. Donor alleles of four markers; *Xgwm99* (1AL), *Xgwm149* (4BL), *Xgwm174* (5DL) and *Xgwm340* (3BL) showed their presence in the resistant pool. These four markers were run on the 11 resistant and 10 susceptible BC_5F_4 progenies. Resistant and susceptible phenotypes showed association with three of the four markers viz., *Xgwm 99*, *Xgwm 174* and *Xgwm 340* as indicated by the Chi square contingency test.

Key words: Karnal bunt resistance, near isogenic lines, molecular markers, *Triticum aestivum*

Introduction

Karnal bunt (KB) caused by *Neovossia indica* (Mitra) Mundkur (syn. *Tilletia indica*) is an important wheat disease with implications for wheat quality and trade. Karnal bunt was first noticed in the samples collected from Karnal (Haryana, India) by Mitra in 1931. The disease, since then, has been of frequent occurrence in North Western India. Karnal bunt was detected in Mexico in 1972 [1] and in 1983, the United States and about 70 other countries placed quarantines on import of wheat from regions where Karnal bunt was known to occur. Thus losses on account of Karnal bunt prevalence are mostly due to trade restrictions. Detection of the disease in Mexico caused an alarm as the germplasm exchange programme of the International Centre for Maize and Wheat Improvement located there could be jeopardized. Brennan *et al.* [2] has evaluated the indirect economic losses incurred on account of this disease in Mexico. The acute perception of Karnal bunt threat was also evident when US Secretary of Agriculture declared a state of emergency in response to the first detection of KB in USA (in Arizona State) in 1995. A wide range of measures were initiated to eradicate the outbreak. The establishment of KB in US would have resulted in a trade embargo of US $ 4.9 billion worth of grains from 22 of the US trading partners [3]. Direct losses from KB become evident beyond about 3% level of grain infection due to changes in colour and palatability of wheat products, rendering them unacceptable for human consumption [4]. The KB infected grains emit a fishy odour due to the presence of trimethyl amine. The global footprint of KB covers parts of several countries including India, Pakistan, Afghanistan, Nepal, Iran, Iraq, Mexico, USA and South Africa [3, 5, 6-9].

Over the last two decades, resistance breeding has emerged as the main strategy for combating KB. However, high degree of resistance was identified in only a few stocks. The genetic analysis of these lines indicated two to three additive genes governing resistance in each of the stocks. Surprisingly, allelic tests revealed a great diversity of loci to be involved in KB resistance [10]. Molecular marker analysis has detected QTL of relatively small effect (generally below 20%) and mostly with significant QTL-Environment interactions [11,12, 13]. NIL based resistance gene mapping followed by use of NIL derived micro RILs for fine mapping can minimize background noise and improve QTL identification. It may as well pave the way for fine mapping and subsequent gene cloning which

[1]Present address: Department of Plant Pathology, Kansas State University, Manhattan, KS 66506, USA.
[2]Corresponding author's e-mail: nsbains@rediffmail.com

would be helpful in understanding the mechanism of resistance. Here we present the results of NIL development using the stock with highest known levels of resistance (KBRL 22) as donor and the most widely grown wheat cultivar in India (PBW 343) as recipient. NILs were subjected to genetic analysis and preliminary marker analysis is also presented.

Material and methods

NIL development and genetic analysis

KBRL 22 was used as the resistance donor for NIL development. This donor was previously derived from a cross of two established KB resistant stocks: HD29/ W485 [14]. KBRL 22 probably combines diverse resistance genes from the two parents as evident from its complete resistance (0% KB) compared to infection levels of up to 5% in the parental stocks [15]. Crosses were made to transfer this high level of KB resistance to the most widely grown wheat cultivar in India, 'PBW343'. PBW343 (an 'Attila' sib = ND / VG1944 // KAL/BB /3/ YACO'S' /4/ VEE#5'S') is moderately susceptible (15 to 20 % infected grains under artificial inoculation). In each backcross generation 70 to 100 plants were simultaneously screened against KB and involved in backcrossing. Backcross seed, which was set on KB-free plants, was retained for growing in the next season. Finally, 80 BC_5F_1 plants derived from backcross of resistant BC_4F_1 individuals were screened for KB resistance (2002-03) and out of 21 resistant plants obtained; only two plants were used to develop two BC_5F_2 populations. The two backcross populations (designated as BC_5F_2-P1 and BC_5F_2-P2) were screened for KB reaction using 3-5 ears per plant (2003-04). Each BC_5F_2 plant was used to derive a BC_5F_3 line. KB screening of 10-12 plants (2-3 ears/ plant) from each BC_5F_3 line in both populations (BC_5F_3-P1 and BC_5F_1-P2**)** provided confirmatory phenotyping (2004-05). Single plants from lines which were uniform for resistance and susceptibility in BC_5F_3 were advanced to BC_5F_4 and screened further for establishment of NILs (2005-06). Genetic analysis of resistance in both the populations was performed in BC_5F_2 and BC_5F_3 generations.

KB screening

The inoculum for screening was based on sixteen *Tilletia indica* isolates representing pathogen variability from KB prone areas of North Western Plains of India [16]. The isolates were maintained from year to year on the susceptible wheat cultivar, WH542 (>40% average KB infection). The cultures of different isolates were multiplied and maintained by frequent subculturing on potato-dextrose-agar medium and were mixed in equal proportions before use. The KB inoculations were performed in the field using the widely followed syringe method [17]. The sowing of the plant material was adjusted to obtain flowering in February which is most conducive period for disease development. Appropriate humidity was created in the field with the use of perfo-sprayer and frequent irrigations during the inoculation period. The inoculated ears were manually threshed at maturity and percent KB infection was calculated on per plant basis.

Marker analysis

Leaf tissue (30-45 DAS) was ground in liquid nitrogen and genomic DNA was extracted using the CTAB-DNA extraction buffer [1.4 M NaCl, 100 mM Tris pH = 8.0, 2 % CTAB (Hexadecyl trimethylammonium bromide), 20 mM EDTA, 0.5 % Na bisulfite, and 1 % 2-mercaptoethanol [18]. Parental survey with 400 SSR markers belonging to *Xgwm, Xwmc* and *Xbarc* series led to identification of 93 polymorphic markers (about 23.25% of the markers surveyed) on 2.3% GenePure HiRes Agarose (ISC Bioexpress, Kaysville, UT, USA) gels. The polymorphic markers were run first on KB resistant pool and subsequently on 11 resistant and 10 susceptible lines from BC_5F_4 generation along with the parents to identify donor chromosome segments inherited by the resistant progenies.

Results and discussion

The resistance in the cross PBW 343/KBRL 22, was known to show almost complete dominance from a previous study and two genes acting in an additive manner were known to confer resistance in F_2 and early backcross generations [15]. The genetic analysis of two BC_5F_2 populations derived from this cross was primarily intended to confirm that the full complement of genes for resistance were passed on during the backcrossing to the two resistant BC_5F_1 plants from which these populations were derived. This would ensure that the NILs developed subsequently carry the genes of interest. For genetic analysis, the BC_5F_2 plants were assigned to parental categories (resistant and susceptible) on the basis of distribution of parental scores and the plants showing intermediate level of resistance falling between the two parental extremes were categorized as moderately resistant in both the populations. The first population (BC_5F_2-P1) consisting of 190 plants was categorized into three classes on the basis of KB scores (Table 1). Seventy-one plants were resistant (0-5% KB

infection), 116 plants were moderately resistant (>5-30% KB infection), and 3 plants were susceptible (>30-70% KB infection). The c^2 value calculated for three additive genes for resistance was clearly acceptable (c^2 = 1.82, p value = 0.4025). An additional gene for resistance was thus being indicated compared to the earlier study [15] with F_2 and early backcross generations. The second population (BC_5F_2-P2) consisting of 200 plants showed a great degree of variability for growth habit and 75 plants were too late to be inoculated. Thus, only 125 plants could be categorized into the three categories, with 43 plants as resistant, 71 as moderate and 11 as susceptible. These proportions do not comply with any simple genetic hypothesis (Table 1). As a large number of plants (75) could not be categorized, the distribution may have got skewed, particularly if one or more resistance loci are in vicinity of chromosomal regions associated with genes controlling flowering time. The presence of extremely late flowering plants was unexpected as both parents have a normal spring habit. Moreover, the late flowering trait did not express in the BC_1F_1 to BC_5F_1 but surfaced in BC_5F_2 only.

In next season (2004-05) BC_5F_3 families were raised in plant to row fashion from both BC_5F_2 populations and designated as BC_5F_3-P1 and BC_5F_3-P2. At least 10 plants were screened for KB resistance in each BC_5F_3 family. On the basis of KB score, BC_5F_3 families were categorized into uniform resistant, intermediate/segregating and uniform susceptible. With 10-12 plants screened per BC_5F_3 line it was also not possible to resolve the one gene segregation for resistance vs. two or three gene segregation. So all heterozygous lines along with intermediate resistance lines were pooled in one category. Thus only the uniformly highly resistant or uniformly highly susceptible lines representing the homozygous extremes served the purpose of genetic analysis. In BC_5F_3-P1, 3 progenies were homozygous resistant, 184 progenies were intermediate and 3 progenies were homozygous susceptible. A non-significant c^2 value indicated the hypothesis of three resistance genes to be valid. Further, this inference was in consonance with inheritance pattern observed in the previous generation.

Table 1. Genetic analysis of KB resistance in BC_5F_2 and BC_5F_3 populations of PBW 343 x KBRL 22

Population	KB reaction categories	No. of BC_5F_2 plants/ BC_5F_3 lines	
		Popu-lation-1	Popu-lation-2
BC_5F_2 plants	Resistant	71	43
	Moderately resistant	116	71
	Susceptible	3	11
	No. of genes postulated	3	-
	c^2 value	1.828	-
BC_5F_3 lines	Homozygous resistant	3	13
	Segregating	184	155
	Homozygous susceptible	3	17
	No. of genes postulated	3	2
	c^2 value	0.001	3.027

Resistant denotes 0-5% KB infection, moderately resistant denotes >5-30% KB infection and susceptible is represented by >30-70% KB infection.

The second BC_5F_3-P2 population was also categorized into similar categories with 13 plants progenies under homozygous resistant category, 155 plants progenies under segregating category and 17 plant progenies under homozygous susceptible category. In this season early planting allowed most of the progenies from the very late flowering BC_5F_2 plants to be screened bringing the population size to 185. Postulating 2 additive genes for resistance the expected number of plants in each category were calculated and c^2 was estimated to check the validity of hypothesis (Table 1). The test showed that the hypothesis was valid and second backcross population thus had 2 genes for resistance to KB from KBRL 22.

In a subsequent experiment the extreme and uniform progenies were advanced to BC_5F_4 to confirm non-segregating families. Two populations were evaluated till the BC_5F_3 but one of the populations (P2) was not used beyond this stage as it showed variation for background traits, including characters such as flowering time, which affect the precision of KB screening. This in turn led to low precision of genetic analysis (Table 1). Thus single plants from the uniformly resistant and susceptible BC_5F_3 lines from P1 population only were advanced to BC_5F_4 for confirmatory evaluation and identification of NILs. Eleven resistant BC_5F_4 lines derived from single plants from the three uniformly resistant BC_5F_3 lines were chosen to represent resistant NILs (Table 2). Likewise the susceptible counterpart was represented by ten susceptible BC_5F_4 lines derived from single plants from three uniformly susceptible BC_5F_3 lines. The KB score of the single plants, the average score of the progenies from which these were chosen along with the score of the parental lines recorded in the previous season is given in Table 2. The clear demarcation of resistant and susceptible progenies as

Table 2. KB score of identified resistant and susceptible BC_5F_4 plants, the average score of the progenies from which these were chosen along with the score of the parental lines

Disease category	Plant design-nation (BC_5F_4)	BC_5F_4 plant lineage (BC_5F_2-BC_5F_3-BC_5F_4)	KB score (%)		
			BC_5F_4 plant	BC_5F_4 line average	BC_5F_3 line average
Resistant	1	11-3-1	0.00	1.14	0.01
	2	11-4-1	0.00	0.71	
	3	11-6-1	0.00	0.00	
	4	11-6-2	0.00	0.00	
	5	12-2-1	0.00	0.00	1.00
	6	12-2-2	0.00	0.00	
	7	12-3-1	0.00	0.00	
	8	12-3-2	0.00	0.00	
	9	32-2-1	0.00	0.00	0.41
	10	32-2-2	0.00	0.00	
	11	32-4-1	0.00	0.62	
Susceptible	12	60-3-5	11.30	9.44	7.30
	13	60-5-5	11.50	8.86	
	14	60-8-2	30.00	9.13	
	15	65-3-2	16.60	7.00	9.30
	16	65-5-4	14.00	11.56	
	17	65-6-4	15.60	7.41	
	18	92-3-3	25.30	8.71	10.90
	19	92-4-4	20.60	10.02	
	20	92-5-1	33.3	15.81	
	21	92-9-1	27.4	15.67	

well as confirmatory support to this categorization from KB scores observed across progenies and seasons is evident. The eleven resistant individuals were first used as part of resistant pool for all polymorphic markers followed by use of all identified BC_5F_4 plants individually against the critical markers. As three genes were found to be necessary for conferring high levels of resistance, presence of an individual resistance gene in an otherwise susceptible individual could not be ruled out. Thus only the resistant pool was constructed from the selected resistant BC_5F_4 progenies and screened for presence of donor alleles of all the 93 polymorphic markers while using the recipient parent as control. Donor alleles of four markers namely X*gwm* 99 on chromosome 1AL, *Xgwm149* (4BL), *Xgwm174* (5DL) and *Xgwm 340* (3BL) showed their presence in the resistant pool. These four markers were run on the 11 resistant and 10 susceptible BC_5F_4 progenies (Table 3, Fig. 1). Resistant and susceptible phenotypes showed a strong association with three of the four markers *viz.*,

Table 3. Analysis of a set of resistant and susceptible BC_5F_4 lines with identified SSR markers

Disease category	Plant design-nation	SSR markers			
		Xgwm 99 (1AL)	Xgwm 149 (4BL)	Xgwm 174 (5DL)	Xgwm 340 (3BL)
Resistant	1	R	R	R	R
	2	S	R	R	R
	3	R	S	R	R
	4	R	S	R	R
	5	S	R	R	R
	6	S	R	R	S
	7	S	R	R	R
	8	S	R	R	S
	9	R	R	R	R
	10	H	S	R	R
	11	H	R	S	S
Susceptible	12	S	R	S	S
	13	S	R	S	S
	14	S	R	S	S
	15	S	S	S	S
	16	S	S	S	S
	17	S	S	S	R
	18	S	S	S	S
	19	S	S	S	S
	20	S	R	S	S
	21	S	S	S	S
c^2 value (2 x 2 contingency test)		7.636	2.290	17.355	8.416
p-value		0.00572	0.13012	0.00003	0.00371

Xgwm 99, *Xgwm 174* and *Xgwm 340* as evident from the significant c^2 values of the 2 x 2 contingency test (using p = 0.05 as the benchmark). These three markers probably mark the regions associated with the three resistance genes revealed by the genetic analysis of this population in BC_5F_2 and BC_5F_3. In this study marker *Xgwm 149* on chromosome 4BL showed relatively low association with KB reaction in the NILs. The 4BL region has however, been reported earlier to play a role in KB resistance [12, 13] along with a significant environmental interaction. The three critical chromosomal regions indicated in the present study were not shown to be significant for KB reaction in an earlier study based on RILs derived from WH542/HD29 and WH542/W485 [13]. Identification of these regions in the present NIL based experiments using a related donor but a different recipient could be attributed to changes in genetic background as well as precision.

The advantage of the present strategy lies in further possibility of dissecting the identified chromosome segments and moving closer to the genes

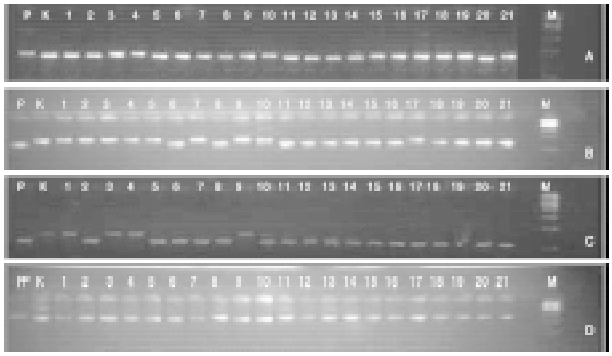

Fig.1. SSR marker alleles amplified in KB resistant and susceptible NILs in PBW 343 background A. *Xgwm* 149-4BL, B. *Xgwm* 340-3BL, C. *Xgwm* 99-1A L, D. *Xgwm* 174-5DL (P = PBW 343, K=KBRL 22, Lanes 1 to 11 = Resistant NILs, Lanes 12 to 21 = Susceptible NILs).

of interest. A BC_5F_6 micro RIL population developed from this material is currently being screened for KB. Using a set of markers for each identified chromosome region would allow for fine mapping and QTL demarcation.

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