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Genetic analysis of Karnal bunt (*Tilletia indica Mitra*) in near isogenic lines of bread wheat (*Triticum aestivum L.*)

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Abstract

Karnal bunt (KB) resistant near isogenic lines (NILs) were developed using six donor stocks viz., ALDAN ' S' / IAS 58 , CMH 77.308, H567.7I/3*PAR, HD29, HP1531 and W485 in the background of susceptible cultivar WH542. The BC_5F_2 and BC_5F_3 populations of six crosses involving these donor stocks with WH542 were screened with a mixture of 17 isolates of *T. indica*. Genetic analysis in BC_5F_2 and BC_5F_3 populations of six crosses revealed involvement of two additive genes in five out of the six crosses viz., WH 542 *5//ALDAN 'S' / IAS 58, WH 542 *5/CMH 77.308, WH 542 *5/H567.7I/3*PAR, WH 542 *5/HP1531 and WH 542 *5/W485 whereas 2-3 additive genes for KB resistance were indicated in WH 542 *5/HD29.

Keywords: Karnal bunt; *Triticum aestivum;* near isogenic lines; resistance; genetics

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Introduction

Karnal bunt (KB) also known as Kernel smut or partial bunt is a fungal disease of bread wheat (*Triticum aestivum* L.), durum wheat (*Triticum durum*), rye and *triticale* (a hybrid of wheat and rye). It is caused by a fungus *Neovossia indica* (Mitra) Mundkar = *Tilletia indica* Mitra. This disease is native to South Asia and was first reported in 1931 by Mitra from Karnal (Haryana, India). Since then, the disease has been of frequent occurrence in Northern India and has been reported in parts of several countries including Afghanistan, Iran, Iraq, Mexico, Nepal, Pakistan, South Africa and the United States of America (USA) (Rush *et al*, 2005). Due to its multiple modes of transmission (seed, soil and air), the management of the pathogen is difficult. Further, several attributes of the etiology of KB and the hardiness and longevity of teliospores make its control very difficult. Identification and deployment of genetic resistance to KB may be the most economical, feasible and eco-friendly approach to manage the disease. The main sources of KB resistance identified to date are of Indian, Chinese and Brazilian origin (Fuentes-Davila and Rajaram 1994). Genetic analysis of KB response is comparatively tedious on account of labour intensive screening procedures. The presence of oligogenic rather than monogenic resistance in the host and the absence of well defined pathogen isolates also hamper precise genetic analysis. Initial work on genetic analysis was based on quantitative genetics for KB resistance and both additive and dominance gene effects were reported (Chand *et al,* 1989; Gill *et al*, 1990; Sharma *et al*, 1991). Later, segregating populations of several resistant x susceptible crosses were subjected to qualitative genetic analysis. In several genetic stocks resistance has been shown to be partially dominant and controlled by 2 -3 additive genes (Singh *et al*, 1995 a; Singh *et al*, 1995 b; Fuentes- Davila *et al*, 1995 a; Fuentes- Davila *et al*, 1995 b; Villareal *et al*, 1995,). Keeping in view the macro and micro environmental influences on KB incidence, genetic analysis has progressively shifted from segregating host populations to stable host populations like RILs which allow repetitive and replicated phenotyping. Singh *et al* (1999) used F_8 RILs derived from a resistant (HD 29) and susceptible (WL711) cross, which were inoculated with two isolates, Ni 7 and Ni 8. Three genes conferred resistance against Ni 7 and two against Ni 8. Later, nine loci were identified by screening 10 RIL populations using mixture of isolates representing genetically heterogenous inoculum, derived from four KB resistant genotypes (ALDAN/IAS 58, H567.71/3*PAR, HD29 and W485) and one susceptible line, WH542 (Sharma *et al,* 2005). For more precise genetic analysis, attempts were made to develop genetically homogeneous inoculum system based on single compatible monosporidial pair and three additive genes were found effective in HD29, W485 and ALDAN/IAS58 (Bodhraj 2005, Sirari 2006 and Sharma *et al,* 2006). A further advance in this regard would be the use of KB resistant near isogenic lines (NILs) - a set of lines with diverse KB resistance genes introgressed into a susceptible genotype. Such a material could lead to molecular tagging of resistance with minimum genotyping efforts. It can also provide opportunity for precise genetic analysis of resistance genes with minimum background noise.

Materials and Methods

The present investigation was carried out at Punjab Agricultural University (PAU), Ludhiana and PAU Off-season Research Station, Keylong, Himachal Pradesh. For this, six crosses viz., WH 542 *5//ALDAN 'S' / IAS 58, WH 542 *5/CMH 77.308, WH 542 *5/H567.7I/3*PAR, WH 542 *5/HD29, WH 542 *5/HP1531 and WH 542 *5/W485 (Table 1) were used. Selected KB resistant single plant from BC_5F_1 derived progenies in BC_5F_2 and BC_5F_3 populations of these six crosses were used for the genetic analysis leading to establishment of KB resistance in near isogenic lines. Sowing of BC_5F_2 populations for different crosses was done in 1m rows in the field with row to row distance of 25 cm,

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thus accommodating about 10-12 plants in each row (2007-08). A part of the BC_5F_2 populations for all the six crosses was advanced to BC_5F_3 at PAU off season research station, Keylong during 2006-07. These BC_5F_3 populations for all the crosses were sown as single plant progenies in the main season 2007-08 at PAU, Ludhiana. Each F_3 progeny was represented by a minimum of 10-15 plants. The total number of plants in BC_5F_2 populations varied from 209 to 378 while the number of BC_5F_3 progenies ranged from 104 to 257 (Table 1).

Seventeen previously collected and well established *Tilletia indica* isolates representing genetic variability prevalent in North Western Plains Zone of India were used for inoculations. The isolation, multiplication and maintenance of pathogen was done on Potato Dextrose Agar (PDA) medium. For isolation, the infected wheat grains were surface sterilized with alcohol avoiding dipping and the teliospores were dusted on the sterilized Petri plate with the help of a sterilized needle. These colonies developed from dusted teliospores were then aseptically transferred to PDA slants under aseptic conditions and incubated at 20-24^{0}C. The pathogen was maintained in sporulating conditions by frequent sub culturing and further use during the period of inoculations. Artificial inoculation of the crop was done following "Syringe inoculation method" given by (Aujla *et al,* 1982) at boot leaf stage. Inoculations were invariably done during the evening hours. To ensure maximum humidity, the frequent irrigation was given in the field. In each population of the six crosses, 2-3 ears per plant along with parents were inoculated in case of BC_5F_2 population while 10-15 plants were inoculated in BC_5F_3 population. The number of plants/progenies in BC_5F_2 and BC_5F_3 populations screened under artificial epiphytotic conditions during 2007-08 are given in Table 1. Inoculated ears were harvested and percentage disease infection was recorded. Data were recorded as percent infection per plant. Plants/progenies within each population of crosses were categorized into different diseases categories. Genetic analysis was carried out based on proportion of Plants/progenies in different disease categories, including the chi-square analysis for goodness of fit of the proposed genetic ratios.

Table 1 BC_5F_2 and BC_5F_3 populations evaluated for Karnal Bunt resistance

Crosses used	Number of plants in BC_5F_2	Number of Progenies in BC_5F_3
WH542*5//ALDAN ' S' / IAS 58	378	177
WH 542 *5/CMH 77.308	307	257
WH 542 *5/H 567.7I/3*PAR	246	196
WH 542 *5/HD 29	221	133
WH 542 *5/HP 1531	307	104
WH 542 *5/W 485	209	182

Results and Discussion

The six resistant parents, ALDAN 'S' / IAS 58, CMH 77.308, H567.71/3*PAR, HD 29, HP 1531 and W 485 showed average KB infections of 2.7 per cent and 2.69 per cent respectively in both the years. The range of KB infection in six resistant parents was from 1.98-2.89 and 2.00-2.86 per cent respectively in both the years. The average infection in the susceptible parent, WH 542 was 40.59 and 43.37 per cent during both the years (Table 2). For genetic analysis, plants in BC_5F_2 populations were categorized into parental categories viz. resistant (R) and susceptible (S) on the basis of parental scores and the plants showing intermediate level of resistance falling between the two parental extremes were categorized as moderately resistant (MR).

The R, MR and S plants showed 0-5% KB, 5-30% KB and > 30% KB infection respectively. BC_5F_3 progenies were categorized into homozygous resistant (HR), heterozygous/ segregating (Het) and homozygous susceptible (HS) the basis of KB score infection. There was a pooling of R and MR categories into one category in BC_5F_2 population and HR and Het into one category for genetic analysis in BC_5F_3 population as no ratio fitted taking them separately. The pooling of categories was done due to large differences in the range of R and S category and further uneven distribution of plants in infection categories. This may be due to the use of mixture of isolates as an inoculum for artificial inoculations. An attempt was made to fit the observed ratios of R+MR and S plants in BC_5F_2 and HR + Het and HS in BC_5F_3 into the nearest expected ratios using chi-square test (Table 3). It was evident from the low frequencies of HS progenies that more than one resistance gene was involved. The proportion of R + MR/HR+ Het: S/HS in all the crosses fitted the hypothesis of 2 additive genes. Accordingly, parents ALDAN 'IAS' 58, CMH77.308, H567.71, HP1531 and W485 were postulated to carry two resistance genes. On the basis of segregation ratios, three additive genes for resistance

Table 2 Karnal bunt infection (per cent) of donor and recipient parents screened with a mixture of 17 isolates of *T. indica* during Rabi 2007-08 and 2008-09

Parents	2007-08 Per cent Karnal bunt infection	2008-09 Per cent Karnal bunt infection
ALDAN ' S' / IAS 58	2.53	2.18
CMH77.308	1.98	2.06
H567.71/3*PAR	2.68	2.00
HD29	2.25	2.62
HP1531	2.89	2.51
W485	2.52	2.86
WH542	40.59	43.37

were postulated in HD 29 but not with consistency across populations. Only BC_5F_3 population of this cross confirmed to three gene models. The calculated chi-squared values and their probabilities are given in Table 3. For the entire set of crosses probabilities were acceptable (>0.05). In the cross WH 542 *5//ALDAN 'S' / IAS 58, BC_5F_2 population consisting of 378 plants was categorized into 353 R+MR and 25 S plants. The postulation of 2 additive genes parent was acceptable with a probability of 0.7702 (2= 0.0853). In BC_5F_3 population, out of 177 progenies, only 5 were HR while 157 Het, these were pooled to 162 progenies. There were 15 HS progenies. These proportions also comply with the two gene hypothesis with a probability of 0.2214 (Table 3). In the BC_5F_2 population of WH 542 *5/CMH 77.308, 289 plants were R+MR and 18 plants were S out of 307. Postulating 2 additive genes for resistance, the expected number of lines in each category was determined and 2 worked out to test the validity of hypothesis. The analysis showed that gene postulation in resistant parent was acceptable with a probability of 0.7797. Similarly in case of BC_5F_3 population there were 238 HR + Het progenies while only 19 were HS out of a total of 257. Thus progenies showed segregation of 238R+MR: 19S which had a good fit (p= 0.4490) for the expected genetic ratio of 15R: 1S (Table 3). This inheritance was in consonance with the inheritance pattern observed in the previous population.

The BC_5F_2 population derived from WH 542 *5/H 567.71/3*PAR containing 246 plants had 228 R+MR and 18 susceptible plants. Chi square analysis (2 = 0.4779, p value =0.4893) showed that the hypothesis of 2 genes for resistance in H567.71/3*PAR i.e. resistance parent was acceptable. In case of the BC_5F_3 population, 186 progenies were HR+Het while 10 were HS out of the total 196 progenies (Table 3). In case of WH 542* 5/HD29, the BC_5F_2 population consisted of 221 plants. Out of these, 204 were categorized as R+MR and 17 as S. The analysis showed that hypothesis of 2 genes for resistance in resistant parent was acceptable with a probability of 0.3757. In case of BC_5F_3 population, 131 progenies were HR+Het while only 2 were HS out of a total of 133 progenies. Thus progenies showed segregation of 131R+MR: 2S which had a good fit (p= 0.9563) for the expected genetic ratio 63R: 1S. The 2 value (Table 3) calculated for three additive genes for resistance was clearly acceptable (2=0.0030, p value =0.9563). An additional gene for resistance was thus being indicated in BC_5F_3 population.

Out of a total of 307 plants in the BC_5F_2 population of WH 542 *5/HP1531, 286 plants were R+MR whereas 21 plants were S. The analysis showed that gene postulation of two additive genes in resistant parent was acceptable with a probability of 0.6691. In BC_5F_3 population, out of 104 progenies, 97 were HR +Het while 7 were HS. These

Table 3 Genetic analysis of Karnal bunt resistance in BC_5F_2 and BC_5F_3 generation of six crosses in the background of WH 542

Cross	Gene-ration	No. of plants/ progenies in KB response categories		Total plants/ progenies	No of resistance gene postulated	Expected ratio	Calculated 2 value	P value**
		R+MR / HR+Het*	S/ HS					
WH542*5// ALDAN ' S' / IAS 58	BC5F2	353	25	378	2	15R:1S	0.0853	0.7702
	BC5F3	5+157 =162	15	177		15HR+Het :1HS	1.4948	0.2214
WH 542 *5/ CMH 77.308	BC5F2	289	18	307	2	15R:1S	0.0782	0.7797
	BC5F3	7+231 =238	19	257		15HR+Het :1HS	0.5730	0.4490
WH 542 *5/H 567.7I/3*PAR	BC5F2	228	18	246	2	15R:1S	0.4779	0.4893
	BC5F3	1+185 =186	10	196	2	15HR+Het :1HS	0.4407	0.5067
WH 542 *5/ HD 29	BC5F2	204	17	221	2	15R:1S	0.7845	0.3757
	BC5F3	5+126 =131	2	133	3	63HR+Het :1HS	0.0030	0.9563
WH 542 *5/HP 1531	BC5F2	286	21	307	2	15R:1S	0.1826	0.6691
	BC5F3	1+96 =97	7	104	2	15HR+Het :1HS	0.0310	0.8602
WH 542 *5/W 485	BC5F2	198	11	209	2	15R:1S	0.3473	0.5556
	BC5F3	6+163 =169	13	182	2	15HR+Het :1HS	0.2475	0.6188

*R=Resistant,MR=Moderately resistant, S=Susceptible, HR= Homozygous resistant, Het= heterozyous , HS= Homozygous susceptible

** $\chi 2$ value for significance of p=0.05 is 3.84. These proportions also comply with the two gene hypothesis with a probability of 0.5067

proportions also comply with the two gene hypothesis with a probability of 0.8602 (Table 3). The BC_5F_2 population of WH 542 * 5/W485 containing 209 plants showed 198 plants as R+MR and 11 plants as S. Chi square analysis (χ^2 = 0.3473, p value = 0.5556) showed that the hypothesis of 2 genes for resistance in W485 was acceptable. In case of the BC_5F_3 population consisted of 182 plants, 169 progenies were HR + Het while 13 were HS. These proportions also comply with the two gene hypothesis with a probability of 0.6188 (Table 3). In a subsequent experiment the extreme and uniform resistant progenies were advanced to BC_5F_4 to confirm non segregating progenies. The single plants from the homozygous resistant BC_5F_3 lines were evaluated for confirmation of resistance in BC_5F_4 during 2008-09 for all the six crosses. In each cross, each progeny is represented by a minimum of 10-15 plants. These progenies showed an average KB infection ranged from 1.37-2.16 percent. These progenies were used for the establishment of NILs in each of the six crosses. In the six donor parents crossed with WH 542, there are two genes with additive effects conferring resistance to KB at both BC_5F_2 and BC_5F_3 levels except HD 29 which showed two genes in BC_5F_2 population and an additional gene was found in BC_5F_3 population. Sharma *et al* (2004) also reported two genes in case of ALDAN 'S' / IAS 58, CMH 77.308, H 567.71/3*PAR, HD 29, HP 1531 and W 485 when crossed with WH 542. Similar results were also reported by Sharma *et al* (2005) on KB resistance studied in populations derived from crosses of four resistant stocks (HD 29, W 485, ALDAN ' S' / IAS 58, H 567.71/3* PAR) and highly susceptible cultivars, WH 542. The study revealed that HD 29, W 485 and ALDAN ' S' / IAS 58 each carried two resistance genes whereas 3 genes were indicated in H 567.71/3 PAR. Similarly, two genes in HD 29 and W 485 with additive effects were also revealed in recombinant inbred lines derived from WH 542 X HD 29 and WH 542 X W 485 when screened with mixture of 16 isolates at boot leaf stage by syringe inoculation method (Sirari *et al,* 2008). Thus genetic analysis of donors ALDAN 'S' / IAS 58, CMH 77.308, HD 29, HP 1531 and W 485 is in conformation with previous studies. Three genes were indicated for resistance in H567.71/3* PAR (Sharma *et al,* 2005) whereas 2 in the present study. The difference in genetic interpretation may be due to the pathogen variation, environmental influences and or dilution effect during backcrossing. Variation in number of genes indicated in HD 29 could be due to different genetic constitution of the population. This variation has been earlier reported (Singh *et al,* 1999) where HD 29 possesses three major genes for resistance to isolate Ni7 and two genes for resistance to isolate Ni8. One of the two genes controlling resistance to Ni8 is common with one of the genes conferring resistance to Ni7. The complexity of KB genetics was shown in the present study although the plant material had minimum genetic background noise and extensive phenotyping was done over two seasons. However the NILs established from material can be used for molecular marker analysis of KB resistance leading to gene tagging and cloning.

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