Genetics of Karnal Bunt Resistance: Use of *Tilletia indica* Populations at Different Levels of Heterogeneity

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Abstract: Genetics of Karnal bunt (KB) resistance in wheat has earlier been investigated using heterogenous pathogen populations of *Tilletia indica* which has applied significance but does not provide clear cut genotypic resolution as in *Puccinia*-wheat systems. Therefore, an attempt was made for the first time to deploy genetically homogenous pathogen population for identifying loci involved in KB resistance. Recombinant inbred lines (RILs) derived from the cross, ALDAN 'S'/IAS 58 (KB resistant) and WH542 (susceptible cultivar) were screened with three inoculum types representing various levels of genetic heterogeneity:(i) mixture of 16 pathogen populations (from different agroclimatic regions representing high genetic heterogeneity), (ii) pathogen population from single geographic region (isolate P_4, Gurdaspur, Punjab, India representing heterogeneity within a single teliospore) and (iii) a pair of monosporidial lines of opposite mating types, derived from single teliospore of an isolate, P_4 (genetically homogenous culture). For genetic analysis, the RILs were grouped into three classes, using the parental scores for demarcating the categories. Using highly heterogenous inoculum, 2 additive genes ($P = 0.947$) and with comparatively less heterogenous inoculum system (isolate P_4) 3 genes ($P = 0.557$) for resistance were postulated. When inoculated with a homogenous pathogen population, the proportion of lines in the resistant category were greatly inflated in comparison to the categories obtained against P_4 isolate as well as mixture of isolates. However, after merging resistant and moderately resistant categories, three genes could be postulated ($P = 0.99$). The additional gene that is observed against less heterogenous inoculum is probably getting knocked out by one or more of the 16 isolates which form a part of the most heterogenous inoculum system. It is inferred that for breeding high yielding KB resistant genotypes, genes effective against a wider spectrum of pathogen populations are of greater practical utility. To understand dynamics of host-pathogen interaction use of homogenous inoculum seems appropriate but it may pose sampling related problems of representing pathogen populations through monosporidial cultures as number of primary sporidia arising from a single teliospore are high and variation exists for pathogenecity alleles in different compatible pairs.

Keywords: genetics; Karnal bunt; resistance; *Tilletia indica*; wheat

Karnal bunt (KB) of wheat caused by *Tilletia indica* is a disease of great concern due to unacceptability of wheat grains by quality conscious countries of the world which have laid legal restrictions for entry of wheat grains from the infested areas. The pathogen perpetuates in the form of teliospores, both in seed and soil. These teliospores germinate in soil and produce infection causing propagules, the allantoid shaped sporidia. These sporidia become air borne and infect the susceptible wheat cultivars at ear head emergence stage. Infection caused by the air borne sporidia can be lowered to a large extent by spraying Triazole group of chemicals at heading and have been recommended for seed production. Spraying of wheat crop with chemicals is, however, cumbersome and not cost effective. Deployment of resistant cultivars is the only remedy to reduce the disease in the endemic areas. Continuous growing of resistant cultivars over years will lower down the soil borne inoculum followed by, if not elimination, drastic reduction of the disease.

A major aspect of resistance breeding for KB involves the understanding of genetics of resistance. Screening of wheat genotypes for identifying resistance to the disease have so far been carried out using mixture of isolates of *T. indica*. This approach is suitable for plant breeding oriented screening work but may not permit precise genetic analysis of host resistance. It needs to be seen if use of appropriate monosporidial cultures can improve the accuracy of genetic analysis of host resistance. Most of the genetic studies based on heterogenous inoculum indicated an oligogenic pattern of resistance. *Tilletia indica* populations have a genetic structure of typical outcrossing (allogamous) organisms. Diploid teliospores, the perpetuating and one of the disease dissemination propagules, are typically heterozygous and on germination segregate to produce haploid primary sporidia by undergoing meiosis followed by mitosis. The primary sporidia produce secondary sporidia which subsequently multiply and at some stage in the life cycle of *T. indica* (yet to be precisely determined), the haploid sporidia/ mycelium carrying dissimilar mating type specificities fuse to form dikaryotic and the actual infective mycelium. Thus genetic heterogeneity of the infective mycelium is ensured. This genetic heterogeneity can be restricted by using a narrow based pathogen population e.g. a population representing one location rather than a wider regional spectrum. Alternately, complete homogeneity may be ensured by deriving monosporidial cultures and using a pair of compatible monosporidial cultures for inoculations. In the present study both the approaches were followed for genetic analysis of resistance in a recombinant inbred line (RIL) population derived from the cross,WH 542 × ALDAN 'S'/IAS 58.

MATERIAL AND METHODS

A set of RILs derived from WH 542 × ALDAN 'S'/IAS 58 was screened for the study of genetics of resistance. WH 542 (Kauz 'S' = BJY/URES// JUP) is a currently recommended cultivar and is highly susceptible to KB. ALDAN 'S'/IAS 58 is an already established KB resistant stock. The RILs were obtained by single seed descent method and were in F_{10} and F_{11} generations in the years 2003–2004 and 2004–2005. These were sown in one-meter row, with 25 cms row to row distance in the month of November. After every twenty lines, susceptible parent, WH 542 was sown which served as check.

In order to employ pathogen populations at different levels of heterogeneity for screening of RILs, three types of inoculum systems were generated. One consisted of mixture of 16 isolates derived from different agroclimaic zones of Punjab, Dhaulakuan (Himachal Pradesh) and Pantnagar – Uttaranchal which represented high degree of heterogeneity in the pathogen population (Sharma *et al.* 1998). Another inoculum system was from the sporidial cultures derived from a single teliospore of a single isolate, P_4 from Gurdaspur district and was the most virulent among all the isolates. This inoculum system represented heterogeneity within teliospore which is comparatively less than the mixture. The third type of inoculum system developed was genetically homogenous and derived from two monosporidia of opposite mating types (PS 18 – mating allel 'a'+ PS 22 – mating allel 'A') of a single germinating teliospore of isolate, P_4.

Single teliospore of pathotype, P4 was isolated by employing dilution method. Sporidial cultures prepared, thereof, were multiplied on PDA and used for inoculations. Further, monosporidia derived from a single teliospore were also isolated by dilution method. Teliospores of P_4 isolate were initially dusted from the bunted grains in 2 ml distilled water contained in test tubes. After five days of incubation under room temperature (18–25°C) it was poured onto autoclaved water agar in Petri dishes. Single germinating teliospores were marked. The bit of water agar having single germinating teliospore was dissected and observed under microscope for confirmation. With the help of forceps the bit having single germinated teliospore was put in a small quantity of autoclaved water and stirred with small camel hair brush so that primary sporidia get detached and spread individually in the water. Using hypodermic syringe, small droplets having primary sporidia were put inside several PDA slants where each droplet either contained single primary sporidia or was devoid of any. The test tubes showing single pin head sized colonies were presumed to have developed from a single primary sporidia. Their monosporidial identity was confirmed by individually inoculating them on to the susceptible host, WH 542. The monosporidia which failed to develop the disease were considered to be of either mating type (mating allel 'a' or 'A').

Compatible types were identified by inoculating the susceptible cultivar with all the possible cross combinations of monosporidial lines. The disease developed in the ear heads inoculated with the opposite mating types and a pair of compatible monosporidia which resulted in the highest infection was employed for inoculation. The monosporidial lines were maintained at low temperature (10–14°C) during off – season and multiplied on PDA for screening the RILs in the month of February/March.

With each type of inoculum system, ten ear heads of the RIL population were inoculated using syringe, as per method of Aujla *et al.* (1982). The experiment was replicated thrice. At maturity, the inoculated ear heads were thrashed manually and percentage of diseased grains per ear were recorded and averaged for each treatment (and of replications and years). For the sake of genetic analysis, the RILs were assigned to the resistant and susceptible disease categories on the basis of distribution of parental disease score. The RILs showing intermediate levels of resistance falling in the zone between the two parental categories were referred to as moderately resistant. Chi square analysis was done for goodness of fit of the proposed genetic ratios and corresponding *P* values recorded.

RESULTS AND DISCUSSION

Screening of RILs with mixture of isolates. When inoculated with mixture of isolates, RILs showed an almost continuous distribution in terms of KB incidence (Figure 1). Distribution of plants of parental lines in different KB response categories is presented in Figure 1. Thus using the parental scores for demarcating three categories, out of 212 RILs, 53 were grouped under resistant category, 104 under moderate and 55 lines under susceptible category. Postulating 2 additive genes for resistance, the expected No. of lines in each category were determined and $\chi2$ worked out (Table 1). The hypothesis turned out to be valid. Several studies conducted at CIMMYT have indicated 1–3 genes governing resistance in KB resistant stocks (Singh *et al.* 1995; Fuentes Davilla *et al.* 1995). Sharma *et al.* (2005) studied KB resistance in RIL populations (inoculated with mixture of isolates) derived from crosses of four resistant stocks (HD 29, W 485, ALDAN 'S'/IAS 58, H 567.71/3 PAR) and a highly susceptible cultivar, WH 542 and indicated two genes in ALDAN 'S'/IAS 58.

Screening of recombinant inbred lines with P_4 isolate. A second set of screening of 206 RILs and parents were based on inoculations with a

Table 1. Genetic analysis for resistance based on recombinant inbred lines (RILs), derived from WH 542 × ALDAN 'S'/IAS 58, falling in different Karnal bunt response categories when inoculated with mixture of isolates, individual isolate, P_4 and a compatible pair of monosporidia of *T. indica*

Obervations/analysis	No. of recombinant inbred lines based on % Karnal bunt infection using three types of inoculum systems								
	mixture of isolates*			single isolate, P_4**			compatible pair of monosporidia*		
Karnal Bunt Response categories	R	M R	S	R	M R	S	R	M S	S
No. of RILs observed in each category	53	104	55	28	148	30	186	–	26
Expected Ratio on the basis of 2 additive genes for resistance	1	2	1	1	6	1	7	–	1
No. of RILs expected in each category		53			25.75			186.50	
		106			154.50			–	
		53			25.75			25.50	
Calculated $\chi2$ value		0.109			1.170			0.010	
P value	0.947			0.557			0.99		

R = resistant, MR = moderately resistant, S = susceptible; $\chi2$ value for significance at *P* = 0.05 is 5.99; *based on inoculation of 212 RILs, **based on inoculations of 206 RILs
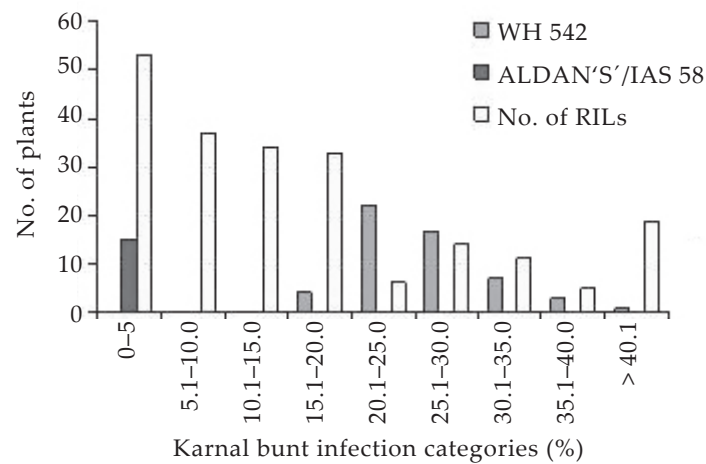
Figure 1. Distribution of parents (WH 542 and ALDAN 'S'/IAS 58) and recombinant inbred lines (RILs) in various Karnal bunt response categories when inoculated with mixture of isolates of *T. indica*

single isolate, P_4. Repeating the same system of classifying RILs into disease categories, genetic analysis of resistance was performed. Distribution of parents and RILs in different KB response categories is given Figure 2. The RILs which remained disease free (resistant parent type) were rated as resistant and the lines scoring 20% and higher disease (susceptible parent type) were rated as susceptible whereas lines showing intermediate level of resistance were referred as moderately resistant. Thus using the parental scores for demarcating these three categories, 28 lines were grouped under resistant category, 148 lines under moderately resistant category and 30 lines under susceptible category (Table 1). The best fitting postulation in this case turned out to be 3 genes for resistance operating in an additive manner. In an earlier study, SINGH *et al.* (1999) indicated four genes in a resistant stock, HD 29 when F_8 recombinant inbred lines derived from the cross of HD 29 (resistant) and WL 711 (susceptible) were inoculated with *T. indica* isolates, Ni 7 and Ni 8. Three genes conferred resistance to Ni 7 and two genes to Ni 8 with one gene being common against both the isolates. The present study was conducted using cultures from a single teliospore of P_4 isolate whereas Ni 7 and Ni 8 referred by SINGH *et al.* (1999) represented cultures from a population of the teliospores of the two isolates. P_4 isolate formed a part of the mixture of inoculum used for the previous analysis in which 2 genes were revealed. These 2 genes could be assumed to be those, which did not overcome by any of the

constituent of the mixture. These genes are probably of the greatest value for resistance breeding purpose as they are likely to be effective against a wide spectrum of races. The use of pure or uniform pathogen population is, however, a requirement of precise genetic analysis. This is expected to take care of the sampling error associated with the use of mixture and bring down the complexity of host pathogen interaction. The use of P_4 isolate with these objectives however provided genetic inferences at variance to the results obtained with the mixture.

Screening of RILs with a single pair of monosporidial cultures. The P_4 isolate represented a narrower spectrum of variation than the mixture of 16 isolates. However, it is not a genetically uniform pathogen population. Creation of genetically uniform pathogen population required a system of maintaining virulent pathotype by combining two compatible monosporidial cultures of opposite mating types (maintained separately in off season). Such a system was devised as the third method of genetic analysis. Distribution of parents and RILs (212) in different KB response categories when inoculated with single compatible pair of monosporidia, is given in Figure 3. Overall, the proportions of lines in the resistant category are greatly inflated in comparison to the categories obtained against P_4 isolate as well as mixture of isolates. The trend does not have a simple genetic explanation. However, after merging resistant and moderately resistant categories, three resistance genes were indicated (Table 1). The low infectivity
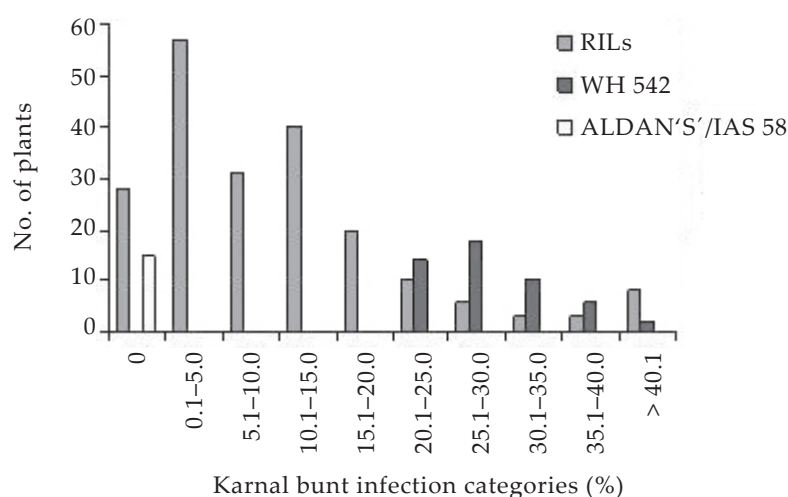
Figure 2. Distribution of parents (WH 542 and ALDAN 'S'/IAS 58) and recombinant inbred lines (RILs) in various Karnal bunt response categories when inoculated with P4 isolate of *T. indica*

of compatible sporidial inoculum is, however, evident from the observed distribution of RILs. There was no earlier report available in the literature regarding screening of any genetic population of wheat including RILs with single pair of monosporidial culture of *T. indica* which represented genetically homogeneous inoculum and also with a single teliospore from one isolate.

The present attempt was made to obtain a more precise genetic analysis. Specifically it was anticipated that the quasi-quantitative nature of disease scores obtained in the screening against KB might become qualitative. This in turn was expected to clarify the gene for gene, host-pathogen interaction. The quasi-continuous distribution

of RILs as in case of screening with mixture of isolate was also observed here. This shows heterogeneity of inoculum is one of the factors but not a primary cause of background noise or the random component of variation for the disease score. The inflation of resistance categories and low infectivity of compatible monosporidial pair is a new observation. Co-culture of monosporidia of opposite mating types for 15–20 days prior to inoculation is likely to take care of this problem as better disease expression was recorded when cultures were used after providing longer duration of mating opportunity.

To sum up, *T. indica* populations are highly heterogenous in terms of their pathogenic po-
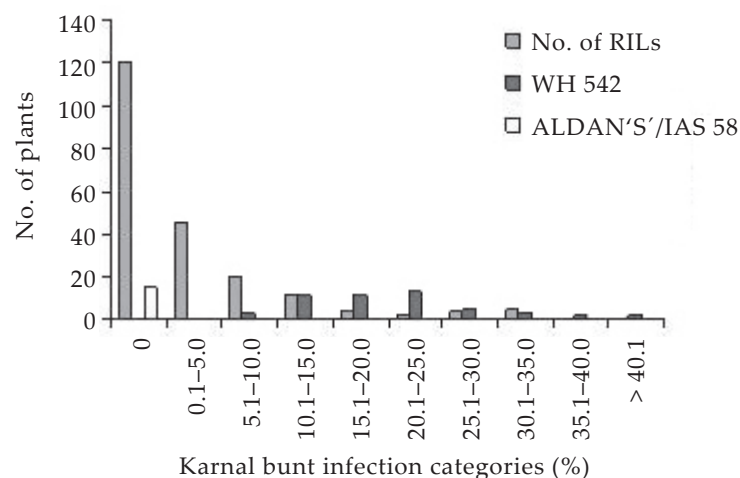

Figure 3. Distribution of parents (WH 542 and ALDAN 'S'/IAS 58) and recombinant inbred lines (RILs) in various Karnal bunt response categories when inoculated with a pair of compatible monosporidia of *T. indica*

tential. Sampling this heterogeneity or creating homogenous cultures results in altered genetic inferences. For breeding high yielding KB resistant genotypes, genes effective against a wide spectrum of pathogen populations is of greater practical utility. Using homogeneous inoculum, dynamics of host-pathogen interactions can be dissected clearly. Multiple screening with a set of homogenous cultures representing a population or even a single isolate is, however, not practically feasible. With these considerations, screening for KB with broad based populations both for resistance breeding and genetic analysis seems reasonable.

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