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Genetics of Karnal bunt resistance in wheat: Role of genetically homogenous *Tilletia indica* inoculum

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Abstract

Use of heterogeneous inoculum for Karnal bunt screening in wheat is often considered to decrease precision of genetic analysis of resistance. In the present study, genetic analysis of KB resistance was carried out based on two inoculum systems. First system was based on mixture of 16 isolates of *Tilletia indica* representing high level of genetic heterogeneity and second consisted of a single compatible monosporidial pair, a specially generated homogeneous inoculum with no within population heterogeneity. Initial attempts at using compatible monosporidial pair by mixing them just before inoculations resulted in markedly poor disease development, thwarting the genetic analysis. Co-culturing of compatible monosporidial pair for 20 days was found to be essential for obtaining adequate level of infection. The two inoculum systems were used on 2 RIL (Recombinant Inbred Line) populations derived from crosses of two established KB resistance donors, HD 29 and W 485 with WH 542, a KB susceptible cultivar. Screening with mixture of isolates indicated two additive genes providing KB resistance in both HD 29 and W 485 whereas three additive genes were found operative against compatible monosporidial pair in both the populations. The additional gene identified with compatible monosporidial pair, is proposed to be overcome by some of the more virulent constituents of mixture of isolates. Mixture of most virulent isolates is, therefore, more useful to identify the KB resistance genes effective against the entire pathogen population in a region.

Key words: Wheat, Karnal bunt, *Tilletia indica*, compatible monosporidial lines, genetics of resistance

Introduction

Karnal bunt (KB) caused by *Tilletia indica* is an important disease of wheat in North Western Plains zone of India which is a major wheat producing belt. The disease impairs the quality of wheat and wheat products when infection is about 3% [1]. Inspite of being second largest producer of wheat in the world, India's participation in international wheat trade is seriously restricted due to prevalence of KB. Several wheat importing countries have imposed embargoes on import from Karnal bunt infested zones.

Owing to multiple modes of transmission (seed, soil and air) chemical control of KB is not very effective. Resistance breeding is regarded as the main option for the management of KB. Keeping in view the poor genotypic resolution and high influence of micro environmental and seasonal effects, genetic analysis for KB resistance has progressively shifted from use of segregating populations like F_2 to stable host populations such as recombinant inbred lines (RILs) which allow repetitive and replicated phenotyping [2, 3]. So far, studies on genetic analysis for KB resistance were based on screening with sporidial cultures derived from mixture of *T. indica* isolates. Though it is well recognized that in case of KB, segregation may not be typically qualitative, use of mixture of isolates for screening also seemed to reduce genetic discrimination and pose problems to some extent in assigning resistant and susceptible classes to the host-population under study.

Teliospores of *T. indica* are diploid in nature. These teliospores upon germination produce haploid primary monosporidia which further multiply to produce haploid secondary sporidia. Secondary sporidia possessing different mating type alleles are compatible and undergo mycelial fusions to form dikaryons which are capable of causing infection on host. This sexual reproduction cycle which is mandatory for infection, ensures genetic heterogeneity in the population. This heterogeneity is

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expected to be even higher when teliospores are derived from mixture of several isolates. Present study was planned to evaluate the effect of genetically homogenous, compatible monosporidial pair on genetic analysis for KB resistance. The homogenous inoculum was generated by combining two compatible primary sporidia, multiplied individually *in vitro*. This approach for genetic analysis has not been used so far in KB-wheat system. Two RIL populations derived from WH 542 x HD 29 and WH 542 x W 485 were screened with a single compatible monosporidial pair. Genetic analysis on the basis of screening with mixture of isolates was also carried out to serve as a benchmark.

Materials and methods

Establishment, maintenance and multiplication of different types of inoculum systems of T. indica

Mixture of isolates

The sixteen isolates of *T. indica* were already collected from North Western Plains of India and established in Wheat Section, Department of Plant Breeding, PAU, Ludhiana [4]. The isolates were maintained from year to year on the susceptible wheat cultivar WH 542 and sporidial inoculum raised from teliospore was multiplied by frequent sub-culturing on potato dextrose agar (PDA) medium. The cultures of the 16 isolates were mixed in equal proportions before use.

Compatible monosporidial lines

A system was developed to establish a set of monosporidial lines from a single teliospore for a separate study aimed at understanding the genetics of mating specificities in *T. indica* [5]. Teliospores from wheat grains infected with already established highly pathogenic isolate, P_4 [4] were dusted on sterilized distilled water in test tubes. The teliospore suspension was then poured on sterilized water - agar contained in autoclaved petri plates under aseptic conditions. The water agar plates containing teliospore suspensions were incubated at 20 ± 1ºC. After 10-15 days, these water agar plates were kept upside down and observed under microscope. Single germinating teliospore with a whorl of primay sporidia was marked on the plate. The water agar block containing single germinating teliospore was cut and removed from the plate, aseptically. It was again observed under microscope to confirm that it contained only a single teliospore. After confirmation, the water - agar block containing single teliospore was transferred to a petri plate containing sterilized distilled water resulting in a suspension of primary sporidia. This sporidial suspension was diluted to a concentration having approximately one sporidia / droplet and transferred to a sterilized 5 ml syringe. Single droplets of sporidial suspension were transferred to PDA slants (one per test tube) under aseptic conditions. After 15 days, PDA slants with a single pin head colony were selected and their identity was maintained by assigning numbers as PS1, PS2, PS3 and so on. These putative monosporidial lines were then multiplied on PDA medium in test tubes and subjected to pathogenecity test to confirm their monosporidial origin. Those lines, which could not produce infection individually on susceptible wheat host WH 542, were considered as true monosporidial lines. Compatibility interactions of these true monossporidial lines were observed by inoculating them on WH 542 in all possible pairwise combinations. Out of several compatible pair, one which gave highest KB score was used in the present study.

Host populations used for genetic analysis

Two sets of 161 and 213 RILs derived from WH 542 x HD29 and WH 542 x W 485 respectively were screened for studying genetics of resistance in the year 2004-05 whereas enlarged sets of 195 and 239 lines were used in the year 2005-06. WH 542 (JUPATECO / BLUE JAY / / URES= KAUZ 'S') is a currently recommended cultivar for timely sown, irrigated conditions in North western Plains of the country and is highly susceptible to KB. Both HD 29 and W 485 are established resistant stocks which have been used in breeding and genetic analysis of KB resistance [2, 6]. The RILs were obtained by single seed descent method and were in F_{10} and F_{11} generations in the year 2004-05 and 2005-06, respectively. These were sown in the month of November in one-meter rows, with 25 cm row to row spacing. After every twenty lines, susceptible parent, WH 542 was sown which served as susceptible check.

Screening of recombinant inbred lines

RILs and parental lines were inoculated with two different inoculum systems at boot leaf stage by syringe inoculation method [7]: i) Heterogeneous inoculum systems based on mixture of isolates ii) Homogeneous inoculum based on single pair of monosporidial lines. For preparation of homogeneous inoculum in the year 2004-05, compatible monosporidial lines were maintained separately and mixed just prior to inoculations. As discussed in the results, the inoculum, thus produced, resulted in very low infection level vitiating the genetic analysis (Table 1). Average of KB infection obtained in both the seasons was taken into account

Table 1. Karnal bunt response of parents of RILs inoculated with different inoculum systems

Parents	% Karnal bunt infection using different inoculum systems					
	Mixture of isolates		Compatible sporidial pair			
			Mixed prior to inoculation		Co-cultured for 20days	
	Average	Range	Average	Range	Average	Range
HD 29	3.8	0-5	0	0	0	0
W 485	4.2	0-5	0	0	0	0
WH 542	38.4	20-70	19.3	10-30	24.7	16-36

for genetic analysis. In an extensive parallel study which was primarily aimed at demarcating the site and stage of dikaryon formation (not discussed here), pairs of compatible monosporidial lines were provided with mating opportunities for different periods. Pairs of compatible monosporidial lines co-cultured for 15-20 days resulted in high disease infection compared to those mixed at the time of inoculations [8]. Therefore, in the second year (2005-06), both the monosporidial lines were co-cultured for 20 days and used for inoculations, resulting in adequate level in parents and RILs.

In all the cases, inoculum density was maintained as 10,000 sporidia/ml of inoculum solution made in water. Appropriate relative humidity (60-100%) was created in the field with the use of mist sprayers and frequent irrigations during the inoculation period. The experiment was replicated thrice and in each replication, 5 earheads were inoculated. After harvesting, percent disease infection was recorded, replication wise, for each treatment.

Results and discussion

The RILs were assigned to resistant and susceptible categories on the basis of benchmark provided by Karnal bunt scores in the resistant and susceptible parents. Average KB scores and range of infection of HD 29, W 485 and WH 542 for 2004-05 and 2005-06 seasons are given in Table 1. These parental lines showed different response to the two inoculum systems as well as to the different methods of preparation of homogeneous inoculum. Average KB infection of resistant parents HD 29 and W 485 was 3.8 and 4.2 % with mixture of isolates whereas both the lines remained free from infection with compatible monosporidial pair. The susceptible parent WH 542 showed 38.4, 19.3 and 24.7% with mixture of isolates, compatible pair mixed before inoculations and co-cultured compatible pair, respectively.

Genetic analysis based on screening with heterogeneous inoculum system

On the basis of parental scores with mixture of isolates, the resistant category was set as 0-5% infection, moderately resistant covered 5.1-20 % and susceptible was represented as >20% infection. Thus, in the first set of RILs (WH 542 x HD 29), 33 lines turned out to be resistant, 92 moderately resistant and 36 susceptible whereas in the second set of RILs, the number of lines in these three classes were 60, 108 and 45 ,respectively in the year 2004-05 (Table 2). The proportion of resistant: moderately resistant: susceptible lines in both the crosses fitted the hypothesis of 2 additive genes for resistance. Similarly in 2005-06, when enlarged sets of RILs were used for genetic analysis with mixture of isolates, 2 additive genes were again postulated for KB resistance in both the parents.

Genetic analysis based on screening with homogenous inoculum system: Compatible monosporidial pair, mixed just prior to inoculations

In the seasons 2003-04 and 2004-05, the homogenous inoculum system was prepared by mixing two compatible monosporidial lines just prior to inoculations. On the basis of parental scores, RILs scoring no disease were grouped under resistant category, moderately resistant covered 0.1-10% infection and susceptible was represented as >10%. The number of RILs in various disease categories is given in table 2. The low infection level produced by this kind of inoculum system resulted in a drastic inflation of resistant category in comparison to that of mixture of isolates (Table 2). The proportion of individuals obtained in different categories did not reflect any simple genetic basis and no genetic inferences could be drawn.

Genetic analysis based on screening with homogenous inoculum system: Co-cultured compatible monosporidial pair

Reasons for low infection potential of compatible pair of monosporidial pair lied in reduced dikaryotization opportunities. Therefore, in the year 2005-06, the homogenous inoculum system was modified by co-culturing monosporidial lines for 20 days before inoculating both the RIL populations. In the first set of RILs (WH 542 x HD 29), 28 lines were grouped under resistant category (0%) whereas 22 lines were grouped under susceptible category (>16%). RILs showing intermediate level of resistance (0.1-16%) numbered 139 and these were grouped under moderately resistant

Table 2. Number of lines in KB infection categories in RILs derived from 'Resistant X Susceptible' crosses with both heterogeneous and homogeneous inoculum systems, genes postulated and chi-square analysis

Cross	Year	Inoculum system	No. of RILs in Karnal bunt infection			Total lines	Expected ratio	No. of resistance genes postulated	Chi square	p value
			R	MR	S					
WH 542 x HD 29	2004-05	Mixture of isolates	33	92	36	161	1R: 2MR:1S	2	3.4	0.18
		Compatible monosporidial pairs (mixed just before inoculations)	105	32	24	161	-	-	-	-
	2005-06	Mixture of isolates	47	98	50	195	1R:2MR:1S	2	0.03	0.98
		Compatible monosporidial pairs (co-cultured for 20 days)	28	139	22	189	1R:6MR:1S	3	0.85	0.65
WH 542 x W 485	2004-05	Mixture of isolates	60	108	45	213	1R:2MR:1S	2	2.15	0.34
		Compatible monosporidial pairs(mixed just before inoculations)	149	24	40	213	-	-	-	-
	2005-06	Mixture of isolates	55	113	69	237	1R:2MR:1S	2	1.95	0.37
		Compatible monosporidial pairs (Co-cultured for 20 days)	26	182	22	230	1R:6MR:1S	3	2.83	0.24

*R = Resistant, MR = Moderately resistant, S = Susceptible; c^2 values for significance at p = 0.05 is 5.99; - Genetic analysis could not be attempted due to inflation of resistance categories

category. The best fitting postulation in this case turned out to be 3 genes for resistance operating in an additive manner with Chi-square value of 0.858 and high probability value of 0.6512. Similarly, in second set of RILs, 26 lines were grouped under resistant category, 182 lines under moderately resistant category, 22 lines under susceptible category (on the basis of parental scores for demarcating these three categories) and 3 additive genes for resistance were postulated at Chi-square value of 2.835, and p value of 0.2423 (Table 2).

In present study, two genes have been postulated governing resistance in KB resistant stocks HD 29 and W 485, when mixture of isolates was used for inoculations. Similar results were also reported by Sharma *et al.* [2] in both the resistant parents HD 29 and W 485 on the basis of screening with mixture of isolates. Thus, inoculum system based on the mixture of isolates led to genetic analysis, which is in conformation with previous studies. These results authenticate the use of this inoculum system as a benchmark for testing the effectiveness of genetically homogenous inoculum system for purpose of genetic analysis.

Initially, low infective potential of uniform population (compatible monosporidial pair) was an unexpected result. When monosporidial lines were mixed just prior to inoculations, there is a possibility that monokaryotic sporidia might have found difficulty in finding their potential mates (sporidia of opposite mating types) as only two mating types were available. Further, these sporidia of two mating types were given mating/ dikaryotization opportunity only on the host surface. In case of co-culturing, though only two mating types were available, enhanced dikaryotization/mating opportunity resulted in higher infection level. Inoculum system based on mixture of isolates represented a co-cultured system of sporidia of several mating types, with greater number of potential mates as well as greater mating / dikaryotization opportunity. No earlier report is available in *Tilletia indica* employing an inoculum system based on co-cultured pair of compatible sporidia for resistance identification. However, attempts have been made to use less heterogeneous population based on single isolate, for screening of RILs derived from WL711 x HD29 [3]. In another Basidiomycete fungus, *Ustilago maydis* causing corn smut, a pair of compatible sporidia was employed for evaluation of variation associated with silk channel inoculations in sweet corn but genetics of resistance has not been studied using such homogeneous inoculum [10].

Generally, the use of homogenous (or single race) inoculum is expected to simplify the genetic analysis. This is typically observed for race specific major gene resistance as in rusts. The results observed in case of KB are due to a different mode of resistance gene action

compared to the rust resistance major genes. KB resistance, as also seen in this study, is generally based on two or more genes with additive effects. Some element of specificity in KB- wheat system is however evident from the present study as exclusion of some pathogen population constituents led to a change in the genes revealed by the genetic analysis. The element of specificity also implies that a large number of compatible monosporidial pairs will be required to represent the pathogen population of a particular geographical region, making the analysis of resistance that is operative in the field very labourious. Under these circumstances, the mixture of most virulent populations should be the preferred inoculum for revealing the comparatively smaller number but effective genes which are of relevance for transfer and commercial deployment.

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