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Research Article

Genetic variability for karnal bunt (*Neovossia indica*) resistance in recombinant inbred lines of wheat

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Abstract
Parental genotypes H567.71 and WH542 and 107 recombinant inbred lines (RILs) were grown in field conditions during the *rabi* season 2010-11 and 2011-12. The numbers of infected grains were recorded and percentage of infected grains and coefficients of infection were worked out. A wide range of variation was observed among the RILs evaluated for Karnal bunt resistance. The distribution of RILs based on Karnal bunt disease was higher towards the resistant parent (H567.71). The population also characterized morphologically for various traits and analysis of variance for morphological characters showed significant variations among the RILs. Seventy SSRs markers were used for screening of the parental genotypes out of these 42 were found polymorphic. These 42 SSR markers were used to screen selected RILs and a total of 88 alleles were detected, number of alleles per locus ranged from 2-3 with an average of 2.09 alleles per locus. The NTSYS-pc UPGMA cluster analysis was used to determine similarity between parental genotypes and selected RILs based on the SSR scoring data. The dendrogram represented two major groups; 1st major group consist of H567.71 (resistant parental genotype) along with resistant RILs and the 2nd major group comprised of WH542 (susceptible parental genotype) and susceptible RILs at the similarity coefficient value of 0.39. Genetic relationship was also assessed by Principal component analysis (PCA) and similar results were obtained.

Key words
Karnal bunt, RILs, percentage of infection, coefficient of infection, genetic similarity.

Introduction

Wheat occupies the prime position among cereals and India is the second largest wheat producer in the world. The world would require around 840 million tonnes of wheat by 2050 from its current production level of 642 million tonnes (FAO 2009). The major wheat producing countries are China, India, USA, the Russian Fedration and Australia. In India, its production is highly concentrated in the northern belt of Uttar Pradesh, Punjab and Haryana, contributing 67 percent of total production. Despite record food production, India continues to be under pressure due to high population growth and there is always a need for enhanced productivity. One of the major constraints in boosting up the wheat yield or quality is its vulnerability to various fungal diseases. Among the fungal diseases, karnal bunt caused by the fungus *Neovossia indica* (*Tilletia indica*) is a wide spread disease of wheat. This disease is native to South Asia and was first reported in 1931 by Mitra from Karnal (Haryana, India). Occurrence of this disease has been reported in northern India, Pakistan, Nepal, parts of Iraq, Mexico, South Africa, Syria and US (Rush *et al*, 2005). Karnal bunt (KB) of wheat is a disease of great concern by quality conscious countries of the world which have laid quarantine restrictions for entry of wheat grains from the infested areas. There is zero tolerance of the disease and even the presence of single teliospore can prohibit the seed shipment. To avoid the establishment of the pathogen many countries of the world such as United States, Canada, Russia and China, have imposed strict quarantine regulations on the import of bunted wheat and require phytosanitary declaration (Sharma *et al.*, 2013).

The disease is difficult to be identified in the field as infected grain shows no symptoms until near maturity. Since the pathogen is seed, soil and air borne, a limited control is achieved through the application of fungicides (Carris *et al.*, 2006). Among several approaches advocated for management of Karnal Bunt, use of resistant varieties has been the most effective and economical method but truly resistant varieties are not available (Sharma *et al*., 2008). The development of Karnal Bunt resistant wheat cultivars take a long time and the field screening for Karnal Bunt is prohibited in some countries. Therefore, biotechnological tools, which provide better understanding of the existing and expanded genetic diversity, should be considered to further accelerate the progress of such breeding programs (Sharma, 2013; Sharma *et al.*, 2015). SSR markers have been successfully used for genetic variability studies in wheat. High polymorphism detection levels, high throughput capability, and low cost compared to other marker systems enhance the utility of microsatellites for mapping genes associated with various important traits (Sharma *et al*., 2015). Keeping in view the importance SSR markers associated with Karnal bunt resistance in wheat, the present investigation was designed to evaluate the genetic variability among the recombinant inbred lines (RILs) of wheat.

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Materials and methods

The present investigation was studied a population of 107 recombinant inbred lines (RILs) of wheat cross: H 567.71 (resistant) × WH 542 (susceptible) which was grown along with parents in a randomized block design in single row plot in three replications during the *rabi* seasons of 2010-2011and 2011-2012. The screening was done under artificial epiphytotic conditions. Inoculum was prepared from 10-12 days old, active culture of *N. indica* containing approximately 10 000 secondary sporidia/ml. Ear heads were inoculated at boot leaf stage by injecting 2 ml of inoculum using hypodermic syringe (Aujla *et al*. 1989). Five to eight spikes of each entry were inoculated. At maturity, the inoculated spikes were harvested individually and threshed manually and percentage of infection and coefficient of infection was worked out as suggested by Aujla *et al.* (1989). Genomic DNA was isolated using CTAB method of Saghai-Maroof *et al*. (1984) from a small amount of fresh leaf tissue (5.0 g) from each of parental genotypes and their 107 RILs. Agarose gel electrophoresis (0.8%) was used to check quality and quantity of genomic DNA. The DNA concentrations were estimated by visual assessment of band intensity in comparison with Lambda (λ) DNA of known concentration.

Molecular marker analysis: A total of 70 SSR primer pairs were used for the molecular characterization of RILs and parental genotypes out of these 42 were polymorphic and used for preparation of SSR database in the above population. The Polymerase Chain Reaction (PCR) amplification conditions were optimized. The PCR reaction was conducted in a reaction volume of 20 µl containing 2ul of 1X PCR buffer, 100 µM dNTPs, 0.5 µl of each primer, 1.5 unit Taq DNA polymerase and 50 ng template DNA. The thermocycling program consisting of an initial denaturation at 95°C for 4 minutes followed by 40 cycles of 95°C for 1 minute, 1 minute and 20 second at annealing temperature (55-63 °C), 1 minute at 72°C and a final cycle of 72°C for 10 minutes was used. Amplified products were resolved on 4% polyacrylamide gels using Amersham Biosciences system. Gels were pre-run until an adequate temperature ($50\text{-}60^0$C) was reached. DNA bands were visualized by using silver staining protocol (SILVER SEQUENCE™ DNA Sequencing System, Promega Inc., Madison, WI, USA) after completion of electrophoresis.

Genetic similarity analysis: The frequency of polymorphism between different lines of wheat for each type of marker was calculated based on presence (taken as 1) or absence (taken as 0) of bands. The 0/1 matrix was used to calculate genetic similarity using 'simqual' subprogram of software NTSYSPC (Rohlf, 1993). The resultant distance matrix was employed to construct dendrograms by the un-weighted pair-group method with arithmetic average (UPGMA) subprogram of NTSYS-PC. Principal component analysis (PCA) was done using the 'CPCA' sub-programme of NTSYS-PC software.

Results and discussion

Screening for Karnal bunt infection: A wide variation was observed among the 107 RILs evaluated for Karnal bunt resistance. RILs with percentage of infection up to 5 % were taken as resistant and those with more than 5% infection were taken as susceptible. There was a variation in expression of disease among RILs during the year 2010 and 2011. Higher number of RILs (85) showed resistant reaction (0-5%) during the year 2010, whereas during the year 2011, 72 RILs were found to be resistant. Number of RILs with 10-15% infection for the year 2010 was only 2 while in year 2011 fourteen RILs were included in this category. For 15-20% infection, there were 11 and 9 RILs in the year 2010 and 2011 respectively. There was no RILs in the year 2010 in higher categories of infection but some RILs were infected in the year 2011 (Figure 1). The distribution of Karnal Bunt disease severity on the RILs was skewed towards resistant parent in both the years, thereby suggesting the segregation of some major additive effects from the parental wheat line H567.71. This is consistent with the results of previous studies by Kumar *et al*., 2007. Most of the genetic studies on Karnal Bunt resistance in wheat indicated one to six major genes conditioning resistance in various wheat germplasms (Goel, 2010). The inheritance of Karnal bunt resistance have also been reported to be controlled by two recessive genes (Sirari *et al*., 2008). Genetics of Karnal bunt resistance was studied in populations derived from crosses of ten resistant stocks (ALDAN, CMH77.308, H567.71, HD29, HD30, HP1531, W485, FRAME, CHIL and MRNG) and a highly susceptible cultivar, WH 542. Karnal bunt resistance was found to be primarily controlled by few major genes along with some minor genes which ultimately affect the disease expression. One common gene for resistance was postulated in all the parents, thereby concluding that additive genes at eleven loci govern the Karnal bunt resistance (Kaur *et al*., 2016). Karnal bunt resistance based on two or more genes with additive effects were also reported by Sirari *et al.,* (2008) using two recombinant inbred lines populations. From these reports it is evident that different number of genes governing resistance may be present in different genotypes and these may interact differently to give the final disease expression.

To further characterize the population under study, data was also recorded on some morphological characters namely plant height (cm), spike length

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(cm), days to flowering, number of tillers/plant, 100 grain weight and grain yield per plant (g). Analysis of variance for different morphological characters showed significant variations among the RILs. The range of different characters were given in the table (Table 1).

Genetic polymorphism: For the SSR marker analysis, twenty wheat RILs were selected which represent the whole population. Out of these 10 were most resistant which had 0.0 % percentage and coefficient of infection and 10 were most susceptible which had percentage of infection 14.89 to 36.46 %. A total of 70 SSRs were used to screen the above RILs as well as parental genotypes. Out of these, 42 primers showed polymorphism among parental genotypes. Database of SSRs was generated using above primers and amplification were scored visually based on presence or absence of the band. SSR analysis revealed a total of 88 alleles. The number of alleles per locus ranged from 2-3 with an average of 2.09 alleles per locus. The overall size of PCR products amplified ranged from 100-300 bp (Table 2). Similarity coefficient data based on the proportion of shared alleles were used to calculate the coefficient values among the selected RILs and parental genotypes. The allelic diversity was used to produce a dendrogram (cluster tree analysis, NTSYS-PC), to demonstrate the genetic relationship among selected RILs and the parental genotypes. Dendrogram showed that all the RILs and their parental genotypes clustered in two major groups at the similarity coefficient of 0.39. Major group I consisted of resistant RILs with resistant parent H567.71 and major group II consisted of susceptible RILs with susceptible parent WH542 (Figure 2). Genetic relationship was also assessed by PCA analysis (NTSYS-PC). Two dimensional PCA scaling exhibited that two parental genotypes were quiet distinct whereas resistant RILs were clustered with resistant parent and susceptible RILs were clustered with susceptible parent, (Figure 3). Silver stained gels showing allelic polymorphism for selected lines using SSR markers.

The results of genetic similarity coefficient analysis showed that extensive genetic diversity (from 39% to 81%, Figure 1) was present among RILs. The DNA polymorphisms between both the parental genotypes obtained by using micro-satellite markers, is a useful tool for genotyping and the assessment of genetic diversity (Sharma, 2013). Microsatellite markers are becoming the markers of choice due to the level of polymorphism, as well as higher reliability (Sharma *et al.,* 2015). Kaur *et al.,* (2016) also studied the 75 recombinant inbred lines with 330 SSR markers and observed that 16% markers showed polymorphism. The results of the present study and those of other studies thus clearly indicate that SSR markers are quite informative (Roder *et al*., 1995; Singh *et al*., 2003). Similar reports were recorded by using microsatellites markers for assessment of genetic diversity among cultivars and their wild relatives of wheat (Kumar *et al.,* 2007). Since microsatellite markers are locus specific only one specific locus is expected to be amplified by each primer. In few earlier studies also, more than one locus per microsatellite primer pair were detected and mapped in bread wheat (Sharma *et al*., 2015). This revealed significant differences in allelic diversity among various microsatellite loci.

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Table 1. Range of various morphological characters in the wheat RILs

S.No.	Trait	H567.71	WH542	Range in RILs
1.	Plant Height (cm)	99.33	80.67	62.0-154.0
2.	Spike length (cm)	12.77	10.40	7.07-18.27
3.	Days to flowering	83.67	73.33	61.67-91.33
4.	Number of Tillers/plant	10.87	11.87	4.07-18.53
5.	Total Grain Yield/Plant (g)	15.73	16.33	4.53-20.53

Table 2. Allelic diversity among parental genotypes H567.71 and WH 542 as assessed by 42 SSR markers

Number of markers	70
Number of polymorphic markers	42
Number of alleles	88
Range of alleles	2-3
Average number of alleles	2.09
Size of PCR products	100-300 bp

Fig. 1. Histogram showing percentage of infection on RILs during the year 2010 and 2011

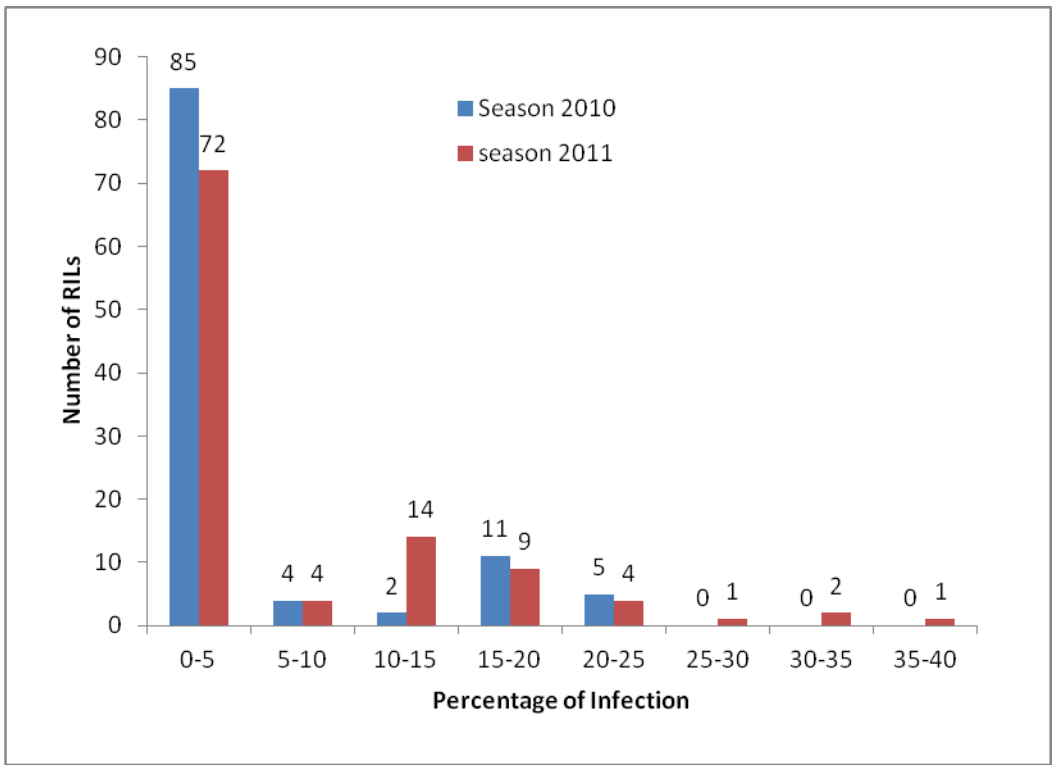

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Fig. 2. Dendrogram of Selected wheat RILs and parental genotypes based on SSR diversity data

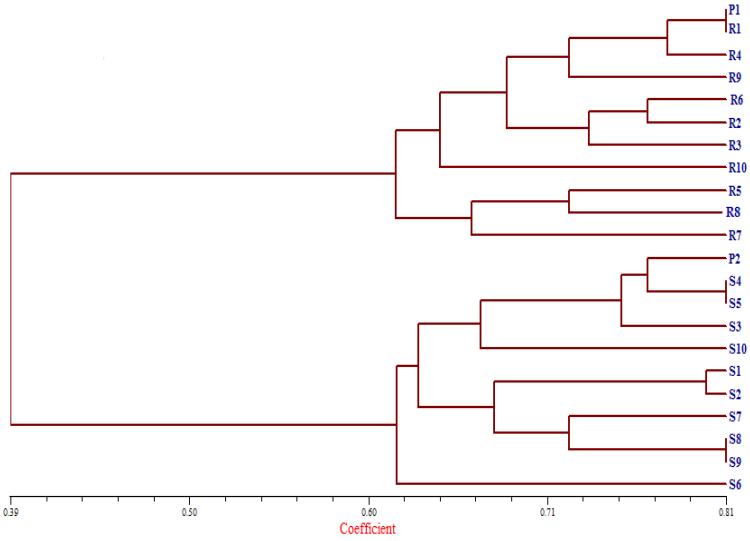

Fig. 3. Two dimensional PCA scaling of selected wheat RILs and parental genotypes based on SSR diversity data