INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 17, No. 5, p. 1-10, 2020
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Genome wide association studies (GWAS) analysis of karnal bunt resistance in Wheat (*Triticum aestivum* L.) germplasm collection from Pakistan

Syeda Qamar Batool[*1], Shazia Iram[1], Rupesh Gaire[2], Mohammad Sajjad[3], Kashif Riaz[4], Syed Mohsin Abbas[5]

[1]*Department Environmental Science, Fatima Jinnah Women University, The Mall, Rawalpindi, Punjab, Pakistan*

[2]*University of Illinois at Urbana, Champaign, IL, United States*

[3]*COMSATS University, Islamabad, Pakistan*

[4]*Department of Plan Pathology, University of Agriculture, Faisalabad, Pakistan*

[5]*Department of Agronomy, University of Agriculture, Faisalabad, Pakistan*

Article published on November 20, 2020

Key words: Candidate genes, Commercial varieties, Karnal bunt, Landraces, SNP markers, *Triticum aestivum*

Abstract

Karnal bunt (KB) disease is one of the most important challenges posed on of wheat (*Triticum aestivum* L.) industry of Pakistan because of itsinclusionin quarantine list around the globe. This disease is caused by the fungus *Tilletia indica* M. (*Neovossia indica*). It affects the grain quality of wheat and hampers its movement in international market resulting in economic losses. Presence of >3% infected grains in wheat lot makes it unsuitable for human consumption. Eradication of this disease is very difficult as no resistant cultivar has been found against KB in Pakistan so far. Genome wide association study (GWAS) was conducted on a set of 199 wheat germplasm collected from Pakistan. In this study 31,000 single nucleotide polymorphism markers were developed by 90K SNP array technology. A linear mixed model in GWAS, accounting for population structure, was fitted to identify significant genomic regions [-log(P) ≥ 4.0] on 6 different chromosomes i.e. 1A, 1D, 2D, 3B, 4A, 5A with novel loci. Candidate genes, through wheat genome assembly, were identified as putative genes related to KB resistance including kinase like protein family. The results of this study can be useful in wheat breeding through marker assisted selection for KB resistant varieties.

***Corresponding Author:** Syeda Qamar Batool ✉ batool.syedaqamar@gmail.com

Introduction

Karnal or partial bunt (KB) is part of the smut and bunt diseases of wheat worldwide and the greatest challenge to wheat industry (Murray & Brennan, 1998; Brar *et al.,* 2018). It is a floret infecting disease and also one of the most devastating in terms of grains quality of bread wheat. Bread wheat is famous for dough quality, but the bunted kernels, more than 3%, can deteriorate the bread quality. A foul fishy smell emits from infected wheat grains on account of presence of chemical trim ethylamine causing the crop to become undesirable for human consumption (Mehdi *et al*., 1973; Mehmood *et al.,* 2014). Among biotic stresses, KB causes not less than 39% yield losses around the world. This disease is also included in quarantine list of more than 70 countries (Pandey *et al.,* 2018). The presence of spores from the causal pathogen of this disease impedes trade of wheat between countries (Rush *et al.,* 2005). Strict quarantine regulations have been imposed on the movement of commercial wheat grains and wheat germplasm in view of the invasion of this pathogen into new areas (Royer & Rytter 1988). Technically, KB disease is least concerned with yield, but it does have serious impact in the international market (Brar *et al.,* 2018).

The possible measures to control KB disease are crop rotation, use of disease free seed, adjustment of irrigations and application of chemical fungicides (Singh, 1985). But the most feasible approach is employment of resistant germplasm in breeding programs. Hence, it is need of the time to explore new sources of resistance and enrich the breeding pool to broaden the genetic base of the resistance.

Genome wide association study (GWAS) is a powerful technological tool for biparental germplasm as well as for those which have been undergoing extensive recombination (Brar *et al.,* 2018). Currently, SNPs markers in GWAS have been used to characterize the genomes of several types of plants and animals but it is still a challenge to use them in wheat crop because wheat has multifaceted genomic construction. Wheat SNP comprising of approximately ninety thousand genes that have been developed for overall exposure of the wheat genome.

This 90K array can be reliably used in both hexaploid and tetraploid wheat for detection of SNPs across worldwide wheat populations (Wang *et al.,* 2014). Different markers have been applied for exploitation of genetic resources in wheat germplasm for KB disease in India (Sharma *et al.,* 2018) and USA (Singh *et al.,* 2002) but no reports have been published from Pakistan on SNP genotyping of wheat populations for KB resistance to date. Keeping in view the importance of SNPs for genome studies, the present study was designed to use SNP markers to identify resistant resources for KB from Pakistan by studying genetic variations within and among *Triticum* spp.

Materials and methods

Experimental Plant Materials and Field Trials

The plant material consisted of 199 wheat genotypes collected from Pakistan including commercial varieties and landraces. The population was planted at the field of Department of Plant Pathology, University of Agriculture, Faisalabad located in central Punjab, Pakistan. The experiments were conducted for two consecutive crop growing seasons (2016-17 and 2017-18) in an augmented design in eight incomplete blocks. Each incomplete block contained 25 entries and 5 susceptible checks of commercial varieties including Faisalabad-2008, Aas-2002, Pakistan-81, Chakwal-50, Inqilab-91. Three rows of each entry were planted containing 10 plants per line, plant to plant distance was 9 cm, row to row distance was 30 cm and 1-meter distance between the blocks was maintained. Local practices were adopted for field management. The plant material was planted by dibbling method (Na-Allah *et al*., 2018) in mid-November in both years.

Phenotyping for karnal bunt infection

Inoculum preparation

Inoculum of *Tilletia indica* was prepared by crushing the karnal bunt infected seeds and mixing them with Tween 20 solution (Wright *et al*., 2003) in a glass tube. The solution was vigorously shaken and filtered and allowed to stand for 24 hours. Sodium hypochlorite 0.6% was added for 2 minutes and centrifuged at3000 rpm for a few seconds.

The supernatant was discarded and the teliospores were rinsed with distilled water and the solution was again centrifuged at 3000 rpm. Rinsing and centrifugation was repeated for one more time. The collected teliospores were transferred to 2% water agar medium and incubated at 18–22°C until germination was observed. To collect secondary sporidia, the pieces of water agar with teliospores were put inversely onto potato dextroseagar (PDA) in Petri plates. After few days, the inoculum was increased by flooding with sterilized water and scraped with a sterilized spatula and transferred the suspension to other Petri plates of PDA. Secondary sporidia were harvested and inoculum concentration was adjusted to 10,000 sporidia/mL using a haemocytometer (Fuentes-Davila, 1994).

Karnal Bunt Inoculations and Disease Scoring

Artificial inoculation was performed by injecting sporidial suspension with hypodermic syringe into boots with emerging awns (Aujla *et al.*, 1981). Artificial infection was completed from January to March at booting stage and appropriate humidity was provided by flooding the field and sprinkling water over the whole field. At harvesting stage, infected tillers were harvested and threshed separately from the healthy tillers. The infected grains were counted and disease severity and incidence were calculated by applying formulae of disease incidence and coefficient of infection (Ziaullah *et al.*, 2012). Mean disease incidence data was used for further analysis.

Statistical Analysis

Data analysis was performed using the R statistical software (R Core Team, 2013). The random effect model was fit to estimate heritability of KB trait in wheat germplasm.

$$Y_{ijkl} = \text{line}_i + \text{year}_j + \text{rep(year)}_{jk} + \text{block(rep)}_{kl} + (\text{year x line})_{ij} + \varepsilon_{ijkl} \quad [1]$$

where Y_{ijkl} is the phenotypic value of i^{th} genotype located in l^{th} block within k^{th} rep in the j^{th} environment, line_i is the effect of i^{th} genotype, $year^j$ is the effect of j^{th} year, $rep(year)_{jk}$ is the effect of k^{th} rep within j^{th} year, $block\ (rep)_{kl}$ is the l^{st} block effect within a rep, and ε_{ijkl} is the residual error.

The variance components extracted from the equation [1] were used to estimate broad-sense heritability on entry-mean basis (H) in the following equation:

$$H = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_{gy}^2/e + \sigma_e^2/er} \quad [2]$$

Where σ_g^2 is the genetic variance, σ_{gy}^2 is the variance of genotype X year interaction, σ_e^2 is the residual error variance, e is the number of year, r is the number of replications. Best Linear Unbiased Estimates (BLUE) of each line were extracted from equation [1] by fitting line as fixed effect. The BLUE estimates were used for downstream analysis.

Genotypic Data and Gwas

Genomic DNA was extracted from two weeks old leaves following CTAB method (Osman *et al.*, 2015). The 199 genotypes were genotyped with the Illumina iSelect 90K SNP chip (Wang *et al.*, 2014) following the manufacturer's protocol. The subsequent SNP calling was completed using the Genome Studio software package (Illumina, 2017). To avoid counterfeit marker trait associations, markers with missing data points >20% and minorallele frequency <0.05 were excluded from analysis. Markers that were monomorphic were also removed from the data set and the remaining array comprised ~31,000 SNP markers across all the chromosomes.

Population Structure and Genome Wide Association Studies

Population structure was analyzed by principal component analysis of marker data. The GWAS was performed by fitting the compressed mixed linear model (Zhang *et al.*, 2010) using the rrBLUP package version 4.5 (Endelman, 2011) in the R environment, version 3.4.0 (R Core Team, 2013). We fitted a mixed model, where markers and population structure was considered fixed effects and polygenic effects of line (kinship inferred from genotype data) and residuals were treated as random effects. In this study, we identified marker trait association (MTA) with –log (P) ≥ 4.

Pairwise LD values were estimated using TASSEL 5.0 among the significant SNP markers identified in the GWAS. Based on the annotated wheat genome IWGSC RefSec v1.0 (Apples *et al.*, 2018), we screened for any gene on the SNP representing the identified MTAs.

Results

Karnal Bunt Resistance In The Wheat Population

The percent KB infection score in the evaluated germplasm collection ranged from 0% to 25.73%. In overall population, disease infection skewed towards lower infection but continuous variation was observed (Fig. 2). Disease score was higher in commercial varieties as disease was more prominent in commercial varieties than landraces in both experimental years. Mean disease incidences are shown in Fig. 1.

Table 1. Identified SNP markers, their positions and Alleles.

Marker Name	Chromosome	Position	-log(P) value	Allele
IWB12518	1A	64623494	7.096258	T/C
IWB60792	1D	67613497	5.08594	T/C
IWB19629	2D	11778498	5.461601	A/G
IWB7061	3B	34632446	5.082242	A/G
WB12426	4A	37703462	4.937749	T/C
IWB12085	5A	32733409	4.873133	A/G

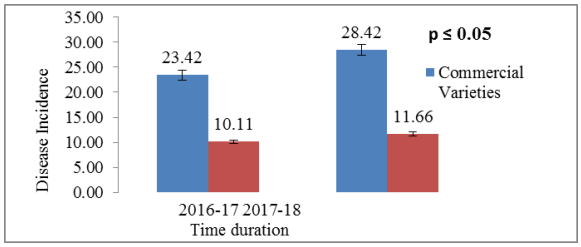

Fig. 1. Mean disease incidence on commercial varieties and landraces in two years (2016-2018).

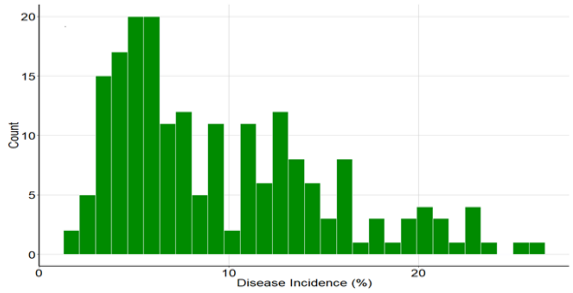

Fig. 2. Histogram of phenotypic data based on disease incidence.

Population Structure

The first two principal components explained more than 30% variation in the genotypic dataset. The first principal component seems to differentiate the population into two main groups: commercial cultivar and landraces (Fig. 3). Six land races clustered together with commercial varieties. Further two clusters were found within commercial varieties.

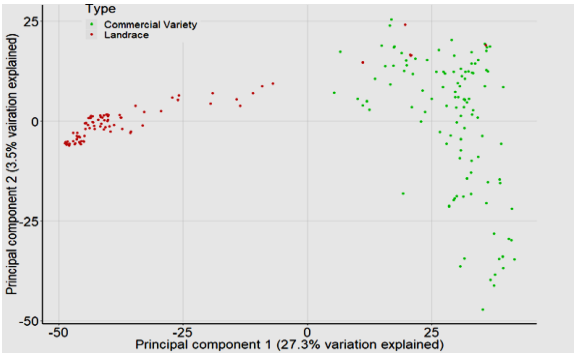

Fig. 3. Population structure of wheat population.

Markers Significantly Associated With Karnal Bunt

The association analysis identified a total of 6 marker-trait associations (MTAs) for disease resistance of KB on 6 chromosomes: 1A, 1D, 2D, 3B, 4A and 5A (Fig. 4). Out of the 6 MTAs, one on chromosome 1A was highly significant due to its highest -log (P) value 7.09.

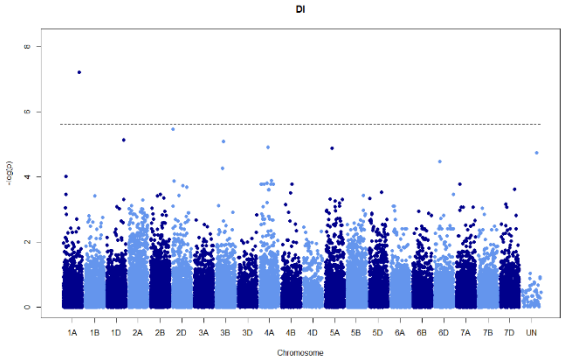

Fig. 4. Manhattan plot consisting of all markers associated with Karnal bunt.

Population Structure and Ld Decay

Linkage disequilibrium value was 0.46 and half LD decay was 0.23. Half decay distance was calculated i.e 191,500 base pairs. Fig. 5 is showing LD among SNP markers.

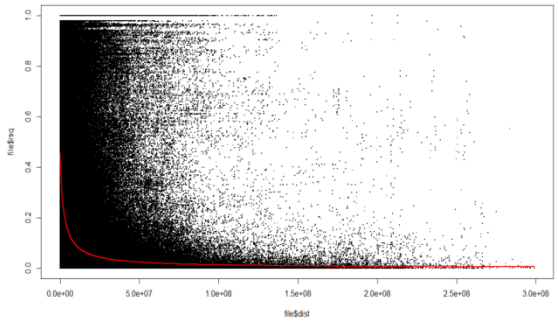

Fig. 5. Linkage disequilibrium among SNP markers.

Putative Genes Associated With Mtas for Karnal Bunt

In this study, another activity of candidate gene identification became possible due to availability of the wheat genome assembly. Searching of gene was secondary objective because we did not functionally characterize these genes due to few limitations. The transition from identification of SNPs and relating the polymorphic sites to candidate genes in self-pollinating plants is a difficult task because of LD across extended stretches of DNA. Another limitation was association of each polymorphic site with many genes which makes it very difficult to find out the candidate genes. Therefore, a follow-up study of these genes is necessary to confirm their involvement in governing the KB disease resistance. In the present study, significant and stable SNPs have been reported on chromosomes 1A, 1D, 2D, 3B,4A and 5A. The SNP marker IWB12518 on position 62643494 on chromosome 1A had the highest log (-p) value and it contained three genes coding for ABC transporter B family, diacylglycerol acyltransferase and reticulon like protein. The chromosome 1D contained SNP marker IWB60792 on position no. 67613497 with one putative gene basic helix loop helix transcription factor. Another significant SNP marker IWB 19629 on chromosome 2D located on position 11778498 encompass fourteen genes coded as cytochrome P450, subtilisin like protease, actin related protein, HR like lesion inducing protein related protein, 2-oxoglutarate (2OG) and Fe (II)-dependent oxygenase superfamily protein putative. Two genes known as non-specific lipid transfer protein were found on chromosome 3B position 34632446.

Nine genes were linked with IWB12426 on chromosome 4A with position no. 37703462 and the genes included threonyl carbamoyl-AMP synthase, AT2G18410-like protein, endo1-4-beta xylanase, adenylyl-sulfate kinase, outer mitochondrial membrane protein porin, dual specificity RNA methyltransferase RlmN, somatic embryogenesis related protein, formin 3 and kinase like. The chromosome 5A contained seven genes named Metallo hydrolase/oxyreductase superfamily protein, methyltransferase, protein kinase family protein, receptor like protein kinase, vacuolar membrane protease, DNA directed RNA polymerase subunit beta and F-box family protein on position 32733409.

Discussions

Across the globe, KB disease of wheat is of prime importance with reference to uncompromising quarantine laws in various countries. It is understood that quantitative relationship in host resistance has been observed in this disease. Very few resistant sources are identified and used in breeding programmes in countries including China, Mexico and India (Sharma *et al*., 2005, Singh *et al*., 2003, Singh *et al*., 2007, Embiri *et al*., 2019).In the current study, germplasm collection from Pakistani wheat accessions was deployed to find new sources of KB resistance. It included both historical and the latest varieties and landraces. Environmental factors are dominant in disease development that is why it becomes a challenge to screen wheat genotypes for resistance sources (Dhaliwal and Singh, 1989).

Genetic exploitation of 199 wheat germplasm was performed using Illumina iSelect 90K wheat SNP chip. After filtering 20% missing values ~31,000 markers were retained for further analysis. Besides genetic variations within and among wheat germplasm, Genome-wide Association Studies were carried out for identification of significant markers and their associated candidate genes for KB disease. Wang *et al*. (2014) developed and suggested that SNP markers, as high-density genotyping arrays, are a powerful tool for studying genomic patterns of diversity, inferring ancestral relationships between

individuals in a population and studying MTAs in mapping experiments. Sets of informative SNPs, selected based on their distribution across the genome, minor allele frequency and intervariant linkage disequilibrium, have been used to design SNP genotyping assays based on various technological principles (Cavanagh *et al*., 2013; Ganal *et al*., 2011; Kim *et al*., 2007; Song *et al*., 2013). Total of 132,000 SNPs markers have been identified to facilitate QTL and association studies of traits (Brenchley *et al*., 2013).

In hexaploid wheat, SNPs marker information is limited (Ravel *et al*., 2006). You *et al*. (2018) reported that a challenge exists for SNP discovery in polyploidy species e.g. wheat but improved and increased number of available analysis tools has made it possible. SNP array technology has been reported as successful technology and getting popularity due to automatic genotyping, cost efficiency and flexibility (Clevengerand Akinz-Ozias, 2015; Song *et al*., 2016).

Control of KB disease is difficult because of its intermittent nature. Spores of *Tilletia indica* remain viable for a long time so it is difficult to eliminate fungus from the field (Sharma *et al*., 2009). The most practicable approach for control of KB disease is to employ host resistance available in the germplasm (Sharma *et al*., 2004). Therefore, the present study was conducted to evaluate resistant sources in wheat germplasm by using 90K SNP chip. Based on phenotypic data of the field trials and 90K SNP chip, wheat germplasm population was clustered into two clearly different groups due to genetic variations found in the germplasm. One group represented landraces and the other indicated commercial varieties. 90K SNP chip used in the present study has been suggested for genotyping of worldwide wheat population including commercial cultivars and landraces. Besides wheat, SNP arrays have been successfully used for other economically important crops including maize and rice (Wang *et al*., 2014).

The results of population analysis showed that 6 landraces were clustered with group of commercial varieties showing genetic resemblance with them. These variations can be assigned to their breeding history and variations in their pedigree and parentage. Another reason is nucleotide diversity in the AABB and DD genomes that has significantly been reduced as compared to ancestral populations, indicating a major diversity bottleneck in the cultivars (Brenchely *et al*., 2012). Modern plant breeding has resulted in narrow based germplasm due to reductions in genetic diversity among wheat cultivars. Genetic diversity can be estimated by using morphological, pedigree and molecular data. Use of DNA based molecular markers; principal component analysis and cluster analysis are meaningful diversity measures for assessment of genetic diversity in a population (Sajjad *et al*., 2018). Wheat landraces collections show a wider genetic diversity than common breeding programmes. This quality has been studied in the countries with improved varieties consisted of landraces. It leads to the transfer of genetic information from landraces to commercial varieties.

There are a number of studies conducted for GWAS in wheat with reference to different aspects including yield components, agronomic traits, *Fusarium* head blight disease, rusts, powdery mildew, grain related traits, wheat growth stages and genomic prediction of quality traits (Sun *et al*., 2017; Wang *et al*., 2017). But no GWAS has been yet published for KB disease in Pakistani wheat germplasm. However, few QTLs from other countries have been identified related to KB disease in hexaploid wheat (Brar *et al*., 2018; Gupta *et al*., 2019).

The GWAS results of present study revealed that 6 significant SNP markers were associated with KB disease based on 90K SNP markers and two years disease incidence data of field trials. The identified markers were located on 1A, 1D, 2D, 3B, 4A and 5A. The chromosome 4B was first time reported for resistant QTL against KB disease on the basis of SSR markers. Several genes/QTLs were suggested for KB resistance in various wheat accessions that can be beneficial for marker assisted selection in breeding programmes (Singh *et al*., 2003). A resistant region located on chromosome 2A was also reported (Gill *et al*., 1993).

In a GWAS study conducted on wheat germplasm from Afghanistan SNPs markers on chromosome 4A were identified for KB resistance. In current study, the physical positions 37703462 and 32733409, 67613497 on chromosomes 4A, 5A and 1D respectively are different from previous reports and could be considered as novel. Chromosome 1A involved in KB resistance was also reported by Embiri *et al.* (2019) and Sehgal *et al.* (2008) but the physical position was different. Similarly, 1D was also reported by Kumar *et al.* (2007) for the first time.

In this study, thirty-two putative candidate genes were identified and previous studies have proved that resistance of Karnal bunt is controlled by one to several genes (Villareal *et al.*, 1995).Chromosome 5A harbors Traes CS5A01G035600 and putative candidate gene in this region is F-box family protein that has also been reported by Gupta *et al.*, 2019. Previously reported kinase like family protein has also been identified in one SNP on chromosome 5A and 4A conferring resistance. Another research identified one region on chromosome 5BL involved in KB disease resistance and seven markers were identified on long arm of chromosome of 5B (Kaur *et al.*, 2016). The SSR markers located on chromosomes 3BL and 4BL were also found in association with KB resistance in inbred lines (Sehgal *et al.*, 2008).

Based on previous studies, the identified significant chromosomes of present study may present novel regions for KB disease resistance in bread wheat. However, 1A chromosome is the most important, due to its log (-p) value, which may have strong influence with KB resistance in hexaploid wheat. Three genes were mapped on chromosome 1A and they are Traes CS1A01G081300 coding ABS transporter B family protein, TraesCS1A01G081400 coding Diacylglycerol acyltransferase and Traes CS1A01G81500 coding reticulon like protein. Chromosome 1D and 3D have been previously reported, for resistance against KB disease, in which these were identified by SSR markers. One SSR marker Xgwm 538 associated with KB resistance was found on 4B chromosome (Kumar *et al.*, 2015). Another SSR marker on long arm of chromosome 5A has been reported by (Vasu *et al.*, 2000).

Linkage disequilibrium value was 0.46 and half LD decay was 0.23. Half decay distance was calculated i.e 191,500 base pairs. The LD decay is relatively low because of the land races. This makes identification of candidate genes feasible as the extent of LD blocks are lower. Different LD values in wheat have been reported depending upon the aspects studied (Wang *et al.*, 2017). Sun *et al.*, 2017 calculated LD in A, B and D genome 231607, 132889 and 173750 respectively. Chao *et al.*, 2007 reported that LD decay occurs at slower rate in self-pollinating plants such as Arabidopsis, rice, barley, wheat and sorghum than out-crossing crops e.g. maize. To interpret pattern of genetic diversity in modern cultivars has become complicated due to strong selection and inbreeding with landraces which elevated the LD value and reduced genetic diversity (Chao *et al.*, 2010). As compared to commercial varieties, landraces are valuable source of genetic diversity due to their specific adaptation to their local environmental conditions and they provide a rich source of genes (Lopes *et al.*, 2015).

There is no previous report for LD of SNP markers implemented for KB disease. However, level of genetic diversity and linkage disequilibrium are affected by various factors including inbreeding, selection of favorable alleles, domestication and out crossing of crop cultivars with landraces (Luo *et al.*, 2007). Genetic diversity plays an important role in improvement of cultivars (Chao *et al.*, 2007). The A, B genome diversity levels are similar but it is reduced in D genome due to breeding history which increased LD in D genome. The results of current study and previous reports indicate diverse resistance sources present in the hexaploid wheat germplasm.

Conclusions

Resistance in Karnal bunt of wheat is a complex trait for which different genes have been identified controlling the resistance trait. The results of current study suggest that variation exists in wheat population and it can be used in breeding programs to convey resistance against KB as few lines have shown <1% karnal bunt infection in two years of experiments.

Single nucleotide polymorphism markers identified on chromosomes 1A, 5A, 2D, 4A, 3B with novel positions are helpful for further studies conducting on marker assisted selection in breeding programs. The identified genomic regions contained many genes which can be investigated further for their role in disease resistance. The present study would be helpful in molecular breeding in wheat breeding programs to eradicate KB disease.

References

Appels R, Eversole K, Feuillet C, Stein N, Feuillet K, Keller B. 2018. Shifting the limits in wheat research and breeding using a fully annotated reference genome. Journal of Science **361,** eaar7191.

Aujla SS, Gill KS, Sharma YR, Singh D, Nanda GS. 1981. Effect of date of sowing and level of nitrogen on the incidence of Karnal bunt disease of wheat in the Punjab. Indian Journal of Ecology **4,** 71-74.

Brar SG, Davilla FG, He X, Sansaloni PC, Singh PR, Singh PK. 2018. Genetic mapping of resistance in hexaploid wheat for a quarantine disease: Karnal bunt. Frontiers in Plant Sciences **9,** 1-14.

Chao S, Dubcovsky J, Dvorak J, Luo MC, Baenziger PS, Matnyazov R, Clark RD, Talbert LE, Anderson JA, Dreisigacker S, Glover K, Chen J, Campbell K, Bruckner PL, Rudd JC, Haley S, Craver FB, Perry S, Sorrells EM, Akhunov ED. 2010. Population and genome specific patterns of linkage disequilibrium and SNP variation in spring and winter wheat (*Triticum aestivum* L.). Journal of BMC Genetics **11,** 1-17.

Chao S, Zhang W, Dubcovsky J, Sorrells M. 2007. Evaluation of genetic diversity and genome wide linkage disequilibrium among U.S. wheat (*Triticum aestivum* L.) germplasm representing different market classes. Journal of Crop Science **47,** 1018-1030.

Dhaliwal HS, Singh DV. 1989. Up-to-date life cycle of *Neovossia indica* (Mitra) Mundkur. Journal of Current Science **57,** 675-677.

Emebiri L, Singh KP, Tan MK, Fuentes-Dávila G, He X, Singh RP. 2019. Reaction of Australian durum, common wheat and triticale varieties to Karnal bunt (*Tilletia indica*) infection under artificial inoculation inthe field. Journal of Crop Pasture and Science **70,** 107-112.

Endelman JB. 2011. Ridge regression and other kernels for genomic selection with R package rrBLUP. Journal of Plant Genome **4,** 250-255.

Fuentes-Davila G, Rajaram S. 1994. Sources of resistance to *Tilletia indica* in wheat. Journal of Crop Protection **13,** 20-24.

Gill KS, Sharma I, Aujla SS. 1993. Karnal bunt and wheat production. Punjab agricultural university, Ludhiana, India pp. 1-153.

Gupta V, He X, Kumar N, Fuentes-Davila G, Sharma KR, Dreisigacker S, Juliana P, Ataei N, Singh PK. 2019. Genome wide association study of karnal bunt resistance in a wheat germplasm collection from Afghanistan. International Journal of Molecular Sciences **20,** 1-15.

Illumina. 2017. Introduction to Genome Studio software. Illumina, San Diego, CA.

Kaur M, Singh R, Kumar S, Mandhan RP, Sharma I. 2016. Identification of QTL conferring Karnal bunt resistance in bread wheat. Indian Journal of Biotechnology **15,** 34-38.

Kumar S, Veena C, Neelam RY, Sharma I, Pawan KY, Kumar S, Behl RK. 2015. Identification and validation of SSR markers for Karnal bunt (*Neovossia indica*) resistance in wheat (*Triticum aestivum*). Indian Journal of Agricultural Science **85(5),** 108-113.

Lopes SM, El-Basyoni I, Baenziger SP, Singh S, Royo C, Ozbek K, Aktas H, Ozer E, Odemir F, Manickavelu A, Ban T, Vikram P. 2015. Exploiting genetic diversity from landraces in wheat breeding for adaptation to climate change. Journal of Experimental Botany **66(12),** 3477-3488.

Mehdi V, Joshi LM, Abrol YP. 1973. Studies on chapati making quality: (vi) effect of wheat grains with bunts on the quality of chapaties. Bull Grain Technol **11,** 195-197.

Mehmood A, Idrees M, Burhan M, Muhammad F, Niaz ZM. 2014. Evaluation of wheat test lines/commercial varieties against Karnal bunt under field conditions. Pakistan Journal of Phytopathology **26(1),** 137-141.

Murray MG, Brennan PJ 1998. The risk to Australia from Tilletia indica, the cause of Karnal bunt of wheat. Journal of Australian Plant Pathology **1998 (27),** 212-225.

Na-Allah MS, Muhammad A, Mohammed IU, Bubuche ST, Yusif H, Tanimu MU. 2018.Yield of Wheat (*Triticum aestivum* L.) as Influenced by Planting Date and Planting Methods in the Sudan Savanna Ecological Zone of Nigeria. International Journal of Life Science Scientific Research **4(5),** 1993-2002.

Osman M, He X, Singh RP, Duveiller E, Lillemo M, Pereyra SA, Westerdijk-Hoks I, Kurushima M, Yau SK, Benedettelli S. 2015. Phenotypic and genotypic characterization of CIMMYT's 15th International *Fusarium* Head Blight screening nursery of wheat. Journal of Euphytica **205,** 521-537.

Pandey V, Manoj S, Dinesha P, Anil K. 2018. Integrated proteomics, genomics, metabolomics approaches reveal oxalic acid as pathogenicity factor in *Tilletia indica* inciting Karnal bunt disease of wheat. Journal of Scientific Reports **8,** 7826.

R Core Team. 2013. R: A language and environment for statistical computing. R Found. Stat. Comp., Vienna.

Royer HM, Rytter JL. 1988. Comparison of host ranges of *Tilletia indica* and *T. barclayana*. Journal of Plant Disease **72(2),** 133-136.

Rush MC, Stein MJ, Bowden LR, Riemenschneider R, Boratynski T, Royer HM. 2005. Status of Karnal bunt of wheat in the United States 1996-2004. Journal of Plant Disease **89(3),** 212-223.

Sajjad M, Saeed A, Muhammad AH, Hafiz GZM, Muhammad N, Muhammad F, Manzoor H. 2018. Incidence of Karnal bunt (*Tilletia indica* Mitra) of wheat in southern Punjab, Pakistan. International Journal of Bioscience **12(2),** 280-285.

Sehgal SK, Kaur G, Sharma I, Bains NS. 2008. Development and molecular marker analysis of Karnal bunt resistant near isogenic lines in bread wheat variety PBW 343. Indian Journal of Genetics and Plant Breeding **68 (1),** 21-5.

Sharma I, Bains NS, Nanda SG. 2004. Inheritance of Karnal bunt-free trait in bread wheat. Journal of Plant Breed **123,** 96-97.

Sharma I, Bains NS, Singh K, Nanda SG. 2009. Additive genes at nine loci govern karnal bunt resistance in a set of common wheat cultivars. Journal of Euphytica **142(3),** 301-307.

Sharma I, Bains NS, Singh K, Nanda GS. 2005. Additive genes at nine loci govern Karnal bunt resistance in a set of common wheat cultivars. Journal of Euphytica **142,** 301–307.

Sharma P, Shefali P, Bharati MKK, Senthil K, Satish S, Mahender S, Sudheer K, Garima S, Sharma I, Gyanendra PS. 2018. Development and validation of microsatellite markers for Karnal bunt (*Tilletia indica*) and loose smut (*Ustilago segetum tritici)* of wheat from related fungal species. Journal of Phytopathology **166,** 729-738.

Singh N, Behl RK, Punia SM, Singh V. 2002. In vitro screening and production of Karnal bunt resistant doubled haploids in wheat. Journal of Wheat Information Service **94,** 5-8.

Singh SL. 1985. Cultural practices and the Karnal bunt (*Neovossia indica*) incidence. Journal of Indian Phytopathology **38,** 594.

Singh S, Brown-Guedira G, Grewal T, Dhaliwal H, Nelson J, Singh H, Gill B. 2003. Mapping of a resistance gene effective against Karnal bunt pathogen of wheat. Journal of Theoretical and Applied Genetics **106,** 287-292.

Singh S, Guerdia-Brown L, Grewal ST, Dhaliwal SH, Nelson CJ, Singh H, Gill BS. 2003. Mapping of a resistant gene effective against karnal bunt pathogen of wheat. Journal of Theoretical and Applied Genetics **106,** 287-292.

Singh S, Sharma I, Sehgal SK, Bains NS, Guo Z, Nelson CJ, Bowden RL. 2007. Molecular mapping of QTLs for Karnal bunt resistance in two recombinant inbred populations of bread wheat. Journal of Theoretical and Applied Genetics **116,** 147-154.

Sun C, Zhang F, Yan X, Zhang X, Dong Z, Cui D, Chen F. 2017. Genome wide association study for 13 agronomic traits reveals distribution of superior alleles in bread wheat from the yellow and Huai valley of China. Journal of Plant Biotechnology **15(2017),** 953-969.

Villareal RL, Fuentes-Davila G, Kazi MA, Rajaram S. 1995. Inheritance of resistance to *Tilletia indica* (Mitra) in Synthetic Hexaploid Wheat x *Triticum aestivum* crosses. Journal of Plant Breeding **114,** 547-548.

Wang SX, Zhu LY, Zhang X, Shao H, Liu P, Hu BJ, Zhang H, Zhang PH, Chang C, Lu J, Xia CX, Sun LG. 2017. Genome wide association study for grain yield and related traits in elite whet vareities and advanced lines using SNP markers. Journal of PlosOne **12(11),** 1-14.

Wang S, Wong D, Forrest K, Allen A, Chao S, Huang EB. 2014. Characterization of polyploid wheat genomic diversity using a high-density 90,000 single nucleotide polymorphism array. Journal of Plant Biotechnology **12,** 787-796.

Wright D, Murray G, Tan KM. 2003. 'National diagnostic protocol for the identification of *Tilletia indica*, the cause of Karnal bunt. (Department of Agriculture, Western Australia).

You Q, Yang X, Peng Z, Xu L, Wang J. 2018. Development and applications of a high throughput genotyping tool for polyploid crops: single nucleotide polymorphism (SNP) array. Journal of Frontiers in Plant Science **9,** 1-15.

Zhang D, Ersoz E, Lai QC, Todhunter JR, Tiwari KH Gore AM, 2010. Mixed linear model approach adapted for genome-wide association studies. Journal of Nature Genetics **42,** 355-360.

Zia MH, Haque MI, Rauf CA, Akhtar LH, Munir M. 2012. Comparative virulence in isolates of *Tilletia indica* and host resistance against karnal bunt of wheat. Journal of Animal and Plant Sciences **22(2),** 467-472.