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D Genome Based Karnal Bunt Resistance in Wheat

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Abstract

Among the most important and widely-cultivated crops in the world, wheat ranks third after maize and rice. The global consumption of wheat increased during the past decades and it is expected that demand for major grains, such as maize, rice, and wheat will increase 48% by 2025. The constraints limiting wheat production are associated with several biotic and abiotic stresses. Karnal Bunt (KB) disease has got a significant importance and plays a major role in deteriorating the quality of wheat grain. Coefficient of similarity and genetic distances among different sets of materials was calculated by using Unweighted Pair Group of arithmetic means (UPGMA) function. For the assessment of variations for rust resistance among cultivar, 52 genotypes were used for RAPD primers. Out of provided primers OpA-15(TTCCGAACCC), OpB-5(TGCGCCCTTC) and OpG-2(GGCACTGAGG) proved result oriented. These primers have detected total 18 loci and out of these, 11 were polymorphic with a polymorphism of 61.11%. The cluster analysis of this whole dendogram reveals that genotypes pop-49 (SABUF/7/ALTAR84/*AE. SQUARROSA*(224)//YACO/6/CROC_1/*AE. SQUARR-OSA*(205)/5/BR12*3/4/…?) and pop-52 (369) BCN/3/68112/WARD//*AE. SQUARROSA)* are showing maximum genetic diversity. These two genotypes are showing a maximum genetic distance of 9.12% as compared with all other genotypes included in analysis.

Key Words: D genome, Karnal Bunt, Wheat, Molecular Markers.
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Introduction

Wheat belongs to the genus *Triticum,* originated about 10,000 years ago in the "Fertile Crescent", which is one of the most diversified regions in the world, comprising a wide array of different habitats (Morris and Sears, 1967). The genomes of bread wheat show a high homology with several diploid and tetraploid wild species due to its allopolyploid nature (Kimber and Feldman, 1987). Consequently, introgression of genes from wild wheat species into cultivated wheat through recombination of the homoeologous chromosomes, and gene linkages of undesirable combinations can often be broken using repeated backcrossing to cultivated wheat (Valkoun, 2001).

Wheat is used mainly for human consumption and supports nearly 35% of the world's population. It is nutritious, easy to store and transport and can be processed into various types of food. The demand for wheat is expected to grow faster than any other major agricultural crop. To meet the needs of the growing human population, the forecast demand for the year 2020 varies between 840 (Rosegrant *et al.,* 1995) and 1050 million tons (Kronstad, 1998). Due to land availability of limited area of cultivation, the enhancement of wheat production must come from higher absolute yields, which can only be met by the concerted action of scientists involved in diverse agricultural disciplines and in particular by increased efforts in plant breeding (Braun *et al*., 1998). For this purpose, the plant breeders have started using DNA markers with the aim to improve the crop plants.

There are three reasons which make development of DNA markers problematic. First, wheat genome size is (16×10^9 bp), compared to barley or maize with 5×10^9 bp), making, application of several marker techniques difficult. Second, wheat is hexaploid in nature which adds complexity to many marker assays. On a gel, three sets of bands usually appear often in the same size range, which makes it difficult to manage and interpret. Third, generally wheat possesses low levels of polymorphism compared to other cereal crops. This implies that a larger number of markers must be screened than in the case of rice, barley or maize (Chao *et al*., 1989; Lui *et al*., 1990). Furthermore, level of polymorphism is not uniform across genomes and crosses. Commonly, the D genome is considered to have the poorest marker coverage (Chalmers *et al*., 2001). To date, most of the work on wheat has used random amplified polymorphic DNA (RAPD), restriction fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLP), and simple sequence repeats (SSRs).

Karnal Bunt or Partial Bunt is a fungal disease of wheat and rye. It was first detected on Durum Wheat in March 1996 in USA, Arizona Department of Agriculture. On March 25, 1996, the Animal and Plant Health Inspection Service (APHIS) issued a federal KB quarantine which directly affected the state of Arizona and six adjacent counties in Texas and New Mexico. Twenty one countries currently list KB as a

quarantine pest. It was first reported in 1931 in experimental wheat at the Botanical station at Karnal, India (Mitra, 1931) and was for many years known in the foothill plains of India and Pakistan. It is a disease of bread wheat (*Triticum aestivum*), durum wheat (*Triticum durum*), and *triticale* (wheat x rye hybrid) (Fuentes-Davilla *et al.,* 1998).

Neovossia indica (*Tilletia indica*, old name), a basidiomycetous fungus causing KB of wheat, was first reported in Northern India by Mitra (1931), subsequently in Pakistan (Munjal, 1975), Nepal (Singh *et al*., 1989), Iraq (CMI, 1989), Mexico (Duran, 1972) and in the USA (Ykema *et al*., 1996). Susceptible host cultivars are infected by air-borne sporidia at the flowering stage, and disease symptoms are evident in the form of teliospores during hardening of the grains (Gill *et al*., 1993). A fishy odour is emitted from infected seeds due to the presence of trimethylamine which makes the products unacceptable for human consumption if more than 1% of the grains are infected (Mehdi *et al*., 1973).

To develop wheat with traits that enable them to yield well under a wide range of adverse conditions, plant breeders draw upon the genetic diversity found in the crop itself, its cultivated relatives, and more recently, uncultivated wild species (Mujeeb-Kazi, 2003).

The main purposes of the study were to;

Test the D genome synthetic hexaploid wheats and their advanced derivatives with bread wheat for KB under inoculated conditions in the field.

Morphologically categorize the test germplasm phenologically for traits that are important for wheat breeding e.g. days to maturity, height at maturity and 1000 kernel weight etc.

Use RAD markers to analyze the diversity in these synthetics and their advanced derivatives in order to facilitate the generation of fingerprinting data and permitting the selection of suitable lines for wheat breeding programs.

Materials and Methods

Pathogen inoculums production

Tilletia indica colonies were developed by germinating teliospores on water agar after treating them in water with a pH of 9. Inoculum from the colonies was multiplied and maintained on Potato Dextrose Agar (PDA). *Tilletia indica* colonies grown on water agar were transferred to lids of the PDA plates. When plates became full of *Tellitia indica* cultures developed by the spores shedding from the colony on lid of the plate, the pieces of PDA baring cultures were then transferred to PDA slants in 250 ml conical flasks. In two to three weeks, slants were fully occupied by the *Tellitia indica* colony developed from shedding secondary sporidia. Slants that were fully colonized by *Tellitia indica* were washed with sterile distilled water to obtain sporidal suspension. The suspensions from different culture lots were centrifuged in a tabletop centrifuge to have sporidial pellets. The pellets were pooled and suspended in water to have diversified inoculum with spore concentrations of 40,000 to 50,000 sporidia/ml.

Boot inoculation technique

The sporidial suspension was injected into each tiller with a hypodermic syringe at the booting stage when awns were just visible. Inoculated plants were placed in glasshouse with day temperature ranging 20-30°C. Plants were lightly showered with water once in a day. Upon maturity, spikes were harvested individually and thrashed manually to record the number of infected seed.

Field screening

Material to be screened under field condition was sown in the research area of National Agriculture Research Center (NARC) Islamabad. Lines were planted in 0.5 m rows with 60cm space between the rows. Plants were inoculated at booting stage by boot inoculation technique. Two to five spikes per line were inoculated. Inoculum was injected with a hypodermic syringe into the boot when the awns were just emerging. The inoculated spikes were covered with glycine bags, properly tagged and marked with entry number and date of inoculation. The inoculated field was showered with water for 30 minutes, three times a day using field rain gun.

Glasshouse screening

For glasshouse screening, the plants were sown in 15cm diameter pots in the glasshouse in first week of January in glasshouse at Crop Disease Research Programme (CDRP) Murree. Plants at booting stage were inoculated with sporidial suspension by boot inoculation technique mentioned earlier. The inoculated spikes were glycine bagged and the glasshouse was maintained at 25-30 °C day and 10-12 °C night temperatures. During the daytime, plants were showered with water using a rain gun fitted in glasshouse at half-hour interval.

Harvesting and scoring

The inoculated heads enclosed in the glycine bags were harvested very carefully at maturity and thrashed manually prior to inspecting them for the presence of KB infection. Total number of grains and number of infected grains were counted and recorded for each line separately. Percent grains infestation in each line was calculated. Disease severity was scored for each sample on a 0-5 severity scale devised by Aujla *et al.* (1989) as shown in Fig. 1.

Molecular diagnostics

DNA based diversity of the material was determined by molecular evaluations using RAPD primers.

DNA extraction

In the growth room 5 to 7 cm long pieces of fresh leaf material were cut from the plants (3 week-old seedlings) and were placed in 1.5 ml eppendorf tubes. In order to rapidly freeze the leaf material, the tubes were then dropped in the liquid nitrogen. The frozen material was then ground to a fine powder with a knitting needle while still inside the tube. 500 µl DNA extraction buffer containing (1% SDS, 100mM tris base, 100mM NaCl, 100mM Na_2EDTA, PH: 8.5 by HCl) was added to each eppendorf tube and the material was mixed well with the help of a knitting needle. In the mixed material, 500 µl of phenol: chloroform: isoamylalcohol (in the ratio of 25:24:1) was added and tubes were shaken until a homogenous mixture is made.

Samples were then centrifuged at 5000 rpm for 5 minutes. The aqueous phase (supernatant) was then transferred to a fresh tube. To precipitate the DNA 50 µl of 3M sodium acetate (pH= 4.8) and 500 µl isopropanol was added to the tube and mixed gently. To make the DNA pellet, samples were centrifuged at 5000 rpm for 5 minutes. The supernant was discarded and the pellet was washed with 70% ethyl alcohol. The pellet was then dried at room temperature for an hour and was resuspended in 40 µl TE buffer (10mM Tris, 1mM EDTA and PH: 8.0). To remove RNA, the sample was treated with 40 µg RNAase-A (0.20 µl of commercially supplied RNAase-A purchased from Gene Link, USA) at 37 °C for 1 hour. Following RNAase-A treatment, DNA was run on 1.0% gel to check the quality of the DNA and then stored at 4ºC. For Polymerase Chain Reaction (PCR) a 1:5 dilution of DNA was made in doubled distilled, deionized and autoclaved water.

Polymerase chain reaction

PCR reactions were carried out in 25 µl reaction containing 50-100 ng total genomic DNA templates, 0.25 µM of each primer, 200 µM of each dATP, dGTP, dCTP, dTTP, 50mM KCl, 10mM Tris, 1.5mM $MgCl_2$ and 2.5 units of Taq DNA polymerase. The amplification conditions were as follows; an initial step of denaturation at 94°C for 1 minute followed by 45 cycles each consisting of a denaturation step at 94°C of 1 minute, an annealing step at 34°C of 1 minute and an extension step at 72°C of 2 minutes. Final extension step at 72°C for 7 minutes was performed to ensure the completion of the primer extension reaction. GeneAmp PCR system 2700 was used for all amplification reactions.

Gel electrophoresis

For electrophoresis of the amplification products, 1.5 % agarose/TBE gel was used. Gels were visualized by Ethidium Bromide under the UV light chamber and observed using the computer program UVIPhotoMW. The GeneRuler™ 1kb DNA Ladder (Catalogue No. SM0313, Lot: 00018968, Concentration: 0.1µg/µl) by Fermentas was used for estimating size and approximate quantification of wide range double stranded DNA fragments on agarose gel. The ladder was premixed with 6X Loading Dye Solution for direct loading on gel.

Statistical analysis

For statistical analysis of RAPD, the scorable bands were considered as a single locus/allele. The loci were scored as present or absent. Bivariate 1-0 data matrix was generated. Genetic distances were calculated using Unweighted Pair Group of Arithmetic Means (UPGMA) procedure as follows (Nei and Li, 1979).

$GD_{xy} = 1- d_{xy}/d_x+d_y-d_{xy}$,

Where GD_{xy} = Genetic distance between two genotypes, x (1) and y (2)

d_{xy} = total number of common loci (bands) in two genotypes,

d_x = Total number of loci (bands) in genotype 1 and

d_y = Total number of loci (bands) in genotype 2.

The 1-0 bivariate data matrix for each set of wheat lines based on the data of RAPD primers were used to construct dendrogram using computer program "Popgene32" version 1.31 (http://www.ualberta.ca./~fyeh/fyeh).

Results

Several lines of synthetic hexaploid wheat crop have been identified which are resistant to Karnal bunt and they were planted at NARC under the Wheat Cytogenetics Program.

Inoculation data from field and from screen house

Screening for Karnal Bunt resistance is a critical process as escape is very common phenomenon in field screening. Successful disease development may not take place if temperature rises during infection process. Successful spore germination, availability of viable inoculum and glasshouse screening thus provided reliable data to identify resistance source. Most of the Pakistani wheat varieties and breeder's material is poor in resistance to Karnal Bunt disease and indentified resistant lines can serve as an excellent source of resistance. Synthetic Hexaploid Lines found resistant in the current study have derived their resistance from their Durum and *A. squarosa* parents.

Wild cross accessions parent diversified source of resistance to Karnal Bunt. Bread-wheat crosses of the synthetic and advanced selections include lines resistant to other biotic stresses and thus serve as valuable germplasm for breeding disease resistant wheat lines. Data from D genome synthetic

can reveal valuable information regarding resistant to *Neovossia indica* (*Tilletia indica*), that can be directly crossed with bread wheat lines for their improvement. Thus revealing the information data were collected from field and from screen house it was determined that synthetic varieties showed maximum resistance to Karnal Bunt disease. Only two lines from field and five lines from screen house exhibited susceptibility.

Molecular diversity evaluation

In this study,, PCR based primers RAPD were used. Randomly Amplified Polymorphic DNA (RAPD) primers were used to detect genetic polymorphism at DNA level in ten karnal bunt synthetic hexaploid wheat and six bread wheat germplasm lines, synthetic genome DD KB, synthetic hexaploid DD KB lines resistant to Karnal bunt. Only the scorable bands were included in the analysis. Every single band was considered as a single locus/allele for all the genetic analysis. The loci were scored as present / absent. Unweighted Pair Group of Arithamatic Means (UPGMA) function (Nei & Li, 1979) was used to estimate genetic distances between the genotypes.

For the assessment of KB resistance variations in 52 genotypes RAPD primers were used. Out of provided primers OpA-15(TTCCGAACCC), OpB-5(TGCGCCCTTC) and OpG-2(GGCACTGAGG) remained result oriented. These primers have detected a total of 18 loci and out of these 11 were polymorphic with a polymorphism percentage of 61.11. The primer that has shown highest number of amplification was Opg-2, having a base sequence (GGCACTGAGG). The number of bands scored for individual primers ranged from one to three. Maximum number of bands three were traced in Sitta, by using decamer RAPD based primer Opg-2. The size of scorable bands in these cultivars ranged from 750-2000bp.

Figure (2) is showing amplification profile of decamer RAPD primer OpA-15 with a base sequence TTCCGAACCC. This primer has traced the maximum three bands in genotypes 14, 15 and 24. These genotypes are monomorphic for the bands of size 750-2000bp. Two bands were traced out by this primer in genotypes 6, 19 and 25 and these genotypes are monomorphic for the bands of sizes 1000-2000bp. One band was scored by this primer in genotypes 8 and 23 with a size of 2000bp. No amplification was observed by this primer in remaining genotypes and therefore are not included in analysis. A band of size 750bp is observed in genotypes 14, 15 and 24 but absent in other amplified genotypes. Therefore, these are polymorphic as compared to other genotypes that were amplified. Similarly genotypes 6, 19 and 25 showed a high degree of polymorphism as compared to 14, 15 and 24 because of a band of size 1000bp.

Decamer RAPD primer OpB-5(GGACTGGAGT) has done amplification only in genotype 12. This primer has scored only one band, with a size in the range of 1000-1500bp. No amplification was observed by this primer in remaining genotypes and subsequently was not included in analysis.

Figure (2) as one of the representative figures, representing the amplification profile of decamer RAPD primer OpG-2 with a base sequence of (GGCACTGAGG). The maximum three bands were shown by this primer in genotypes 35, 36, 37, 38, 40, 43, 46, 47, 49, 51 and 52. These genotypes are monomorphic for band sizes of 750-2000bp. However, a single band of size 750bp is only scored in genotypes 34 and 41. Genotypes 34 and 41 are monomorphic for this band. Two bands with a size in the range of 1000-2000bp were not traced in genotypes 34 and 48 but present in all other amplified genotypes.

Similarity matrix

According to the table of similarity matrix, the value of similarity coefficient ranged from 0.6111(61.11%) to 1.00(100%). The minimum similarity of 61.11% exists between the genotypes:

- Pop-2 and pop-49
- Pop-3 and pop-52
- Pop-4 and pop-49

Dendogram explaination

Unweighted Pair Group of Arithamatic Means (UPGMA) function (Nei & Li, 1979) was used to estimate genetic distances between the genotypes. The dendogram shows the precise analysis of genetic distances by performing the grouping of genotypes on the bases of similarities and differences. Dendogram was divided into five clusters A, B, C, D and E for an easy interpretation. In cluster, identical genotypes are placed in the smallest group of two genotypes. Cluster analysis was also performed for an easy interpretation of dendogram.

Cluster A: This cluster includes a total of five genotypes i.e pop-1, pop-2, pop-3, pop-4 and pop-5. Within this cluster pop-2 and pop-4 are showing a genetic distance of 0.00 % so they are exactly similar. Pop-3 is showing a genetic distance of 2.86 % with pop-2 and pop-4. Similarly, in this cluster pop-1 and pop-5 exhibited a genetic distance of 2.865 with one another compared with other genotypes of the cluster.

Cluster B: this cluster includes a total of 18 genotypes i.e pop-6, pop-9, pop-15, pop-22, pop-24, pop-33, pop-34, pop-35, pop-37, pop-38, pop-40, pop-42, pop-43, pop-44, pop-45, pop-48, pop-50 and pop-51. All these genotypes show a genetic distance of 0 %, hence they are showing 100% similarity.

Cluster C: this cluster includes total eleven genotypes i.e pop-7, pop-8, pop-10, pop-11, pop-12, pop-31, pop-32, pop-39, pop-41, pop-46 and pop-47. Within this

cluster the genotypes which showed a minimum genetic distance of 0 % are,

- pop-8 an pop-32
- pop-46 and pop-47

In this cluster pop-31 exhibited a maximum genetic distance of 6.54% with other genotypes pop-8 pop-10, pop-11, pop-12, pop-31, pop-32, pop-39, pop-41, pop-46, pop-47 and cluster B. so this genotype is highly diversified.

Cluster D: this cluster includes a total of nine genotypes i.e., pop-13, pop-14, pop-16, pop-18, pop-19, pop-20, pop-21, pop-23 and pop-28. Within this cluster all the genotypes with the exception of pop-16 exhibited minimum genetic distance of 0 % hence they are similar. In this cluster pop-16 is presenting a maximum genetic distance of 2.86 % compared to other genotypes of the cluster.

Cluster E: this cluster also includes a total of nine genotypes i.e., pop-17, pop-25, pop-26, pop-27, pop-29, pop-36, pop-49 and pop-52. Within this cluster genotypes pop-17, pop-25, pop-26, pop-27, pop-29 and pop-36 showed a minimum genetic distance of 0 % with each other hence these genotypes are similar. Pop-36 exhibited a genetic distance of 2.71% with pop-17, pop-25, pop-26, pop-27 and pop-29. Cluster D and E showed a genetic distance of 0.67 %. Similarly cluster B and Cluster C showed a genetic distance of 1.05 % with genotypes of cluster D and E. while genotypes of cluster A exhibited a maximum diversity of 4.95 % compared to all other genotypes of remaining clusters.

The cluster analysis of whole dendogram revealed that genotypes pop-49 and pop-52 exhibited maximum genetic diversity. These two genotypes showed a maximum genetic distance of 9.12 % compared with all other genotypes included in analysis.

Discussion

For the improvement of resistance in the present cultivars against biotic and abiotic stresses, alternative genetic resources possessing diversity are required. These valuable genetic resources for wheat improvement are found among different species in the *Triticeae* tribe and reside within the three gene pools of this family (Mujeeb-Kazi *et al*., 1998). The A-genome strategy cover the D genome that demonstrates a genetic diversity for stresses tolerance as observed in the AABBDD synthetic (Mujeeb-Kazi, 2001a). Crossing of resistant synthetic hexaploids (SH) with elite but susceptible bread wheat (BW) genotypes has yielded resistant BW/SH derivatives (Mujeeb-Kazi *et al*., 2001a, 2001b).

Promise also exists for resistances and tolerances in this SH germplasm for leaf rust, stripe rust, mineral toxicities, drought, salinity (Pritchard *et al*., 2001), heat, cold, sprouting, water logging (Villareal *et al*., 2001), high molecular weight (HMW)/low molecular weight (LMW) quality subunits, powdery mildew, loose smut, cereal cyst nematode (CCN), yield and its components. The least accessional diversity observed so far in the D genome is for scab (less than 1.0 %), however under evaluation tests conducted at one location in Mexico, the observed scab resistance is promising superior than the leading bread wheat cultivars Frontana and Sumai-3 with their assemblage of four genes (Van-Ginkel *et al*., 1996).

Karnal Bunt (KB) or partial Bunt is the most recently described smut disease of wheat (Mitra, 1931; Mundkur, 1943; Bedi *et al*., 1949) that restricts the free movement of wheat grains from one country to another. Yield losses resulting from KB are generally lesser. Survey in India was conducted which determined that in a heavy disease year it loses 0.5%, However in few cases 89% of the field is affected the and the total losses ranged from 20-40 % (Fuentes-Davilla, 2006). Coghlan, (2006) illustrated that the synthetic germplasm is an enormous wealth of genetic diversity that is being utilized internationally for wheat improvement and with its diversity for Karnal bunt forms the research material choice.

Mujeeb-Kazi, (2003) has documented the contribution of synthetics towards Karnal bunt that supports additional depth in experimentation under Pakistani conditions where Karnal bunt is a wheat production constraint and factored into new varietal releases. Durum wheats with field resistance to KB were susceptible under greenhouse inoculation tests. Hence, resistant SH wheats were interpreted in the same way because of the involvement of the respective *Aegilops tauschii* accession. Resistance genes from the SH have been transferred successfully to elite bread wheat cultivars. Homoeology of the wheat genomes with those of wild and cultivated diploids in the tribe *Triticeae* makes the latter especially *Aegilops squarrosa* ready sources of genes for wheat improvement. Accessions of several *Aegilops* species were screened for KB resistance under green-house conditions (Warham *et al*., 1986) via boot inoculation. In nine species, all tested accessions showed complete resistance. It was also observed in five of 12 *Ae. squarrosa* accessions. Among cultivated diploid *Triticeae*, all barley (*Hordeum vulgare* L.) and many rye (*Secale cereale* L.) accessions proved highly KB-resistant (Warham, 1988), indicating their potential use as resistance sources.

Fuentes-Davilla *et al*. (1992) has documented that at CIMMYT, breeding for genetic resistance to *Tellitia indica* is based on the identification of sources of resistance and hybridization in order to incorporate

resistance genes into agronomically suitable genotypes, and evaluation and selection of plant progenies to develop advanced lines. Among the sources evaluated are *Triticum aestivum, Triticum turgidum, Triticosecale* and synthetics of '*Aegilops tauschii / Triticum turgidum*. Resistance diversity is based upon the disease screening data over several years in a testing location within Mexico. Selected immune SH from diverse *Aegilops tauschii* accessions were crossed with elite but KB-susceptible bread wheat cultivars. Progeny was advanced by the pedigree method leading to the selections of advanced derivatives that were freed threshing and resistant to KB. The cluster analysis of this whole dendrogram reveals that genotypes pop-49 (SABUF/7/ALTAR 84/ *AE. SQUARROSA* (224)//YACO/6/CROC_1/ *AE. SQUARROSA* (205)/5/BR12*3/4/…?) and pop-52 (36 9) BCN/3/68112/WARD// *AE. SQUARROSA)* exhibited maximum genetic diversity. These two genotypes showed a maximum genetic distance of 9.12 % as compared with all other genotypes included in analysis. This diversity is incorporated from *Aegilops tauschii,* as indicated by their pedigree. These can be bred with our current cultivars to make them resistant against Karnal bunt.

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References

Aujla, S.S., I. Sharma and B.P. Singh. (1989). Rating scales for identifying wheat varieties resistant to Neovossia indica, *India Phytopathol.,* 41: 161-162.Bedi, S.K.S., M.R. Sikka and B.B. Mundkur. (1949). Transmission of wheat bunt due to Neovossia indica. *Indian Phytopathol.,* 2: 20-26.

Braun, H.J., T.S. Payne, A.I. Morgounov, M.V. Ginkel and S. Rajaram. (1998). The challenge: One billion tons of wheat by 2020. pp. 33-40. In A.E. Slinkard (ed.) Proc. Int. Wheat Genet. Symp. 9th, Saskatoon, Canada. 2-7 Aug. 1998. Extension Division, University of Saskatchewan, SK.

Chalmers, K.J., A.W. Campbell, J. Kretschmer, A. Karakousis, P. Henschke, S. Pierens, N. Harker, M. Palotta, G.B. Cornish, M.R. Shariflou, L.R. Rampling, A. McLauchlan, G. Daggard, P.J. Sharp, T.A. Holton, M.W. Southerland, R. Appels and P. Langridge. (2001). Construction of three linkage maps in bread wheat, *Triticum aestivum*. Aust. J. Agr. Res., 52:1089-1119.

Chao, S., P.J. Sharp, A.J. Worland, E.J. Warham, R.M.D. Koebner and M.D. Gale. (1989). RFLP-based genetic linkage maps of wheat homoeologous group 7 chromosomes. Theor. Appl. Genet., 78: 495-504.

CMI. (1989). Distribution map of plant diseases Tilletlia indica map no 173. CAB International Wallingford UK.

Coghlan, A. (2006). Synthetic wheat offers hope to the world. New Scientist, 11th February 2006.

Duran, R. (1972). Further aspects of teliospore germination in North American smut fungi. Can. J. Bot., 50: 2569–257.

Fuentes-Davilla, G., R. Delgado and A. Mujeeb-Kazi. (1998). Karnal bunt resistance in D genome synthetic hexaploid (SH) and in their susceptible bread wheat /SH cross derivatives. Agronomy abstracts. Am. Soci. Agro., 78.

Fuentes-Davilla, G. (1992). Identification of sources of resistance in *Tilletia indica*. Update on Karnal bunt research in Mexico. Wheat special Report No. 7, 12-13, CIMMYT, Mexico.

Gill, K.S., I. Sharma and S.S. Aujla. (1993). Economic importance of Karnal bunt. In: K.S. Gill, I. Sharma and S.S. Aujla. (eds) Karnal bunt and wheat production. PAU Press, Ludhiana, India, 20–23.

Kimber, G. and M. Feldman. (1987). Wild wheat. An introduction. pp. 129-131. Special Report 353, College of Agriculture, University of Missouri, Colombia.

Kronstad, W.E. (1998). Agricultural development and wheat breeding in the 20th century. PP. 1-10. In Braun HJ, Altay F, Kronstad WE, Benival SPS, McNAb (eds.) Wheat: Prospects for global improvement. Proc. Of the 5th Int. Wheat Confernce, Ankara, Turkey, Developments in Plant Breeding v. 6. Kluwer Academic Publishers, Dordrecht.

Lui, Y.G., N. Mori and K. Tsunewaki. (1990). Restriction fragment polymorphism (RFLP) analysis in wheat. I. Genomic DNA library construction and RFLP analysis in common wheat. Jpn. J. Genet., 65: 367-380.

Mehdi, V.L.M., Y.P Joshi and K. Abrol. (1973). Studies on chapati making quality: (vi) effect of wheat grains with bunts on the quality of chapaties. Bull. Gra. Tech. 11:195–197.

Mitra, M. (1931). A new bunt on wheat in India. Ann. Appl. Biol., 18:178–179.

Morris, R. and E.R. Sears. (1967). The cytogenetics of wheat and its relatives. In Quinsberry KS. Reiz LP. (eds.). Wheat and wheat

improvement. AgronomyMonograph 13. Am. Soci. Agro. Inc., Madison, WI.

Mujeeb-Kazi, A., L.I. Gilchrist, G. Fuentes-Davila and R. Delgado. (1998). Production and utilization of D-genome synthetic hexaploids in wheat improvement. In. Jaradat AA, ed. Proc. 3rd Int. Triticeae Symp. ICARDA, Aleppo, Syria, 369-374. Science Publishers.

Mujeeb-Kazi, A. (2001a). Synthetic hexaploids for bread wheat improvement. In 4th Int. Triticeae Symp. Abstract, University of Cordoba, Cordoba, Spain, 10-12 Sept. 37.

Mujeeb-Kazi, A. (2001b). Intergeneric hybrids in wheat: current status in CIMMYT. In 4th Int. Triticeae Symp. Abstract, University of Cordoba,Cordoba, Spain, 10-12 Sept. 111.

Mujeeb-Kazi, A. (2003). New genetic stocks for durum and bread wheat improvement. Tenth International Wheat Genetics Symposium, Paestum, Italy. 772-774.

Mundkur, B.B. (1943). Studies in cereal smuts. V. Mode of transmission of the Karnal bunt of wheat. Indian. J. Agr. Sci. 13: 54-58.

Munjal, R.L. (1975). Status of Karnal bunt (*Neovossia indica*) of wheat in northern Indian during 1968-1969 and 1969-1970. Indian. J. Myc. Plant. Pathol. 5(2): 185-187.

Nei, N and W. Li. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. P. Natl. A. Sci., 76: 5269-5273.

Pritchard, D.J., P.A. Hollington, W.P. Davies, J. Gorham, J.L. Diaz-de-Leon and A.Mujeeb-Kazi. (2001). Ann. Wheat Newslett., 47: 103-104.

Rosegrant, M.W., A. Agcaoili-Somlilla and N. Perez. (1995). Global food projections to 2020. Discussion paper v. 5. IFPRI, WA.

Singh, D.V., R. Aggarwal, J.K. Shreshtha, B.R. Thapa and H.J. Dublin. (1989). First report of Neovossia indica on wheat in Nepal. Plant Dis., 73:277.

Valkoun, J.J. (2001). Wheat pre-breeding using wild progenitors. Euphytica. 119:17-23.

Van-Ginkel, M., W. Van-Der-Schaar, Y. Zhuping and S. Rajaram. (1996). Inheritance of resistance to scab in two wheat cultivars from Brazil and China. Plant Dis., 80: 863–867.

Villareal, R.L., K. Sayre, O. Bañuelos and A. Mujeeb-Kazi. (2001). Registration of four synthetic hexaploid wheat (*Triticum turgidum*/*Aegilops tauschii*) germplasm lines tolerant to water logging. Crop Sci., 41: 274.

Warham, E.J., A. Mujeeb-Kazi and V. Rosas. (1986). Karnal bunt (*Tilletia indica*) resistance screening of Aegilops species and their practical utilization for *Triticum aestivum* improvement. Can. J. Plant Pathol., 8: 65-70.

Warham, E.J. (1988). Screening for Karnal bunt (*Tilletia indica*) resistance in triticale, rye, and barley. Can. J. Plant Pathol., 10: 57–60.

Ykema, R.E., J.P. Floyd, M.E. Palm and G.L. Peterson. (1996). First report of Karnal bunt of wheat in the United States. Plant Dis., 80:1207.

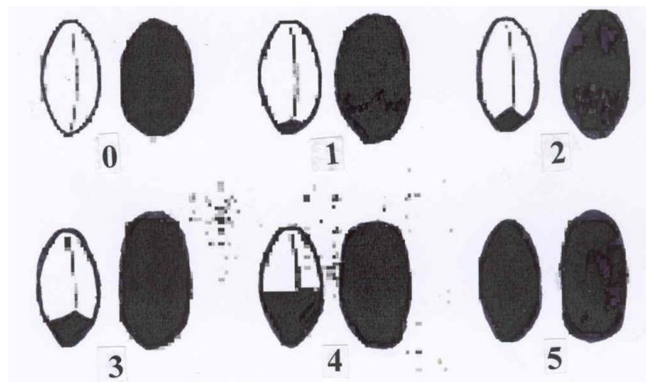

Fig. 1: Rating scale of Karnal Bunt. Severity is increasing from 0-5. 0 is not infected and 5 is highly infected.

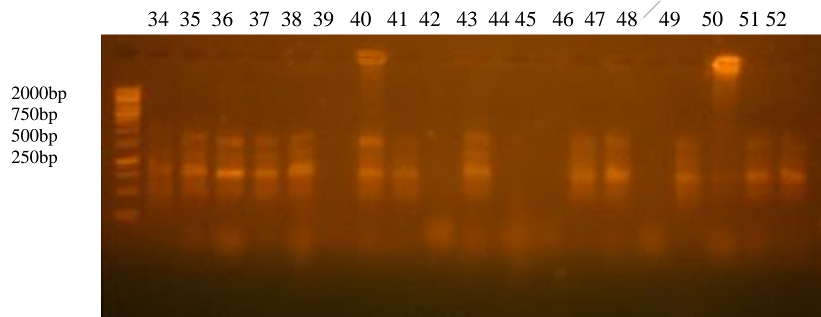

Fig. 2: Agarose (1.7 %) gel representing PCR amplification products of decamer primer OpG-2 (GGCACTGAGG) from DNA of various wheat genotypes resistant to Karnal bunt. Lane 0 is molecular weight marker (GeneRuler^{TM} 1kb DNA Ladder, Cat # SM0313, Fermentas) and lanes 34-52 are representing the genotypes in the same order as indicated in Table1.

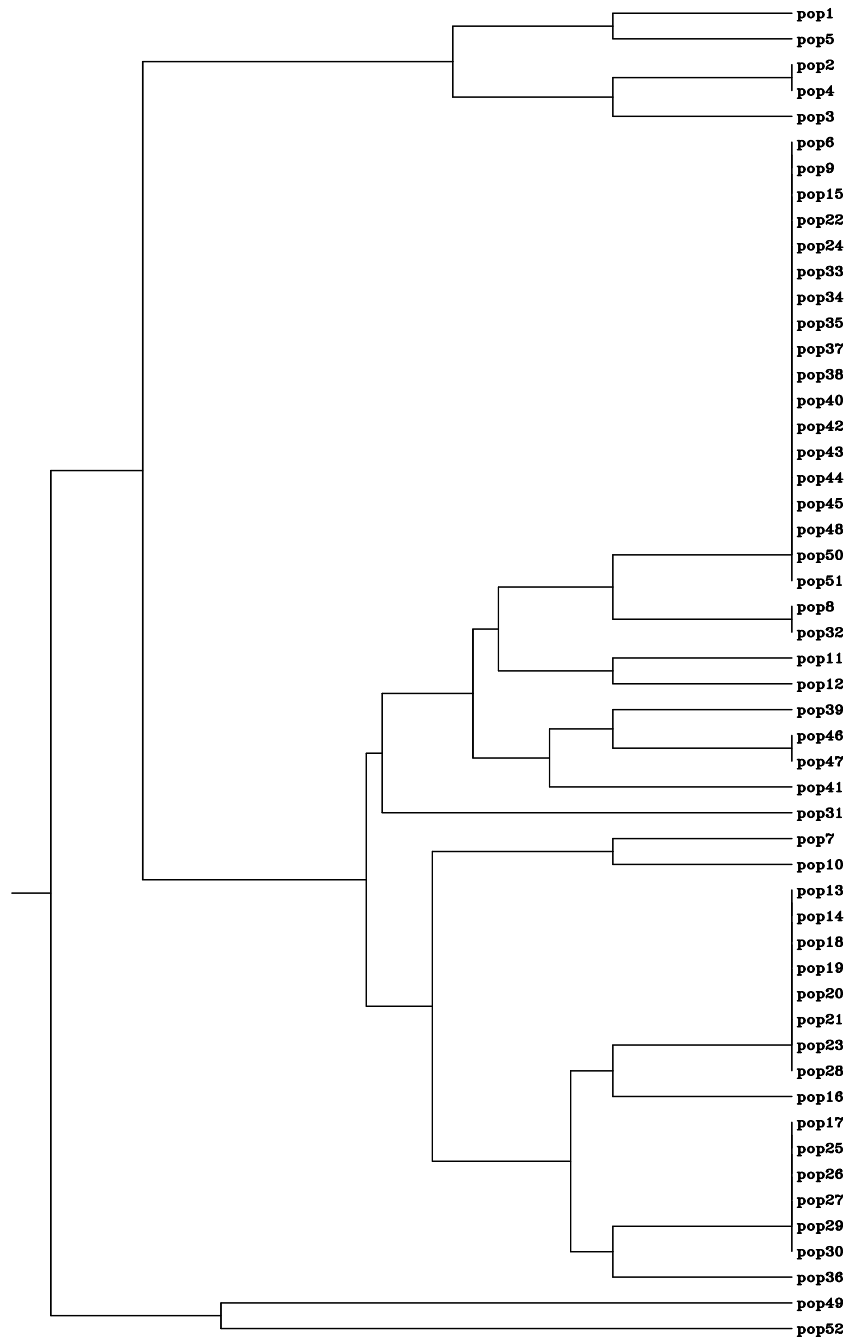

Fig. 3, Clusters formation of 52 genotypes

Table 1: Pedigree of Ten Karnal bunt synthetic hexaploid wheat and six bread wheat germplasm lines

1. CETA/*AE. TAUCHII* (174)+
2. ALTAR 84/ *AE. TAUCHII* (188)
3. ALTAR 84/ *AE. TAUCHII* (192)
4. YUK/ *AE. TAUCHII* (217)
5. YAV2/TEZ// *AE. TAUCHII (249)*
6. *DOY 1/ AE. TAUCHII* (447)
7. *DOY 1/ AE. TAUCHII* (458)
8. *SCA/ AE. TAUCHII* (518)
9. *YAR/ AE. TAUCHII* (518)
10. 68.111/RGB-U//WARD/3/FGO/4/RABI/ *AE. TAUCHII* (629)
11. CROC 1/ *AE. TAUCHII* (205)//FLYCATCHER
12. CROC 1/ *AE. TAUCHII* (224)//KAUZ
13. ALTAR 84/ *AE. TAUCHII* (221)//YACO
14. CROC1/ *AE. TAUCHII* (205)//KAUZ
15. CROC 1/ *AE. TAUCHII* (205) //BORLAUG 95
16. CROC 1/ *AE. TAUCHII* (213)//PAPAGO M86

Synthetic genome dd r. Kb

17. DOY/*AE. SQUARROSA* (188)
18. ALTAR 84/ *AE. SQUARROSA* (192)
19 ALTAR 84/ *AE. SQUARROSA* (193)
20 ALTAR 84/ *AE. SQUARROSA* (205)
21 CPI/GEDIZ/3/GOO//JO69/CRA/4/ *AE. SQUARROSA (208)*
22 ALTAR 84/ *AE. SQUARROSA (211)*
23 D67.2/P66.270// *AE. SQUARROSA (217)*
24 YUK/ *AE. SQUARROSA* (217)
25 D67.2/P66.270// *AE. SQUARROSA* (218)
26 ALTAR 84/ *AE. SQUARROSA* (219)
27 ALTAR 84/ *AE. SQUARROSA (220)*
28 DVERD_2/ *AE. SQUARROSA* (221)
29 ALTAR 84/ *AE. SQUARROSA* (221)
30 ACO89/ *AE. SQUARROSA* (309)
31 GARZA/BOY// *AE. SQUARROSA* (311)
32 68112/WARD// *AE. SQUARROSA* (369)
33 68112/WARD// *AE. SQUARROSA* (369)
34 doy1/ *AE. SQUARROSA* (511)
35 doy1/ *AE. SQUARROSA* (515)
36 68.111/RGB-U//WARD/3/FGO/4/RABI/5/ *AE. SQUARROSA* (629)

Synthetic hexaploid dd karnal bunt

37 LTAR 84/ *AE. SQUARROSA* (198)
38 DVERD_2/ *AE. SQUARROSA* (221)
39 ALTAR 84/ *AE. SQUARROSA* (223)
40 CROC_1/ *AE. SQUARROSA* (224)

Lines resistant to Karnal bunt

41 LTAR 84/ *AE. SQUARROSA* (219)//OPATA/3/ALTAR 84/ *AE. SQUARROSA* (191)//OPATA
42 CROC_1/ *AE. SQUARROSA* (205)//KAUZ
43 CROC_1/ *AE. SQUARROSA* (205)//FCT
44 CROC_1/ *AE. SQUARROSA* (205)//FCT
45 CROC_1/ *AE. SQUARROSA* (205)//2*FCT
46 DVERD_2/ *AE. SQUARROSA* (221)/3/SHAB//PRL/VEE#6
47 CN//CETA/ *AE. SQUARROSA* (895)
48 SABUF/3/BCN//CETA/ *AE. SQUARROSA* (895)
49 SABUF/7/ALTAR 84/ *AE. SQUARROSA* (224)// YACO/6/CROC_1/ *AE. SQUARROSA* (205)/ 5/BR12*3/ 4/
50 ZHONG 4//2*BUC/PVN
51 BCN*2//DVERD_2/ *AE. SQUARROSA* (247)
52 (36 9) BCN/3/68112/WARD// *AE. SQUARROSA*