Aegilops tauschii: a valuable source for karnal bunt resistance

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ABSTRACT

Karnal bunt (KB) of wheat caused by *Tilletia indica* adversely affects international wheat trade and the movement of germplasm due to quarantine restrictions. The genetic base of cultivated wheats for Karnal bunt resistance is extremely narrow to achieve the required zero tolerance. The wild germplasm of wheat, however, represent a rich and still unexploited source for KB resistance. Here we report the evaluation of *Aegilops tauschii* germplasm for KB resistance, identification of resistance sources and transfer of resistance to *T. aestivum*. A collection of 183 accessions of *Ae. tauschii*, were screened under artificial inoculation with a mixture of nine KB isolates for 2-3 years in a specially designed screen house. Twenty-two accessions of *Ae. tauschii* were found to be highly resistant whereas 10 were moderately resistant. For transferring KB resistance to hexaploid wheat, a synthetic amphiploid between a KB resistant *Ae. tauschii* accession (#3743) and KB susceptible *T. durum* cultivar was crossed with KB susceptible elite wheat cultivar. Homozygous introgression lines (ILs) derived from this cross were evaluated extensively for KB resistance under field as well as screen house conditions for four years (2004-2007). Mean KB incidence in KB resistant ILs ranged from 0-1.2% compared to 10.7% in the recipient parent and 30% in a highly susceptible cultivar. Molecular characterization and graphical genotyping of the ILs using 60 D-genome specific polymorphic markers detected introgressions of *Ae. tauschii* specific alleles on chromosomes 1D, 2D, 4D and 6D. The number of introgressed segments, however, varied from line to line. The work for further tagging and marker assisted pyramiding of the introgressed resistance in elite wheat cultivars is in progress.

INTRODUCTION

Karnal bunt (KB), caused by *Tilletia indica* was first discovered in Karnal, India (Mitra, 1931) and is an important disease of wheat resulting in grains filled with masses of fungal spores. It has been reported in several countries including India, Nepal, Pakistan, Afghanistan, Iran, Iraq, South Africa, Mexico and U.S.A. The disease can be damaging even at a low incidence due to the discolouration of flour and the generation of a fishy odour (Mehdi *et al*., 1973). The disease has become important worldwide due to the strict international quarantine measures imposed by number of countries (Herrman *et al.,* 2003). The pathogen is soil, seed and air borne in nature, it is difficult to control once introduced and established in an area. The host genetic resistance is the most effective, economical and eco-friendly method of KB management. The genetic base of KB resistance in bread wheat is extremely narrow. The wild relatives of wheat represent a rich source of genes for resistance to various wheat pathogens (Gale and Miller, 1987; Jauhar, 1993; Jiang *et al*., 1994; Singh and Dhaliwal, 2000). Progenitor species comprising the primary gene pool of wheat offer an advantage over non-progenitor species for the transfer of useful variability since their chromosomes recombine freely with wheat chromosomes.

Ae. tauschii, the D genome donor of wheat, represent a valuable source of useful genetic variability. Nine leaf rust resistance genes have been transferred from *Ae. tauschii* to hexaploid wheat (McIntosh et al. 2007). Because of its importance as a potential donor of desirable genes for the improvement of cultivated wheats, this species has been the object of intense genetic studies. The present investigation reports the identification of new sources of KB resistance from *Ae. tauschii* germplasm and its transfer to cultivated wheat background.

IDENTIFICATION OF KB RESISTANT AE. TAUSCHII ACCESSION

Ae. tauschii germplam was evaluated for KB resistance in a specially designed screen house where the optimum environmental conditions conducive for KB development were simulated with controlled temperature, humidity, fogging and shading. In the KB screen house, *Ae. tauschii* lines were inoculated at the boot stage with a mixture of nine KB isolates representing pathogen variability from North Western Plains of India (Sharma *et al*., 1998). Boot inoculations were done with the sporidial suspensions consisting of 10,000 sporidia/ml of water following the syringe method (Aujla *et al.,* 1982). Susceptible check variety WL711 was inoculated each day to ensure the infection ability of the inoculum. A KB resistant check HD29 was also included for comparison. A total of 25-30 spikes from each accession were inoculated. These were harvested in bulk and thrashed manually and percentage of diseased grains was recorded.

Because of limited space available in the screen house, it was not possible to test all the accessions in any one year, hence a limited number of accessions were tested each year. The accessions identified as resistant in any year were retested in the following years. KB incidence in the *Ae. tauschii* accessions in the screen house ranged from 0-77%. Overall, a total of 183 accessions were

screened for KB resistance during the crop seasons 2003-04, 2004-05 and 2005-06. Based on repeated screenings over three years, seven accessions (pau#14095, 14106, 14130, 14178, 14195, 14233 and 14245) were identified as having high level and four (pau#14091, 14160, 14228 and 14249) having moderate levels of KB resistance (Chhuneja *et al.* 2008). An additional 15 accessions showing a high levels of resistance and six with moderate resistance were identified based on two years of screening. Two accessions (14130 and 14195) showed no detectable infection over all the three years when the resistant check HD29 recorded 2.1-8.1% infection.

TRANSFER OF KB RESISTANCE TO CULTIVATED WHEAT BACKGROUND

For the transfer of KB resistance from *Ae. tauschii* to bread wheat, an amphiploid involving a susceptible *T. durum* cultivar WH 890 and KB resistant *Aegilops tauschii* accession PAU#3743 was synthesized. The synthetic hexaploid was crossed with a KB susceptible "Veery" line and subsequent generations screened for KB resistance. A selected derivative with high level of KB resistance were recovered and crossed with PBW343, a widely grown cultivar, to transfer KB resistance to desirable agronomic background. Homozygous introgression lines (ILs) were selected in the advanced self generations and evaluated for KB resistance. The same inoculation procedure as described above was followed for KB screening of the ILs from the cross *T. durum*-*Ae. tauschii*/Veery#5//PBW343 in the field except that a mixture of four isolates was used for inoculations.

Twelve selected homozygous introgression lines derived from the cross *T. durum* cv. WH890-*Ae.tauschii*/Veery#5//PBW343 were screened extensively against KB through artificial inoculations under field conditions as well as in the KB screen house during 2004-07 along with the recipient parent and a susceptible check WL711. The KB incidence recorded in the field inoculations is summarized in Table 1.

The recipient parent PBW343 and susceptible check WL711 had mean KB incidence of 7.6-10.7% and 27.4-60.2%, respectively. The PBW343-*Ae. tauschii* introgression lines 2544 and 2550 were found to have highest level of KB resistance with KB incidence <1.0% in all the three screenings followed by ILs 2535, 2536, 2568 and 2611 with <2% KB incidence. Two of the introgression lines 2516 and 2528 were found to be moderately susceptible with >5% KB. Based on the plant type and KB incidence ILs 2544 and 2568 have been selected for developing mapping populations for mapping of the KB resistance of *Ae. tauschii*. These introgression lines are also being used as donor stocks for transferring this new KB resistance to elite wheat backgrounds.

Table 1. KB incidence in introgression lines derived from the cross WH890-*Ae.tauschii*/Veery#5// PBW343

Genotype	Mean KB incidence (%)			KB reaction[1]
	2004-05	2005-06	2006-07	
PBW343	7.6	8.5	10.7	MS
WL711	60.2	27.4	33.0	S
2516	8.3	0.8	0.36	MS
2522	2.6	0.3	0.58	MR
2528	12.3	0.3	3.60	MS
2535	1.5	0	0.86	R
2536	1.2	0	0.94	R
2540	3.7	0.1	1.54	MR
2544	0.7	0	0	R
2550	0.4	0.9	0.07	R
2556	2.1	0	2.13	MR
2568	1.1	0	0.76	R
2596	3.0	0	0	MR
2611	1.1	0	0.18	R

[1]ILs with KB incidence of <2% were classified as resistant (R) , ones with 2-5% as moderately resistant (MR), with 5-10% as moderately susceptible (MS) and with >10% as susceptible (S). Highest KB incidence in any of the years was used for assigning KB reaction

MOLECULAR CHARACTERIZATION OF INTROGRESSION LINES

Eight resistant (2522, 2535, 2536, 2544, 2556, 2568, 2596, 2611) and one moderately susceptible IL (2516) were selected for molecular characterization. For this, DNA from all the ILs was isolated using the CTAB method. A total of 120 D-genome specific SSR markers spanning all the seven linkage groups were used for identifying the introgressed segments in the selected PBW343-*Ae. tauschii* introgression lines. Sixty primers (50.0%) showed polymorphism between both the parents in 2.5% agarose gels. Maximum polymorphic primers were detected on chromosome 1D and minimum on chromosome 3D. The selected ILs were analysed with polymorphic SSR markers for the identification of introgressed regions from *Ae. tauschii*. Introgressions of *Ae. tauschii* specific alleles were found on chromosomes 1D, 2D, 4D and 6D. Number of introgressed segments varied from line to line (Fig. 1). Introgression regions on chromosome 1D represented by SSR markers *Xcfd15* and *Xcfd61* and on 2D represented by *Xcfd56* were present in six of the eight KB resistant ILs but absent in the KB susceptible line. One region on chromosome 6D spanning *Xcfd13* was present in all the seven KB resistant ILs but absent in susceptible line. Based on the comparison of introgressed regions in KB resistant and susceptible ILs, it is suggested that the KB resistance

gene(s) in these wheat-*Ae. tauschii* introgression lines may be located on chromosome 1D, 2D or 6D.

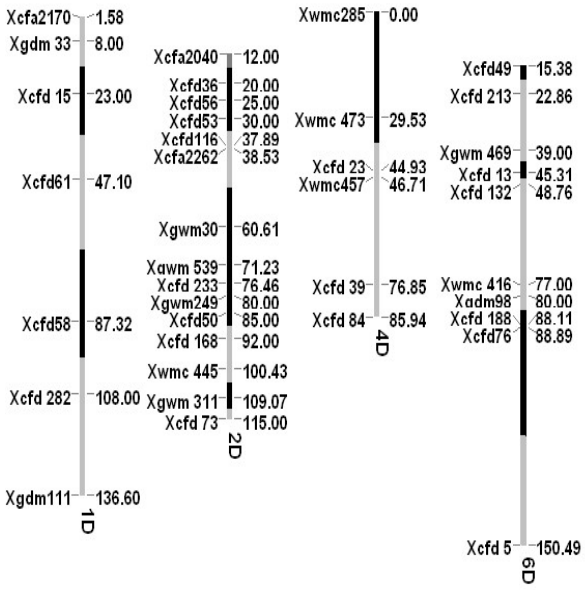

Fig. 1. Graphical genotyping of KB resistant wheat-*Ae. tauschii* introgression line 2544 using D genome specific SSR markers. Grey areas represent wheat specific alleles and black areas indicate introgression of *Ae. tauschii* specific alleles on chromosomes 1D, 2D, 4D and 6D. Map distances are according to Komugi composite wheat map (http://www.shigen.nig.ac.jp/wheat/komugi/maps and Somers et al. 2004.

Mapping populations are being developed for mapping of KB resistance transferred from *Ae. tauschii* acc. 3743 to PBW343 using the introgression profiling of these ILs. The PBW343-*Ae. tauschii* ILs are also being used for wheat germplasm enhancement for KB resistance. The KB resistant *Ae. tauschii* accessions identified during the present investigation are being used for transferring additional KB resistance genes to bread wheat background using *T. durum* as bridging species.

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