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Development of Karnal bunt resistant bread wheat doubled haploid lines

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Abstract

A set of 151 doubled haploid lines were generated from crosses between elite bread wheat lines and Karnal bunt resistance donors using wheat x maize system. Donor alleles of three markers; *Xgwm99* (1AL), *Xwmc 445* (5AL) and *Xgwm340* (3BL) known to be associated with KB resistance were run on the DH lines. After preliminary screening for resistance and plant type, 24 lines along with four checks were chosen for yield testing and confirmatory screening against Karnal bunt. Seventeen of the doubled haploid lines showed Karnal Bunt score of less than 5% whereas check varieties, PBW 343 (18.3%), PBW 550 (20.4%), PBW 621 (20.1%) and HD 2967 (13.6%) showed higher levels of Karnal Bunt infection. A Karnal Bunt score of less than 1% was observed in three doubled haploid lines, namely BWL 0009 (0.60%), BWL 0001 (0.67%) and BW 9996 (0.87%). Two doubled haploid lines (BW 9986 and BW 9991) combining Karnal Bunt resistance (< 2%) and high yield were identified and promoted to multilocation evaluation.

Key Words: Wheat, Karnal bunt, doubled haploids, *Neovossia indica*

Introduction

India is the second largest producer of wheat in the world. India's participation in the international wheat trade however is not commensurate with being a major wheat producer. This may, to some extent, be attributed to prevalence of Karnal bunt (KB) in the major wheat growing zone of the country. Internationally, more than twenty countries have placed an embargo on the import of Karnal bunt infected wheat. Karnal bunt caused by *Neovossia indica* (Mitra) Mundkur (syn. *Tilletia indica)* was first noticed in the samples collected from Karnal (Haryana, India) by Mitra in 1931. Since then, the disease has been of frequent occurrence in northern India and has been reported in parts of several countries including Afghanistan, Iran, Iraq, Mexico, Nepal, Pakistan, South Africa, and the USA (Bonde *et al.*, 1997; Rush *et al.,* 2005). Karnal bunt was detected in Mexico in 1972 and in 1983, the United States and about 70 other countries placed quarantines on import of wheat from regions where Karnal bunt was known to occur. Thus losses on account of Karnal bunt prevalence are mostly due to trade restrictions. Detection of the disease in Mexico caused an alarm as the germplasm exchange programme of the International Centre for Maize and Wheat Improvement located there could be jeopardized. Brennan *et al.* (1990) has evaluated the indirect economic losses incurred on account of this disease in Mexico. The acute perception of Karnal Bunt threat was also evident when US Secretary of Agriculture declared a state of emergency in response to the first detection of KB in USA (in Arizona State) in 1995.

The fungus attacks the developing kernels where it produces a sorus containing black teliospores. KB is seed and soil-borne and also has an airborne sporidial stage (Carris *et al.,* 2006; Garrett and Bowden 2002).The KB infected grains emit a fishy odour due to the presence of trimethyl amine. Mehdi *et al.* (1973)observed that even 3% infected grains change the colour and palatability of wheat meal and products. Widely grown varieties such as PBW 343 in the major wheat growing area of the country are, however, susceptible to the disease. The development of disease resistant varieties through conventional breeding methods is hampered because of the complex genetics of KB resistance and tedious screening procedure. Extensive work on the development of KB screening method (Aujla *et al.,* 1982), identification of resistance donors and genetics of resistance (Sharma *et al.,* 2005) has been carried out at Punjab Agricultural University Ludhiana. Unlike rusts, subjecting a large amount of segregating material to KB screening may not be practically feasible.

The opportunity offered by the doubled haploid approach can be relevant in this context. Homozygous lines can be derived from crosses performed for the incorporation of KB resistance in one step. This not only allows rapid mobilization of KB resistance genes into high yielding backgrounds but also facilitates the screening for resistance

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and confirmation of gene transfer. Adequate ground work in terms of development of wheat x maize system of doubled haploid production has already been undertaken in the wheat section of Department of Plant Breeding and Genetics (Bains *et al.* 1998; Sood *et al.,* 2003; Srivastava *et al.*, 2012). Karnal Bunt resistant versions of PBW 343 were developed at Punjab Agricultural University but they could not be deployed commercially due to stripe rust susceptibility. Considering these points, the present study was planned to combine KB with stripe rust resistance and other useful traits using doubled haploid technique.

Material and methods

The plant material used for the study included (i) established KB resistance donors e.g., HD 29, MRNG 's', Frame; (ii) donors representing KB resistance in high yielding backgrounds e.g., BW 5183, KBLR 22, KB 34-2; (iii) donor lines derived from 'resistant x resistant' crosses and thus carrying high levels of resistance e.g., AMSEL/ALDAN, ALDAN/W 485, HD 29/W 485 and W 485/HD 29; (iv) stocks with new, effective rust resistance genes e.g., BIO 582; (v) advanced breeding lines and (vi) released cultivars as listed below:

Lines/Stocks used for doubled haploid production and their brief description

BW 5183	PBW 343+ KB resistance
KBLR 22, KB 34-2	PBW 343+KB resistance +*Lr 24*
BIO 582	WL 711+*Lr 57/Yr 40* (Rust resistance genes from *Aegilops ovata*)
DBW 16, PBW 343, PBW 550	Released cultivars
DBW 18, BW 3558, BW 3652, BW 5221	Rust resistant and high-yielding advance breeding lines
KBD, AMSEL/ALDAN, ALDAN/W 485, FRAME, HD 29/W 485, W 485/ HD 29,MRNG 'S'	Donor stocks For KB resistance

DH production: The wheat plants were sown in first week of September during 2007-08 at PAU, Ludhiana. The potted plants were maintained in cold chamber at about 12^{0}C at night and moved out into the open on trolleys during the day. Wheat x maize crosses were performed from mid November onwards. Wheat tillers were emasculated and detached thereafter. Pollinations with maize pollen were done on detached tillers maintained indoors in sulphurous acid (0.8%) containing nutrient medium. Embryo rescue was performed 15-18 days after pollination. Regenerated plants were transplanted into the soil in January and on emergence of 2-3 tillers, they were treated with 0.05% colchicine for chromosome doubling. Treated plants were transplanted back into the field and the seed from the doubled sectors were harvested in early May. The protocol development and detailed description is available in Ragupathy, 2008. Seed was multiplied at off season location during 2008.

*Karnal bunt screening***:** Screening was performed during 2008-09 followed by confirmatory screening on a selected set of lines in 2009-10. Doubled haploids were screened for Karnal bunt resistance using a mixture of isolates. The inoculum for screening was based on different *T. indica* isolates representing pathogen variability from North Western Plains of India (Sharma *et al.,* 1998). The isolates were multiplied and maintained by frequent sub culturing on potato dextrose agar medium and mixed in equal proportions before use. The KB inoculations were performed in the field by syringe method (Aujla *et al.,* 1982) using approximately 10,000 sporidia/ml of inoculum solution made in water. About 20 ears of each DH line were inoculated. Appropriate relative humidity (60-100%) was maintained in the field with the use of mist-sprayer and frequent irrigations during the inoculation period. At maturity, the inoculated ears were harvested and threshed by hand. Percentage of diseased grains per plant was recorded.

Yield trial: The material was planted in a yield trial with plot size of 1.38m x 4.5m and three replications during 2009-10 crop season. Observations were recorded on yield and other agronomic traits (days to flowering, plant height, spikelets per spike, grains per spike and thousand grain weight).

Marker analysis: SSR markers, *Xgwm99* (1AL), *Xwmc 445* (5AL) and *Xgwm340* (3BL), known to be associated with KB resistance in the donor stocks were used to screen the set of 151DH lines.

Results and discussion

Fourteen cross combinations were used for deriving the 151 DH lines used in this study. The parental stocks carrying resistance to KB are unique materials developed over several years of work and represent incorporation of KB resistance into high yielding backgrounds (Sharma *et al.,* 2003; Sharma *et al.,* 2004). However, PBW 343 which had been the main recipient succumbed to new races of stripe rust and the current cycle of improvement was mainly aimed at improving this aspect in the shortest possible time. The DH production was based on wheat x maize crosses employing a special crossing schedule. In this schedule wheat plants are raised ahead of the normal wheat season by planting in first week of September with the help of a growth chamber at least for the first 30-40 days. This allows wheat x maize crosses to be made in November and first half of December. Consequently the regenerated plants can be chromosome doubled and raised to maturity within the wheat growing season itself. Due to late planting of DH (in January), plant growth is restricted and only small amount of seed is obtained. In the present study, the DH seed harvested in 2007-08 main season was planted at the off-season location during summer of 2008. Sufficient seed of 151 DH lines could be obtained for preliminary evaluation and screening for KB resistance in the 2008-09 crop season.

Three SSR markers which were shown to be associated to some extent with KB resistance in an earlier study (Sehgal *et al.,* 2008) were assayed on the DH lines. The number of DH lines produced from each cross and their marker profile is given in table 1. For *Xgwm99* present on chromosome 1AL, 55.6% of the lines carried the donor allele whereas 59.6% and 49.0% of the DH lines carried the donor alleles for *Xwmc 445* (5AL) and *Xgwm340* (3BL) respectively. These observations seem to be within the expected zone, as about half of the lines are likely to carry the donor allele at any locus. The presence of these alleles was however not highly predictive of resistance to KB in the DH lines (data not given here). Failure of the identified markers to explain a large share of the genetic variation for KB has been reported (Singh *et al.*, 2007). The promotion of the lines was thus by and large determined by the actual disease score and plant type in the field. Out of the 151 DH lines 24 were short listed for yield evaluation and confirmatory screening for KB resistance. Seventeen of the doubled haploid lines showed Karnal Bunt score of less than 5% whereas check varieties, PBW 343 (18.3%), PBW 550 (20.4%), PBW 621 (20.1%) and HD 2967 (13.6%) showed higher levels of Karnal Bunt infection. A Karnal Bunt score of less than 1% was observed in three doubled haploid lines, namely BWL 0009 (0.60%), BWL 0001 (0.67%) and BW 9996 (0.87%).

Table 1. Number of DH lines produced from different crosses and their marker profile for three SSR markers associated with KB resistance

S. No.	Cross	No of DH line	*Xgwm 99*		*Xgwm 340*		Xwmc 445	
			Donor allele	Recipient allele	Donor allele	Recipient allele	Donor allele	Recipient allele
1	DBW 18 / KB 34-2	7	5	2	3	4	3	4
2	DBW 16 / BW 5183	21	9	12	15	6	7	14
3	BW 3558 / BW 5183	8	2	6	5	3	4	4
4	BW 5221 / BW 5183	14	10	4	9	5	6	8
5	BW 3652 / BW 5183	3	1	2	1	2	0	3
6	KBD/5* PBW 343	47	31	16	26	21	26	21
7	AMSEL/ ALDAN //5* PBW 343	6	5	1	3	3	5	1
8	ALDAN/ W485//4* PBW 343	3	1	2	0	3	0	3
9	FRAME/4* PBW 343	1	0	1	0	1	1	0
10	HD 29 / W485//4* PWB 343	13	7	6	11	2	7	6
11	W485 / HD29//4* PBW 343	8	8	0	5	3	6	2
12	KBLR 22/BIO 582	6	1	5	4	2	3	3
13	KBLR 65/ PBW 550	2	0	2	1	1	0	2
14	MRNG'S'/ PBW 343	12	4	8	7	5	6	6
	Total	151	84	67	90	61	74	77
	DH lines with donor marker	-	55.6%	-	59.6%	-	49.0%	-

Two doubled haploid lines (BW 9986 and BW 9991) combining Karnal Bunt resistance (< 2%) and high yield were identified and promoted to multilocation evaluation. The yield superiority of the selected lines was mainly due to high tillering and higher thousand grain weight.

The utility of the doubled haploid approach for breeding Karnal bunt resistant lines is amply demonstrated by the present study, where products from new crosses could be taken to multilocation evaluation in three years time. Though KB resistance is widely recognized for its complex inheritance, it was encouraging to find a significant proportion of lines with high levels of resistance in this small set of derivatives. The present report thus serves as an excellent pilot study, which needs to be scaled up for future work.

Table 2. Performance of selected DH lines for yield, yield components, stripe rust and KB score.

S. No.	Genotype	Parentage	DTF	Tillers/ mt	Pl ht (cm)	Spklts /spk	Yield /plot (kg)	Gr/ spike	1000-GrWt (g)	Stripe rust	KB Score
1	BW9986	DBW 16 /BW 5183	101.0	117.8	90.7	14.7	3.57	44.2	33.8	20S	1.0
2	BW9987	BW 3558 / BW 5183	101.6	115.3	93.8	16.8	2.93	47.3	33.1	20S	3.7
3	BW9988	BW 3558 /BW 5183	97.0	107.8	89.5	30.2	2.78	45.1	38.9	0	3.1
4	BW9989	BW 5221 / BW 5183	101.0	114.3	92.6	17.5	3.23	45.5	32.9	0	5.0
5	BW9990	BW 5221 /BW 5183	103.0	115.3	86.4	17.5	2.72	48.5	27.8	0	9.3
6	BW9991	KBLR 22 / BIO 582	104.6	125.3	84.8	17.2	3.39	45.9	33.4	0	1.2
7	BW9992	KBLR 22 / BIO 582	99.6	86.3	65.0	18.3	1.57	33.6	30.3	10S	5.4
8	BW9993	KBLR 22 / BIO 582	102.6	106.1	85.4	19.2	3.46	37.0	33.7	0	6.2
9	BW9994	KBLR 22 / BIO 582	104.3	105.0	89.5	18.8	2.58	45.8	31.6	0	8.0
10	BW9995	DBW 16 /BW 5183	100.6	117.1	83.3	16.6	2.88	44.9	36.5	10S	1.7
11	BW9996	DBW 16 /BW 5183	102.3	109.0	92.6	16.2	3.28	41.2	35.9	5S	0.87
12	BW9997	DBW 16 /BW 5183	101.6	115.3	87.4	17.0	3.39	44.9	33.4	0	3.0
13	BW9998	DBW 16 /BW 5183	102.3	110.0	86.8	18.4	2.58	54.3	26.2	20S	6.7
14	BW9999	BW 3652 /BW 5183	103.0	114.6	83.1	17.1	3.31	51.3	35.1	20S	1.0
15	BWL0001	DBW 18 /KB 34-2	94.3	91.10	88.20	16.3	3.18	51.8	38.6	20S	0.67
16	BWL0002	DBW 18 /KB 34-2	101.3	107.3	94.80	20.4	2.64	59.1	27.4	20S	2.4
17	BWL0003	DBW 18 /KB 34-2	98.3	106.6	94.80	19.8	2.61	50.7	32.4	20S	2.7
18	BWL0004	BW 3558 /BW 5183	95.0	83.3	90.7	18.1	3.04	43.4	40.8	10S	7.3
19	BWL0005	BW 5221 /BW 5183	104.6	101.5	91.3	17.9	2.58	50.9	30.6	10S	1.1
20	BWL0006	BW 5221 /BW 5183	105.3	123.1	86.2	18.8	2.95	48.3	32.5	10S	1.3
21	BWL0007	BW 5221 / BW 5183	90.0	92.8	91.3	17.0	2.66	46.4	35.7	0	4.0
22	BWL0008	BW 5221 / BW 5183	93.0	93.00	91.5	17.0	2.81	46.7	41.8	0	1.0
23	BWL0009	BW 5221 / BW 5183	103.3	109.5	84.8	18.2	2.41	49.3	26.6	0	0.60
24	BWL0010	BW 5221 / BW 5183	96.3	90.6	86.6	20.2	2.18	54.8	31.0	5S	4.0
25	PBW 550	Check	95.6	96.5	84.2	17.4	3.25	49.5	36.6	0	20.4
26	PBW 621	Check	106.0	94.1	94.3	19.8	3.03	58.7	27.7	0	20.1
27	PBW 343	Check	99	90.1	78.5	18.3	2.85	48.6	35.2	10S	18.3
28	HD 2967	Check	103.3	96.0	96.5	20.0	3.56	58.4	39.4	0	13.6
	CD	-	2.0	13.3	3.90	2.71	0.28	1.82	5.5	-	-

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