

## Development of wheat lines carrying stripe rust resistance genes *Yr10* and *Yr15* in productive genetic backgrounds

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### 1. Introduction

Wheat is India's second most important crop after rice. Among the major constraints to wheat production in India are the biotic stresses. Stripe rust caused by *Puccinia striiformis f.sp. tritici* is the most important disease of wheat. Only few stripe rust resistance genes are currently being used in wheat breeding programmes. The dominant gene *Yr10* was originally found in Turkish wheat line P.I.178383 (Temel *et al* 2008). Microsatellite marker *Xpsp3000* was found linked to *Yr10* gene at a genetic distance estimated at 1.2 cM (Wang *et al* 2001). *Yr10* was also the first yellow rust resistance gene which was sequenced. It is located in the short arm of chromosome 1B, is 3630 bp long and consists of two exons interrupted by an intron (Temel *et al* 2008). The second gene *Yr15* is derived from *Triticum dicoccoides* accession G-25, which was shown

### Abstract

Stripe rust caused by *Puccinia striiformis f. sp. tritici*, is a major problem of wheat production in many parts of the world and is an important disease of wheat in North Western plains of India. One of the resistance breeding options is based on major, marker tagged genes with the ultimate objective of pyramided, complete and long lasting resistance. Two such genes, *Yr10* and *Yr15*, effective against stripe rust races prevalent in India had been previously introgressed from Avocet based stock into variety PBW 343 which served as a parent for further crosses with variety PBW 621 (Also known as DPBW 621-50). In the present study, BC<sub>1</sub>F<sub>5</sub> derivatives of crosses PBW 621/4/PBW 343//*Yr10*/6\* Avocet/3/4\*PBW 343/5/PBW 621 and PBW 621/4/PBW 343//*Yr15*/6\* Avocet/3/4\*PBW 343/5/PBW 621 were screened for presence of *Yr10* and *Yr15* using closely linked markers E1 and *Xgwm* 498 respectively. The gene positive lines were evaluated for yield performance and stripe rust resistance over two years. A set of important agronomic traits including yield components was recorded. Two lines, namely BWL 3284 (carrying *Yr10*) and BWL 3287 (carrying *Yr15*) showed yield advantage over best commercial check varieties in both years and are deemed to constitute a suitable parental pair for pyramiding of these two genes.

Keywords: *Puccinia striiformis*, resistance breeding, marker assisted selection, gene introgression, PBW 621

to be highly resistant to more than 20 stripe rust races from six countries (Gerechter-Amitai and Stubbs 1970). Further studies showed that this resistance was conferred by one dominant gene, designated as *Yr15* (Gerechter-Amitai *et al* 1989). Based on genetic analysis, McIntosh and Arts (1996) revealed that the stripe rust resistance gene, *Yr15*, resides in the short arm of chromosome 1B of wheat at a distance of about 7 cM from the centromere. A codominant marker, *Xgwm498* was found closely linked to this gene (Yaniv *et al* 2015).

PBW 621 (DPBW 621-50) is a wheat variety released in 2011, for irrigated timely sown conditions of North Western Plains zone and was known to show good level of 'adult plant resistance', owing to presence of minor rust resistance genes. Presently, however, the resistance has

succumbed to the increasingly aggressive stripe rust races. The present study evaluates BC<sub>1</sub>F<sub>5</sub> lines carrying *Yr10* and *Yr15* in a background which is expected, on the average, to have 75% of the PBW 621 genome complement. The remaining component is primarily from PBW 343. After thorough selections in the segregating phase for rust resistance and plant type, a promising set was subjected to evaluation of yield and related traits in an effort to identify genotypes for promotion to multilocation trials as well as for further breeding work.

## 2. Materials and methods

**2.1 Plant material:** Originally the stripe rust resistance genes *Yr10* and *Yr15* were transferred into the background of PBW 343 from Avocet+*Yr10* and Avocet+*Yr15* respectively by employing four backcrosses. Then, these versions, PBW 343+*Yr10* and PBW 343+*Yr15* were crossed with PBW 621 to make F<sub>1</sub>s separately. These F<sub>1</sub>s were then backcrossed with PBW 621 (recipient parent) to get BC<sub>1</sub>F<sub>1</sub>s. Further, BC<sub>1</sub>F<sub>1</sub>s underwent selection cycles and selfings till BC<sub>1</sub>F<sub>5</sub>. As a result of this, a set of 10 backcross inbred lines (BILs) having *Yr10* gene (BWL 3283, BWL 3284, BWL 3564, BWL 3565, BWL 3566, BWL 3567, BWL 3568, BWL 3569, BWL 3570, S. MULT-K 13 S.NO. 73) and 3 with *Yr15* gene (BWL 3562, BWL 3563, BWL 3287) were obtained. The parentage of these two sets may be represented as:

- PBW 621/4/PBW 343//*Yr10*/6\* Avocet/3/4\*PBW 343/5/PBW 621 - 10 lines.

**Table 1.** DNA sequence of primers used for *Yr10* and *Yr15* linked markers

S. No.	Genes	Origin	Location	Markers	Primer sequence
1	<i>Yr10</i>	Moro	1BS	E1	5'CTTGCTGGCGACCTGCTTA3' 5'TGTTTCGCTCCACGCTGACT3'
2	<i>Yr15</i>	<i>T. dicoccoides</i>	1BS	<i>Xgwm 498</i>	5'GGTGGTATGGACTATGGACT3' 5'TTTGCATGGAGGCACATACT3'

**2.3 Field evaluation:** Eleven of the thirteen lines were subjected to replicated trial during 2013-14 and 2014-15 crop seasons. The trials were sown in first week of November during 2013-14 and last week of November during 2014-15 with a plot size of 5.4 square meters (6 rows of 4.5 metres each) in three replications during both the crop seasons. Standard recommended practices of seed rate, fertilizer application and irrigation etc. were followed.

- PBW 621/4/PBW 343//*Yr15*/6\* Avocet/3/4\*PBW 343/5/PBW 621 - 3 lines.
- PBW 621 (= DPW 621-50) is a wheat variety released in 2011 for timely sown irrigated conditions of North Western Plains of India. (Parentage: Kauz//Altar 84/Aos/3/Milan/Kauz/4/Huites)
- PBW 343 is mega wheat variety released in 1995 and cultivated widely upto 2006- 07. It is highly susceptible to stripe rust (race 78S84) after breakdown of resistance.
- *Yr10*/6\*Avocet is near isogenic stock of Australian cultivar Avocet, used as initial donor of *Yr10* gene
- *Yr15*/6\*Avocet is near isogenic stock of Australian cultivar Avocet, used as initial donor of *Yr15* gene

**2.2 Molecular characterization for rust resistance genes:** The single stripe rust resistance gene (*Yr10* and *Yr15*) BILs were screened for the presence of the genes by using PCR markers E1 (Temel *et al* 2008) and *Xgwm 498* (Yaniv *et al* 2015), which are closely linked to *Yr10* and *Yr15* respectively. Plant DNA was extracted using a modified CTAB method (Saghai- Maroof *et al* 1984). Appropriate temperature profile for 20 µl PCR reaction (containing the template genomic DNA, MgCl<sub>2</sub>, forward and reverse primers, dNTPs, PCR buffer and *Taq* polymerase) was provided and amplified DNA products were resolved on 2% agarose gels in case of marker E1 and 6% poly acrylamide gel in case of *Xgwm 498*. The marker primer sequence is given in table 1.

Observations were recorded on days to flowering, plant height, tillers per metre row length, spike length, 1000 grain weight and plot yield. Average of five counts per replication (plot) were taken for days to flowering, plant height, tillers per metre row length and spike length. Analysis of variance was performed using SAS ver. 9.3 to determine differences among the lines for various traits and identification of the superior genotypes.

**2.4 Screening against stripe rust:** Observations on stripe rust were taken in a separately planted disease nursery. Artificial disease epidemic was created using race 78S84 of stripe rust. Disease severity was recorded using the modified Cobb's scale.

### 3. Results and discussion

During material generation the selections were performed on basis of plant type and stripe rust score. Stripe rust reaction gave good indication of the presence of gene *Yr10* or *Yr15*. However, confirmation needed to be performed with molecular markers before entering into yield trials. Molecular screening of 13 chosen lines (10 for *Yr10* and 3 for *Yr15*) was done with the respective markers as outlined in the materials and methods.

The eleven gene tag positive lines were subjected to yield evaluation trials for two years. Plot yield and disease score

from the screening nursery are given in Table 2. All the test lines were completely free of stripe rust in both the years. The checks, particularly PBW 343, DPW 621-50 and HD 2967 showed high rust infestation. The trials also showed rust infestation and lowering of yield in the susceptible checks. So for comparison of new lines the disease resistant check HD 3086 was considered more appropriate. It also gave the highest yield among the check varieties. Line BWL 3284, positive for *Yr10* gene was superior for yield over best check HD 3086 during both years. During 2013-14 a yield superiority of 32.54% was registered while the advantage was 29.05% during 2014-15. Small plots may not provide an accurate assessment of yield potential but a significant yield advantage is indicated for BWL 3284. For *Yr15* positive lines, BWL 3287 shows superiority over best check in both years. The margin is impressive for at 22.78% for year 2013-14. The overall yield of 2013-14 trial was comparatively lower due to late planting.

**Table 2.** Yield performance (kg/plot) and rust reaction of *Yr10* and *Yr15* positive lines

Genotypes	Presence of Yr genes Yr10/ Yr15	2013-14		2014-15	
		Rust score	Yield	Rust score	Yield
BWL 3564	<i>Yr10</i>	0	3.35	0	2.48
BWL 3283	<i>Yr10</i>	0	4.04	0	2.44
BWL 3284	<i>Yr10</i>	0	4.48	0	3.02
BWL 3565	<i>Yr10</i>	0	3.43	0	2.58
BWL 3566	<i>Yr10</i>	0	2.6	0	2.24
BWL 3567	<i>Yr10</i>	0	3.23	0	2.85
BWL 3568	<i>Yr10</i>	0	2.97	0	2.68
BWL 3569	<i>Yr10</i>	0	3.17	0	2.57
BWL 3570	<i>Yr10</i>	0	2.63	0	2.51
BWL 3287	<i>Yr15</i>	0	4.15	0	2.51
BWL 3562	<i>Yr15</i>	0	3.07	0	2.41
PBW 343	-	80S	0.89	80S	0.17
PBW 621	-	60S	2.27	60S	1.63
HD 2967	-	40S	1.97	40S	1.56
WH 1105	-	20S	3.02	20S	2.04
HD 3086	-	5R	3.38	5R	2.34
CD			0.58		0.16

Table 3 provides the information on yield components. The yield superiority of BWL 3284 seems to accrue from higher 1000 grain weight (43.33 gm). The yield superiority

of BWL 3287 is not explained well by the component traits. This is likely due to the fact that in 2014-15, when component traits were recorded, yield advantage of

BWL 3287 was quite small (less than 1%). The plant height of BWL 3284 (81cm) and BWL3287 (83 cm) is comparable with parental check PBW 621 (85 cm). For days to flowering, which is another important agronomic trait, line BWL 3284 is comparable to parental check PBW 621 while BWL 3287 has a comparatively longer duration (111 days).

**Table 3.** Phenotypic evaluation of stripe rust resistance single gene lines during 2014-15

Genotypes	Days to flowering	Plant height (cm)	Tillers per metre	Spike length (cm)	Spikelets/ spike	1000 grain weight (gm)
BWL 3564	98	82	104	8.67	17	35.02
BWL 3283	111	89	100	9.0	19	38.02
BWL 3284	105	81	106	9.0	19	43.33
BWL 3565	102	82	92	8.67	19	38.25
BWL 3566	102	87	85	10.0	19	33.9
BWL 3567	107	86	104	8.67	19	33.18
BWL 3568	100	83	88	9.0	19	34.87
BWL 3569	100	90	90	8.67	19	40.08
BWL 3570	107	86	94	9.67	19	35.1
BWL 3287	111	83	93	9.67	19	32.98
BWL 3562	107	86	97	9.33	19	32.35
PBW 343	93	67	31	6.67	13	17.03
PBW 621	106	85	84	9.4	19	30.57
HD 2967	107	90	79	9.67	19	33.15
WH 1105	104	84	92	9.53	21	30.35
HD 3086	97	86	110	9.0	17	34.93
CD	1.25	4.46	12.89	1.01	1.86	5.7

The study is thus effective in identifying two parental lines for a pyramiding cross. The superiority of the introgression lines may also trace back to positive complementation of PBW 621 (approximately 75%) and PBW343 (approximately 25%) genetic backgrounds. It is also evident that the resistance genes do not seem to carry a yield penalty. This is expected as the gene *Yr10* is derived from a cultivated wheat stock. Similarly *Yr15* comes from *T. dicoccoides* which is wild species but nevertheless carries the same two genomes as present in cultivated wheat. Experience has shown that some resistant cultivars containing single resistance genes were not effective for long periods; thus the need arose to pyramid genes against particular rusts into a single cultivar

(Schnurbusch *et al* 2004). The two identified lines with single stripe rust resistance genes are excellent parental lines for gene pyramiding crosses with further possibilities of complementation for productivity genes as well.

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