

## Chromosomal location of non-hypersensitive leaf rust resistance genes in bread wheat cultivar PBW65 using microsatellite markers

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Microsatellite or simple sequence repeat (SSRs) markers have been powerful tool for genetic mapping in wheat. Indian bread wheat (*Triticum aestivum* L.) cultivar PBW65 has shown significant level of resistance to most virulent race 77-5 of leaf rust (*Puccinia triticina*). It has been indicated that PBW65 expresses non-hypersensitive type of resistance against race 77-5. F<sub>2</sub> and F<sub>3</sub> crossing of PBW65 with WL711, a leaf rust susceptible wheat cultivar, and allelic tests with such already known genes (present in cultivars RL 6058 and HD 2009) revealed that cultivar PBW65 could be a potential source of novel non-hypersensitive leaf rust resistance genes. So far, only non-hypersensitive leaf rust resistance gene *Lr34* was found to be effective under Indian conditions. Attempts to locate such durable leaf rust resistance genes in PBW65 through microsatellite markers showed 2B, 2D and 3D as critical chromosomes for PBW65. The primer *Xgwm341* (3D) was found located 41.5 cM away from gene *LrPBW1* in PBW65.

**Keywords:** Bread wheat, leaf rust, microsatellite markers, non-hypersensitive resistance, cv. PBW65, *Puccinia triticina*, *Triticum aestivum*

### Introduction

World over, bread wheat (*Triticum aestivum* L.) is prone to leaf rust fungus *Puccinia triticina* Erickss. Depending on the severity and duration of infection and local factors, the loss can go up to 50%<sup>1,2</sup>. Although, timely application of the fungicides against obligate parasites can provide some control, their use not only adds to production costs but also considered unfriendly to the environment. Use of disease resistance genes is viable and acceptable strategy to minimize yield losses as it saves input costs and is environmentally safe<sup>3</sup>. Adopting hypersensitive (race-specific) resistance is common for wheat improvement due to crop cleanliness and easy incorporation in wheat germplasm. However, hypersensitive resistance genes lead to high selection pressure on the pathogen races and, thereby, evolution of new races. Breeders are increasingly focusing on identification and incorporation of non-hypersensitive (race non-specific) resistance genes that provide only partial resistance but when used in combination with other genes can condition highly effective resistance<sup>4</sup>.

The non-hypersensitive resistance is often characterized by its inability to evoke hypersensitive response, slow growth of the pathogen, partial resistance phenotype, additive nature<sup>5</sup>, optimal expression at adult plant stage and long term effectiveness (durability)<sup>2,6-11</sup>. Since only two genes *Lr34* (7DS) and *Lr46* (1BS) are known to elicit durable non-hypersensitive type of resistance<sup>12</sup>, there is a need to establish chromosomal location of more such genes. The present study is focused on the chromosomal location of non-hypersensitive leaf rust resistance genes present in cultivar PBW65 using microsatellite markers.

### Materials and Methods

#### Plant Material

Bread wheat cultivar PBW65 (USA225/K816/3/S738//C306/Kalyansona) was sourced from the All India Coordinated Wheat Improvement Project, Directorate of Wheat Research, Karnal, India. It has been shown that cultivar PBW65 carry non-hypersensitive leaf rust resistance. Cultivar PBW65 was crossed to a universally leaf rust susceptible wheat cultivar WL711 (S308/Chris/ Kalyansona) to generate the mapping population for assigning chromosomal location for such genes using 536

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microsatellite markers selected from consensus maps of wheat by Roder *et al.*<sup>14</sup>. In wheat, microsatellite or simple sequence repeat markers (SSRs) have been shown to be a powerful tool for genetic mapping<sup>13-15</sup>.

#### Leaf Rust Evaluation

The parents and progeny population of the cross (F<sub>1</sub>, F<sub>2</sub> & F<sub>3</sub>) were evaluated for leaf rust disease against the leaf rust pathotype 77-5 (*syn.* 121 R63-1), the most virulent and prominent pathotype of *P. triticina* in Southeast Asia<sup>15</sup>. The disease severity was recorded when susceptible infector rows recorded leaf rust severity of 80S to 90S using modified Cobb scale as described by Peterson *et al.*<sup>13</sup>.

#### DNA Extraction

The plant material was grown in bread boxes and fresh leaf samples were ground in liquid nitrogen. Total genomic DNA was extracted from 2 g of powdered leaf tissue as described in Saghai-Marooof *et al.*<sup>16</sup>. For PCR (polymerase chain reaction) amplification, DNA concentration of each sample was adjusted to 25 ng/μL by dissolving required quantities of DNA in fixed volume of sterile double distilled water.

#### Bulk Segregant Analysis

The microsatellite markers linked to the non-hypersensitive leaf rust resistance genes in cultivar PBW65 were identified using bulked segregant analysis (BSA)<sup>17</sup>. Two contrasting DNA bulks were made by pooling equal amount of DNA from randomly chosen 10 F<sub>3</sub> homozygous resistant (HR) and 10 F<sub>3</sub> homozygous susceptible (HS) families. DNA samples of resistant and susceptible parents along with resistant and susceptible bulks were screened for polymorphism with microsatellite markers.

#### Microsatellite Screening

A total of 536 microsatellite primers selected from consensus maps of Roder *et al.*<sup>14</sup> and Somers *et al.*<sup>18</sup> and procured from Illumina, Inc., 9885 Towne Centre Drive, San Diego, CA 92121, USA ([www.oliagator.com](http://www.oliagator.com)) were screened on two bulks as well as parents. The PCR reactions were performed in Mastercycler (Eppendorf AG 22331, Hamburg, Germany). The 20 μL reaction volume contained 2 μL of genomic DNA samples (25 ng/μL), 2 μL primer (5 μM), 0.2 μL Taq polymerase (3 U/μL from Bangalore Genei Pvt. Ltd.), 4 μL of 2.5 mM dNTPs and 2.5 μL of 10× Taq buffer (100 mM Tris pH 9.0,

15 mM MgCl<sub>2</sub>, 500 mM KCl and 0.1% gelatin). The solution was overlaid with one drop of low mol wt mineral oil (Sigma Pvt. Ltd.).

#### Linkage Analysis

The linkage analysis was performed on F<sub>2</sub> derived F<sub>3</sub> families of the cross PBW65×WL711, which segregates for two non-hypersensitive adult plant leaf rust resistance genes. The microsatellite markers showing linkage with the homozygous resistant and homozygous susceptible bulks were screened on F<sub>3</sub> families to assign location of those genes. The linkage analysis between the phenotypic and molecular data between F<sub>3</sub> families was done using the software MAPMAKER version 3.0b<sup>19</sup> with a LOD threshold of 3.0. The distance between the resistance gene and the linked primer was also deduced by using this software.

#### Results

The F<sub>2</sub> and F<sub>2</sub>-derived F<sub>3</sub> families from the cross of bread wheat cultivars PBW65 and WL711 were fitted to digenic segregation ratios of 15:1 and 7:8:1, respectively<sup>20</sup>. Of the 536 primers tested for parental polymorphism, 166 (31%) recorded polymorphism between PBW65 and WL711. The minimum percentage (15.5%) of parental polymorphism was observed for D genomes, while A and B genomes observed parental polymorphism of 35.5 and 42.5%, respectively (Fig. 1). The highest percentage of parental polymorphism was observed for chromosomes 7A (59%), 7B (61%), 2B (53%) and 3B (56%) and lowest for chromosome 5D (4.2%). No polymorphism was reported for chromosome 4D. Those primers recorded polymorphism with parents were tested on HR and HS bulks. Of 166 primers that showed parental polymorphism, 12 primers recorded polymorphism for HR and HS bulks (Table 1). And of the 12 identified primers polymorphic on F<sub>3</sub> family

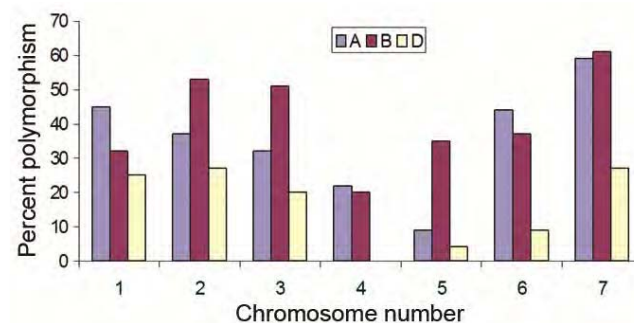


Fig. 1—Chromosome wise percentage of parental polymorphism between PBW65 and WL711

Table 1—List of primer sequences showing polymorphism with HR and HS bulks of PBW65 and WL711

No.	Primer	Chromosome no.	Sequence of forward primer	Sequence of reverse primer
1	<i>Xgwm210</i>	2B	TGC ATC AAG AAT AGT GTG GAA G	TGA GAG GAA GGC TCA CAC CT
2	<i>Xwmc154</i>	2B	ATGCTCGTCAGTGTTCATGTTTG	AAACGGAACCTACCTCACTCTT
3	<i>Xwmc265</i>	2B	GTGGATAACATCATGGTCAAC	TACTTCGCACTAGATGAGCCT
4	<i>Xgwm261</i>	2D	CTC CCT GTA CGC CTA AGG C	CTC GCG CTA CTA GCC ATT G
5	<i>Xgwm341</i>	3D	TTC AGT GGT AGC GGT CGA G	CCG ACA TCT CAT GGA TCC AC
6	<i>Xgwm644</i>	6B	GTG GGT CAA GGC CAA GG	AGG AGT AGC GTG AGG GGC
7	<i>Cfa2019</i>	7A	GACGAGCTAACTGCAGACCC	CTCAATCCTGATGCGGAGAT
8	<i>Cfa2028</i>	7A	TGGGTATGAAAGGCTGAAGG	ATCGCGACTATTCAACGCTT
9	<i>Xgwm332</i>	7A	AGC CAG CAA GTC ACC AAA AC	AGTGCTGGAAAGAGTAGTGAAGC
10	<i>Dupw398</i>	7B	CTGAGCCCTCTTTGCTATGC	TCGGTGAGATTGAAAGGTCC
11	<i>Xgwm400</i>	7B	GTG CTG CCA CCA CTT GC	TGT AGG CAC TGC TTG GGA G
12	<i>Xgwm350</i>	7D	ACC TCA TCC ACA TGT TCT ACG	GCA TGG ATA GGA CGC CC

bulks, only 3 primers, viz., *Xwmc154* (2B), *Xgwm261* (2D) and *Xgwm341* (3D), were identified as segregating in F<sub>3</sub> families. The linkage studies between the resistance genes and the primers along those critical chromosomes (2B, 2D and 3D) using computer software MAPMAKER could establish the linkage between the primer *Xgwm341* (3D) and the gene *LrPBW1*. The primer *Xgwm341* (3D) was found 41.5 cM away from the gene.

### Discussion

The percentage of polymorphism between two cultivars depends on many factors, which include their evolutionary history, genotype and environmental conditions. The genome wise percentage of parental polymorphism revealed minimum polymorphism for D genome of leaf rust resistant cultivar PBW65 and susceptible cultivar WL711. The bread wheat being an allohexaploid consist of A, B and D genomes. Evolutionary history of bread wheat depicts that these A, B and D genomes combined at different points of time to give rise to presently cultivated bread wheat. The D genome entered late in the bread wheat and, thus, shares much similarity with it. This explains the low level of polymorphism for D genome of wheat. Bossolini *et al*<sup>11,21</sup> also reported low levels of polymorphism for D genome of wheat.

The highest percentage of parental polymorphism revealed for chromosomes 7A, 7B, 2B and 3B can be explained on the basis of more number of recombination events, while low and no polymorphism reported for chromosome 5D and 4D could be due to very few and low recombination events on these chromosomes during the development of respective resistant and susceptible cultivars. The polymorphism

of primers with HR and HS bulks depicts the possibility of linkage of such primers with non hypersensitive leaf rust resistance loci present in resistant cultivar PBW65.

The segregation of the primers in the population reveals their tentative linkage with the non hypersensitive leaf rust resistance genes in respective cultivars. Such linkage could be confirmed by knowing the exact distance in centiMorgan (cM) between the resistant gene and primer. The computer software MAPMAKER version 3.0b<sup>19</sup> compares phenotypic and molecular data of individual genes for getting exact distance between marker and the gene in question. The establishment of linkage between the primers and the genes of interest is of paramount importance in marker assisted selection. This could be used in detection and efficient utilization of such genes in future breeding programmes.

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