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Molecular Evidence for distribution of Rht-B1b (Rht1) and Rht-D1b (Rht2) Genes in few Indian Wheat Cultivars

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Abstract
Over 70% of wheat (*Triticum aestivum*) cultivars grown worldwide have a semidwarf phenotype controlled by the major genes Rht-B1, Rht-D1, and Rht8c. Rht-B1 and Rht-D1 encode for DELLA proteins which act to repress GA-responsive growth by repressing GA-signaling. The mutant alleles Rht-B1b and Rht-D1b confer dwarfism by producing more active forms of DELLA growth repressors. The objective of this study was to determine the frequency of occurrence of Rht-B1b and Rht-D1b genes in some of wheat cultivars grown in India. Sixteen Indian wheat cultivars were evaluated with PCR-based molecular markers for Rht-B1b and Rht-D1b. These PCR-based markers can detect the point mutations responsible for the two major semidwarfing genes Rht-B1b (Rht1) and Rht-D1b (Rht2) in wheat. Fourteen cultivars were found to have both Rht-B1b and Rht-D1b genes whereas two cultivars were identified that did not carry any of the two semi-dwarfing genes.

Keywords: Wheat (Triticum aestivum), semidwarf phenotype, DELLA, GA-responsive, growth repressors, PCR-based markers.

1. Introduction
Dwarfing or reduced height (*Rht*) genes have been found to be associated with large increases in the yield potential of cereals and have been a key component of the Green Revolution since they were introduced in wheat and rice breeding programmes in the past century [10]. Till date, 21 *Rht* genes with major effects on decreasing plant height have been identified in wheat [20, 9]. These genes were assigned designations from *Rht1* to *Rht21* [11]. Among the 21 *Rht* genes, *Rht-B1b* (*Rht1*) and *Rht-D1b* (*Rht2*), were the major factors in the Green Revolution and more than 70% of the wheat cultivars all over the world contain atleast one of them [20]. These 21 *Rht* genes were classified into two groups based on their response to exogenous gibberellins (GA) phytohormone. One group includes the GA-insensitive dwarfing genes of wheat which are present in two homoeologous allelic series located on the short arms of chromosomes 4B and 4D [4, 5, 12]. Other group includes GA-responsive dwarfing genes which have been located on chromosomes 2A [18], 2DS [17], 7BS [19] and 5A [16]. The *Rht-B1b* (*Rht1*) and *Rht-D1b* (*Rht2*) semi-dwarfing genes were introduced into commercial wheat cultivars from the Japanese variety "Norin10" in the 1960s as part of wheat improvement programs in the USA and at CIMMYT, Mexico. The mutant alleles of the genes *Rht-B1 and Rht-D1* leading to dwarfism in wheat, as well as the maize gene *dwarf-8* (*d8*), are orthologues of the *Arabidopsis Gibberellin Insensitive (GAI)* gene [8]. The *Rht-B1b* (*Rht1*) and *Rht-D1b* (*Rht2*) alleles produce 20% reduction in plant height. Reduced plant height conferred through the introduction of semidwarfing *Rht* genes *Rht-B1b* and *Rht-D1b*, is due to decreased responsiveness to GA phytohormones [7, 14]. The *Rht-B1b* and *Rht-D1b* genes encode for DELLA proteins, transcriptional regulators which act to repress GA signalling. The DELLA protein belongs to a plant specific family of transcription factors known as GRAS. The mutant *Rht* alleles *Rht-B1b* and *Rht-D1b* have single nucleotide substitution which led to non-sense mutation i.e. introduction of premature stop codons in the N-terminal coding region [8]. This led to the termination of translation within the DELLA region, resulting in production of N-terminally truncated proteins, which are more active growth repressors and confer dwarfism by repressing GA signaling. A reduction in plant height improved lodging resistance and partitioning of assimilates to the developing grain [2]. The large increases in yield that followed the introduction of these dwarfing genes led to widespread adoption of the dwarfing genes throughout the world [6].

The presence of these dwarfing genes can be determined by testing seedlings for the lack of responsiveness to GA [3-15]. Although relatively easy, this test is time-consuming, not always reliable, and does not discriminate between *Rht-B1b* and *Rht-D1b*. So, these limitations can be overcome by using PCR-based molecular markers for these dwarfing genes. PCR-based specific markers have been developed to discriminate between the dwarf genes *Rht-B1b* and *Rht-D1b* and their wild-type, tall alleles *Rht-B1a* and *Rht-D1a* [1]. Sixteen Indian wheat cultivars were evaluated with PCR-based molecular markers for *Rht-B1b and Rht-D1b.* Fourteen cultivars were found to have both *Rht-B1b and Rht-D1b* genes whereas two cultivars were identified that did not carry any of the two semi-dwarfing genes.

Materials and Methods

Plant Material

A range of bread (*Triticum aestivum*) wheat varieties were obtained from Punjab Agricultural University, Ludhiana, Punjab.

DNA Extraction and PCR-based Molecular Marker Analysis

Genomic DNA was extracted from young wheat leaves using a cetyl-trimethyl-ammonium bromide extraction buffer (CTAB method).

The following oligonucleotide primers were used [13]

- Rht-B1b F (5′-CAC TAC TAC TCC ACC ATG TTC GAT TCT CTG-3′)
- Rht-B1b R (5′- GCG GCA GGA GCA GCA GCC -3′)
- Rht-D1b F (5′- CCA CGA GAC GCT GGG -3′)
- Rht-D1b R (5′- CCT TCC TTC TCC TCC ACC TTG TAG -3′)

PCR conditions were as follows (20µl total volume): 1X PCR Buffer, 60ng of template DNA, 0.5mM of dNTPs, 0.375mM each of the forward and reverse primers, 1 unit of *Taq* polymerase and 1.5mM of $MgCl_2$.

For the primer *Rht-B1b*, amplification was carried out on a polymerase chain reaction (PCR) express cycler running the following program: 5 min at 95 °C, 42 cycles of 20 s at 94 °C, 30 s at 63 °C, 10 s at 72 °C, and a final step of 72 °C for 2 min.

For the primer *Rht-D1b*, amplification was carried out on a polymerase chain reaction (PCR) express cycler running the following program: 5 min at 95 °C, 42 cycles of 20 s at 94 °C, 30 s at 58 °C, 10 s at 72 °C, and a final step of 72 °C for 2 min.

PCR products were separated on 2.5% agarose gels and visualized after ethidium bromide staining using standard procedures.

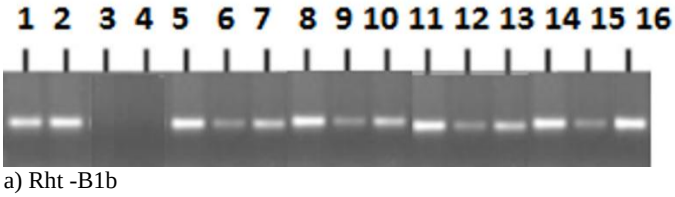
Results and Discussions

PCR analysis of selected wheat varieties

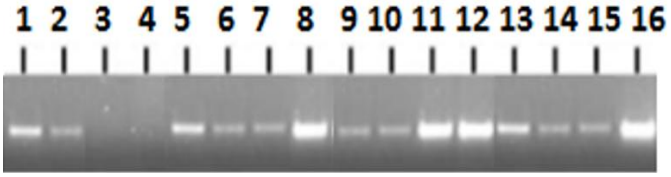
A range of wheat varieties were selected (Table 1) and DNA samples from these lines were analysed by PCR using the *Rht-B1b*- and *Rht-D1b*-specific markers (Fig. 1). Fourteen varieties gave the amplification product with the *Rht-B1b* and *Rht-D1b* specific markers (Table 2). Two varieties were found to be *Rht-B1b* and *Rht-D1b* negative with no amplification product.

Table 1. List of wheat cultivars.

S.No.	Variety	Source
1.	WH 1105	PAU, Ludhiana
2.	HD 3086	PAU, Ludhiana
3.	C 323	PAU, Ludhiana
4.	C 591	PAU, Ludhiana
5.	CB 324	PAU, Ludhiana
6.	CB 313	PAU, Ludhiana
7.	DBW 88	PAU, Ludhiana
8.	CB 315	PAU, Ludhiana
9.	CB 320	PAU, Ludhiana
10.	PBW 321	PAU, Ludhiana
11.	CB 318	PAU, Ludhiana
12.	CB 314	PAU, Ludhiana
13.	CB 322	PAU, Ludhiana
14.	CB 273	PAU, Ludhiana
15.	5218	PAU, Ludhiana
16.	5219	PAU, Ludhiana



a) Rht -B1b



b) Rht -D1b

Fig 1: Visualization of PCR products on 2.5% agarose gel
a) Rht -B1b b) Rht -D1b

Table 2: List of wheat cultivars with their Rht genotype

S. No.	Variety	Rht genotype
1.	WH 1105	Rht-B1b, Rht-D1b
2.	HD 3086	Rht-B1b, Rht-D1b
3.	C 323	Tall
4.	C 591	Tall
5.	CB 324	Rht-B1b, Rht-D1b
6.	CB 313	Rht-B1b, Rht-D1b
7.	DBW 88	Rht-B1b, Rht-D1b
8.	CB 315	Rht-B1b, Rht-D1b
9.	CB 320	Rht-B1b, Rht-D1b
10.	PBW 321	Rht-B1b, Rht-D1b
11.	CB 318	Rht-B1b, Rht-D1b
12.	CB 314	Rht-B1b, Rht-D1b
13.	CB 322	Rht-B1b, Rht-D1b
14.	CB 273	Rht-B1b, Rht-D1b
15.	5218	Rht-B1b, Rht-D1b
16.	5219	Rht-B1b, Rht-D1b

The effect of the presence of *Rht-B1b* and *Rht-D1b* genes on the phenotype of the cultivars containing these genes was studied by taking the data related to coleoptile length and also the plant height measurements (Table 3). The phenotypic variation in the coleoptile length and plant height was observed among the tall and dwarf cultivars (Figure 2). The tall genotypes have longer coleoptiles and longer plant height as compared to dwarf genotypes in which dwarfism is conferred by *Rht-B1b* and *Rht-D1b* genes.

Table 3: Comparison of coleoptiles length and plant height of dwarf and tall cultivars

S. No.	Variety	Rht genotype	Coleoptile length (Cm)	Plant height (Cm)
1.	WH 1105	Rht-B1b, Rht-D1b	13.7±2.40	100±3.40
2.	HD 3086	Rht-B1b, Rht-D1b	10.4±1.46	103±2.00
3.	C 323	Tall	19.4±3.20	125±1.41
4.	C 591	Tall	26.8±3.06	128±2.82

Fig 2: Comparison of coleoptiles length of dwarf and tall cultivars growing in rolled filter-paper 'cigars'. Tall cultivars (C 323 and C 591) have longer coleoptiles while dwarf cultivars (HD 3086 and WH 1105) have shorter coleoptiles.

Conclusion

Rht-B1b and *Rht-D1b* genes are responsible for dwarfism in wheat. The dwarf genotypes were differentiated from tall genotypes by using PCR-based molecular markers. Also, the effect of these genes on the phenotype was studied by analyzing the data related to coleoptiles length and plant height.

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