

## Microsatellites Based Genetic Linkage Map of the *Rht3* Locus in Bread Wheat

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**Abstract** The wheat reduced height genes (*Rht*) played an important role in “The Green Revolution” by reducing damage due to lodging and making wheat more responsive to fertilizer applications. The successful use of cultivars containing *Rht-B1b* and *Rht-D1b* around the world compelled the scientists to identify and generate height mutants through induced mutation in wheat. There are many *Rht* genes known in wheat, however genetic and molecular characterizations of many *Rht* genes are lacking. The *Rht3* gene was originally found in the cultivar, Tom Thumb, and resulted in 46% reduction in plant height compared to the wild type allele, *rht3*. The objectives of our investigation were to identify SSR markers linked to *Rht3* and to develop a genetic linkage map of the region containing the *Rht3* gene. The *Rht3* gene was located on chromosome 4B using bulked segregant analysis. Genetic linkage mapping using an F<sub>2</sub> population placed *Rht3* between markers wmc125 and gwm149 with a genetic distance of 14.4 cM and 23.6 cM, respectively. Identification of SSR markers associated with *Rht3* may help breeders to more efficiently screen for reduced height genes in breeding programs with diverse environmental conditions.

**Keywords** Wheat; Reduced height; Simple sequence repeat (SSR); Genetic linkage map; *Rht3*

### Introduction

Wheat is an important cereal crop in the sustenance of human populations across the globe. As the third most globally produced cereal crop, its supply has to keep up with increasing demand of expanding populations across the world (Rajaram, 2001). Varieties of wheat that provide high yields are crucial to farmers facing increasing demand. Height is an essential factor in the yield of wheat crop and the ability for farmers to manipulate height is indispensable. Reduced height (*Rht*) genes were first utilized after 1945 when Norin-10, a dwarf wheat variety from Japan, was crossed with Brevor-14 and the progenies were crossed to varieties adapted to grow in warm climates (Mathews et al., 2006). Most commercial varieties grown around the world today contain Norin-10 derived *Rht* genes: *Rht-B1b* and *Rht-D1b* (McVittie et al., 1978). A total of twenty-two genes, which affect wheat height, have been assigned designations (Ross et al., 1997). The *Rht3* allele was originally found in the Tom Thumb variety as a semi-dominant allele; it has a severe effect on the phenotype of wheat and reduces plant height by 46% compared to the wild type *rht3* allele (Flintham and Gale, 1982).

While many reduced height mutants are effective in achieving dwarfed phenotypes, many are of little use due to their effect on grain yield. *Rht7*, notable for its highly dwarfed phenotype, maps to chromosome 2A but is not advantageous in breeding programs due to its negative effect on yield (Worland et al., 1980). However, *Rht3* is of particular interest because the mutant variety neither reduces yield nor has adverse effects on bread making quality, as measured by sedimentation and baking tests (Flintham and Gale, 1983). *Rht3* may provide added advantages by reducing damage during preharvest sprouting due to an inhibited release of  $\alpha$ -amylase in germinating seed (Gale and Marshall, 1973). This proves that *Rht3* may have some value to breeders as a dwarfing tool.

Many reduced height genes including *Rht3* are known to be GA insensitive but that does not fully explain their phenotype (Peng et al., 1999). Responsiveness to GA in the *Rht3* mutant leads to the conclusion that the *Rht3* mutant is a result of the cessation of cell elongation (Tonkinson et al., 1995). Reduced height genes *Rht-B1b* and *Rht-D1b*, however, are found to code for DELLA proteins and are known to be negative

regulators in the Gibberellins (GA) biosynthesis pathway (Peng et al., 1997). A single base substitution difference in *Rht-B1b* and *Rht-D1b* resulted in early stop codons within the DELLA region, resulting in N-terminal truncated polypeptides (Peng et al., 1999). Because GA insensitive genes increase endogenous terminal GA levels, their mechanisms are more complex than hormone response, as in the case for *Rht3* (Crocker et al., 1990). Furthermore, the delayed induction of  $\alpha$ -amylase activity and inhibited choline uptake is the result of GA insensitivity in *Rht* genes, but does not fully explain the significant height reduction in *Rht3* (Flintham and Gale, 1982).

Molecular markers have been used extensively to incorporate qualitative and quantitative traits into new cultivars (Ellis et al., 2005). *Rht* genes have a strong foundation in wheat; semi-dwarf alleles were an important component of the Green Revolution, which helped avoid major worldwide food shortages (Wilhelm et al., 2013). Simple sequence repeat (SSR) markers are frequently used in the mapping of novel genic regions of cereal crops including wheat, and are essential in many breeding programs worldwide (Hargrove and Cabanilla, 1970). Identification of SSR markers linked to *Rht3* may be instrumental in screening for reduced height genes in breeding programs. The objectives of our investigation were to identify SSR markers linked to *Rht3* and to develop genetic linkage map of the region containing the *Rht3* locus.

## 1 Results and Discussion

Plant height was evaluated in the growth chamber on the  $F_2$  population generated for the *Rht3* gene. Average heights for the tall parent (Indian) and the short parent (Burt *Rht3*) were 104 cm and 42 cm, respectively. The  $F_2$  population consisted of 139 plants and segregation displayed a range in height from 31 cm to 92 cm. The height-range in the segregating population showed a skewed distribution

towards reduced height as compared to a previous study (Flintham and Gale, 1983). The observation suggests involvement of additional modifier genes in different backgrounds. Forty seeds from each  $F_{2:3}$  family were grown in the field to determine genotypes of the  $F_2$  plants. In  $F_2$ , it was difficult to classify plants into three discrete classes, however in the  $F_{2:3}$  generation, families were easily classified into homozygous tall, heterozygous and homozygous short categories. Based on the  $F_{2:3}$  segregation,  $F_2$  plants displayed a 1:2:1 ratio for homozygous tall, heterozygous and homozygous short with a  $p$  value of 0.81 (Table 1). The  $F_2$  and  $F_{2:3}$  segregations confirmed semi-dominant monogenic inheritance of the *Rht3* gene (Table 1). These observations are in agreement with previous studies showing monogenic inheritance of *Rht3* (Flintham and Gale, 1983; Wan et al., 2001).

The bulked segregant analysis (BSA) method was used with the tall and short bulks to locate the *Rht3* locus to the wheat chromosome, using 800 SSR markers. SSR marker, wmc125, showed clear polymorphism between the bulks (Figure 1A). In BSA, polymorphic markers between the bulks indicate proximity of the markers to the gene of interest. Association between wmc125 and *Rht3* was confirmed by using wmc125 on 10 short  $F_2$  plants (Figure 1B). Of the 20 gametes studied in 10 short plants, 3 displayed recombination, suggesting linkage between *Rht3* and wmc125 (Figure 1B). The marker wmc125 is located on chromosome 4B (Somers et al., 2004). Twenty markers present on chromosome 4B were tested on the parents for polymorphism. Of these, three (gwm149, gwm113 and barc20) showed polymorphism between the parents. Polymorphic markers were tested on the entire  $F_2$  population. Linkage analysis showed that the *Rht3* locus was flanked by wmc125 and gwm149 (Figure 2). The closest marker was wmc125 that was 14.4 cM from the gene. The location of *Rht3* is in the same region

Table 1 Segregation patterns, Chi-square and  $P$ -values for *Rht3* mapping population

Cross	No. $F_{2:3}$ families			$\chi^2_{(1:2:1)}$	$P$ -value
	Homozygous tall	Heterozygous	Homozygous short		
Burt <i>Rht3</i> $\times$ Indian	30	68	33	0.42	0.81

Note: Genotypes of the  $F_2$  plants were determined by progeny testing in  $F_{2:3}$  and are represented in 1:2:1 segregation

where *Rht-B1b* is located (Peng et al., 1999) suggesting that either these genes are closely linked to each other or they are allelic. The *Rht3* mutation has been shown to reduce GA-binding ability of the protein suggesting distinct mechanism as compared to *Rht-B1b* and *Rht-D1b* (Ross et al., 1997). Identification of SSR markers closely associated with *Rht3* genes may assist breeders in early identification of dwarf lines in breeding populations. In addition, phenotype produced by *Rht3* may be affected by background and environmental conditions; in those conditions PCR based selection may be easier and more precise.

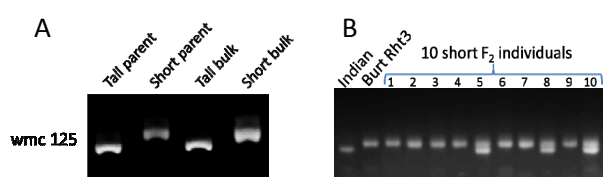


Figure 1 Identification of SSR marker linked to *Rht3* using bulked segregant analysis. A) SSR marker wmc125 shown on tall parent, short parent, tall bulk, and short bulk indicating that wmc125 is closely associated with *Rht3*. B) SSR marker wmc125 was tested on tall parent Indian, short parent Burt *Rht3* along with 10 short F<sub>2</sub> plants that constituted the short bulk. Seven of the 10 short F<sub>2</sub> plants showed band pattern same as short parent. Plant number 5, 8 and 10 showed recombination

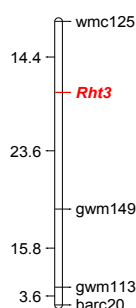


Figure 2 Genetic linkage map of chromosome 4B showing location of the *Rht3* locus. Genetic distances are represented in centiMorgans (cM)

Characterization of genes involved in height will be instrumental in designing new strategies for yield improvement in wheat and other cereal species. In addition, isolation and characterization of other players in this pathway may help us decipher its complexity.

## 2 Materials and Methods

### 2.1 Plant Materials

A mapping population (A11-200) consisting 139 F<sub>2</sub> plants was generated by crossing cultivar Burt *Rht3* (*T. aestivum*; Cltr 17786; *Rht3Rht3*) with the tall line Indian (*T. aestivum*; Cltr 4489; *rht3rht3*) using standard crossing techniques at University of Wisconsin-Stevens Point. Burt *Rht3* was developed in 1978 by Dr. Calvin Konzak at Washington State University. Cultivar Indian was developed in 1915 and registered 1926 as a tall bread wheat line (Clark et al., 1926). The resulted F<sub>2</sub> population was grown under controlled conditions in a growth chamber under 16 h day (18 °C) and 8 h (14 °C) night. Plant height of each F<sub>2</sub> phenotype, excluding awns, was recorded at maturity to the nearest cm.

### 2.2 Bulk Segregant Analysis

For the mapping population, leaf samples were harvested three weeks after germination and ground in liquid nitrogen before DNA isolation. CTAB method was used for DNA extractions (Sandhu et al., 2004). BSA was used to find the chromosomal location of *Rht3* (Michelmore et al., 1991). Tall and short bulks for BSA were prepared by mixing DNA from either 10 homozygous tall or 10 homozygous short F<sub>2</sub> plants. Each bulk was diluted to a final concentration of 50 ng of DNA/μl.

### 2.3 SSR Analysis

For SSR analysis, 30 ng DNA was used as the template in a 10 μl reaction containing 1x reaction buffer (10 mM Tris-HCl, 50 mM KCl, pH 8.3), 2.0 mM MgCl<sub>2</sub>; 0.25 μM of each primer; 200 μM of each dNTP and 0.25 units of *Biolase* DNA polymerase (Bioline, USA Inc., Taunton, MA). The PCR conditions consisted of: 94° C for three minutes, 11 cycles of 94°C for 30 s, 58°C for 30 s with an increment of -1°C per cycle and 72°C for one min, 35 cycles of 94°C for 30 s, 46°C for 30 s, and 72°C for one min, and a final ten minutes at 72°C. The PCR products were separated on a 4% agarose gel at 150 V for one to three hours. The primer sequences are shown in Table 2. Sequence information for developing SSR markers was obtained from <http://wheat.pw.usda.gov/cgi-bin/graingenes/report.cgi>. The Mapmaker 2.0 was used to determine genetic linkages and genetic distances (Kosambi, 1944; Lander et al., 1987). Marker order was determined at a LOD threshold of 3.0. Software “MapChart” was used to create maps (Voorrips, 2002).

Table 2 SSR markers used to map *Rht3* locus. All primers are represented 5' to 3' from left to right

Marker	Forward Sequence	Reverse Sequence
wmc125	ATACCACCATGCATGTGGAAGT	ACCGCTTGTCATTTCTTCTGT
gwm149	CATTGTTTTCTGCCTCTAGCC	CTAGCATCGAACCTGAACAAG
gwm113	ATTGAGGTTAGGAGGAAGAGG	GAGGGTCGGCCTATAAGACC
barc0020	GCGATCCACACTTTGCCTCTTTTACA	GCGATGTCGGTTTTTCAGCCTTTT

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