GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

A new reduced height gene found in the tetraploid semi-dwarf wheat landrace Aiganfanmai

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Genet. Mol. Res. 10 (4): 2349-2357 (2011)
Received October 8, 2010
Accepted June 3, 2011
Published October 5, 2011
DOI http://dx.doi.org/10.4238/2011.October.5.5

ABSTRACT. Aiganfanmai is a dwarf tetraploid wheat landrace (*Triticum turgidum* var. *turgidum*) that stably produces the semi-dwarf trait. Plant height varies from 80-105 cm under cultivation. Compared with tall durum wheat (*T. turgidum* var. *durum*) variety Langdon, we found it to have short spikes and seeds, besides a semi-dwarf character. We crossed Aiganfanmai with Langdon to analyze the genetic basis of the semi-dwarf trait. The F_2 population segregated at a 1:3 ratio for the short trait to the normal, which demonstrates that Aiganfanmai carries a recessive reduced height (Rht) gene. This gene was found to be located between the molecular markers Xgwm471 and Xgwm350 on chromosome arm 7AS by microsatellite analysis. No Rht gene had been reported from this chromosome; we designated it as Rht22. Rht 22, unlike other previously reported Rht genes, does not reduce internodal cell length. Reduced cell numbers might explain the short stem trait.

Key words: Wheat; *Triticum turgidum*; Dwarf; Rht22; Cell dimension; Landrace

INTRODUCTION

Wheat (*Triticum* L.) is the foremost food crop in the world, nearly 35% of the global population uses it as a staple food (www.idrc.ca/en/ev-31631-201-1-DO_TOPIC.Html). With the global population growing and arable land limited, wheat production and yield improvement become even more important (Ruttan, 1993; Mitchell et al., 1997; Rajaram, 2002). Since the Green Revolution, semi-dwarf wheat sources have been emphasized in most wheat improvement programs (Borlaug, 1968; Reitz and Salmon, 1968; Singh et al., 2001), and they have replaced the old tall wheat mostly in irrigated and high yielding regions of the world (Byerlee and Moya, 1993). As the semi-dwarf trait could provide lodging tolerance (Allan, 1989), it showed a positive response to higher doses of fertilizers under irrigated conditions without lodging (Rajaram et al., 1997). Although it cannot be concluded that tall wheat would not have performed better than dwarf wheat, there are currently almost no programs breeding tall wheat.

It was previously thought that there was a positive correlation between plant height and grain yield, which hampered the breeding of short-stem wheat to reduce lodging susceptibility and minimize yield loss (Law et al., 1978). This correlation was broken by introducing reduced height (Rht) genes to wheat in the first half of the twentieth century, which contributed significantly to a worldwide increase in wheat yield since the 1960s (Gale and Youssefian, 1985; Evans, 1993; Calderini et al., 1995). So, the Rht genes were called "Green Revolution genes" (Hedden, 2003), correspondingly, investigation of Rht gene resources became a hot field for wheat research. It was reported that twenty Rht genes had been found and studied in dwarf wheat (Konzak, 1988; McIntosh et al., 2003); however, few valuable Rht genes were found intrinsically in tetraploid wheat (*T. turgidum*) species, so most durum wheat breeders will have to use hexaploid wheat dwarf germplasm for tetraploid wheat breeding by interspecies crossing. Improving the lodging resistance of tetraploid wheat by this method is very time-consuming, since many artificial backcrosses for progenies will have to be conducted with tetraploid wheat to eliminate the genetic constitution of hexaploid wheat other than the Rht gene and thus is ineffective. So intrinsic dwarf germplasm in tetraploid wheat is highly desirable.

Fortunately, Aiganfanmai, a tetraploid landrace native to China, shows the semi-dwarf trait, with normal chromosome number and normal chromosome constitution (Peng, 1998). Our previous investigation indicated that its semi-dwarf trait is determined by a single-recessive Rht gene (Peng et al., 1999). This paper intended to reveal the chromosome location of the Rht gene as well as to describe phenotypic characterizations of Aiganfanmai.

MATERIAL AND METHODS

Materials

The semi-dwarf teroploid wheat (*T. turgidum* L. var. *turgidum*) landrace Aiganfanmai, native to Shaaxi, China, was collected in 1950s. Ten years ago, it was kindly provided by the Triticeae Research Institute, Sichuan Agricultural University, China, and since then it has been cultivated in the experimental field of the China West Normal University. In addition to the semi-dwarf tetraploid wheat landrace, the tall tetraploid wheat variety Langdon (*T. turgidum* L. var. *durum*) was used as the control.

To prepare the Rht gene mapping populations, Aiganfanmai was crossed with Lang-

don, and the F_1 plants were selfed to obtain F_2 seeds. The seeds of the two parents, F_1 and F_2, were sown in the experimental field on the same day during the 2007-2008 growing season. Plants were kept 10 cm apart. All the seeds of each population were randomly planted with the aid of labels for identification.

Phenotype record

Toward harvest time, the phenotypes for the two parents, F_1 and F_2, were recorded. The F_2 plants were used as a gene mapping population in this study. Considering that the plant height is affected both by the environment and the genotype, the F_3 families were used to verify the phenotype of F_2 plants during the 2008-2009 growing season. The described phenotype in this paper was investigated at the experimental field during the 2008-2009 growing season.

Cell dimension

The central section of fully expanded internodes and seeds was taken and fixed in formalin-acetic alcohol (FAA). Following the potassium hydroxide technique (D'Ambrogio, 1986), epidermal peels were prepared from the median region of the top internode surface of the sampled plants, and the pericarp was taken from the seeds in wax rip stage. Epidermal peels of internodes and pericarp were attached to a microscope slide, stained with safranin and mounted in clear resin. Following the classification of cell types by Wenzel et al. (1997) in barley, lateral cells of the stomata rows on internode surfaces were measured. Cell length and width were measured using the projected Olympus Microscope field (Image-Pro Plus) following the method by Miralles et al. (1998), and 150 cells per organ were measured. The comparison of cell dimensions between Aiganfanmai and Longdon was preformed with LDS by SPSS v15.0.

Genomic DNA extraction

Genomic DNA was extracted from the young leaves with the CTAB method (Clark, 1997). Extracted DNA samples were dissolved in TE buffer, pH 8.0, and visualized after electrophoresis on 1% agarose gels in 1X TBE. DNA purity and concentration was measured with a UV spectrophotometer. The DNA was adjusted to a final concentration of 50 ng/μL and stored at -20°C for use.

Microsatellite marker analysis

A total of 160 microsatellite primer pairs on genomes A and B were selected to evaluate their linkages with the Rht gene in Aiganfanmai. The microsatellite primer sequences are the same as those published by Röder et al. (1998) and Somers et al. (2004), respectively, and were synthesized by the Shanghai Sangon Co. (Shanghai, China). Prior to use in the experiment, they were diluted to 10 μM. Microsatellite PCRs were performed according to Röder et al. (1998). The PCR products were separated on 8% denatured polyacrylamide gels. The gels were run in 1X TBE buffer (0.09 M Tris-borate and 0.002 M EDTA) at 300 V for 3 h. The products were visualized by silver staining using the method described by Bassam et al. (1991).

Linkage analysis

Bulk segregant analysis (Michelmore et al., 1991) was applied to detect markers in specific chromosomal regions. Linkage relationships between the Rht gene and microsatellite markers were calculated with the MAPMAKER 3.0b program (Lincoln et al., 1993). The Kosambi (1944) mapping function was used to convert recombination data into centimorgans (cM). The linkage map was obtained by selecting 0.5 as the maximum recombination fraction and 3.0 as the minimum logarithm of the odds (LOD) ratio.

RESULTS

Characterization of some agronomic traits

The mean height of 110 Aiganfanmai mature plants was 97.2 ± 1.14 cm in 2010. It varied from 80-105 cm depending on the supply of water and artificial fertilizers in different cultivated seasons. However, the semi-dwarf landrace Aiganfanmai was much shorter than that of the tall cultivar Langdon (138.7 ± 1.68 cm, 108 plants investigated in 2010) in the same season (Figure 1a). So, the semi-dwarf trait of Aiganfanmai should be ascribed to a genetic basis. Like other wheat plants, Aiganfanmai possessed seven internodes. The measured results indicated that all the internodes of Aiganfanmai were statistically shorter than those of the tall landrace, Longdon. Besides the short stems, Aiganfanmai expressed some differential phenotype from the tall variety. For example, its spike length was obviously shorter than that of Langdon (Figure 1b). Compared with Langdon, Aiganfanmai showed more florets per spikelet (Figure 1c), which lead to more seeds setting per spike. In addition, seeds of Aiganfanmai were plump but shorter than Langdon, as shown in Figure 1d.

Figure 1. Plant morphology of the Aiganfanmai (left) and the control Langdon (right). **a.** Picture of the semi-dwarf trait (the ruler is 100 cm long); **b.** Picture of the shorter spike and awn; **c.** Picture showing more florets per spikelet; **d.** Picture of the short seeds.

Cell dimensions

The epidermal cells of internodes and seeds in Aiganfanmai were scrutinized under a microscope, as a result, they showed the same cell types and arranged normally compared to those in the tall variety. In contrast to the control variety, cell shape variation of Aiganfanmai was not observed under a light microscope, but its epidermal cell dimensions were different from those in Langdon (Figure 2). As listed in Table 1, statistical analysis revealed that the Aiganfanmai showed longer internodal epidermal cells and pericarp cells, although it expressed short stems and seeds (Figure 1). As for the cell width, no significant difference was observed in internodal cells between Aiganfanmai and Langdon. In contrast, Aiganfanmai has wider pericarp cells than Langdon (Table 1).

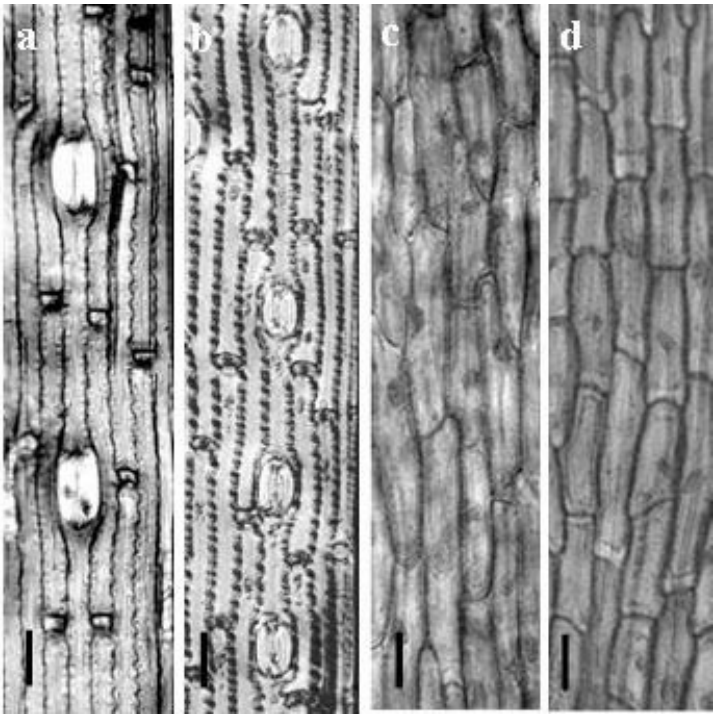

Figure 2. Epidermal cells of internode and pericarp in Aiganfanmai and Langdon sampled plants. **a.** Epidermal cells of internode in Aiganfanmai; **b.** Epidermal cells of internode in Langdon; **c.** Epidermal cells of pericarp in Aiganfanmai; **d.** Epidermal cells of pericarp in Langdon. Bar = 100 μm.

Table 1. Epidermal cell dimensions of Aiganfanmai and Langdon plants.

	Cell length (μm)		Cell width (μm)	
	Internode	Pericarp	Internode	Pericarp
Aiganfanmai	558.3 ± 11.9	475.0 ± 12.5	59.3 ± 0.6	86.1 ± 1.2
Longdon	498.5 ± 11.7	326.1 ± 5.1	60.0 ± 0.6	78.7 ± 1.0
Significance	0.0004	0.0000	0.4780	0.0000

Significant difference level at 0.05.

Microsatellite mapping of the gene for semi-dwarf trait

The plant heights of reciprocal F_1 hybrids were investigated in 2010. One hundred and seven F_1 hybrids (Aiganfanmai × Langdon) showed mean plant height of 140.3 ± 2.03 cm, and 98 F_1 hybrids (Langdon × Aiganfanmai) showed mean plant height of 139.6 ± 1.89 cm. The difference was not statistically significant. This result indicated that the cytoplasm effect on plant height was not obvious. The plant height of the F_1 progeny is much higher than that of Aiganfanmai, and closer to that of Langdon, indicating that the dwarf trait of Aiganfanmai is recessive. Analyzing 128 F_2 hybrid plants found that they segregated to 34 short and 94 high plants, statistically following the Mendelian 1:3 ratio (P = 0.683) for the short trait to the normal, which confirmed that the semi-dwarf trait of Aiganfanmai was determined by a single-recessive Rht gene.

Of the 160 microsatellite markers tested, 75 markers showed polymorphism and were used to establish the linkage group. The segregation ratios of all the markers confirmed the expected 1:2:1 ratio, with χ^2 values ranging from 0.702 to 2.426 (d.f. = 2). This result means that the F_2 population segregation was normal, and the F_2 hybrids could be applied as a mapping population.

To detect linkage relationships between the Rht gene and these markers, bulk segregate analysis was performed. The results indicated that the Rht gene was linked to 3 microsatellite markers, Xgwm471, WMC497 and Xgwm350. As these markers are located on the short arm of chromosome 7A, it could be concluded that the Rht gene of Aiganfanmai must be on the 7AS chromosome arm. Based on the recombination data calculated with the MAPMAKER 3.0b program, the linkage relationships are mapped in Figure 3.

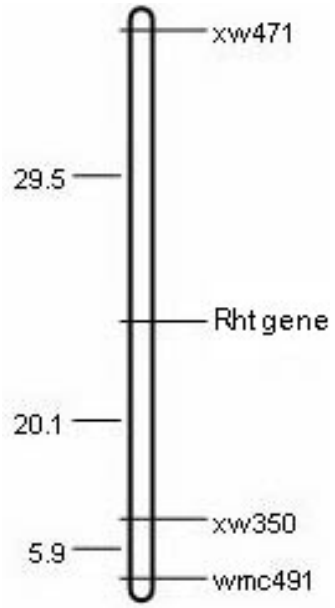

Figure 3. Linkage maps of the Rht gene with microsatellite markers Xgwm471, WMC497 and Xgwm350 on chromosome 7AS.

DISCUSSION

The semi-dwarf trait of landrace Aiganfanmai was found by an agronomist in the 1950s when Chinese government launched a landrace collection campaign all over the country. The Chinese word prefix "Aigan" means dwarf, which indicated that its semi-dwarf trait was determined at that time. But it is a pity that no breeder has utilized it in a wheat improvement program up to now. There are probably two reasons. One reason is that China has hardly any tetraploid wheat breeders presently. Chinese farmers cultivated tetraploid wheat landrace widely before 1950, but after that time turned to plant hexaploid wheat cultivars instead. The other reason could be lack of genetic research on this landrace. Our previous investigation found that Aiganfanmai expressed high crossability with rye (Peng et al., 1998), and noted its semi-dwarf trait at that time. This led to the preliminary hereditary study, which concluded that a single recessive *Rht* gene controls the semi-dwarf trait (Peng et al, 1999). This paper conducted a further study that revealed the location of the gene Rht 22 on chromosome arm 7AS and links with molecular markers by microsatellite analysis. Molecular markers associated with agronomic traits can provide breeders with rapid, cost-effective and nondestructive screening tools that are independent of the environment (Gupta et al., 2010). This study will help breeders to select Rht genes for particular environments, and thus Aiganfanmai is good for use in breeding durum wheat varieties.

Up to now, six reduced height genes have been reported in tetraploid wheat. Among them, five genes, Rht14, Rht15, Rht16, Rht18, and Rht19, were obtained in durum wheat by induced mutation via radiation and chemicals (Konzak, 1987). Only one spontaneous mutant Rht gene of tetrploid wheat was reported in *T. turgidum* ssp *polonicum* semi-dwarf accession IC12196. It is a dominant gene Rht-B1 (IC12196) at the Rht-B1 locus of chromosome 4B (Watanabe, 2004). According to the literature, a total of 21 Rht genes have been reported in tetraploid and hexaploid wheats (McIntosh et al., 2003), and some Rht genes were linked to molecular markers in wheat (Korzun et al., 1997, 1998; Ellis et al., 2005). But none were located on chromosome 7A, or linked with the markers Xgwm471, WMC497 or Xgwm35. Therefore, the reduced height gene in tetraploid landrace Aiganfanmai could be considered a new one. The gene symbol Rht 21 was used to name the dwarfing gene in common wheat variety XN0004 by Yang et al. (1993). Although Borner and Worland (2002) concluded that there is no Rht 21 gene in XN0004 at all, Rht 21 could not be reapplied to notate the new gene in Aiganfanmai. Following the Recommended Rules for Gene Symbolization in Wheat (McIntosh et al., 2003), the Rht gene on chromosome 7AS in Aiganfanmai was tentatively designated as Rht 22.

It is likely that the Rht 22 gene reduced plant height through differential mechanisms by comparing it with others reported before. Previous research leads us to the conclusion that Rht genes could reduce epidermal cell length without affecting cell width and division (Keyes et al., 1989; Hoogendoorn et al., 1990; Miralles et al., 1998; Botwright et al., 2005). The small cell sizes associated with Rht genes produce concomitant reductions in the length of internodes and coleoptiles, and leaf area of wheat seedlings (Allan, 1989; Botwright et al., 2001). On the contrary, the semi-dwarfing landrace Aigangfanmai carrying Rht 22 showed longer internodal epidermal cells than the control tall cultivar Langdon. The results suggested that the Rht 22 gene could not reduce the cell length, and the short stem trait might be contributed to by the depletion in cell numbers.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Wan Y (Plant Science Department, Rothamsted Research, UK) and Prof. Yang J (China West Normal University) for the thorough revision of the manuscript. Research supported financially by the National Natural Science Fund of China (Grant #30871533).

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