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ABSTRACT

Haque MA (2016) Microsatellite mapping of the semi-dwarf gene *Rht-Leeds* using bulk segregation analysis in durum wheat. *Int. J. Sustain. Crop Prod.* 11(1), 18-22.

Many wheat breeding programs in the world utilize giberellic acid insensitive and sensitive semidwarf germplasm to improve productivity and lodging resistance. Leeds mutant (*Rht-Leeds*) one of the height reducing recessive GA_3-sensitive mutant can reduce plant height without reducing or restricting coleoptile elongation in durum wheat. Bulk segregation analysis revealed that the marker *Xbarc147* on chromosome 3BS showed distinct polymorphism between the two parents, between the two bulks, and between individual semi-dwarf and tall plants in the F_2 population of Leeds mutant × LD222. Microsatellite mapping indicated that the *Rht-Leeds* allele was found between the SSR markers *Xbarc102* and *Xbarc147* on chromosome arm 3BS. The allelic relationship indicated that *Rht-Leeds* and *Rht5* were located on the same locus. *Rht-Leeds* would be help to further improvement of GA_3-sensitive semidwarf wheat.

Key words: *Triticum durum* L., *emergence, coleoptiles length, plant height, reduced height gene*

INTRODUCTION

Historical comparisons of wheat varieties reveal moderate changes in plant height as a consequence of selection for semi-dwarfism. Older wheat varieties are tall and prone to lodging in favorable environments, whereas current varieties are shorter, less prone to lodging and have good dry-matter partitioning to the grain. The shorter height of current wheat varieties is due to the use of GA_3-insensitive height-reducing genes *Rht-B1b* or *Rht-D1b* associated with large increases in the yield potential of cereals (Richards 1992). These genes decrease plant height by reducing cell size in leaf and stem tissue (Hoogendoorn *et al*. 1990). These semi-dwarf genes located on homologous chromosome 4BS or 4DS, other heights reducing genes have been reported by Konzak (1988) which listed 21 *Rht* genes.

Studies show that wheat height can also be reduced by the use of GA_3-sensitive genes for plant height (Yamada 1990). A number of alternative dwarfing genes (*Rht4* to *Rht20*) have been reported to reduce plant height in wheat but show sensitivity to exogenous gibberellic acid (GA_3) (Gale and Youssefian, 1985; Ellis *et al*. 2004). These genes reduce plant height to levels equivalent to that plants of containing GA_3-insensitive *Rht* genes yet are less likely to reduce coleoptile length or leaf area at seedling stage (Allan 1980; Whan 1976). Leeds mutant (*Rht-Leeds*) is one of the height reducing recessive GA_3-sensitive mutant (Ellis *et al*. 2004) has the ability to reduce plant height without reducing or restricting coleoptile elongation in durum wheat. The selection of SSR markers was one of obligation to made linkage map of Leeds mutant gene. The PCR-based Bulked Segregant Analysis (BSA) has been used in several organisms as a reliable tool to accomplish this process (Michelmore *et al*. 1991; Matsuda *et al*. 2002; Nanda *et al*. 2002). The purpose of present study is to determine the chromosomal location of semi dwarf GA_3-sensitive gene *Rht-Leeds* in durum wheat.

MATERIAL AND METHODS

Semi-dwarf material

Leeds mutant is a mutant of Leeds induced by methylnitrosourea at USDA-ARS Washington, United States. Culm lengths of Leeds mutant was 92 ± 2 cm and tall check variety LD222 was150.1 ± 8 cm, respectively (Fig. 1). The semi-dwarf Leeds mutant sensitive to GA_3 (Ellis *et al*. 2004), and genes in this material was designated as *Rht-Leeds*.

Fig. 1. Plants morphology of Leeds mutant (Left) and LD222 (Right).
Leeds mutant was semi-dwarf, whereas LD222 was tall

To construct linkage map of *Rht-Leeds*, F_2 population was developed from the cross of Leeds mutant x LD222 and B_3F_2 populations was also developed from Marfed ERT1 × LD222 to compare segregation results. The

culm length of individual plants was measured at three weeks after transplantation. F_2 populations of Leeds mutant x Marfed ERT1 and Marfed ERT1 x Leeds mutant were also grown to explore allelic relationship between *Rht-Leeds* and *Rht5* and culm lengths of individual plants were measured after ripening. All populations were grown in the experimental field at the college of Agriculture, Ibaraki University, Japan.

Bulked Segregant Analysis (BSA) was performed as described by Haque *et al*. (2011). For the bulk segregation analysis (BSA), two poll homozygous bulk samples equivalent aliquots of DNA from semi-dwarf and tall homozygous plants were prepared from Leeds mutant and LD222. Each bulk consisting of 8 homozygous semi-dwarf height ranges were 80 to 117 cm and tall height ranges were 147 to 158 cm, respectively.

Allelism test

Semi-dwarfing bread wheat *Triticum aestivum* Marfed ERT1 (*Rht5*) was used to investigate the allelic relationships with recessive GA_3-sensitive Leeds mutant (*Rht-Leeds*) at 3BS locus. The possible allelic relationships between *Rht-Leeds* and *Rht5* were tested in F_2 populations of Leeds mutant x Marfed ERT1 and Marfed ERT1 x Leeds mutant. The F_1 plants were sown and were selfed by covering the spike with a glassine bag prior to flowering. F_2 plants from these crosses were sown in the field, and the segregation of culm length was analysed in the bulks (off spring of single F_1 plants) and classified as either tall or semi-dwarf.

Assessment of the GA_3 response in semi-dwarf accessions

To evaluate the response of accession Leeds mutant to GA_3, seedlings of Leeds mutant and those of LD222 controls were sown in 6-cm pots, each containing a 50:50 mixture of vermiculite and perlite. The pots were then placed in plastic trays (25-cm width × 50-cm length × 4-cm depth) containing water (8-mm depth) at 26°C, with three replications. A GA_3 solution (0.04 mg/l) was sprayed daily on the leaves of the plants in each tray and distilled water was sprayed as control. The plants were harvested at the 3-leaf stage, and the length of the first leaf and distance from the seedling base to the ligule of the first leaf were measured.

Microsatellite analysis of the GA_3-sensitive *Rht* genes

Genomic DNA extraction was done from seedlings of 94 individuals per F_2 hybrid population according to the method of Dellaporta *et al*. (1983). The microsatellite markers *Xgwm*, *Xbarc*, and *Xhbg* located on chromosome 3B were used to map *Rht-Leeds* genes. Markers of 3B chromosome chosen based on the results of BSA and the potentially allelic relationship detected between the semi-dwarf genes under study. These markers were obtained from Röder *et al*. (1998), Song *et al*. (2005), and Torada *et al*. (2006). PCR analysis was performed as described by Kosuge *et al*. (2008). Linkage values in centiMorgans (cM) were calculated using Map Manager QTX (http://mapmgr.roswellpark.org/). Minimum LOD score of at least 3.0 were used to develop the linkage map. The software calculated genetic distances in cM using the Kosambi (1944) mapping function.

RESULTS

Microsatellite analysis of GA_3-sensitive *Rht-Leeds* gene

The length of the first leaf and the length from seedling base to ligules of the first leaf of *Rht-Leeds* were significantly different between the GA_3 treatment and control indicated that *Rht-Leeds* is sensitive to GA_3. In the F_2 populations of Leeds mutant × LD222 were not significant different from a model of 1 semi-dwarf: 3 tall ($\chi 2$ = 1.716; df =1) confirmed that the semi-dwarf *Rht-Leeds* is a recessive gene, similar culm length segregation found in the BC_3F_2 of Marfed ERT1 × LD222 ($\chi 2$ = 1.420; df =1) populations is shown in Fig. 2.

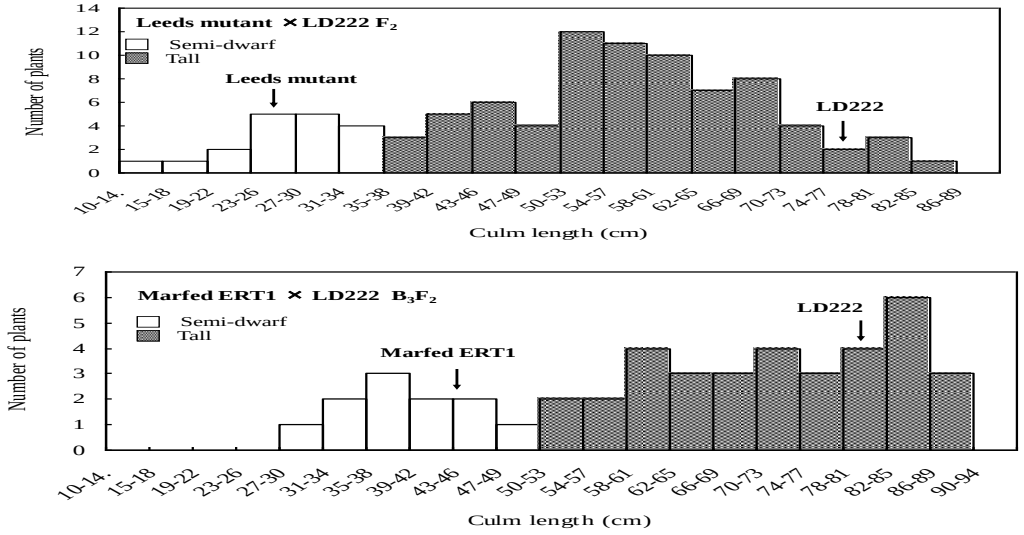

Fig. 2. Segregation of culm length in F_2 of Leeds mutant × LD222 and B_3F_2 of Marfed ERT1 × LD222. Two categories of culm length are shown in different texture. Arrows indicate the mean parental culm length

Total of 87 SSR microsatellite markers were chosen from seven different A and B genomes (*Triticum durum* is a tetraploid and contains only genomes A and B) and were used to the detection of polymorphism between the two parental genotypes to map *Rht-Leeds*. Of the above markers, only thirty eight showed polymorphism between the parental genotypes of Leeds mutant and LD222. Using these polymorphic markers, BSA was conducted with two pooled DNA samples, each consisting of 8 homozygous semi-dwarf and tall. BSA indicated that marker *Xbarc147* on chromosome 3B was polymorphic between the two parents, between the two bulks, and between individual semi-dwarf and tall plants in the F_2 population of Leeds mutant × LD222 (Fig. 3).

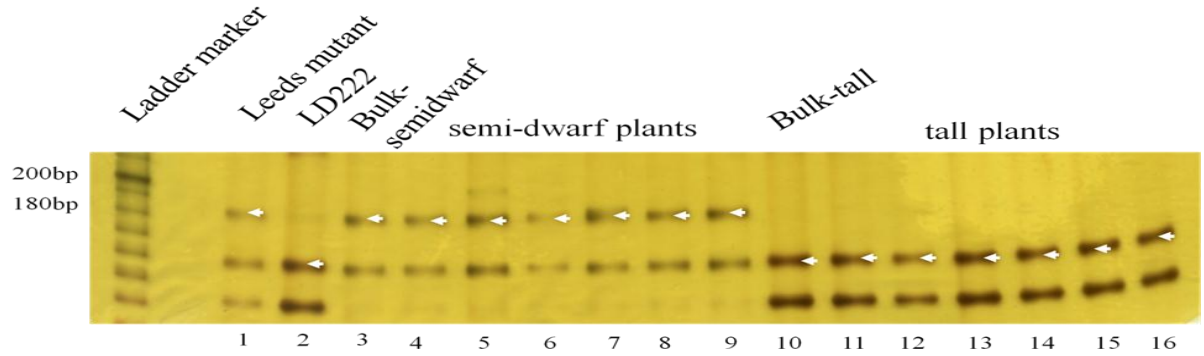

Fig. 3. Amplification profile of microsatellite marker Xbarc147-3B by bulk segregation analysis in F_2 population of Leeds mutant × LD222. Left to right: Semi-dwarf parent (lane 1), LD222 (lane 2), bulk of semi-dwarf plants (lane 3), individual semi-dwarf F_2 plants (lane 4 to 9), bulk of tall plants (lane 10), and individual tall F_2 plants (lane 11 to 16). Arrows indicate bands common between in given parent, individual homozygous progeny with the same phenotype as the parent and the corresponding bulk.

These results indicated that *Rht-Leeds* is located on chromosome 3B. Among 35 microsatellite markers on chromosome 3B, seven were polymorphic and used to map *Rht-Leeds* more precisely. Microsatellite marker analysis indicating the *Rht-Leeds* allele was localized to a region on chromosome 3BS bracketed by *Xbarc102* and *Xbarc147* (Fig. 4). *Rht5* was mapped to the same region as *Rht-Leeds*, between *Xbarc102* and *Xbarc147* on chromosome 3BS.

No tall plants were observed in F_2 population derived from the cross combinations of Marfed ERT1× Leeds mutant (n= 134) and Leeds mutant × MarfedERT1 (n= 142). The allelic relationship further indicating that *Rht-Leeds* and *Rht5* are located on the same locus.

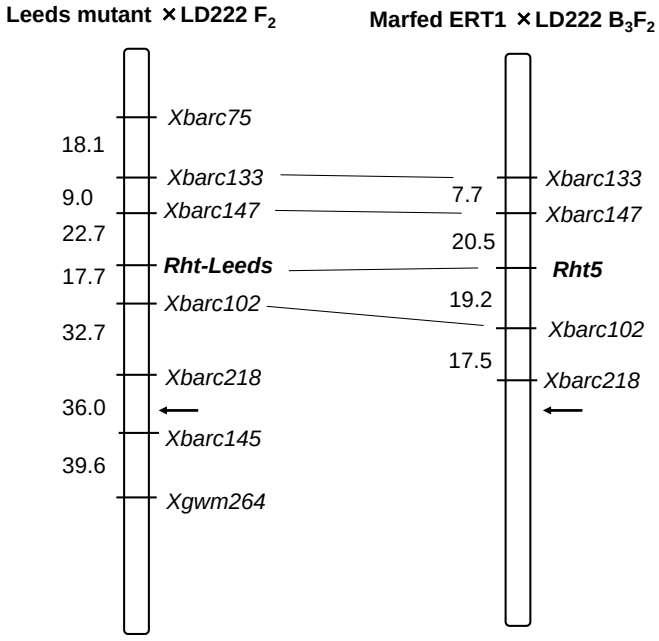

Fig. 4. Linkage maps for semi-dwarfism in chromosome 3BS. Arrows indicate the supposed position of the centromere. Distances between markers are shown in cM

DISCUSSION

GA_3-insensitive dwarfing genes *Rht-Blb* (*Rht1*) and *Rht-Dlb* (*Rht2*) reduce coleoptile length slowing early leaf growth to reduce crop establishment and vigour (Richards 1992; Rebetzke *et al*. 1999). GA_3-sensitive dwarfing genes are responsible to overcome the problems of poor establishment and low vigour in durum wheat (Giorgi *et*

al. 1984). The present study was focused to map GA_3-sensitive dwarf Leeds mutant (*Rht-Leeds*) followed by bulk segregant analysis. *Rht-Leeds* gene was located on the short arm of chromosome 3B which was allelic with *Triticum aestivum cv.* Marfed ERT1 at same locus. These finding was analogous with allelic variation in *Rht-B1* and *Rht-D1* loci, which were contained by the most widely utilized semi-dwarf wheat cultivars.

Rht-Leeds was mapped to chromosome 3BS, between markers *Xbarc147* and *Xbarc102*. The recessive, GA_3-sensitive gene *Rht5* is also located on chromosome 3B and was reported to be approximately 10 cM from *Xbarc102* (Ellis *et al*. 2005). To develop a semi-dwarf NIL for *Rht5* from Marfed ERT1, a backcross was made between Marfed ERT1 with tetraploid wheat LD222 as a recurrent parent. The linkage map derived from the BC_3F_2 population of Marfed ERT1 × LD222 showed that the 4 markers (*Xbarc102*, *Xbarc147*, *Xbarc133*, and *Xbarc218*) were polymorphic and linked with *Rht5*, whereas three more distant markers (*Xbarc145*, *Xbarc75*, and *Xgwm264*) were monomorphic and could not be scored in this population. In the F_2 population of Leeds mutant × LD222, all 7 markers were polymorphic and could be mapped. These results suggest that, in the case of the Marfed ERT1 × LD222 BC_3F_2 population, the semi-dwarf plant BC_3F_2 chosen for the mapping; cross was already fixed for several marker alleles from LD222, but still carried the marker alleles from Marfed ERT1 that were most closely linked to *Rht5*. The *Xbarc102* marker was located approximately the same map distance from both *Rht-Leeds* (17.7 cM) and *Rht5* (19.2 cM), suggesting that *Rht-Leeds* and *Rht5* may be allelic.

CONCLUSION

Microsatellite marker analysis through bulk segregation method confirmed the GA_3-sensitive gene *Rht-Leeds* on chromosome 3BS may be useful for further wheat breeding program. This finding is similar to that of the multiple alleles discovered at the *Rht-B1* locus and at the locus where *Rht14*, *Rht16*, and *Rht18* are located.

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