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Selective recombination close to the semi-dwarfing locus through bulk-population in the hybrid between *Triticum aestivum* and *Triticum polonicum*

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Abstract

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The *Rht-B1* locus on the short arm of chromosome 4B has multiple alleles that give phenotypic expressions ranging from semi-dwarfism to extreme dwarfism. Allelic variation at *Rht-B1* locus was also found in semi-dwarf *Triticum polonicum* L. cv. IC12196. The semi-dwarf allele from IC12196 could be an alternative source of reduced height gene. The experimental lines derived from the cross between *Triticum aestivum* L. cv. Novosibirskaya 67 (N67) and IC12196 were analyzed concerning the introgression of semi-dwarf allele from IC12196. The aim of the present study was to assess the selection for the semi-dwarfism and recombination around the locus of semi-dwarf gene using microsatellite markers on the short arm of chromosome 4B. The *Rht-IC12196* allele was allelic to *Rht-B1b* allele, and microsatellite markers indicated that the *Rht-IC12196* allele was linked with the markers, *Xbarc1045* and *Xgwm113* on the short arm of chromosome 4B. Out of 112 hexaploid introgression lines, 29 lines were semi-dwarf. The *Xgwm113* and *Xhbg226* markers flanking the *Rht-B1* locus were replaced by N67 allele in the majority of introgression lines. Although it did not intend, the region around the *Rht-B1* locus was highly selectable, but on short arms of chromosome 4B, *Rht-IC12196* gene near the locus *Xgwm113* explained a large part of the phenotypic variation originated from N67-allele and non-parental allele in tall accessions. The *Rht-B1a* allele was favorable in the consequence of bulk population breeding and the frequency of *Rht-B1a* allele was 73% in the hexaploid introgression lines.

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Introduction

A number of current wheat cultivars possess the genes for semi-dwarfism, *Rht-B1b* (*Rht1*) and *Rht-D1b* (*Rht2*), originating from *Triticum aestivum* L. cv. Norin 10. They are commonly associated with increased yields in bread wheat (Perry and D'Antuono 1989) and in durum wheat (Waddington *et al.*, 1987). Height reductions associated with attributed to a shorter yet stronger culm that accommodates high yields and prevents lodging in favourable environments (Ellis *et al.*, 2004; Chapman *et al.* 2007). The utilization of the *Rht* genes in many open public as well as personal reproduction applications changed higher cultivars through shorter ones (Richards, 1992). The *Rht-B1* and *Rht-D1* loci are contained in the most widely utilized semi-dwarf wheat cultivars. The *Rht-B1* locus on the short arm of chromosome 4B and *Rht-D1* locus on the short arm of chromosome 4D have multiple alleles that give phenotypic expressions ranging from semi-dwarfism to extreme dwarfism (Börner *et al.*, 1996). Allelic variants at the *Rht-B1* locus were found in common wheat (Worland, 1986; Worland and Petrovic 1988; Worland and Sayers, 1995), in *T. aethiopicum* Jakubz. (Börner *et al.* 1996), and also in *Triticum polonicum* L. cv. IC12196 (Watanabe, 2004).

It is interesting that the semi-dwarf allele of *T. polonicum* might be an alternative source of reduced height gene. Kang *et al.* (2009) were produced a

synthetic hexaploid between *T. polonicum* collected in the region of Xinjiang, China, whose plant height was semi-dwarf, and *Aegilops tauschii* Coss., the D-genome progenitor of common wheat. Kosuge *et al.* (2010) suggested that hybridization of *T. aestivum* 'Novosibirskaya 67' - *Triticum polonicum* L. (2n = 4x = 28, BBAA genome) 'IC12196' can rapidly give rise to a new landrace due to selective introgression of the *P* gene for long glumes. IC12196 is characterized by semi-dwarfism and long glumes (Watanabe, 2004). The aim of the present study was to assess the selection and recombination for the semi-dwarfism in the progenies between *T. aestivum* and *T. polonicum* using microsatellite markers on the short arm of chromosome 4B.

Materials and Methods

Plant materials

To map the GA₃-insensitive semi-dwarf gene, tentatively designated as *Rht-IC12196*, and microsatellite markers on chromosome 4BS, produced F₂ hybrid of *T. aestivum* 'Novosibirskaya 67 (N67)' - *T. polonicum* 'IC12196' and F₂ hybrids of *T. polonicum* 'IC12196'/*Triticum durum* Desf. 'LD222'. Plant height of LD222 was tall. To investigate the allelic relationship between *Rht-IC12196* and *Rht-B1b* alleles, F₂ hybrids of *T. polonicum* 'IC12196'/*T. durum* 'Cando' were also obtained. Cando has the *Rht-B1b* allele derived from

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Norin 10. Using the F₂ generation of N67/IC12196, a small-scale bulk breeding method was applied to develop introgression lines. The procedure was described by Kosuge *et al.* (2010). Two hundred F₂ plants were grown in an area of 20 m². All the seeds were harvested from the F₂ plants as a bulk. Subsequently, 200 F₃ seeds were randomly chosen from the bulk, and were grown in the same area size in the subsequent cropping cycle. This method was applied until reaching the bulked F₆ generation. Single seed descents of 132 F₆ plants were used to advance the population from F₇ through F₁₀ generation. One-hundred and twelve hexaploid F₁₀ lines were available for the present experiment. All populations were grown in the experimental field at the College of Agriculture, Ibaraki University, Japan. Culm lengths of individuals were measured after ripening.

Microsatellite mapping of the *Rht-IC12196* and allelic composition of the experimental lines

An intended to map of *Rht-IC12196* using the F₂ hybrid of N67/IC12196. However, it was found that segregation for plant height showed irregularity, probably due to aneuploidy in the F₂ generation. The chromosome numbers of the F₂ of N67/IC12196 was expected to vary from 2n = 28 to 42, depending on the transmission of the D-genome univalents (Alston and Jones, 1968). Alternatively, there are used F₂ hybrids of IC12196/LD222 to establish a template linkage map of chromosome 4BS including the semi-dwarf locus. It was expected that the ratio of IC12196 and non-IC12196 (N67 and non-parental) allele at the given locus would be 1:1 in the F₁₀ generation provided there was lower natural selection during segregating generations. It was supposed non-parental allele was caused by a spontaneous mutation at the given locus.

Genomic DNA was extracted from seedling leaves of N67, IC 12196, LD222, F₂ hybrids of IC12196/LD222 and 112 hexaploid introgression lines, according to Dellaporta *et al.* (1983). To map the *Rht-IC12196* gene and allelic composition of the introgression lines, used the wheat *Xgwm*, *Xbarc* and *Xhbg* microsatellite markers located on chromosome 4BS. The *Xgwm* microsatellite markers were developed by Röder *et al.* (1998). The *Xbarc* and *Xhbg* microsatellite markers were available from Song *et al.*, (2005) and Torada *et al.* (2006), respectively. The PCR conditions, electrophoresis of PCR products and detection of amplified fragments followed Kosuge *et al.* (2008). Multipoint linkage values were calculated using Map Manager QTX (Manly *et al.*, 2001). Minimum LOD scores of >3.0 were used to develop the linkage map. The software calculated genetic distances in centiMorgans (cM) by applying the Kosambi (1944) mapping function.

Results and Discussion

Twenty-nine semi-dwarf and eighty three lines were hexaploid introgression lines (Table 1) and Fig. 1 shows

the distribution of culm length of hexaploid introgression lines. In the consequence of introgression of reduced height gene from IC12196, culm length of 29 lines was 67–106 cm. Culm length of tall introgression lines was comparable with N67 (Fig. 1). The transmission ratio (27%) of semi-dwarf gene (*Rht-IC12196*) was remarkably less than the ratio of the gene for long glume (P1) found by Kosuge *et al.* (2010), who obtained around 50% of long-glumed introgression lines. Khalifa and Qualset (1974, 1975) found that the proportion of dwarf genotypes in the mechanical mixture and hybrid bulks was gradually decreased from generation to generation under the supposed inter-genotypic competition between tall and dwarf genotypes in *T. aestivum*. It was expected that alleles closely linked to *Rht-IC12196* allele may be eliminated. The percentage of the IC12196-allele around the *Rht-IC12196* locus was about 50%. The reports suggested that intended selection was required to obtain more recombinants for semi-dwarfism.

Table 1. Number of tall and semi-dwarf introgression lines derived from the cross of N67/ IC12196

Chromosome number ¹⁾ (2n)	Culm length		Total
	Semi-dwarf (67-106 cm)	Tall (115-154 cm)	
42	29	83	112
28	3	3	6
Total	32	86	118

¹⁾Chromosome numbers of individual lines were derived from Kosuge *et al.* (2010).

Table 2. Distribution of allele type of 19 markers in semi-dwarf, hexaploid introgression lines

Semi-dwarf lines	Type of allele		
	IC12196	N67	Non-parental
7029	10	2	7
7041	8	8	3
7018	6	7	6
7001	5	12	2
7007	4	9	6
7009	4	15	0
7011	4	14	1
7022	4	13	2
7025	4	13	2
7037	4	14	1
7060	4	10	5
7069	4	12	3
7088	4	14	1
7104	4	11	4
7120	4	11	4
7051	3	12	4
7075	3	12	4
7078	3	12	4
7085	3	12	4
7097	3	15	1
7012	2	15	2
7039	2	16	1
7050	2	15	2
7117	2	16	1
7118	2	14	3
7125	2	14	3
7028	1	17	1
7033	1	18	0
7127	1	16	2

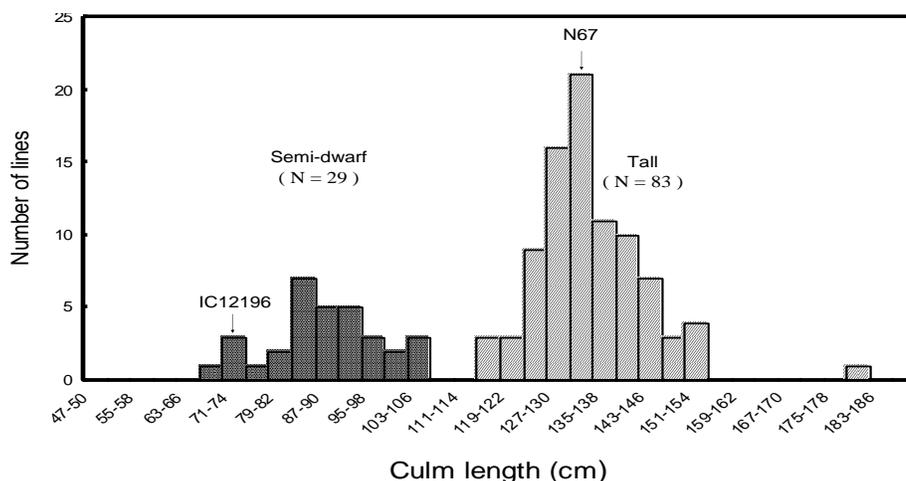


Fig. 1. Distribution of culm length of introgression lines. Arrows indicate mean values of culm length; N67 (132 cm) and IC12196 (74 cm)

Microsatellite mapping of the gene for *Rht-IC12196*

F₁ hybrid between IC12196 and Cando was semi-dwarf. If the loci determining the culm length were different, the segregation would be expected to be 13 semi-dwarf: 3 tall in F₂ progenies but any tall plants were not found in one hundred thirty F₂ plants. It was confirmed that the semi-dwarf gene of IC12196 was allelic to *Rht-B1b*. The dominant alleles of IC12196 were temporarily designated as *Rht-IC12196*. F₂ hybrid of IC12196/LD222 was segregated for plant height (Fig. 1). Segregation of culm length (Table 1) fit a 3:1 ratio ($\chi^2 = 0.434$, $df = 1$). *Rht-IC12196* was linked with markers, *Xbarc1045* and *Xgwm113* on chromosome 4BS (Fig. 2). Except for *Xgwm495* ($\chi^2 = 4.487$, $P < 0.05$), segregation for five markers, *Xbarc1045*, *Xhbg226*, *Xgwm113*, *Xwmc48* and *Xwmc47*, fitted 3: 1 ratios. The χ^2 values ranged from 1.294 to 3.710 ($df = 1$). An inaccurate genetic map of the region of *Rht-B1* locus would modify the expected effects of recombination and linkage. Indeed, conflicting orders of markers in this *Rht-B1* region reported in the ITMI reference map cited in Raquin *et al.* (2008) were not polymorphic around the *Rht-B1* locus and covered a larger genetic distance.

introgression lines and their parents, N67 and IC12196. They were scored for allelic composition at the mapped and un-mapped marker loci on the short arm of chromosome 4B (Fig. 2). Right-handed side of Fig. 2 indicated the type of allele linked with mapped marker in semi-dwarf lines and tall lines. It was supposed that non-parental alleles were not caused by any mixture. Probably they were due to outcross with surrounding plots, because, early generations (F₂ and F₃) were relatively sterile due to the presence of aneuploids. The short arms of chromosome 4B were replaced by N67-allele and non-parental allele in tall accessions. In the great number of introgression lines, the *Xgwm113* and *Xhbg226* markers flanking the *Rht-B1* locus were replaced by N67-allele. It suggested that N67-alleles were more favorable than IC12196-alleles even in semi-dwarf introgression lines. In other words, tall phenotypes were more favorable than semi-dwarf ones during bulk breeding process, and N67-alleles were selectively saved in the progenies. However, the IC12196-alleles at proximal markers were not selective in both tall and semi-dwarf introgression lines.

Allelic composition in the short arm of chromosome 4B of N67/ IC 12196 introgression lines

The introgression lines were designated as IL 7001 to IL 7118 and focused on analysis of 112 hexaploid

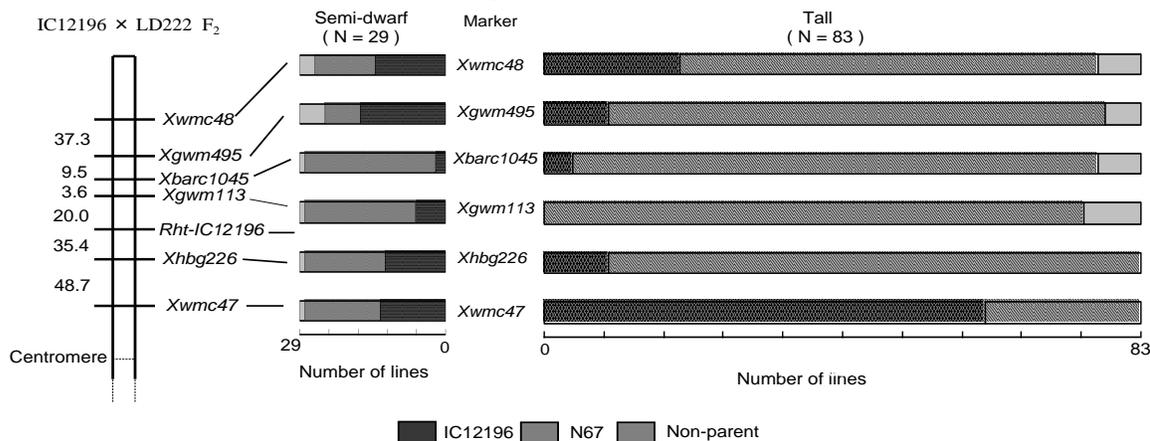


Fig. 2. Linkage map of the short arm of chromosome 4B (left-hand side) and the frequency of allele in hexaploid introgression lines (right-hand side)

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