QTLs Associated with Plant Height and Related Characters in Hexaploid Wheat

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Two genetic systems are involved in the control of the plant height in wheat: *Rht* genes which exert a major effect, and many other genes which usually exert only minor effects on plant height. The purpose of the present study was to identify RFLP markers linked with quantitative trait loci (QTLs) with minor effects on plant height. To remove the strong effect of *Rht* genes, a population of recombinant inbred lines (RILs) from a cross between hexaploid wheat, *T. aestivum* cv. Chinese Spring and *T. spelta*, was used, where neither parents harbor the *Rht* alleles. Seven RFLP markers on chromosomes 1A, 1D, 2B, 2D, and 4B were linked with plant height. In addition, QTLs associated with morphological characters related to plant height could be identified.

Key Words: linkage analysis, QTL, plant height, recombinant inbred lines, RFLP markers, wheat.

Introduction

Plant height is controlled by many genes that regulate development, morphology and vigor. In wheat a series of genes for dwarfness have been listed in the catalogue of gene symbols for wheat (McIntosh *et al.* 1998), in which the genes can be classified into two types based on their reaction to exogenous gibberellic acid (GA) (Gale and Youssefian 1985). In breeding, emphasis had been placed mainly on GA-insensitive genes. However, these genes shorten the coleoptile length, causing problems in deep sowing in dry land. It was reported that deep-seeding and ethylene stimulate the elongation of the first internodes in wheat seedlings (Suge *et al.* 1998). Since the effect of GA-insensitive genes is sometimes too strong to enable the adaptation to stress, many other GA-sensitive genes should be considered for wheat breeding world-wide.

To analyze the complex interaction of these genes, Kuspura and Unrau (1957) used three sets of homoeologous chromosome substitution lines and found that eight chromosomes carried genes for plant height. Snape *et al.* (1977) revealed that 17 chromosomes displayed a genetic variation

for plant height by using the complete set of single chromosome substitution lines.

Recently, the development of molecular genetic markers has stimulated the interest in detecting a linkage between marker loci and quantitative trait loci (QTL) because even if the marker loci do not exert a direct effect on the quantitative traits, they could be used for increasing the response to selection. Cadalen *et al.* (1998) used molecular markers and could detect three genomic regions and one interaction influencing plant height. These regions, located on the chromosome arms 4BS, 7AL, and 7BL, explained 5% to 20% of the variability for this trait.

We analyzed the plant height and its related characters in a population of recombinant inbred lines derived from a cross between common wheat cv. Chinese Spring and spelt wheat *T. spelta* var. *duhamelianum*, neither of which carried major *Rht* genes. By utilizing the RFLP markers, we were able to identify new QTLs for plant height along with QTLs for other morphological characters related to plant height.

Materials and Methods

Plant materials

The F₈ population of recombinant inbred lines (RILs) derived from the cross between *Triticum aestivum* cv. Chinese Spring (CS) and *T. spelta* var. *duhamelianum* (Sp) was used for this investigation (Sasakuma and Tsujimoto, unpub.). Sixty-six RILs in addition to the parents (CS and Sp) were grown in the experimental field of Kihara Institute for Biological Research, in a randomized complete block design (RCBD) with two replications in 1998.

Evaluation of plant height and its related characters

Plant height and its related characters were analyzed in three plants of each replication after complete heading: Plant height (PH) was measured from the ground level to the top of the tallest three tillers in each plant (cm). First internode length (1I) and second internode length (2I) referred to the length of the first and second internodes from the top (cm). Culm diameter (CD) was measured at 10 cm below the rachis node (cm). Number of tillers (NT) corresponded to the total number of tillers with spikes in each plant. Flag leaf length (FL) and flag leaf width (FW) were defined as the length and the maximum width of the flag leaf (cm).

Analysis of variance (ANOVA) for each character was carried out. The differences between the means of lines

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were tested by using the Least Significant Difference test (LSD).

Mean squares (MS) obtained from ANOVA were partitioned into expected mean squares (EMS) to calculate genotypic and phenotypic variances. Heritability (h^2) was calculated from the following formula:

$$h^2 = \sigma^2 G / (\sigma^2 G + \sigma^2 E),$$

where $\sigma^2 G$ is the genotypic variance and $\sigma^2 E$ is the environmental variance.

DNA hybridization and RFLP mapping

The probes used in this study as well as the techniques for DNA extraction, digestion, electrophoresis, blotting and Southern hybridization have been described in detail in our previous paper (Ahmed *et al.* 2000).

One hundred three among the polymorphic RFLP probes revealed 155 marker loci. They were tested on the 66 RILs of CS and Sp. Linkage analysis of the loci was performed with a computer program, MAPMAKER ver. 2.0 for Macintosh (Lander *et al.* 1987). The mapping function of Kosambi (Kosambi 1944) was used. The linkage groups were assigned to the wheat chromosomes according to the published wheat maps (GrainGenes database) and RFLP patterns of nulli-tetrasomic lines of Chinese Spring. The LOD threshold used was 3.0 and the value decreased to 2.5 where the unlinked markers could be added to the linkage groups.

QTL analysis

The QTL analysis was performed as a single marker analysis using the QGENE computer program (Nelson 1997). This program was used to determine the association

between individual marker loci and putative QTLs by using individual marker-genotype groups as class variables for the detection of linkage between markers and putative QTLs. Whenever the F-test exceeded a value necessary for a probability value less than 0.02, the QTLs were considered to be significant. This significance level was chosen to maximize the identification of putative QTLs. The phenotypic effect was calculated as the difference between the class means of CS and Sp.

Results

Variability and correlation of plant height and related characters

The RILs showed large variations in plant height and its related characters (Fig. 1). The analysis of variance indicated that the genetic variations among the recombinant inbred lines for the plant height and its related characters were highly significant ($P < 0.01$) (Table 1). The heritability of these characters, especially plant height and flag leaf length, was very high (Table 2). LSD indicated that the plant height of 26 lines was significantly taller than that of CS at the 5% level, while no lines were significantly taller than Sp.

Plant height was significantly correlated with the other related characters except for the culm diameter (Table 3). The first internode length was the most significant component of plant height ($r = 0.826$) among the related morphological characters. The plant height was also significantly correlated with the heading date ($r = 0.576$) reported in our previous paper (Ahmed *et al.* 2000).

Table 1. Analysis of variance for the plant height and its related characters in RILs

S.O.V	df	Mean Square						
		PH	II	2I	CD	NT	FL	FW
Rep.	1	0.03	26.8	3.0	0.01	3.0	3.5	0.004
Lines	67	373.0**	98.0**	13.5**	0.004**	186.8**	46.6**	0.10**
Error	67	39.3	11.1	3.9	0.001	59.8	4.6	0.01

Note) The abbreviations of the characters are as follows: plant height (PH), first internode length (II), second internode length (2I), culm diameter (CD), number of tillers (NT), flag leaf length (FL), and flag leaf width (FW).

**, highly significant at the 1% level.

Table 2. Means, variance, and heritability for plant height and its related characters in RILs

	PH	II	2I	CD	NT	FL	FW
Means							
RIL	101.2	42.8	26.5	0.32	22.9	30.9	1.83
CS	90.3	39.2	27.5	0.27	13.2	29.8	1.85
SP	122.8	49.8	26.9	0.30	23.3	31.0	1.77
Variance							
$\sigma^2 G$	167.2	43.5	4.8	0.002	63.5	20.9	0.04
$\sigma^2 E$	39.3	11.1	3.9	0.001	59.8	4.7	0.01
$\sigma^2 P$	206.5	54.6	8.7	0.003	123.3	25.6	0.05
h^2	80.9	79.7	55.2	65.4	51.5	81.9	74.3

Note) The abbreviations of the characters are the same as those indicated in Table 1.

Fig. 1. Distribution of plant height and its related characters in RILs. The performance of CS and Sp is indicated by arrow heads.

Single marker analysis

Out of 233 RFLP probes used, 103 (44.2%) showed a polymorphism between the parental lines with at least one enzyme. These probes revealed 155 loci, which were employed for the single marker analysis to check the linkage between the markers and the phenotype of the plant height and its related characters.

A total of eighteen significant marker-trait associations were identified for plant height and its related characters on the chromosomes of the homoeologous groups 1, 2, 3, 4, and 5 (Table 4). Some of them were found to be linked with more than one character at the same time (Fig. 2).

QTLs for plant height were associated with seven markers linked with plant height on chromosomes 1A, 1D, 2B,

2D, and 4B. The alleles of Sp in four markers were associated with a taller plant height (*Xpsr934*, *Xpsr131a*, *Xpsr135*, and *Xcs22-2*) (Table 4), while those in three markers (*Xcdo1160*, *XksuF11b*, and *XksuI27a*) with a shorter height.

Discussion

QTLs for plant height

In the present study we could identify seven RFLP markers associated with plant height. These markers were located on five chromosomes of three homoeologous groups (Fig. 2).

Two markers (*Xcdo1160* and *XksuI27a*) were located on chromosomes 1A and 1D, respectively. On the consensus

Table 3. Correlation coefficient among plant height and its related characters in the RIL population

	PH	II	2I	CD	NT	FL	FW
II	0.826**						
2I	0.553**	0.393**					
CD	ns	ns	ns				
NT	0.559**	0.373**	0.398**	ns			
FL	0.510**	0.446**	0.390**	0.337**	0.523**		
FW	0.403**	ns	0.266*	0.569**	0.390**	0.444**	
HD	0.576**	0.351**	0.250*	0.284*	0.659**	0.613**	0.516**

Note) The abbreviations of the characters are the same as those indicated in Table 1, in addition to HD, which refers to the heading date.

*, **: significant at the 5% and 1% levels, respectively. ns: not significant.

Table 4. RFLP markers associated with QTLs for plant height and its related characters

Trait	Marker name	Chromosome	P value	CS type ¹⁾	Sp type ¹⁾	Phenotypic effect ²⁾
PH	<i>Xpsr934</i>	2D	0.0011	96.6	107.7	-11.1
	<i>Xpsr131a</i>	2B	0.0016	95.9	106.3	-10.4
	<i>Xcdo1160</i>	1A	0.0021	107.0	96.1	10.9
	<i>XksuF11b</i>	2D	0.0024	105.0	94.6	10.4
	<i>Xpsr135</i>	2B	0.0068	96.4	105.3	-8.9
	<i>Xksu127a</i>	1D	0.0129	106.0	97.6	8.4
	<i>Xcs22-2</i>	4B	0.0169	97.5	105.6	-8.1
II	<i>Xcdo1160</i>	1A	0.0006	46.0	39.9	6.1
	<i>Xksu127a</i>	1D	0.0029	45.4	40.3	5.1
	<i>Xpsr131a</i>	2B	0.0050	40.3	45.2	-4.9
	<i>Xpsr934</i>	2D	0.0099	40.8	45.5	-4.7
	<i>Xpsr370</i>	5D	0.0103	40.6	45.3	-4.7
2I	<i>Xpsr934</i>	2D	0.0015	25.5	27.6	-2.1
	<i>Xcdo1160</i>	1A	0.0035	27.4	25.5	1.9
	<i>Xpsr754</i>	3A	0.0094	27.3	25.7	-1.6
	<i>Xpsr901</i>	2D	0.0105	27.2	25.6	1.6
	<i>Xpsr1</i>	2A	0.0111	27.5	25.8	1.7
	<i>Xpsr544</i>	1B	0.0167	27.3	25.8	1.5
CD	<i>XksuD18c</i>	4B	0.0069	0.30	0.33	-0.03
	<i>XksuD14b</i>	1D	0.0070	0.33	0.30	0.03
	<i>XksuG9b</i>	1D	0.0090	0.31	0.34	-0.03
	<i>Xpsr102a</i>	2B	0.0152	0.34	0.31	0.03
NT	<i>Xpsr135</i>	2B	0.0002	18.3	27.2	-8.9
	<i>Xpsr131a</i>	2B	0.0002	18.2	27.1	-8.9
	<i>Xpsr126b</i>	2B	0.0010	18.5	26.2	-7.7
FL	<i>XksuG9b</i>	1D	0.0004	29.2	33.9	-4.7
	<i>Xpsr131a</i>	2B	0.0021	28.7	32.6	-3.9
	<i>XksuF11b</i>	2D	0.0026	32.4	28.7	3.7
	<i>Xpsr164</i>	5A	0.0030	32.7	29.0	3.7
	<i>XksuE16b</i>	2D	0.0120	32.3	29.2	3.1
FW	<i>XksuF11b</i>	2D	0.0009	1.89	1.72	0.17
	<i>Xpsr131a</i>	2B	0.0035	1.74	1.90	-0.16
	<i>Xpsr934</i>	2D	0.0079	1.76	1.90	-0.14

¹⁾ Average values of the sub-populations of CS type and Sp type.

²⁾ Phenotypic effect=means of CS sub-population-Sp sub-population.

The abbreviations of the characters are the same as those indicated in Table 1.

Fig. 2. RFLP linkage map of wheat chromosomes concerning with plant height and its related traits. The names of the markers and their map positions (in cM based on Kosambi function) are shown on the left and the right sides of the chromosomes, respectively. The approximate position of the centromeres, indicated by arrows, was deduced from published wheat maps. White and black boxes in front of the markers, indicate the QTLs detected for the traits in which Cs and Sp increased the performance, respectively. The abbreviations of the traits are the same as those indicated in Table 1. As for heading date, only QTLs that were located on the chromosomes carrying the respective QTLs for plant height and its related traits are shown. Other QTLs for heading date were reported by Ahmed *et al.* (2000).

map of the homoeologous group 1 chromosomes (GrainGenes database), these two markers were tightly linked in the distal part of the long arm, suggesting that these QTLs display a homoeologous relationship. At these QTLs the alleles of CS increased the plant height. The effect of group 1 chromosomes on the plant height has been revealed using nullisomic lines (Sears 1954) and RFLP markers (Cadalen

et al. 1998). Cadalen *et al.* (1998) reported the presence of significant loci on the chromosome arms 1AS and 1BL.

Two markers *Xpsr131a* and *Xpsr135* on 2B, linked with each other at 12.5 cM, and the allele of CS reduced the plant height. It is assumed that one QTL may be located in the region between the markers. In addition, the CS allele of one of the two markers on chromosome 2D (*Xpsr934*) re-

duced the plant height. On the other hand, the CS allele of the other marker (*XksuF11b*) increased the plant height. The three markers (*Xpsr131a*, *Xpsr135*, and *XksuF11b*) affected the heading date as well. Ahmed *et al.* (2000) reported that they were linked with genes for earliness *per se*. The CS alleles which led to early heading (*Xpsr131a* and *Xpsr135*) reduced the plant height, while the CS alleles which led to late heading (*XksuF11b*) increased the plant height. These findings may account for the fact that the plant height showed a highly significant positive correlation with the heading date (Table 3). Therefore these QTLs exert pleiotropic effects on the heading and plant height. However, we could not easily determine the cause effect relationship between the traits because not all the QTLs that affected the plant height exerted an effect on the heading date and vice versa. The QTL detected on 2B exerted pronounced phenotypic effects on the plant height, first internode length, number of tillers, flag leaf length and flag leaf width (Table 4). We could not map an RFLP marker *psr109* which was reported to be linked with *Ppd-B1* (Worland 1996). The QTL detected here in this study (*Xpsr131a* and *Xpsr135*) was located on the short arm within about 30 cM from the centromere. Worland (1996) reported that gene(s) for earliness *per se* were associated with the group of centromeric RFLP markers. On the other hand, the short arm of chromosome 2B carried a strong photoperiod gene (*Ppd-B1*) at about 40 cM from the centromere (Graingenes database). Therefore, it could not be determined presently whether the QTL on 2BS represents a single *per se* gene or exerts a pleiotropic effect of *Ppd-B1*. However, it is important to know that a strong gene (or genes) on 2BS affects several morphological traits at the same time.

For the QTL with the marker (*Xcs22-2*) on chromosome 4B, the allele of CS reduced the plant height. Cadalen *et al.* (1998) reported the presence of two QTLs on chromosome 4B in the cultivar "Courtot", and stated that there may be two copies of *Rht-B1* genes in this cultivar. Our marker associated with the QTL was located in a region near the centromere in which one of the *Rht* genes of the cultivar "Courtot" is located. Neither CS or Sp harbours the *Rht-B1* gene. However, the present QTL may be attributed to the differences in the recessive alleles of *Rht* between CS and Sp.

Among the six QTLs for plant height, the alleles for the increased height were found in the shorter parent (CS), whereas some for height decrease were derived from the taller parent (Sp) (Table 4). These results are in agreement with the presence of transgressive lines (shorter than CS or higher than Sp) in our RIL population.

QTLs for related characters

Five and six markers were found to be associated with the first and second internode length, respectively. Since these two traits are direct components of the plant height, many of these markers were common to those for height. However, we were able to detect QTLs which specifically

acted for internodes: *Xpsr370* associated with the first internode length only, and *Xpsr754*, *Xpsr901*, *Xpsr1*, and *Xpsr544* associated only with the second internode length. Although all of the specific markers were significant at the 2% level, the phenotypic effects were less pronounced compared with those of plant height. Thus, they did not reflect the plant height.

Unlike the first and second internode length, none of the four markers associated with the culm diameter were linked with either the plant height or the first and second internode length. This finding is in agreement with the correlation analysis between the culm diameter and plant height (Table 3). Chromosome 1D carried two QTLs for culm diameter showing an opposite effect although these markers were linked with each other.

We detected strong QTL(s) for the number of tillers on chromosome 2B. Two of the markers were linked with plant height as well, and one marker *Xpsr126* showed only a QTL for the number of tillers.

The QTLs for plant height on the 2B and 2D chromosomes that are related to heading (earliness *per se* genes) affected the flag leaf length. The QTL for late heading, and thus tallness increased the length. Because CS and Sp may carry different alleles for opposite effects on their 2B and 2D chromosomes, many transgressive types (shorter than CS and longer than Sp) appeared in the RILs. In addition, we detected a strong QTL for the flag leaf length on chromosome 1D which affected also the culm diameter. The allele of CS led to a shorter leaf and thin culm. All of the QTLs for the flag leaf width were involved in plant height.

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