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RESEARCH ARTICLE

Inheritance of (1-3)(1-4)-Beta-D-Glucan Content in Barley (*Hordeum vulgare* L.)

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Abstract

β-glucan is the soluble dietary fiber component and occurs at its highest in barley. This study aims to evaluate the inheritance of β-glucan content in barley grains and to map quantitative trait loci (QTL) associated with this trait. F_5-derived 107 lines from the cross of the six-rowed waxy hulless barley, 'Yonezawa Mochi' and the six rowed non-waxy hulless barley, 'Neulssalbori' were measured for their agronomic traits and β-glucan level at four different environments. These recombinant lines showed significant genotypic variation ($P < 0.01$) and normal distribution for β-glucan content with a range of 43.6 - 62.1 g kg^{-1} across environments. A significant genotype-by-environment interaction was also found. The broad-sense heritability estimates for β-glucan content ranged from 0.42 to 0.82 across environments. Using one-factor analysis and composite interval mapping, a main effect of QTL associated with β-glucan content was identified in the genomic region near waxy gene (*wx*) and HVM4 on chromosome 7H. The major QTL at this region explained on average 44.4% of the variation for the mean of β-glucan content across environments with LOD values that ranged from 5.7 in Suwon in 2001 to 13.9 in Suwon in 2003. Two minor QTLs were identified but their significance of association with β-glucan content was inconsistent across environments..

Key words: barley, β-glucan, DNA marker, mapping, QTL

Introduction

Barley has been one of the major food crops and a main source of starch. Recently, a lot of interest has been placed on barley because of its nutritional function. This trend is quite prominent in the world where it has been mainly used as either animal feed or for brewery. In Korea, barley has long been cultivated since the fifth century B.C. and consumed as a staple food.

Barley cereals are especially good source of water-soluble dietary fibers. As one of these fibers, β-glucan is a major structural component in the cell walls in barley endosperm and in aleurone tissues. Structurally, β-glucan is a polymer of beta-D-glucose with about 70% of the glucose residues 1,4-linked, and about 30% 1,3-linked. The overall size of the molecule and the relative order of 1,4 and 1,3 linkages can vary (Roubroeks et al. 2001).

The amount of β-glucan is one of the important factors affecting barley grain quality associated with its nutritional and therapeutic values. As compared to other cereals, barley has relatively high content of β-glucan between 2 and 10% (Bamforth 1982). As a healthy diet for humans, this fiber is known to have hypocholesterolemic effects with decreased serum and low-density lipoprotein cholesterol (Klopfenstein and Hoseney 1987). On the other hand, barley β-glucan is an undesirable component for brewery and animal feed. It contributes considerable viscosity to the mash and may cause slow wort filtration and haze formation in the beer. The β-glucan in barley may also cause sticky

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droppings and decreased growth rates in poultry (Gohl et al. 1978).

With the development of various molecular markers and extensive genome studies, quantitative trait loci were mapped in the barley genome and a few closely flanking markers associated with traits of interest have been identified. As for β-glucan content of barley grains, a few regions of the genome across all seven chromosomes were found with significant associations depending on different genetic background of two or six-rowed barley (Gao et al. 2004; Han et al. 1995; Igartua et al. 2002; Jeung 2000; Li et al. 2008; Mather et al. 1997; Molina-Cano et al. 2007). These molecular genetic information and marker technologies offer plant breeders a new challenge to manipulate β-glucan content more efficiently for target selection in barley.

Genetic enhancement for β-glucan content is one of the major goals of the Korean barley breeding program which aims to improve the end-use quality and functional property for human health. When evaluated, the Korean waxy barley genotypes have higher β-glucan content level than non-waxy genotypes by 0.59% on average (Kim et al. 2006). In particular, it is notable that consumption of waxy barley as food has been on the rise due to its better palatability, nutrition, and cooking-related properties. The study aims to determine genetic basis of β-glucan content of barley grains in relation to the impact of waxy genetic background and to present the results from mapping QTLs that control β-glucan level.

Materials and Methods

Plant material

A population of F_5-derived lines derived from the cross between a Japanese barley cultivar with high β-glucan content, 'Yonezawa Mochi' and a Korean barley cultivar with low β-glucan content, 'Neulssalbori' was developed and used for map construction and QTL analysis. Both parents are six-rowed and hulless barley genotypes carrying different qualitative genes of each other; 'Yonezawa Mochi' has both waxy and uzu (*uz*) genes, but 'Neulssalbori' doesn't have any. The uzu gene is responsible for a semi dwarf phenotype in barley with such characteristics as dark green leaves, short awns and panicles.

Field trials

One hundred seven F_5-derived lines and their parents were grown in four different environments in Suwon from 2001 to 2003 and Jinju in 2002. Experimental plots were arranged in a randomized complete block design with two replications. The plants of individual lines were sown in the plots with 3-m row length and 40-cm row spacing. Cultivation of plant materials including the quantity of sowed seeds and fertilization followed the standard cultivation method for winter cereal crops developed by Rural Development Administration (RDA) in Korea. In general, seeds were sown at a rate of 130 - 140 kg ha^{-1}. Individual genotypes were evaluated for agronomic traits such as heading date and plant height in the field.

Measurement of β-glucan content

Bulked seeds of individual lines harvested from each replicate at a given location/year environment were determined for β-glucan content. For balanced sample preparation, 5-grams of seed was ground in a ball mill with a 0.5-mm mesh screen. Total β-glucan content of each line was determined by McCleary method which was dependent on a streamlined enzymatic reaction and absolutely specific for mixed-linkage β-glucan (McCleary and Mugford 1997).

DNA marker analysis

Genomic DNA was isolated from a bulk of fresh leaf tissues of 2-week-old plants by a modified CTAB procedure (Saghai Maroof et al. 1984). RAPD and SSR analyses were performed as described by Kim et al. (2005) and Kim et al. (2002), respectively.

For RAPD analysis, 10 ng of genomic DNA of each genotype was amplified with random decamer primers (Operon Technologies Inc., Alameda, CA, USA) using a thermal cycle profile of 5 min at 93ºC followed by 45 cycles of 1 min at 93ºC, 1 min at 36ºC, 2 min at 72ºC, and an additional 10 min at 72ºC. PCR bands were visualized on a 1.4% (w/v) agarose horizontal gel by ethidium bromide staining.

For SSR analysis, a set of 37 primer pairs were selected as anchor markers assigned to individual chromosomes. PCR amplification conditions were optimized depending on primers following the protocols described in Becker and Heun (1995) and Liu et al. (1996). The PCR product was separated on the 5% polyacrylamide gel, and was visualized by the silver staining method (Promega, Madison, USA).

For STS analysis, anchor primer sets were applied with thermal cycle profile of 5 min at 94ºC followed by 35 cycles of 30 s at 94ºC, 30 s at 50ºC, 1 min at 72ºC, and additional 5 min at 72ºC. Whenever the size of PCR bands was expected to be larger than 1.0 kb, annealing and extension times were changed to 1 and 2 min, respectively (Jeung 2000). Polymorphism of STS bands was visualized on the 6% polyacrylamide gel by ethidium bromide staining.

AFLP analysis was conducted as described by Vos et al. (1995) with some minor modifications (Jeung 2000). After digestion of genomic DNA with restriction enzymes of *Pst*I and *Mse*I, ligation was conducted with *Pst*I adaptor (5'-CTC GTA GAC TGC GTA, CAT, GCA; CAT CTG ACG CAT GT-5') and *Mse*I adapter (5'-GAC GAT GAG TCC TGA G; TAC TCA GGA CTC AT-5'). The first amplification was conducted with *Pst*I and *Mse*I with pre-selective primers (G and A for *Pst*I, C for *Mse*I) as described by Vos et al. (1995). Second amplification of the first amplified DNA was then conducted with *Mse*I and *Pst*I primers with three selective nucleotides (Jeung 2000). The AFLP-PCR product was separated on the 5% polyacrylamide gel, and was visualized by the silver staining method as described above.

Table 1. Average values of agronomic traits of 'Yonezawa Mochi', 'Neulssalbori' and their progeny F_5-derived population across environments

	Heading date (M/d)	Maturing date (M/d)	Plant height (cm)	Head length (cm)	No. of kernels per head	1,000-seed weight (g)
Yonezawa Mochi	4/27	6/5	88.5	5.3	65.4	27.3
Neulssalbori	4/26	6/2	85.1	5.6	58.7	27.2
Yonezawa Mochi/Neulssalbori F_5-derived population						
Average	4/28	6/3	89.1	6.2	61.3	29.2
Maximum	5/1	6/4	99.3	7.6	67.6	33.7
Minimum	4/23	6/1	69.4	4.3	54.5	22.1
LSD 0.05	1.6	1.8	6.7	0.7	5.4	2.4

Statistical analysis

Analysis of variance was conducted on the field data of phenotypic traits and β-glucan content using PROC GLM of SAS (SAS Institute 1985). F_5-derived lines, years, locations, replications, and blocks were analyzed as random effects. Each location/year combination was considered as one environment. Data from each environment were analyzed separately, while a combined analysis of variance for all environments was conducted. Estimates of variance components and broad-sense heritability values of traits were calculated for each and across environments.

Segregation data of 88 DNA markers and two morphological markers were analyzed to test linkages among them using a computer program MAPMAKER/EXP3.0 (Lander et al. 1987). A minimum likelihood of odds (LOD) of 3.0 and maximum distance of 50 cM was used as the linkage criteria. Linkage groups were assigned to the barley chromosomes on the basis of anchor markers of the barley frame map. For single-marker approaches to detect QTL, significant associations of β-glucan content and individual marker loci on linkage groups were tested with one-factor analysis of variance using PROC GLM of SAS.

For resolution of location of QTLs, composite interval mapping (CIM) was performed using the default parameters of forward/backward regression (forward $P < 0.01$, backward $P < 0.01$) in the Windows QTL Cartographer ver. 2.5 (Basten et al. 2007) to detect QTL controlling β-glucan content. In order to determine significant thresholds for precise inference of QTLs and to control type-I error, empirical LOD thresholds were calculated through 1,000 permutations at $P = 0.05$ and $P = 0.1$ for CIM. The walking step for the mapping was set at 0.5 cM.

Results

Agronomic traits

The parents, 'Yonezawa Mochi' and 'Neulssalbori' and a population of 107 F_5-derived lines were evaluated for some important agronomic traits at all four environments. Table 1 shows the average, minimum and maximum values of parents and population lines across environments. 'Yonezawa Mochi' matured 3 days earlier and its kernel numbers per head were greater than 'Neulssalbori'. Heading date and 1,000-grain weight of both parents were similar. One hundred-seven lines of population were significantly different for the agronomic traits in all four environments from 2001 to 2003. Also, the mean values of all lines in the population across environments were significantly different for the agronomic traits as shown in Table 1. Significant interactions of environments with genotypes were observed in the variance of heading date and 1,000-grain weight (data not shown).

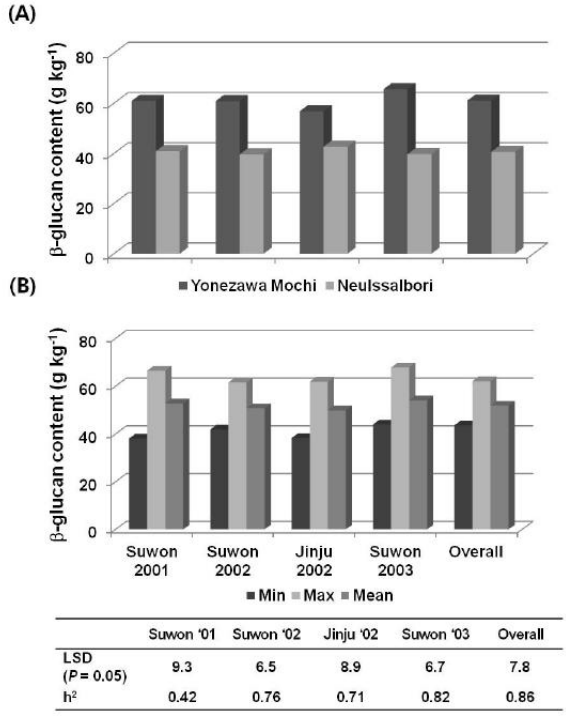

Fig. 1. Comparisons of parental cultivars, 'Yonezawa Mochi' and 'Neulssalbori', for β-glucan content (A), minimum, maximum and mean values of β-glucan content, broad-sense heritability estimates for the F_5-derived population for individual environments and across four environments (B).

β-glucan content

The parent, 'Yonezawa Mochi' exhibited significantly higher β-glucan content ($P < 0.05$) than 'Neulssalbori' at each environment and across environments (Fig. 1). The difference in β-glucan content between two parents ranged from 14.1 to 25.9 g kg^{-1} over environments. F_5-derived population from the cross of 'Yonezawa Mochi' x 'Neulssalbori' showed a continuous distribution for β-glucan content with the average of all lines non-significantly different from that of the parents for each and across environments (Fig. 1). There was a significantly transgressive segregation with lines from the population having higher or lower β-glucan content than 'Yonezawa Mochi' and 'Neulssalbori', respectively. In particular, two waxy lines of the population, YN047 and YN006 have higher β-glucan content than 'Yonezawa Mochi' by an average of 0.8 - 0.9 g kg^{-1} over environments.

There was significant ($P < 0.01$) genotypic variation for β-glucan among the lines in the population at each environment and across environments. The genotype x environment interac-

Table 2. Correlations between β-glucan content and agronomic traits on a pair-wise basis across environments

Trait	Suwon'01-'03	Suwon '01	Suwon '02	Suwon '03
Heading date (HD)	-0.06	-0.10	-0.07	0.11
Maturity date (MD)	0.03	0.07	-0.06	0.15
Maturity period (MD-HD)	0.08	0.19	0.05	0.00
Plant height	0.01	0.02	0.00	0.02
Head length	0.10	0.18	0.02	0.11
Kernel numbers per head	-0.07	0.03	-0.04	-0.12
1,000-grain weight	0.14	0.36	0.05	0.07

Table 3. Markers significantly associated with β-glucan content of barley grains at the 0.01 probability level in each and across environments based on one-factor analysis. The estimates shown in the table were calculated with the average β-glucan content values for individual lines across environments 2001 - 2003

Marker	LG	Chr	Pr	R^2 (%)	Genotypic means‡ (g kg^{-1})		
					YY	YN	NN
Waxy	1	7H	***	45.9	55.2	53.7	47.9
HVM4	1	7H	***	52.7	55.1	53.2	47.8
HVM49	2	7H	**	15.2	54.2	50.8	50.5
					YY	Y_ / _N	NN
OpU01	1	7H	***	24.5		54.1	49.4
AFYN47_1	1	7H	***	14.1		53.9	50.4
AFYN29_3	7	?	***	14.8	54.6	51.0	
AFYN33_1	8	?	**	10.3		53.7	50.7

, * significant at 0.01 and 0.001 probability levels, respectively.
‡ YY designates homozygous 'Yonezawa Mochi' class; YN designates segregating class; NN designates homozygous 'Neulssalbori' class; Y_ designates segregating and homozygous 'Yonezawa Mochi' class; _N designates segregating and homozygous 'Neulssalbori' class.

tions, i.e. genotype x locations for 2002 or genotype x years for 2001 - 2003, for β-glucan content were also significant. The broad-sense heritability estimates for β-glucan content were relatively high ranging from 0.42 to 0.82 for individual environments (Fig. 1). The correlations for β-glucan content of lines were highly significant between the means of individual environments with a range of 0.6 - 0.73. The majority of correlations between β-glucan content and agronomic traits on a pair-wise basis did not exist or were inconsistent across environments (Table 2). In most cases, positively or negatively very low correlations were observed except for 1,000-grain weight (0.36) in the environment of 2001 Suwon.

QTL mapping of β-glucan content

The parents of the population were tested with SSR, STS, RAPD, and AFLP markers to identify polymorphisms. For the SSR markers, 10 (27.8%) out of the 36 SSR primer pairs tested revealed polymorphisms between the parents with the acrylamide gel system. For RAPDs, 28 (7.5%) out of the 373 decamer primers revealed polymorphic fragments between the parents. For STS-PCR, 8 (4.8%) out of the 167 primer pairs revealed polymorphisms. For AFLP, 53 marker loci derived from 31 selective primer pairs were polymorphic between parents. For marker genotyping, the population was scored for 2 morphological marker loci, 8 SSR, 2 STS, 24 RAPD, and 53 AFLP DNA marker loci. Linkage analysis of the 89 marker loci resulted in the formation of 13 linkage groups (LG) with 17 markers remaining unlinked.

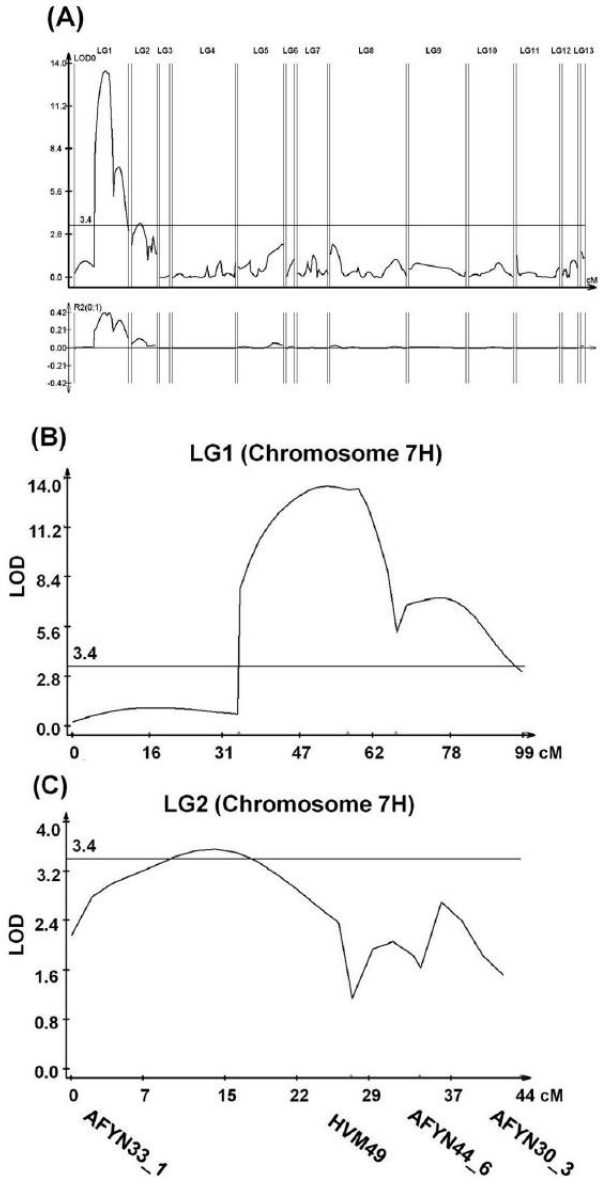

Fig. 2. Results of QTL analysis using composite interval mapping approach for the average barley β-glucan content across environments 2001 – 2003 : Quantitative trait loci likelihood map indicating likelihood of odds (LOD) score profiles (above) and the plot of the estimated R^2 values indicating the proportion of phenotypic variance explained by the QTL at the position (bottom) for 13 linkage groups (A), Distribution of DNA markers along linkage group 1 assigned to chromosome 7H and their LOD scores (B), Distribution of DNA markers along linkage group 2 assigned to chromosome 7H and their LOD scores (C).

Single-factor analysis of variance was conducted for individual markers mapped to the linkage groups to identify their significant association with β-glucan content. Seven markers consisting of one morphological trait (waxy gene), 2 SSR, 1 RAPD, and 3 AFLP markers were found to be significantly ($P < 0.01$) associated with β-glucan content in each and across environments (Table 3). In particular, the SSR marker HVM4 on LG1 which was assigned to barley chromosome 7H had the highest association with β-glucan content, explaining 52.7% of the variation in the population. The mean β-glucan content of individu-

Table 4. QTL and markers significantly associated with average β-glucan content of F_5-derived population of the 'Yonezawa Mochi' x 'Neulssalbori' cross in each and overall environments on the basis of composite interval mapping analysis

Environment	Chromosome	Marker	LOD	Position (cM)	Additive effect	Dominance effect	Variance explained
Overall	7H	HVM4	13.5	52.3	0.35	0.08	44.4
	7H	opU01	7.25	74.8	0.29	0.29	33.8
	7H	HVM49	3.57	13.9	0.16	-0.16	11.3
SW03	7H	HVM4	13.88	52.1	0.44	0.06	42.6
	7H	opU01	6.95	76.8	0.34	0.14	27.2
	7H	AFYN30_3	3.78	39.9	0.18	-0.05	8.2
SW02	7H	HVM4	10.79	56.7	0.34	0.19	38.7
	7H	opU01	6.65	72.7	0.28	0.62	37.6
	7H	AFYN30_3	3.23	38.0	0.15	0.38	11.7
JJ02	7H	wx	9.64	46.3	0.39	0.27	36.4
	7H	HVM4	9.37	59.1	0.35	0.51	32.7
	7H	opU01	7.12	71.0	0.30	0.79	31.6
SW01	LG 5	HVM3	3.9	71.4	-0.24	-0.09	13.0
	7H	HVM4	5.7	50.2	0.27	-0.17	29.2
	7H	AFYN33_1	4.16	2.1	0.14	-0.74	25.0

als homozygous for HVM4 marker allele from 'Yonezawa Mochi' was 55 g kg^{-1}, which was higher than that of 'Neulssalbori' (48 g kg^{-1}) across all environments. AFLP markers such as AFYN37-1, AFYN60-1, AFYN61-1, and AFYN64-2 were significant only at Jinju environment in 2002.

Composite interval mapping (CIM) analyses of QTLs were conducted, and their results from different environments are shown in Table 4. As revealed by single factor analysis, significantly increasing effect attributed by the QTL at chromosome 7H was identified for β-glucan content in each and combined environments. In particular, QTL with consistent major effect could be placed in the genomic region near waxy gene and HVM4 at which LOD ranged from 5.7 at Suwon 2001 to 13.9 at Suwon 2003. The QTL at this region had the greatest association with β-glucan content, explaining on average 44.4% of the variation in the population across combined environments. As shown in the Table 4 and Fig. 2, the additive effect of the allele of 'Yonezawa Mochi' at the locus of HVM4 was estimated to be 0.35, with the dominance deviation of 0.08 for across environments. One more peak with significant but lower LOD level over all environments was near opU01 on LG1 (chromosome 7H), suggesting that there may be two separate QTLs on this linkage group. LG2 contained two peaks with a LOD of 3.0 at the interval of two markers, AFYN33_1 and HVM49 for Suwon 2003 and a LOD of 3.8 near AFYN30_3 for Suwon 2003.

Unlike the major genetic effect of waxy gene on the β-glucan content, the significance of the putative QTLs on LG2 (chromosome 7H) and on LG5 was inconsistent depending on the environment (Table 4). These two QTLs near AFYN30_3 and HVM3 were significantly associated with β-glucan content of grains harvested in Suwon 2002 and Jinju 2002, respectively.

Discussion

Even though barley β-glucan is a polygenic trait, the future potential of genetic gain by selection for this trait in the breeding program can be expected with moderate to high values of heritability estimated in this study. Different interactive behaviors of genotypes depending on the environments with respect to β-glucan content may influence on the limit of possibility of genetic improvement. With this study, the genetic correlations for β-glucan in different environments were significant but not high enough. In this case, it is assumed that direct selection on the single plant basis is possible to manipulate the β-glucan level on a long term goal.

Barley grain β-glucan content might not be influenced by agronomic traits with no significant correlation. As described in the findings of others, inconsistent correlations between β-glucan content and agronomic traits were observed (Peterson et al. 1995). Hang et al. (2007) reported that seed yield and test weight were weakly and negatively correlated with β-glucan in barley. Güler (2003) found positive correlations between β-glucan and protein content, but significantly negative correlation between β-glucan and 1,000-grain weight. These findings, including the result of this study suggested that it was not likely that selection of higher or lower β-glucan in the breeding program should have influence on the genetic shift of other agronomic traits as a correlated response.

Genetic mapping based on mean β-glucan content across environments resulted in the finding of significant contribution of the genomic regions around waxy gene locus originated from 'Yonezawa Mochi'. When considering small population size in this study, the magnitude of the QTL identified in the chromosome 7H might be over-estimated (Beavis 1998). Significant impact of waxy gene on the rise of β-glucan level in barley grains has already been observed in previous studies (Jeung 2000; Wood et al. 2001).

Except for the QTL in the chromosome 7H, inconsistent QTLs were detected for β-glucan content over the environments. This indicates that the magnitude of their influence on β-glucan content varied due to environmental impact on the expression of genetic loci as verified by the significant genotype x environment interactions. Another possible reason is that minor QTL

with small effect on the β-glucan may not be detected due to the masking effects of major QTL within the epistatic QTL-QTL interactions. As compared to the results of β-glucan content associated with monogenic-like or most QTL represented by waxy gene, however, a few QTL with different genetic effects have been detected in all seven chromosomes by using the various mapping populations constructed from the parents carrying normal starch but different level of β-glucan in former studies.

The introduction of waxy gene is important in Korean barley breeding program since it changes physico-chemical properties of barley grains to improve cooking quality in boiled rice and barley mixture. Waxy barley grains are characterized by lower initial gelatinization temperature and higher maximum viscosity with improved swelling power and water-binding capacity. As proven in our studies, the waxy gene derived from 'Yonezawa Mochi' is genetically associated with an increase in β-glucan content. This indicates that the candidate gene for waxy grain phenotype may affect biosynthesis of variant forms of starch components and also atypical regulation of photosynthate product allocation into synthesis of cell wall polysaccharide. The molecular characteristics of waxy allele resulted from genetic mutation of a deletion of about 400 base pairs in Granule Bound Starch Synthase I (GBSSI) locus in starch synthesis has been partly well disclosed (Patron et al. 2002). GBSSI, encoded by the waxy gene, is a key enzyme of starch synthesis and determines the accumulation of amylose in the starch granules. This GBSSI waxy mutant allele might have a pleiotropic effect on β-glucan. Though, little is known about the physiological molecular mechanism of waxy gene in this locus for the increase of β-glucan content. The waxy gene conferring on the β-glucan content was quite effective, but can only explain about 50% of the total phenotypic variation.

Consumption of β-glucan-enriched foods and supplements has been on the rise as its benefits as a healthy diets becomes widely known (Ames and Rhymer 2008). This offers major opportunities to the barley industry where many forms of barley or barley extracts will be produced for the growing market. In line with this, various researches and breeding programs will be encouraged aiming for the improvement of hulless and waxy barley cultivars worldwide. Genetic markers mapped in these genomic segments may be available for selection of QTL associated with barley β-glucan content in early plant growth stage. It is likely that putative QTL may be more precisely assigned for their location as our linkage map will be more densely saturated with genetic markers.

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