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Genomic regions influencing β glucan content in barley (*Hordeum Vulgare L)*

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Abstract: *This study aims to evaluate the β-glucan content in barley grains and to localize genomic regions influencing this trait in F3-derived recombinant inbred lines from a cross DWR30/Shebec. Biochemical assay was performed to measure the â-glucan content of each individual RIL that ranges from 3.0% (Shebec) to 6.4% (DWR30) in recombinant lines as compared to 3.0% and 7.5% for contrasting parental lines. Molecular analysis was performed using closely linked DNA based markers viz. Amy2, Brz, Bmag603 and HvBKASI for β-glucan content trait. During multiple regression analysis, Brz (7HL) was found to be closely associated with β-glucan content trait. This analysis indicates the involvement of genomic regions of longer arm of chromosome 7H in the inheritance of β-glucan content in barley. This information also provides an opportunity to barley breeders to use this marker for screening barley genotypes for low β-glucan content during malt barley breeding program.*

Keywords: *Barley, malt quality, β-glucan, molecular markers, regression analysis*

INTRODUCTION

Barley (*Hordeum vulgare* L.) is an important cereal grain crop grown chiefly in the northern plains and hilly areas in India for feed, food and malt. It is one of the most important cereal crops majorly used as primary ingredients in brewing industries for beer production in most of the world breweries. The rising up demand of malt based product attracts the attention of plant breeders to enhance the quality of barley grain. During malting, the stored mixed linked β glucan is partially depolymerized by enzyme synthesized during seed germination that can be utilized by yeast during brewing (Jones 2005a). Malting quality is a complex phenotype that combines a large number of interrelated components, each of which shows complex inheritance (Hayes and Jones 2000). Mixed-linked (1-3, 1-4)-β-D glucans are the major components of starchy endosperm and aleurone cell walls in barley grain. β-glucan is a non-starch polysaccharides which is a polymer of D-glucose residues with about 70% glucose residue 1-4 linked and about 30% are 1-3 linked. High levels of β-glucan have deleterious effects in both malting and brewing processes and it may reduce the rate of endosperm modification, by forming a barrier to the enzymes responsible for protein solubilization and starch breakdown. In addition, if high levels of β-glucan persist into the final malt and are solubilized during hot water extraction, they cause increases in wort viscosity which may lead to filtration problem (Bamforth and Barcaly 1993).

β-glucan is the major quantitative trait of commercial importance in brewing industry and has attracted plant breeders for genetic studies to identify genomic regions/QTLs involved in inheritance of this trait in barley. The amount of β-glucan was reported to be regulated by several QTLs located on chromosome 2H and 5H (Han *et al;* 1995), 4H (Wei *et al;* 09), and 7H (Li *et al*; 2008). Similarly Han *et al.* (1995) reported that chromosomes 2, 1, 4 and 7 harbored loci having largest effects on barley β-glucan and malt β-glucan during quantitative trait loci (QTL) analysis from the cross of Steptoe/Morex. In another study on marker-assisted selection for malting quality traits, two major quantitative trait loci (QTL) regions were identified in six-row barley for malt extract percentage, alfa-amylase activity, diastatic power, and malt β-glucan content on chromosomes 7H (QTL1) and 3H (QTL2) with flanking markers, Brz and Amy2, and WG622 and BCD402B, for these regions in

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doubled haploid population derived from cross of Steptoe/Morex (F. Han *et al.* 1997). This study is conducted with the objective of scanning the genome of barley for closely linked molecular marker which gives close information to biochemical characterization of recombinant inbred line, provided as a resource.

MATERIAL AND METHODS

Plant material

Recombinant inbred lines (RILs) population was developed from a cross DWR30/Shebec. DWR30 and Shebec are two row barley genotypes identified for between a high (DWR30) and low β glucan (Shebec) contents, respectively. Seed material of 44 RILs of $F_{3:4}$ generation were used as a resource for conducting the following experiment. The seed material of RIL lines was procured from Barley Section of Indian Institute of Wheat & Barley research, Karnal.

Biochemical assay

β-glucan content of each line was determined by McCleary enzymatic method given by McCleary and Codd (1991) (AOAC method 995.16) which was dependent on a streamlined enzymatic reaction and absolutely specific for mixed-linkage â-glucan. 500mg of seeds of each individual line was ground in a ball mill with a 0.5-mm mesh screen. 0.5g of finely ground flour dispersed in 5.0 ml of sodium phosphate buffer (20mM, ph- 6.5). After incubation in boiling water bath the solution was subjected to lichenase treatment at optimum 40 °C for 1 hour, supernatant was recovered by centrifugation of 30ml solution that was adjusted with distilled water. the biochemical reaction was carried out in three set of test tube in which one set was taken as control having 0.1ml of samples and 0.1ml sodium acetate buffer (50mMph 4.0) other set of tubes in duplicate was having 0.1ml sample and 0.1ml β-glucosidase enzyme to break β-oligosaccharide and recovered individual glucose residuals. Standard and blank was also prepared simultaneously. Pink color was developed by using glucose oxidase peroxidase reagent which was read at 510nm.

Molecular screening of RILs

Molecular marker

Molecular markers reported to be closely linked with malt quality and β-glucan traits in barley in earlier reports were used for molecular screening of RILs and parental lines (Table 1). The sequences of these SSR/STS markers were obtained from *www.graingenes.com* and oligos were synthesized from Sigma Aldrich, India.

DNA extraction and PCR Amplifications

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). The isolated DNA was qualitatively and quantitatively checked on 0.8% agarose gel. DNA of concentration 50-100ng suitable for PCR amplification was prepared and PCR amplifications were carried out in 10 µl volume of PCR buffer (0.01 M Tris, 0.05 M KCl, 1.5 mM MgCl2, 0.01% gelatine), approximately 100 ng of genomic DNA, 0.2 mM of dNTPs, 0.2 lM of each primer and 1 U of Taq polymerase. PCR amplifications were done as initial denaturation at 94° for 5 minutes followed by 35 cycles at 94^0 for 45 second, annealing at 55, 58 or 60 depending upon the Tm of SSR/STS markers, for 45 seconds, extention at 72^0 for 45 seconds and final extention at 72^0 for 6 minutes BIORAD S 1000 thermocycler. PCR products were resolved by electrophoresis on 2% agarose gels (HiMedia) at 4v/cm in 0.5 X TBE buffer. Fragment sizes were approximately calculated by interpolation from the migration distance of marker fragments of 100-bpDNA ladder (NEB, UK) and corroborated with the reported amplified fragment size of respective molecular marker. The occurrence of 'null' alleles was verified by re-amplification under similar PCR conditions. Gels were stained with ethidium bromide (0.5ug/ml) and DNA banding patterns were visualized under UV light (Syngene Synoptics Ltd. USA). Differences in the banding pattern were qualitatively scored from gel photographs for the presence (1) and absence (0) of bands assuming that each band represents a unique genetic locus. Scoring was done for clear, unambiguous amplicons ranging from 100bp to 600bp. The band size was determined by comparing with 100bp DNA ladder.

Statistical analysis

For identification of association between SSR/STS molecular markers and biochemical data, multiple regression analysis was performed using SAS statistics (Version 9.1.3.) software to examine associations that are more likely to be based on repeat length variation of STS. Multiple linear regression analysis was performed to examine the effect of adding extra molecular markers that were previously reported to be linked with β glucan trait. The adjusted R^2 values obtained for the two alleles of marker that detected heterozygotes were different.

Table1
Details of STS/SSR markers used for screening RILs in this study

Molecular marker	*Chr*	*Tm (^{0}c)*	*Allele size (bp)*	*Reference*
Brz	7H	58	300	F. Han *et al*. 1997
Amy2	7H	58	500	F. Han *et al*. 1997
Bmag603	3H	55	120	Han F *et al*. 1995
HvBKASI	2H	58	197	K. Pillen *et al*. 2003
WG622	4H	60	161	F. Han *et al*. 1997

RESULTS

Barley lines with varying level of β glucan content have different fate like high β-glucan is favorable for food but low amount of β glucan is desirable for malt production. This study is conducted to make use of closely linked molecular markers to characterize genomic region influencing inheritance of β-glucan trait in barley in Indian conditions.

Variation in β glucan content in RILs

Forty four F_3 derived RILs developed from a cross DWR30/Shebec subjected to biochemical analysis which resulted in wide range of β-glucan content across entire population. As expected variable distribution was observed in selected RILs segregating for β-glucan content ranging from 3.1 to 7.2 as compared to 3.0% (Shebec) and 7.5% (DWR30) for contrasting parental lines as shown in figure 1. Most of segregating lines contained intermediate level of β-glucan content ranging from 4.0 to 5.0% followed by 5.0 to 6.0% and then 6.0 to 7.0% categories therefore, showing continuous variation quantitative trait in segregating population. These RILs were grouped for low (3.0 to 4.5%) and high level (4.6 to 7.5%) β-glucan content categories for multiple regression analysis.

Screening of RILs with closely linked STS/SSR markers

Total 5 STS/SSR markers which have been reported closely linked for β-glucan and malt quality traits were selected to characterize the genomic regions influencing β-glucan content in barley. Amplification profiles of contrasting parental lines were used to compare the RILs segregating for β-glucan trait as of high and low β-glucan content category. Polymorphism shown by amplified bands for respective STS/SSR markers suggested that trait is segregating in mapping population. Out of 44 selected recombinant lines, 22 barley lines amplified the characteristic larger DNA band (300 bp) as same for parental genotype having high β glucan content and band (280bp) for low β glucan parental genotype for molecular marker Brz. Other molecular markers also showed significant polymorphism among parental and RILs population which is then subjected to statistical analysis to found out closely linked molecular markers to the â glucan trait in barley.

Regression Analysis using biochemical and molecular data

Data collected by biochemical analysis and molecular data generated from amplified bands were subjected to analysis of variance (ANOVA) involving multiple regression analysis for β-glucan trait using SAS statistics version 9.1.3. Means for each STS marker were separated by the least significant difference (LSD) at $P \leq 0.05$ and $P \leq 0.01$. During single marker regression analysis, three markers viz. Bmag603, Brz and WG622 were used individually on 1-0 binary data with β-glucan content measured by biochemical assay. STS marker Brz regressed significantly with β-glucan content trait in RIL populations derived from cross DWR30 X Shebec. This information was further validated using ANOVA involving multiple regression analysis that assess the segregation of β-glucan content with respect to a marker genotype to indicate which markers are associated with the quantitative trait of interest and, therefore, point to the existence of potential QTL. The magnitude of the marker-associated phenotypic effect was described by the coefficient of determination (R^2) which is a fraction of the total variance accounted by the marker genotype. The molecular data on Bmag603, Brz and WG622 loci and percent β-glucan content of individual recombinant inbred line were subjected for QTL analysis through multiple regression method. The regression of β glucan content on Brz marker was found almost significant, indicating the association between the molecular marker and β-glucan trait. The R^2 value of 0.417 suggested that the QTL on 7HL region is linked with STS marker Brz and is contributing almost 41.7% of total variation (%) among RILs population as shown in table 2.

Table 2
Details of analysis of variance (ANOVA) involving multiple regressions for molecular markers and β-glucan content for RIL population derived from cross DWR30 X Shebac

R^2 = 0.4170285566 *Source*	*SS*	*df*	*MS*	*F*	*P*	
Total	42.469565217	45				
Regression	17.711021482	3	5.9036738274	10.014898429	.0000	***
V2	6.5875017253	1	6.5875017253	11.174933204	.0018	**
V3	10.816559858	1	10.816559858	18.349040189	.0001	***
V4	0.3069598993	1	0.3069598993	0.5207218933	.4745	ns
Error	24.758543735	42	0.5894891366			

Significant at $P \leq 5$ and 1%

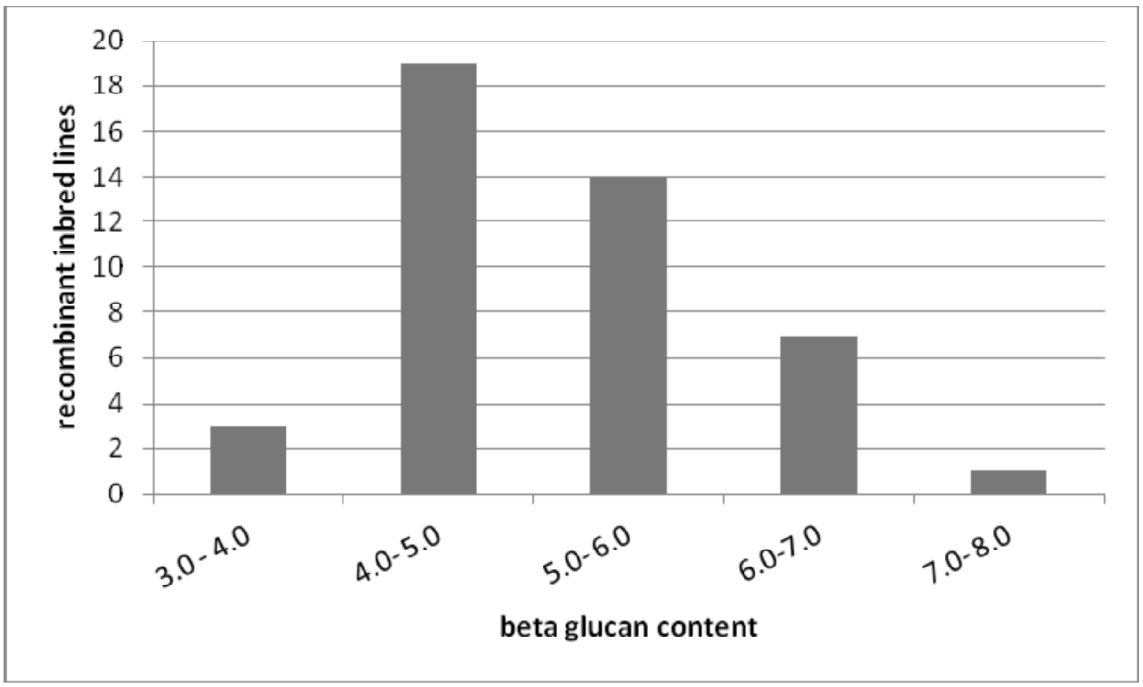

Figure 1: Frequency distribution graph showing β-glucan range distributed in recombinant inbred lines of DWR30/Shebec

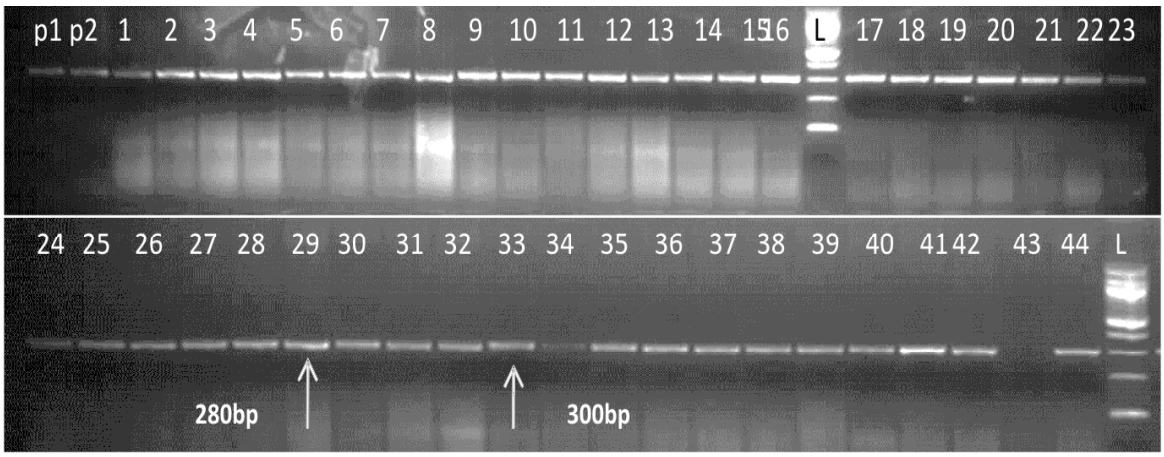

Figure 2: Amplified PCR product obtained with STS marker Brz in parents and recombinant inbred lines (RILs) of DWR30/ Shebec. Well p1: DWR30, p2: Shebac, 1-44: RILs and L: 100bp DNA ladder

DISCUSSION

β-glucan content in barley is an important traits which affect the malt quality, malt extract value and ultimately quality of beverages especially beer. β-glucan content is quantitative trait i.e. controlled by more than one gene and each gene loci have their individual additive effect. Quantitative trait locus (QTL) analysis provides a powerful tool to locate the genes controlling quantitative traits on chromosomes. Several QTL studies have been conducted in regard to malting quality traits using various genetic markers (Han *et al.*, 1995; Panozzo *et al.*, 2007; Pillen, 2009; Castro *et al.*, 2013). In the present study previously identified molecular markers linked with β-glucan trait were used to verify the association of these markers with selected quantitative trait using regression analysis. Three markers viz. Bmag 603, Brz and WG622 were subjected to single marker regression analysis and only STS marker Brz regressed significantly with β-glucan content trait. This result suggested that STS marker Brz located on 7H chromosome is found to be closely linked and segregating with β-glucan content trait. ANOVA involving multiple regressions was conducted for these three markers and malt quality determining trait β-glucan content for validation. It revealed significant regression for STS marker Brz located on 7H chromosome whereas other two markers were found non-significant. This marker explained 41.7% variation for β-glucan content in RIL populations of cross DWR30 X Shebec. Similar results were reported for multilocus QTL with a correlation coefficient value of 0.40 identified on chromosome 7H with in the Brz-ABC156D mapping interval linked to malt β-glucan trait. This complex QTL region also showed the largest and the most consistent effects for these traits over multiple locations and years in cross double haploid population of Steptoe/Morex (Han *et al.*, 1995), which makes this region a good candidate for malt quality studies. Our findings were further supported by a study reporting main effect of QTL associated with β-glucan content in the genomic region near waxy gene (wx) and HVM4 on chromosome 7H. This major QTL at this region explained on average 44.4% of the variation for the mean of β-glucan content from the cross of the six-rowed waxy hulless barley, Yonezawa Mochi and the six rowed non-waxy hulless barley, Neulssalbori (Hong-Sik Kim *et al.*, 2011).

CONCLUSION

Conclusively, our finding will help in deciphering bin location for β-glucan content trait inheritance controlling QTL on chromosome 7H since Brz is STS marker previously reported to be linked with quality trait in barley. Therefore, present study will assist in locating QTLs involved in inheritance of β-glucan content that is critical in determining malt quality for brewing purposes and owns a significant commercial value. This marker may prove efficient in identifying the genomic region which regulates the β-glucan trait in barley and is expected to be very useful for marker assisted selection (MAS).

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