Journal of Cereal Science 48 (2008) 647–655

Contents lists available at ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs

Identification of quantitative trait loci for β-glucan concentration in barley grain

Jingzhao Li [a], Monica Båga [a], Brian G. Rossnagel [b], William G. Legge [c], Ravindra N. Chibbar [a,*]

[a] Department of Plant Sciences, University of Saskatchewan, 51 Campus Drive, Saskatoon, Saskatchewan S7N 5A8, Canada
[b] Crop Development Centre (CDC), University of Saskatchewan, Saskatoon S7N 5A8, Canada
[c] Brandon Research Centre, Agriculture and Agri-Food Canada (AAFC), Brandon, Manitoba R7A 5Y3, Canada

ARTICLE INFO

Article history:
Received 13 November 2007
Received in revised form 17 February 2008
Accepted 22 February 2008

Keywords:
β-glucan concentration
QTL mapping
barley (Hordeum vulgare L.)

ABSTRACT

The amount of (1 → 3),(1 → 4)-β-D glucan (β-glucan) accumulated in cell walls of barley (*Hordeum vulgare* L.) kernel is an important determinant for grain end-use. Grain β-glucan concentration is affected by environmental and genetic factors and usually varies from 3 to 6%. In this study, we have analyzed the β-glucan trait in a doubled-haploid (DH) population of 170 lines grown in three separate field trials. Most of the DH lines showed β-glucan values that ranged from that of the low β-glucan parent (cultivar CDC Bold; ~3.3%) to that of the high β-glucan parent (breeding line TR251; ~5.4%). Eighty-eight lines of the DH population were genotyped using simple sequence repeat (SSR), amplified fragment length polymorphism (AFLP) and diversity array technology (DArT) markers, which were subsequently integrated into a barley genetic map spanning 1059 cM. Interval mapping and multiple-QTL-mapping (MQM) of quantitative trait loci (QTL) from the three trials indicated seven genomic regions associated with low grain β-glucan concentration. For all putative QTLs, the low β-glucan concentration was contributed by alleles from CDC Bold except for two loci on chromosomes 5H that were derived from TR251. A major QTL located to the centromere region of chromosome 7H was identified by both mapping methods for all three trials. The 7H QTL explained up to 39% of the β-glucan concentration and genetic markers associated with the locus may be used to aid selection of high and low β-glucan barley lines.

1. Introduction

The major storage components in cereal grains are carbohydrates, of which water-insoluble starch is the predominant form in the endosperm. The less abundant complex carbohydrates, e.g. arabinoxylans and β-glucans are concentrated in cell walls and constitute a substantial amount of soluble dietary fiber in cereal grains. In barley about 70% of total soluble dietary fiber is derived from β-glucan molecules (Fincher, 1975).

β-glucan is a linear polymer made up by 3-D-glucopyranosyl molecules that can form β-1,4 and β-1,3 linkages. The β-1,4 linked molecules constitute primarily cellotriose and cellotetraose units (90–95%), which are joined by β-1,3 linkages in a ratio of about 2.5:1 (Buckeridge et al., 2001). The amount of β-glucan in the kernel is one of the determining factors for the end-use of the grain. In the malting industry, malt with low β-glucan concentration is preferred, as the breakdown of β-glucans increases the viscosity of wort, which negatively affects brewing efficiency and beer filtration rates (Stewart et al., 1998). High viscosity is also obtained upon digestion of β-glucan-rich diets in animals and humans leading to lower absorption of nutrients in the digestive tract. The low nutritious value associated with β-glucan-rich grain is a problem for the monogastric animal feed industry, but for human food, the reduced uptake of nutrients in the small intestine is considered beneficial to health. Lower postprandial blood sugar (Cavallero et al., 2002) and serum LDL-cholesterol (Hecker et al., 1998) levels are associated with β-glucan-rich diets, which therefore may reduce the risk of coronary heart disease and type II diabetes (Poppitt et al., 2007).

The amount of β-glucan deposited during grain filling is affected by the environment and multiple genetic factors which contribute to the quantitative trait (see reviews by Fox et al., 2003 and Zale et al., 2000). The study of quantitative traits in crops has rapidly evolved during the last two decades, due to the emergence of molecular markers, which have become increasingly useful tools for accelerating crop improvement. In barley, a large number of restriction fragment length polymorphism (RFLP), random amplified polymorphism DNA (RAPD), amplified fragment length polymorphism (AFLP), sequence tagged sites (STS), simple sequence repeats (SSR) and single nucleotide polymorphisms (SNP) markers are available for mapping (http://wheat.pw.usda.gov). In addition, a recently developed high-throughput and low cost diversity array technology (DArT®) based on DNA hybridization, is applicable to whole genome scanning in barley (Wenzl et al., 2004, 2006).

* Corresponding author. Tel.: +1 306 966 4969; fax: +1 306 966 5015.
E-mail address: ravi.chibbar@usask.ca (R.N. Chibbar).

0733-5210/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jcs.2008.02.004

Genotyping of various barley mapping populations using genetic markers has led to construction of many genetic linkage maps with increasingly higher precision over the years (reviewed by Backes et al., 2006).

Grain β-glucan concentration is often included in the malting quality evaluation of barley. Malting quality is a complex characteristic, which encompasses malt extract percentage, total and soluble protein content, soluble/total protein ratio, kernel plumpness, α- and β-amylase activities, diastatic power, dormancy, and malt β-glucanase activity in addition to β-glucan concentration in grain and malt. The first QTLs for malting quality were identified for α-amylase activity on chromosomes 2H, 4H and 6H (Hayes et al., 1993). By the year 2000, a total of 168 QTLs for 19 malting quality traits had been identified among nine populations (Zale et al., 2000). All seven barley chromosomes are associated with the β-glucan concentration in grain or malt (Ayoub and Mather, 2002; Gao et al., 2004; Han et al., 1995; Igartua et al., 2002; Mather et al., 1997; Molina-Cano et al., 2007), and some of the individual QTLs are affected by environmental conditions.

Barley grain β-glucan concentration normally ranges from 3 to 6% (Izydorczyk et al., 2000), but mutant lines with concentrations up to 19% have been reported (Seefeldt et al., 2007). Low starch barley lines, such as high amylose or waxy barley often show β-glucan concentrations above 6% (Izydorczyk et al., 2000), which may indicate a partial reallocation of photosynthate products from starch biosynthesis to cell wall polysaccharides in these mutants. To avoid the confounding effects of starch mutations on β-glucan biosynthesis, we have for this study chosen barley lines that show normal starch characteristics, but differ in β-glucan concentration. The DH mapping population used was derived from a cross between CDC Bold (~3.3% β-glucan) and breeding line TR251 (~5.4% β-glucan) and derived DH lines showed a 2.5% range in β-glucan concentration. Three field trials were conducted for collection of phenotypic data and genomic regions associated with low β-glucan concentration were identified by QTL mapping.

2. Materials and methods

2.1. Barley genotypes

A barley doubled-haploid (DH) population of 170 lines was developed at Agriculture and Agri-Food Canada (AAFC), Brandon Research Centre, Brandon, Manitoba, Canada, from a cross between a moderately high β-glucan parent, two-rowed breeding line TR251 (AAFC, Brandon Research Centre, Brandon, Manitoba, Canada), and a low β-glucan parent, two-rowed semi-dwarf cultivar, CDC Bold. TR251 is derived from a cross between TR229 and AC Oxbow/ND7556. CDC Bold was developed at the Crop Development Centre (CDC), University of Saskatchewan, Saskatoon, Canada, and registered (Canadian Food Inspection Agency registration # 4951, June 15, 1999) for sale in Canada in June 1999. CDC Bold was selected from the cross SB88403/Tyra, where SB88403 was a CDC breeding line and Tyra is a semi-dwarf variety from Norway. The DH population of 170 lines and parents were planted in randomized blocks with repeated checks in New Zealand (2004) and Saskatoon, Canada (2005 and 2006). The 191 blocks included 170 DH lines, six repeats for each parent, and nine checks (CDC Gainer).

2.2. Grain β-glucan concentration phenotyping

The total β-glucan concentration in harvested grains was determined from 15 kernels that were cut in halves and ground into a fine powder using a WIGL BUG grinding machine improvised in our laboratory. Three samples of 50 mg of flour each were used for extraction of total β-glucans, which were analyzed by the calcofluor flow injection method (Aastrup and Jorgensen, 1988; Switala et al., 1989) using a flow-injection-analyzer (Eppendorf, Madison, WI, USA) for quantifications. The amount of total β-glucan was calculated from a standard curve established using a commercial β-glucan standard (Megazyme International Ireland, Ltd.) and values were expressed as percent β-glucan in dry grain. One-way ANOVA was performed to test the significance of differences using three field trials as replications (SPSS).

2.3. Genetic mapping

The parents and population lines were planted in soil in growth chambers maintained at 20 °C in 16-h light at 320 μmol m^{-2} s^{-1}. Leaf samples were harvested at the 5–10 leaf stage, frozen in liquid N_2, and stored at −80 °C. DNA was extracted from the leaf tissues using a CTAB method (Doyle and Doyle, 1990) and quantified by UV spectrophotometry.

A total of 194 microsatellite markers (Liu et al., 1996; Ramsay et al., 2000) were tested for polymorphisms between the parents TR251 and CDC Bold. The PCR reactions consisted of 100 ng template DNA, 5 pmol of each primer, 10 mM Tris–HCl pH 8.3, 3.5 mM $MgCl_2$, 25 mM KCl, 20 mM dNTP, 0.5 U of Red Taq Polymerase (Sigma) in a total volume of 12.5 μl. Amplifications were done in a MyCycler thermal cycler (Bio-Rad Lab. Hercules, CA, USA) programmed for an initial denaturation at 94 °C for 5 min followed by 32 cycles of 45 s denaturation at 94 °C, 20 s annealing at optimized temperature and 90 s extension at 72 °C. In the final cycle, the 72 °C elongation step was extended by 10 min. A 2.5 μl aliquot of the PCR reaction products was mixed with 2.5 μl 2×concentrated loading buffer (95% w/v formamide, 10 mM NaOH, 0.05% w/v bromophenol blue, and 0.05% w/v xyanol blue) and denatured at 95 °C for 2 min. Samples of 3.0 μl were loaded onto 6% w/v denaturing polyacrylamide gels, and DNA fragments were separated using a Gibco BRL S2 sequencing apparatus. Silver staining following the Silver Sequence protocol (Promega, Madison, WI, USA) was performed to visualize separated DNA fragments. When the size difference between polymorphic alleles was >10 bp, the PCR products were separated by 2% w/v agarose gel electrophoresis and fragments were visualized by ethidium bromide staining.

Amplified fragment length polymorphism (AFLP) analysis was done essentially as described by Vos et al. (1995) using 64 EcoRI/MseI primer combinations. Separation of AFLP fragments, scoring and size determinations were as described (Båga et al., 2007).

The genotype data using diversity arrays technology (DArT) markers were generated by Triticarte Pty Ltd. (Canberra, Australia; www.triticarte.com.au), as described (Wenzl et al., 2004, 2006).

2.4. Map construction and QTL analysis

Joinmap3 software (van Ooijen and Voorrips, 2001) was used to construct a barley genetic map. Markers were assembled into linkage groups at likelihood ratio statistic (LOD) ≥6.0 and assigned to the seven barley chromosomes based on published data for microsatellite and DArT markers (Ramsay et al., 2000; Wenzl et al., 2006). Recombination frequencies were converted into centiMorgan (cM) map distances using the Kosambi mapping function (Kosambi, 1943). Segregation ratios for each locus and deviations from expected ratios were determined using the chi-square (χ^2) test.

The MapQTL5 software (van Ooijen, 2004) was used to identify QTLs by simple interval mapping (SIM) and multiple-QTL-mapping (MQM) methods. The significant genome-wide LOD threshold at *p*-value of 0.05 was determined by 1000 permutation tests (Churchill and Doerge, 1994). A marker with the highest LOD score as determined by SIM was used as a co-factor in the MQM mapping analyses. The MQM process was repeated until the remaining QTL associated markers were below the LOD threshold score set for the trial.