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Efficient selection of β-glucan content enhances wider utilisation of barley grain

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SUMMARY – β-glucan is the major constituent of barley endosperm cell walls. High levels obstruct modification during malting, while β-glucan, solubilised during malt extraction, increases viscosity, slowing filtration. While low β-glucan is thus required for malting, high levels are desirable, for lowering cholesterol, in barley for food. Enzymic or fluorescence techniques enable phenotypic selection, but β-glucan is a polygenic character, with significant environmental effects. Different germplasm and environments have given inconsistent QTL location. DH lines, from Beka x Logan, were grown in contrasting environments in North-East Spain and Eastern Scotland. Three QTLs were located at both sites, the extent of genetic variation, explained by each locus, differing between environments. The QTLs differed from others detected previously, indicating that only some of the loci, contributing to β-glucan content, demonstrate functional diversity in specific populations. Understanding the function and location of these genetic factors will facilitate breeding strategies for both high and low β-glucan.

Introduction

The polysaccharide (1-3), (1-4) β-D-glucan is the major constituent of barley endosperm cell walls (Fincher, 1975), (Forrest and Wainwright, 1977). For barley breeders aiming to improve malting quality, reduction of β-glucan has been a consistent target as high levels have deleterious effects in both malting and brewing. During malting, they may reduce the rate of endosperm modification (Martin and Bamforth 1980), by forming a barrier to the enzymes responsible for protein solubilisation and starch breakdown. In addition, if high levels of β-glucan persist into the final malt and are solubilised during hot water extraction, they cause increases in wort viscosity which may lead to filtration problems (Bamforth and Barclay ,1993).

Where barley is a food ingredient, however, high β-glucan content is desirable, for its effect in lowering cholesterol (Hecker *et al*., 1998). Bhatty (1996) suggested that naked (hull-less) barley was better suited to a range of food uses than hulled barley, traditionally favoured for malting, so the development of high β-glucan, hull-less varieties could encourage wider utilisation of barley. Although direct consumption of barley, as human food, is very much lower than that of other cereals, e.g. wheat maize and rice, the inclusion of a portion of hull-less barley flour into food products such as bread, pasta and noodles has been successfully demonstrated (Izydorczyk *et al*., 2001), (Cavallero *et al*., 2004).

Phenotypic selection for β-glucan content

Selection for low β-glucan, in malting barley breeding programmes, was not possible prior to the 1970s, as methods were too time-consuming and required large grain samples (Greenberg and Whitmore, 1974). Initial screening methods did not measure β-glucan directly, but determined the viscosity of barley flour extracted in weak acid (Greenberg and Whitmore, 1974). Subsequently, a fluorometric method was developed that enabled the breakdown of β-glucan during malting to be visualised (Aastrup and Erdal, 1980), but this was influenced both by grain β-glucan content and by the rate and extent of β-glucanase production during malting. A major development in measurement of grain β-glucan was the enzymic method (McCleary and Glennie-Holmes, 1985), which employed lichenase and β-glucosidase to break β-glucan down to glucose, the content of which was determined

and used to calculate the initial amount of β-glucan. This method, which was suited to a large throughput of small samples, thus facilitated both selection within breeding programmes and genetic studies.

Genetics of grain β-glucan content

Grain β-glucan content is a quantitative trait, which Powell *et al*. (1985) suggested was determined by the additive effects of between three and five genetic factors. Subsequently, a number of studies have attempted to locate the genetic factors and Han *et al*. (1995) detected three QTLs for grain β-glucan and six for malt β-glucan in lines from a cross between the six-row varieties Steptoe and Morex. The largest effect on grain β-glucan was associated with a locus on chromosome 2H (Han *et al*., 1995) and Burton *et al*. (2006) established the biochemical basis for this association. Using the markers flanking the QTL, they identified a syntenic region of the rice genome, which they found to contain a group of six cellulose synthase-like genes. Subsequently, they were able to transform *Arabidopsis*, the cell walls of which do not, naturally, contain β-glucan and demonstrate β-glucan synthesis, confirming the action of the candidate gene.

Han *et al*. (1995) had advocated selection for optimal expression at the locus on chromosome 2H as a viable breeding strategy for low β-glucan, but Meyer *et al*. (2000), looking at a cross between two-rowed genotypes grown in the UK, found the largest effect on grain β-glucan adjacent to the *sdw1* dwarfing locus on chromosome 3H. Thomas (2003) noted that QTLs associated with quality parameters can vary considerably between populations, which may reflect the loci at which there is functional variation between the parental genotypes or indicate differences in genetic mechanisms controlling particular parameters.

Environmental effects on grain β-glucan

In the endosperm of mature barley grains, β-glucan exists in two forms, classed according to solubility or insolubility in water, which can be assessed separately (Aman and Graham, 1987). Morgan and Riggs (1981) suggested that although total β-glucan content was increased when plants were grown in hotter, drier conditions; it was the soluble portion, in particular, which was raised. This was supported by Swanston *et al*. (1997), who compared barleys grown in Spain and Scotland, and found Spanish grown samples to have higher levels of total β-glucan, but lower levels of the insoluble portion. Differences in the pattern of β-glucan deposition during grain filling were also observed. In Scotland there was rapid accumulation until ~ 600 degree-days after anthesis, then levels remained fairly constant until harvest ripeness, whereas in Spain levels rose virtually throughout the growing season, explaining the higher β-glucan levels attained compared to Scotland. These finding suggest that genetic factors may be expressed to differing extents under contrasting environmental conditions, increasing the complexity of phenotypic expression of β-glucan content.

European x North American malting barley population

The French variety Beka has been widely grown in Spain as a malting variety, due to its good adaptation to Mediterranean environments (Moralejo *et al*. 2004). It was crossed to the N. American malting variety Logan, in an attempt to introduce a genetic factor that would reduce protein content and the location of factors affecting protein content, in the resultant DH lines, was determined (Moralejo *et al*., 2004). As a fairly extensive genetic map had been constructed, using a variety of molecular markers, it was decided that this population would also be suited to determining the location of factors influencing β-glucan content. To study the effect of environment, two highly contrasting sites were used for replicated field trials, Lleida in NE Spain and Dundee, Scotland, with grain harvested in 2002. In Lleida, the seed was sown in late autumn and harvested in early summer, whereas in Dundee, the crop was sown in early April and harvested in late August. Following harvest, a sample of grain from each plot was finely milled to pass through a 0.5 mm sieve and the resultant flour used for estimation of total β-glucan content, by the enzymic method of McCleary and Glennie-Holmes (1985).

Beka had slightly lower β-glucan content than Logan at both sites (Fig. 1), but differences between the parental varieties were not significant. Both parents were, however, significantly lower at Dundee compared to Lleida. Analysis of variance of the DH lines (data not shown) indicated highly significant effects of genotype and site and there was also significant genotype x site interaction. Although the mean value of the DH lines was higher at Lleida compared to Dundee, the ranking order, within the lines, differed between the two sites. The same three QTLs were detected at both sites (Table 1), but all three had a larger effect in Spain compared to Scotland. The QTL with the largest effect was found below the centromeric region of chromosome 7H (77 cM), marked by the SSR Bmag516, with the increasing allele contributed by Beka. This QTL is also located in the vicinity of MWG808, which belongs, together with the naked grain gene *nud*, to BIN 7 in the Oregon Wolfe Barley map (www.barleyworld.org). The second QTL was located on the centromeric region of 5H (22 cM) and is marked by the SSRs Bmag337 and Bmac96, i.e. in the same region as a QTL for grain protein (Moralejo *et al*., 2004). For both protein and β-glucan, the increasing allele was contributed by Beka. Of possibly most interest, however, is the QTL located on the distal part of the long arm of chromosome 1H (183 cM), which co- segregated with the EST Ctig8484, now called scssr04163, whose sequence has been identified as UDP-glucose4-epimerase (UGE) (Moralejo *et al*., 2004). UGE catalyses the interconversion of UDP-D-galactose and UDP-D-glucose (Relter and Vanzin, 2001), both of which act as activated sugar donors for the biosynthesis of cell-wall polysaccharides such as cellulose, xyloglucans, (1,3;1,4)-β-D-glucans and pectins.

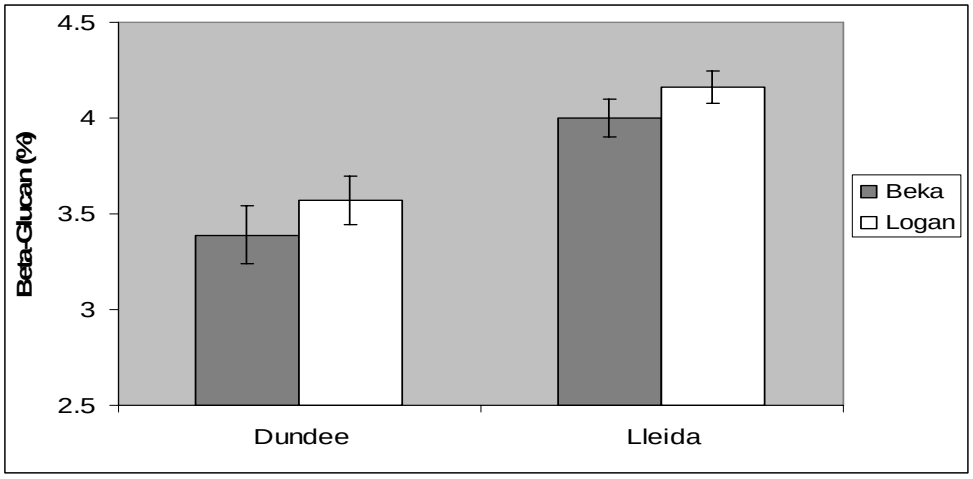

Fig.1. Mean β-glucan contents of the 2 parental varieties at the 2 sites. Vertical lines indicate standard errors.

It is still to be established, however, whether the gene is expressed in barley grain tissue and whether there is functional as well as allelic variation between the parents. This will be the subject of future research.

Table 1. Chromosomal location of 3 QTLs detected and their relative contributions to β-glucan content at the Dundee and Lleida sites

Chromosome	Closest Marker†	Position (cM)	Effect of Beka allele (% β-glucan)		Variance explained (%)	
			Spain	Scotland	Spain	Scotland
7H	Bmag516	77	0.13	0.05	21.4	10.3
5H	Bmag337	22	0.10	0.04	13.3	9.6
1H	Ctig8484	183	-0.10	-0.04	15.2	7.7

†See Moralejo *et al*. (2004) for chromosome map.

Discussion

The observation of a QTL for β-glucan content in close proximity to the location of the naked (hull-less) locus offers the potential to select parents with the desired combination and fix both characters in the homozygous state, during the early stages of breeding programmes. This will facilitate the breeding of varieties for human food use, extending the utilisation of the crop.

In addition, the potential to map ESTs represents the first step in the identification of candidate genes and, subsequently, obtaining a far greater understanding of the biochemical as well as the genetic basis of a desirable trait. For this purpose, maximising the differences between parental varieties is advantageous and has clearly led to the identification of an important step in the synthesis of β-glucan (Burton *et al*., 2006). However, the QTL with greatest effect described in Steptoe x Morex (Han *et al*., 1995) has not been detected in this study, or in a previous one with European malting barley (Meyer *et al*., 2000). The varieties Steptoe and Morex were released in 1973 (Muir and Nilan, 1973) and 1978 (Rasmusson, 2000) respectively and are parents for the type of cross, between sources of yield and quality, being made about thirty years ago. Contemporary breeding makes greater use of elite germplasm (Thomas, 2003) into which major genes for quality will almost certainly have been incorporated, through phenotypic selection, as described above. QTL studies can inform marker assisted selection, but they must utilise populations that are appropriate to the location and purpose. This will require a better understanding of the genetic factors present in elite cultivars and the choice of parents and selection strategies that will deliver optimal expression at all relevant loci.

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