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Genetic variants of *HvGlb*1 in Tibetan annual wild barley and cultivated barley and their correlation with malt quality

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ABSTRACT

Improvement of malt quality is the most important objective in malt barley breeding. The current experiments investigated the variation of malt quality characters among barley genotypes and the difference in genetic variants of *HvGlb*1, encoding β-glucanase isoenzyme I, between Tibetan annual wild barley and cultivated barley. The correlation between the gene variants and malt quality showed that there was a large difference in the four malt quality parameters, i.e. Kolbach index, diastatic power (DP), viscosity and malt extract, among the analyzed barley cultivars. Kolbach index was negatively and positively correlated with viscosity and malt extract, respectively, while malt extract was negatively correlated with viscosity. Malt β-glucan content was a major determinant of malt quality, and was significantly correlated with Kolbach index (−0.633), malt extract (−0.333) and viscosity (0.672). On the other hand, malt β-glucan content was mainly controlled by malt β-glucanase activity. The correlation analysis showed that the *HvGlb*1 gene was correlated with malt β-glucan content and three of four main malt quality parameters, except DP. In addition, we also found that the *HvGlb*1 of Tibetan barley had wider diversity in haplotype than that of the cultivated barley, supporting the hypothesis that Tibet is one of the original centers of cultivated barley.

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1. Introduction

Barley (*Hordeum vulgare* L.) is the fifth most important cereal crop in terms of total world production (FAOSTAT, 2006), and its most important end use is the production of malt as substrate for brewing beer and distilling whisky. Malt quality of barley grain involves a number of complex quantitative traits (Ullrich et al., 1997). Diastatic power (DP), malt extract, viscosity and Kolbach index are the most important quality parameters for malt identification. The Kolbach index refers to the ratio of soluble protein to total nitrogen, which is closely related to the modification of malt. DP refers to the starch-degrading capacity of malt, and represents the complementary actions of at least four enzymes on starch to produce sugars and low-molecular weight dextrins. The malt extract efficiency can be measured by malt extract, which measures the amount of fermentable sugars. It determines the amount of alcohol that can be made from a given number of grains. The higher the extract level is, the more alcohol that can be made. Historically, β-glucan largely contributes to the reduced beer filtration efficiency, highly viscous wort, and it may cause chill haze (Bamforth, 1982; Palmer, 1975).

High β-glucan content in malt has the tendency to increase the viscosity of beer by forming gels, consisting primarily of large β-glucan molecules (Kruger et al., 1989). Incomplete degradation of the cell walls during malting leads to poor modification of endosperm, potentially resulting in low wort extract and the presence of high levels of β-glucan in the wort (Brennan et al., 1997). On other hand, insufficient degradation of β-glucan can also lead to viscous initial extracts. Increased viscosity, which is attributed to the high molecular weight and extreme asymmetry of the polysaccharides, can lead to filtration difficulty at various points during the brewing process (Bamforth and Barclay, 1993).

Wei et al. (2009) found that Tibetan annual wild barley contained higher grain β-glucan content than cultivated barley. The malt β-glucan content was more dependent on malt β-glucanase activity than the original level of β-glucan in grains (Wang et al., 2004). There are two β-1,3-1,4-glucanase isoenzymes in barley, which were nominated as EI and EII. *HvGlb*1 encoding EI was located on chromosome 1H, and *HvGlb*2 encoding EII was located on chromosome 5H. They play a major part in the hydrolysis of the endosperm cell wall (Stuart et al., 1986). The assessment of malt quality parameters by micromalting and micromashing is time

Abbreviation: DP, Diastatic power; SSCP, Single strand conformation polymorphism.

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consuming, labor intensive, and often has limitations on seed availability. Thus, marker assisted selection of malting quality is required for early generation selection in malt barley breeding programs.

A study comparing nucleotide polymorphism of genes between cultivated cultivars and wild relatives showed substantial loss of diversity during domestication (Glémin and Bataillon, 2009). Tibetan annual wild barleys (*Hordeum vulgare* subsp. *spontanum*) originated in Qinghai-Tibet Plateau, are regarded as one of the progenitors of cultivated barley and wild barley from the Fertile Crescent has been the subject of much research (Nevo, 1992). However, few studies have been done on genetic diversity of malt quality traits in Tibetan annual wild barley, thus restricting its utilization in genetic improvement of barley.

In the current study, a wide variation was observed for malt quality, β-glucan content in grains and malt among barley genotypes. The relationships between *HvGlb*1 and β-glucan content in malt, malt quality were analyzed in the hope of providing markers, which may be used in breeding programs for barley quality improvement.

2. Experimental

2.1. Plant materials

Eighty cultivated barley cultivars and 80 Tibetan annual wild barley accessions were planted in Huajiachi campus of Zhejiang University (Hangzhou 120.2°E, 30.5°N) in 2008 and 2009. All genotypes were sown in early winter and grown in two replicates of two-row plots, 2 m long, 24 cm apart between rows and 40 seeds per row. All agronomic managements including fertilization, weed and disease control were the same as applied locally. The leaves of each genotype were collected for DNA extraction. At maturity, 30 spikes of main shoots were harvested and utilized for subsequent analysis. The harvested seeds were dried at 37 °C for 48 h, milled to pass through a 0.5 mm screen and stored under 4 °C before malting.

2.2. Malting and quality analysis

Grain samples (200 g of each cultivar) were micro-malted in a Phoenix System Micro-malting Apparatus using the regime: 6 h steep, 14 h air-rest, 8 h steep, 14 h air-rest and 4 h steep followed by 96 h germination – all under 15 °C. The malts were then kilned at 65 °C for 24 h, de-rooted and milled using a Tecator Cyclone mill fitted with a 0.5 mm screen. The malt quality parameters, including malt extract, Kolbach index, viscosity and DP, were determined according to Analytica EBC Official Methods (European Brewery Convention, 1975).

2.3. Measurement of β-glucan content

Total β-glucan content in grains and malt was analyzed according to McCleary and Codd (1991), using a commercial kit (Megazyme Ltd., Ireland), and presented on the basis of dry mass with 12% of moisture content. Three replications were taken for each measurement.

2.4. Measurement of β-glucanase activity in malt

β-Glucanase was assayed by the method of McCleary and Shameer (1987) using Megazyme kits (Megazyme Ltd. Ireland). A unit (U) of activity is defined as release of 1 Lmol/min of reducing sugar at 30 °C and pH 4.6.

2.5. DNA extraction and PCR reaction

Genomic DNA from the young leaves of barley seedlings was isolated as described by Uzunova et al. (1995). In brief, an initial grinding stage with liquid nitrogen was employed to break down the leaf cell walls and allow access to the DNA, while harmful cellular enzymes and chemicals remained inactivated. Once the leaf tissues were ground, the resulting powder was re-suspended with CTAB (Hexadecyl trimethylammonium bromide) buffer (pH 5.0). Each 100 ml CTAB buffer contained 2.0 g CTAB, 10.0 ml 1 M Tris (pH 8.0), 4.0 ml 0.5 M EDTA (Ethylenediaminetetra Acetic acid Disodium salt) (pH 8.0), 28.0 ml 5 M NaCl, 40.0 ml H_2O, and 1 g PVP 40 (polyvinyl pyrrolidone-vinylpyrrolidine homopolymer, MW 40,000). To purify the DNA, insoluble particulates were removed through centrifugation, while soluble proteins and other material were separated through mixing with chloroform: phenol (1:1) and centrifugation. DNA was precipitated from the aqueous phase and washed thoroughly to remove contaminating salts.

The PCR reaction contains 20 mM Tris-HCl, 50 mM KCl, 2.0 mM $MgCl_2$, l M of each dNTP, 5 pmol of each primer, 50–100 ng of genomic DNA and one unit of Taq DNA polymerase. The reaction was initially denatured at 95 °C for 5 min, followed by 35 cycles of 95 °C for 45 s, 57 °C for 45 s and 72 °C for 1 min. The PCR was terminated at 72 °C for 10 min.

2.6. SSCP analysis

All cultivated and wild barley genotypes were used in the analysis of single strand conformation polymorphism (SSCP). SSCP was considered as a simple and efficient technique for the detection of single base substitutions. The SSCP method followed previously described procedures (Martinslopes et al., 2001; Savov et al., 1992; Zietkiewicz et al., 1997) and was optimized with a 12% polyacrylamide gel (acrylamide/bisacrylamide ratio of 37.5:1) in 0.5xTBE and run at room temperature for 22–32 h. The PCR products were evaluated for purity by agarose gel electrophoresis before being loaded onto an SSCP gel, as any unwanted PCR products may interfere with gel analysis.

2.7. Data analysis

Diversities of genomic DNA and SSCP were analyzed with TASSEL (Bradbury et al., 2007) and DnaSP (Rozas et al., 2003), respectively. Association analysis followed the unified mixed model using Sigmaplot 10.0. The haplotype analysis was conducted using DnaSP 4.0.

3. Results

3.1. The difference of four malt quality parameters among genotypes and years

The four malt parameters (Kolbach index, DP, viscosity and malt extract) in 34 barley cultivars were assessed over two successive years (Fig. 1). There were significant genotypic differences in the four malt parameters. The DP ranged from 254.42 WK in 2008 to 515.71 WK in 2009, with a mean of 388.65 WK in 2008 and 373.20 WK in 2009. Meanwhile, the mean and CV of viscosity in 2008 were higher than those in 2009, whereas the mean and CV of malt extract in 2008 were lower than those in 2009. The Kolbach index in 2008 had higher CV and lower mean than those in 2009.

The data in 2008 explaining the variation of the data in 2009 varied from 33.32% to 55.11% ($R^2 = 0.3332$ for DP; $R^2 = 0.5511$ for Kolbach index; $R^2 = 0.3922$ for malt exact and $R^2 = 0.4871$ for viscosity). Thus, it suggests that the four malt quality parameters

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