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QTLs for malting flavour component associated with pre-harvest sprouting susceptibility in barley (*Hordeum vulgare* L.)

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ABSTRACT

Lipoxygenase (LOX) is a key factor affecting quality of beer in terms of foam stability and flavour. Low LOX content is a desirable trait for malting quality. A doubled haploid (DH) population was made from a cross of Australian malting barley Stirling and Canadian malting barley Harrington and mapped with 513 molecular markers. The 120 DH lines with their parents were planted in field trials and the harvested grains were micro-malted for analysis of LOX content in two consecutive years. LOX content was controlled by both genetic effects and environment conditions. Three QTLs were consistently detected. One QTL flanked by the markers E6216 and SCssr03907 at the telomere region of chromosome 5HL contributed 39% of genetic variation in LOX content. The second QTL close to the centromere region of chromosome 5H accounted for 17% of genetic variation. A minor QTL on chromosome 2H explained 6% of genetic variation but was significant in both years. The Australian variety Stirling contributed to higher LOX content for the three QTLs. The two QTLs mapped at chromosome 5H for LOX content coincided with the QTLs for seed dormancy/pre-harvest sprouting from the same population. The pre-harvest sprouting susceptible alleles were associated with low LOX content, which indicated that the low LOX QTL from the Canadian malting barleys are only useful in the barley growing areas where the pre-harvest sprouting risk is low. New genetic sources for low LOX should be exploited in different germplasm with different mechanisms.

1. Introduction

Lipoxygenases (linoleate:oxygen oxidoreductase, LOX, EC 1.13.11.12) form a family of non-heme-iron-containing fatty acid dioxygenases that are widely distributed in plants and animals. The enzymes catalyze the peroxidation of polyunsaturated fatty acids containing a 1Z, 4Z-pentadiene system to yield the (S)-configured hydroperoxy fatty acids and are involved in the first reaction for synthesis of compounds derived from polyunsaturated fatty acids, collectively called oxylipins (Feussner and Wasternack, 2002). They are involved in several plant metabolic processes, such as seed development, germination, vegetative growth, wounding, stress responses, senescence and cell signaling (Porta and Rocha-Sosa, 2002). Many isoenzymes of LOX have been found in plants.

Three isoforms of LOX have been described in barley. Through lipoxygenase reactions, the fatty acids can be converted to a variety of secondary metabolites which are considered to play a role in staling and off-flavor formation in beer (Feussner et al., 2001; Kuroda et al., 2003a). Besides favouring the yeast performance during fermentation, lipids can negatively influence filtration rates, decrease foam stability, and may give rise to the development of off-flavor compounds (Doderer et al., 1992). A lipoxygenase (LOX1) in barley was first reported by Franke and Freshe (1953). Subsequently a second isoenzyme (LOX2), which generally appears to develop only after germination, was documented (Baxter, 1982; Doderer et al., 1992; Yabuuchi, 1976). LOX-1 is already present in quiescent grains and catalyzes the formation of (9S)-9-hydroperoxy-10E, 12Z-

Abbreviations: cM, Centimorgan; DH, Doubled Haploid; DHLs, Doubled Haploid Lines; LOX, Lipoxygenase; QTL, Quantitative Trait Loci; (S)-9-HPODE, (9S)-9-hydroperoxy-10E, 12Z-octadecadienoic acid; (S)-13-HPODE, (13S)-13-hydroperoxy-9Z, 11E-octadecadienoic acid; THOD, Trihydroxyoctadecenoic acid; 9-HPL-like, Hydroperoxide Lyase-like.

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octadecadienoic acid [(S)-9-HPODE]. LOX-2 is a germination-associated LOX isoform, which is formed during germination and catalyzes the formation of (13S)-13-hydroperoxy-9Z, 11E-octadecadienoic acid [(S)-13-HPODE] with linoleic acid as the substrate (Doderer et al., 1992; Holtman et al., 1997; Van Mechelen et al., 1999). In mature plants its distribution is similar to that of LOX-1 with the highest activity in leaves and roots (Holtman et al., 1996). Expression of the third LOX isoform has been detected only after germination and is similar to that of LOX-2, although in mature vegetative tissues it is present only at low levels (Van Mechelen et al., 1999). LOX-1 is of great interest because 9-HPOD forms beer-deteriorating substances such as trans-2-nonenal and trihydroxyoctadecenoic acid (THOD) during further reactions in the brewing process (Kobayashi et al., 1993; Kuroda et al., 2002, 2003b). Trans-2-nonenal is known as a major component of the cardboard flavour in aged beer (Drost et al., 1990; Meilgaard, 1975). THOD is known to have an adverse effect on the quality of beer in terms of foam stability and flavour (Bauer et al., 1977; Kaneda et al., 2001; Kobayashi et al., 2002; Yabuuchi and Yamashita, 1979).

The genes encoding LOXs were cloned and then mapped to the LoxA locus on chromosome 4H and the LoxC locus on chromosome 5H, respectively (Van Mechelen et al., 1995, 1999). LOX-1 provides the predominant LOX activity in malt and has a relatively low pI compared to LOX-2 (Yang et al., 1993; Yang and Schwarz, 1995). In contrast to these functional characterisations of LOX, little is known about its genetic variation in barley. Recently, LOX-1 null mutants were explored to reduce LOX content in new malting barley varieties (Hirota et al., 2006). Malting barley lines with the mutant gene demonstrated significant improvement of flavour and foam stability (Hirota et al., 2006).

The test of wort nonenal potential, which is widely used by brewers to estimate quality of malting related to beer flavour stability, is a kind of forcing test to measure the potential of wort to form 2(*E*)-nonenal. Recently a successful malt selection and malting methods for improving the flavour stability of beer was identified, which involved in the production of 2(*E*)-nonenal during mashing. LOXs, which are nonheme ferrus proteins and catalyze the hydroperoxidation of polyunsaturated fatty acids with a 1.4-cis–cis-pentadiene structure, play an important role in this process. It was found that 2(*E*)-nonenal is produced by the cascade reaction of barley LOX and malt 9-fatty acid hydroperoxide lyase-like activity (9-HPL-like activity) in malt (Founier et al., 2001; Larsen et al., 2001). Thus, LOX activity has been chosen as an indicator for beer quality. However, measurement of LOX activity is a time consuming and labour intensive process as demonstrated in the material and methods section in this paper. Furthermore, the requirement of malting barley grain before the enzyme assay also restricts the use of the chemical LOX method for early generation selection of LOX in breeding programs.

It has been a market mystery that the Canadian malting barley has better malting flavour. In our barley breeding program, significant variation has been observed for LOX content among barley varieties. In general, the Canadian malting barley varieties and the varieties derived from the Canadian varieties showed lower LOX content comparing to Australian malting barley varieties. In this study, we examined LOX content in a DH population from a cross between Australian and Canadian malting barley varieties and identified QTLs for LOX content which will provide tools for marker-assisted selection for low LOX in the breeding programs.

2. Experimental

2.1. Plant material

A population of 185 doubled haploid lines (DHLs) was generated by anther culture from a Stirling/Harrington cross and kindly provided by Kirin Australia. This population was previously used to map QTLs controlling seed dormancy/pre-harvest sprouting. Details of the map construction and seed dormancy analysis were reported in the previous publication (Bonnardeaux et al., 2008).

2.2. Field experiment

The Stirling/Harrington DH population was planted in 2005 and 2006 at the Wongan Hills Research Station, Department of Agriculture and Food WA (DAFWA), 188 km north-east of Perth. The field plot was planted in a randomised complete block design with plots of 1 by 3 m^2. Control plots of Stirling (parental line) plants were sown in the first and last rows (rows 1 and 19) and Hamelin (progeny of Stirling/Harrington cross) control plots were sown in the middle of the field plot (row 11). The control varieties were used for spatial adjustment of the experimental data. The second parental line, Harrington, was planted in an individual plot in the next block in one season.

2.3. Micro-malting process

120 DH lines were sub-sampled from this population for micro-malting and QTL analysis of LOX content. Barley samples were cleaned and sieved over a 2.2 mm screen prior to micro-malting in a Joe White Systems micro-malting unit without the use of additives. A standard malting schedule was used: Steeping - 19 °C, 7 h wet, 8 h air rest, 3 h wet, 4 h air rest, 1 h wet. Germination was total 96 h (48 h at 18 °C followed by 48 h at 16 °C), moisture adjusted to 46% at 24 h. Kilning was 2 h at 45 °C, 3 h at 50 °C, 4 h at 55 °C, 3 h at 60 °C, 3 h at 65 °C, 3 h at 70 °C, 2 h at 75 °C, and 4 h at 80 °C. Malt rootlets were removed using a custom made rootlet removing machine (Fraser Fabrications P/L, Malaga, Western Australia).

2.4. Lipoxygenase assay

We used the Joe White Malting revised version of the Malt Lipoxygenase (LOX). The assay was originally from Baxter (1982). All processes were completed on ice unless otherwise indicated.

2.4.1. Preparation of substrate solution (2.5% linoleic acid)

A 5 mL of 0.05 M-borate buffer (pH 9.0) was added to a volumetric flask (10 mL) followed by adding 0.25 mL Tween20, 0.25 mL Linoleic acid and 0.65 mL 1 M NaOH. The contents were shaken gently in the ultrasonic bath with ice water until the solution became clear, then distilled water was added to 10 mL.

2.4.2. Enzyme extraction from finished malt

Finished malts were milled in a Retsch ZM200 centrifugal mill (Retsch GmBH, Germany) with a 1.0 mm screen and 5 g of milled malt was transferred to 100 mL flask. 50 mL of acetate buffer (pH5.0) containing 0.1 M NaCl was added and kept in ice water bath for 15 min with occasional shaking. The resulting solution was transferred into a 1.5 mL eppendorf tube and centrifuged for 5 min at 10,000 rpm. The supernatant was subsequently transferred to a new eppendorf tube and stored on ice.

2.4.3. Enzyme assay

The temperature of the cell holder and phosphate buffer (0.1 M, pH 6.8) was equilibrated to 25 °C by water circulation. 100 μL enzyme extract and 2850 μL phosphate buffer (0.1 M, pH 6.8) was added to 50 μL substrate solution, mixed, returned to the cell holder and the absorbance recorded at 1 min and 4 min at 234 nm. The blank absorbance was measured using 50 μL substrate solution and 2950 μL phosphate buffer at 1 min.