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The relationship of limit dextrinase, limit dextrinase inhibitor and malt quality parameters in barley and their genetic analysis

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ABSTRACT

Limit dextrinase (LD) and limit dextrinase inhibitor (LDI) are the two important traits affecting malt quality. The genetic variation and controlling of LD activity and LDI content in barley grains and malt are not well understood. In this study, we measured LD activity and LDI content in both grains and malt of 68 cultivated barley genotypes. The results show that there is a wide difference among barley genotypes in both LD activity and LDI content. LD in malt is not correlated with LD in grains, but negatively correlated with LDI in malt. LD in malt is positively correlated with diastatic power (DP), Kolbach index (KI) and soluble nitrogen content (SN), and negatively correlated with viscosity (VC). LDI in malt is positively correlated with DP and total nitrogen (TN), and negatively correlated with KI. Association analysis identifies 5 QTLs associated with LD and 3 QTLs associated with LDI in malt. Three major QTLs controlling LD in malt account for 35.7%, 35.7% and 28.4% of phenotypic variation, respectively. A total of 17 QTLs associated with malt quality are identified. The current results address the importance of both LD and LDI in affecting malt quality and the identified QTLs could be useful in barley breeding.

1. Introduction

Limit dextrinase (LD) (EC 3.2.1.142) is an important quality trait for malt barley, as it cleaves alpha-1-6 linkages in branched amylopectin, thereby contributing to complete degradation of starch (MacGregor, 2003). LD is mainly synthesized in aleurone layer of germinating barley grains, and exported to endosperm, where it facilitate the production of fermentable sugars (Burton et al., 1999), necessary for brewing. However, LD is also detected at a low level in embryo and endosperm of developing grains (Sissons et al., 1993), where its role is proposed to produce dextrin primers for starch synthesis (Burton et al., 1999).

LD may exist in free and bound forms in both mature and germinating grains. The bound LD is inactive due to its binding with an endogenous inhibitor, named as limit dextrinase inhibitor (LDI) (Macri et al., 1993). *In vitro*, LD and LDI interact with each other by formation of 1:1 M ratio complex (MacGregor, 2003). LDI is synthesized during grain development, and accumulated mainly in the out region of endosperm (Stahl et al., 2007). The function of LDI in developing grains may be participated implicated in formation of type B starch granule, as antisense down-regulation of LDI synthesis resulted in reduction of type B granule in endosperm (Stahl et al., 2004). LDI may degrade rapidly in germinating grains (Ross et al., 2003; Stahl et al., 2007) through both hydrolysis by cysteine proteases and disulfide reduction by thioredoxin (Heisner and Bamforth, 2008; Jensen et al., 2012).

LD is thought to be a limiting factor in complete starch breakdown (Evans et al., 2005). The presence of LDI may lead to reduction of free LD activity because of formation of LD-LDI complex (Ross et al., 2003), thus resulting in reduced production of fermentable sugars. On the other hand, the formation of LD-LDI complex may protect LD from degradation by high temperature in kilning and mashing (Sissons et al., 1995; Walker et al., 2001; Stenholm and Home, 1999). Therefore, the balance of LD activity and LDI content should be important for starch breakdown during malting and mashing. On the other hand, diastatic power (DP), malt extract (ME), Kolbach index (KI), viscosity (VC), soluble nitrogen content in wort (SN) and total nitrogen content in malt (TN) are important malt quality parameters (Jin et al., 2013). DP represents the ability in producing fermentable sugars, and ME represents the amount of the soluble substance in malt. Higher levels of DP and ME mean higher production of fermentable sugars. KI is the percentage of soluble nitrogen content to total nitrogen content. However, the effect of LDI alone, or the combined LD and LDI on

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these quality parameters is still not clear.

LD activity in malt seems to be controlled by a major locus in chromosome 7H, which could be *HvLD* gene (Burton et al., 1999). The expression of *HvLD* is low in developing grains, and increases dramatically in germinating grains (Burton et al., 1999). The mechanism in the regulation of *HvLD* expression during grain development and germination is not understood yet. Although the biochemistry and genetics of malt LD has been widely studied, little is known about the genetics of LD in grains. LDI content in grains is controlled by a major gene *HvLDI* in chromosome 6H. Meanwhile, a minor QTL was also reported to be associated with LDI content in grains of Tibetan wild barley (Huang et al., 2014). However, the genetic basis of LDI content in malt is not understood yet.

In this study, we determined LD activity and LDI content in both grains and malt of 68 cultivated barley genotypes, and their potential correlation with malting quality parameters. Moreover, we identified the QTLs associated with LD, LDI and malting quality parameters using association analysis.

2. Materials and methods

2.1. Plant materials

Sixty-eight barley genotypes were grown at the experimental farm of Zhejiang University (Hangzhou, China, 120.2°E, 30.5°N). Each genotype was planted in a 2-m × 0.25-m row with three replications. The plants were managed as locally. At maturity, grains were harvested and stored at 4 °C.

Barley grains were micro-malted according to Cai et al. (2015). Briefly, 150 g of grains were micro-malted in Phoenix System Micro-malting Apparatus (Joe & White, Adelaide, Australia), with the settings as below: (1) steeping stage (wet stage 17 °C, 5 h; air-rest stage 17 °C, 8 h; wet stage 16 °C, 8 h; air-rest stage 16 °C, 12 h; wet stage 15 °C, 4 h; air-rest stage 15 °C, 5 h; wet stage 15 °C, 2 h); (2) germination stage (16 °C, 48 h; 15 °C, 48 h); (3) kilning stage (50 °C, 2 h; 55 °C, 4 h; 60 °C, 6 h; 65 °C, 4 h; 70 °C, 1 h; 75 °C, 1 h; 80 °C, 1 h; 82 °C, 2 h). The obtained malt was de-rooted and stored in 4 °C for further analysis. The barley grains and malt were finely grounded and filtered with a 0.5 mm screen.

2.2. Measurements of LD, LDI activity and malt quality parameters

LD in grains and malt was determined using the Limit-Dextrinase assay kit (Megazyme International, Bray, Ireland) according to the instruction of the manufacturer.

Partially purified LD is an essential reagent in measurement of LDI activity. Thus, LD was partially purified from malt as described in a previous study (Huang et al., 2014). Briefly, the supernatant of malt was passed through the ion exchange chromatography (DEAE Sepharose™ Fast Flow, GE Healthcare, USA), and the collected solution was passed through gel filtration chromatography (Sephacryl™ S-200 High Resolution, GE Healthcare, USA). The fraction with LD activity was collected and stored at −20 °C.

LDI content was determined according to MacGregor et al. (1994) with some modification. Grain or malt powder (0.1 g) was extracted with 1 ml 0.1 M sodium acetate (pH 5.5) containing 10 mM 1,10-phenanthroline at 4 °C for 30 min, followed by heating at 70 °C for 40 min. The extract was centrifuged and the supernatant was collected. Twenty micrograms of protein of LDI extract was mixed with approximately 10 mU partially purified LD, and the final volume was made up to 0.5 ml with 0.1 M maleic acid (pH 5.5, with 0.02% Na azide). After 30 min of reaction, the LD activity in the mixture was determined using the Limit- Dextrinase assay kit (Megazyme International, Bray, Ireland). The same amount of LD was mixed with 0.1 M maleic acid (pH 5.5, with 0.02% Na azide) and adjusted to the volume of 0.5 ml, then the activity of LD was assayed. The LDI activity was calculated as the reduced LD activity per microgram protein.

Malt quality parameters, including ME, KI, VC, SN, TN and DP, were determined according to Analytical EBC Methods (European Brewery Convention, 1998).

2.3. Association mapping of LD, LDI and malt quality parameters

Association analysis was performed by TASSEL v3.0 (http://www.maizegenetics.net) using 758 DArT (Diversity Arrays Technology) markers. Four different models, including naïve approach, Q model, K model and Q + K model, were employed in the association analysis according to the previous studies with some modifications (Cai et al., 2013; Yu et al., 2006). To reduce false positive results caused by population structure, structure matrix

Table 1
The correlation among LD activity and LDI content in grains and malt.

	LD_M	LDI_M	LD_G
LDI_M	−0.344**		
LD_G	0.086	0.219	
LDI_G	−0.095	0.349**	−0.133

Note: LD_G: LD activity in grains; LD_M: LD activity in malt; LDI_G: LDI content in grains; LDI_M: LDI content in malt. **Represents significant at $P < 0.01$ level.

Table 2
The correlation between LD activity, LDI content and malting quality parameters.

	LD_M	LDI_M	LD_G	LDI_G
DP	0.344**	0.289*	0.346**	0.000
KI	0.575**	−0.564**	−0.374**	−0.018
ME	−0.013	−0.116	−0.402**	0.122
VC	−0.504**	0.168	0.315**	−0.145
SN	0.552**	0.166	0.124	0.225
TN	0.178	0.573**	0.435**	0.235

Note: LD_G: LD activity in grains; LD_M: LD activity in malt; LDI_G: LDI content in grains; LDI_M: LDI content in malt; DP: diastatic power; KI: Kolbach index; ME: malt extract; VC: Viscosity; TN: total nitrogen content in malt. * and ** Represents significant at $P < 0.05$ and $P < 0.01$ level, respectively.

Table 3
List of DArT markers associated with LD activity and LDI content in grains and malt.

Trait	Marker	Chromosome	Genetic position (cM)	$-\log_{10}(P)$	R^2
LD_G	bPb-6822	2H	114.4	2.38	0.213
	bPb-3630	3H	111.7	2.09	0.180
	bPb-8569	4H	14.6	2.99	0.353
	bPb-6135	5H	115.3	2.62	0.245
	bPb-0071	5H	126.8	2.23	0.200
	bPb-7179	6H	58.6	2.01	0.170
	bPb-9155	7H	27.2	3.19	0.363
LD_M	bPb-0631	1H	128.5	2.02	0.171
	bPb-3533	2H	157.1	3.49	0.357
	bPb-4925	3H	118.6	3.49	0.357
	bPb-2910	3H	51.6	2.06	0.178
	bPb-8049	7H	53.4	2.92	0.284
LDI_G	bPb-5778	6H	84.6	2.09	0.181
	bPb-5348	7H	82.6	2.10	0.185
LDI_M	bPb-8734	2H	156.4	2.12	0.183
	bPb-7561	5H	75.1	2.67	0.251
	bPb-5532	5H	86.3	2.54	0.244

Note: LD_G: LD activity in grains; LD_M: LD activity in malt; LDI_G: LDI content in grains; LDI_M: LDI content in malt. These markers were identified using *k* model with significance threshold as $P < 0.01$. R^2 (Marker) represents the contribution of marker for phenotypic variation.