Journal of Zhejiang University SCIENCE B
ISSN 1673-1581
http://www.zju.edu.cn/jzus
E-mail: jzus@zju.edu.cn

Relationship between malt qualities and β-amylase activity and protein content as affected by timing of nitrogen fertilizer application*

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Received Aug. 25, 2005; revision accepted Oct. 5, 2005

Abstract: The effects of different timing of N fertilizer application at the same rate on grain β-amylase activity, protein concentration, weight and malt quality of barley were studied. Grain β-amylase activity and protein concentration were significantly higher in treatments where all top-dressed N fertilizer was applied at booting stage only or equally applied at two-leaf stage and booting stage than in the treatment where all top-dressed N fertilizer was applied at two-leaf age stage only. On the other hand, grain weight and malt extract decreased with increased N application at booting stage. There were obvious differences between barley varieties and experimental years in the grain and malt quality response to the timing of N fertilizer application. It was found that grain protein concentration was significantly and positively correlated with β-amylase activity, but significantly and negatively correlated with malt extract and Kolbach index. The effect of grain protein concentration on malt quality was predominant over the effect of grain β-amylase activity.

Key words: Barley, Nitrogen fertilizer, β-amylase, Malt quality
doi:10.1631/jzus.2006.B0079 **Document code:** A **CLC number:** S512.3+1

INTRODUCTION

Barley β-amylase is synthesized during grain development and stored in mature grains. It plays an important role in determining good malting quality (Ziegler, 1999; Evans *et al*., 2003). Georg-Kraemer *et al*.(2001) found that β-amylase activity was a better predictor of diastatic power (DP) than α-amylases in barley grains, and increased markedly during germination. High level of DP was required in brewing processes and was an important characteristic for estimating the quality of malt for beer production (Evans *et al*., 1995). Grain protein content is a key "gateway" characteristic for malting quality. Excessively high protein level is commonly associated with lower soluble substance content and malt extract quality, resulting in unacceptable malt quality. Grain weight was also reported to be related to malt extract (Erkkila *et al*., 1998; Swanston and Molina-Cano, 2001), so that, these characteristics are closely correlated with malt quality.

Several studies showed that β-amylase activity, grain protein content and weight were influenced to large extent by environmental factors, although they are mainly controlled by genetic factors (Bathgate, 1987; Smith, 1990). High quality of malting barley is reflected by optimal protein content (9.5%~11.5%), higher β-amylase activity and grain weight. Grain protein content is greatly affected by N availability in soil, and closely related to the rate and timing of N fertilizer application (Birch *et al*., 1997; Eagles *et al*.,

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* Project supported by the National Natural Science Foundation of China (No. 30270779) and the Special Research Foundation for Doctor Discipline of Chinese Universities (No. 20020335028)

1995).

It is often difficult to keep protein content below the upper limit to ensure the malt quality. In southern China, it is a regular practice to top-dress N fertilizer at booting stage in order to realize yield potential. This results in malters and brewers complaining about unstable malt quality and low extract yield from grains grown in this area. However, high N fertilization rate or ratio of N application in the late barley growth stage markedly increases β-amylase activity through promoting synthesis and accumulation of grain protein (Arends *et al*., 1995). Therefore the interaction of N fertilizer application with malt quality is quite complex, as a consequence of the balance of these positive and negative effects.

Despite the importance of N application, there are few reports on the influence of N fertilizer application on β-amylase activity, protein content and their relationship to malt quality. The present investigation uses two local commercial malt barley cultivars, differing in yield components and malt quality in field experiments to determine the influence of different N application timing on β-amylase activity and malt quality, and also examines the relationship between some malt quality characters.

MATERIALS AND METHODS

Two commercial malt barley cultivars, Xiumai 3 (with more tillers and smaller grains) and 92-11 (with higher grain weight and fewer tillers) were used in this study. The field experiment was conducted at the experimental farm of Huajiachi campus, Zhejiang University in 2000~2002. The experiments were arranged in a completely random block design with three replications. Plots consisted of eight, 1.67 m long rows spaced 0.25 m apart. In each row 100 seeds were sown. All plots were supplied with 150 kg/ha of N. Apart from 40 kg/ha of N as compound fertilizer applied before seeding, N_1 and N_3 treatments received another 110 kg/ha of N as urea at 2-leaf and booting stage, respectively. In N_2 treatment 55 kg/ha of N as urea was top-dressed at 2-leaf and booting stages, respectively. In addition, 180 kg/ha of potassium chloride was applied to all plots prior to seeding. Other field management practices were the same as those locally recommended.

At maturity, grains were randomly sampled for chemical analysis. Grain samples were ground to pass through a 0.5-mm sieve in a Cyclotec mill (Tecator AB, Hoganas, Sweden). β-amylase was assayed according to McCleary and Codd (1989) using a commercial Betamyl kit (Megazyme International, Ireland, Ltd.). Cysteine was added to buffer solution in order to extract total β-amylase (Evans *et al*., 1997). Grain protein concentration was determined by a near infrared reflectance analyzer (Model 5000, FOSS Co. Denmark) with the calibration established in a previous study (Yin *et al*., 2002). Barley grains from the 2000~2001 experiment were passed through over a 2.2 mm sieve, with the grains remaining on the 2.2 mm sieve being used for micro-malting. Samples (about 200 g) were micro-malted in a Phoenix System Micro-malting Apparatus (Adelaide, Australia) with the following regime: steeping (6 h, 16 °C), air-rest (14 h, 16 °C), steeping (8 h, 16 °C), air-rest (14 h, 16 °C), steeping (4 h, 16 °C); germination for 96 h at 15 °C; kilning for 24 h at 65 °C; followed by de-rooting. The malt quality parameters, including extract, Kolbach index, viscosity and DP were determined according to Analytica EBC Official Methods (EBC, 1975).

All data presented are the mean values. Statistical analysis was carried out by one-way ANOVA using Student's *t*-test to test significance of the difference between means. Means were considered significantly different for $P \leq 0.05$.

RESULTS

Grain β-amylase activity

Timing of N fertilizer application affected significantly grain β-amylase activities of both barley cultivars (Table 1). N_1 treatment, in which all top-dressed N fertilizer was applied at the 2-leaf stage, had the lowest β-amylase activity among three N application treatments. However, there was no significant difference between grain β-amylase activity in N_2 and N_3 treatments, which indicated that applying N at late (booting) stage may increase β-amylase activity. In terms of difference between the two cultivars, 92-11 had significantly higher β-amylase activity, consistently in the two experimental years.

Table 1 Effect of timing of N application on β-amylase activity in barley grains (U/g)

N treatment	2001		2002	
	92-11	Xiumai 3	92-11	Xiumai 3
N_1	1054.4 b*	925.8 b	979.8 b	951.6 b
N_2	1338.7 a	1056.7 a	1153.9 a	1101.3 a
N_3	1348.9 a	1054.4 a	1212.7 a	1136.2 a

* Different letters after data within a column represent significant difference at 95% probability

Grain protein concentration

Grain protein concentration showed dramatic response to the timing of N fertilizer application (Table 2). Treatment N_1 resulted in significantly lower grain protein concentration than that in N_2 and N_3, regardless of cultivars. The difference between N_2 and N_3 varied with the experimental years. Thus significant difference was found in 2001, with N_3 grain protein concentration being higher than that in N_2, while there was no significant difference between the two treatments in 2002. It was also found that there was great difference in grain protein concentration of both cultivars between two experimental years, with grain protein concentration in 2002 being distinctly higher than that in 2001. The difference between the grain protein concentrations of the two cultivars also varied with the experimental year. In 2001, 92-11 grain protein concentration was consistently higher than that in Xiumai 3 in all three N treatments, while in 2002 little difference in grain protein concentration was found between the cultivars.

Table 2 Effects of timing of N application on protein content of barley grains (%)

N treatment	2001		2002	
	92-11	Xiumai 3	92-11	Xiumai 3
N_1	10.0 c*	9.4 c	10.9 b	11.6 b
N_2	11.1 b	10.7 b	13.3 a	13.3 a
N_3	12.3 a	11.0 a	13.4 a	13.3 a

* Different letters after data within a column represent significant difference at 95% probability

Grain weight

The response of grain weight to timing of N application was also significant (Table 3). The treatment N_1 had the largest barley grain weight among the three N treatments regardless of the two experimental years and cultivars, although there was no significant difference with N_2 in 2001. Between the two cultivars, 92-11 barley grain weight was consistently greater than that of Xiumai 3 in the three N treatments and the two experiments. Between the experimental years, grain weight in 2001 was significantly greater than that in 2002. The results showed that grain weight was reduced with increasing ratio of N fertilizer applied at booting stage, which was presumably the result of more spikes per plant and grains per spike. We observed that treatment N_3 resulted in significantly more spikes per plant and grains per spike than N_2, and in turn than N_1, which might reduce available carbohydrates for grain filling.

Table 3 Effects of timing of N application on barley thousand grain weight (g)

N treatment	2001		2002	
	92-11	Xiumai 3	92-11	Xiumai 3
N_1	46.1 a*	41.4 a	42.3 a	37.1 a
N_2	45.2 ab	41.3 a	39.9 b	33.5 b
N_3	44.7 b	37.6 b	37.5 c	31.2 c

* Different letters after data within a column represent significant difference at 95% probability

Malt quality

Timing of N fertilizer application had significant influence on all malt quality parameters assessed in this study, except viscosity (Table 4). For malt extract, the two cultivars showed the same trend of decreasing extract with increased ratio of N fertilizer applied at booing stage. In contrast, diastatic power showed the opposite response to the N treatment compared to malt extract, i.e. N_3 had the highest value, followed by N_2, although the difference between N_1 and N_2 was not significant. The influence of N application timing on Kolbach index varied with cultivar. For 92-11, there was no significant difference among the 3 N treatments, while for Xiumai 3, N_3 was significantly lower than the other two N treatments. In addition, Xiumai 3 had higher malt extract, DP and Kolbach index, and lower viscosity than 92-11 at all N application rates.

Correlations between grain protein concentration, weight, β-amylase activity and malt properties

Analysis of correlation among grain protein concentration, weight, β-amylase activity and malt properties showed that grain weight was negatively

associated with malt extract, diastatic power and Kolbach index (Table 5). As expected, protein concentration was negatively correlated with malt extract and Kolbach index, and significantly and positively correlated with diastatic power. β-amylase activity was negatively correlated with malt extract and Kolbach index, and positively correlated with viscosity, protein concentration and grain weight. It was shown that high grain protein concentration benefited increase of β-amylase activity, and as a result promoted diastatic power. However, it would cause a significant reduction in malt extract.

DISCUSSION

Previous studies showed that increasing N application rate at late growth stage of barley generally increased grain protein content and decreased grain weight (Xie and Ding, 1996; Zhao *et al*., 1988). The present results of two-year experiments indicated that N top-dressing at early stage (2-leaf stage) benefited improvement of malt quality due to increasing grain weight and decreasing grain protein concentration, in comparison with the treatment in which more N top-dressed at late growth stage (booting stage).

β-amylase activity in grains was significantly increased when barley plants were top-dressed with more N fertilizer at booting stage. β-amylase is predominantly synthesized and accumulates during grain maturation up to 1%~2% of total barley protein (Hejgaard and Boisen, 1980). As such, it is one of the most common protein components in barley grain. Thus the amount of β-amylase in barley grains is largely dependent on the conditions under which the grain develops, in particular on nitrogen metabolism and protein synthesis. It was found that there was a close association between β-amylase activity and grain nitrogen/protein content (Rutger *et al*., 1967; Hayter and Riggs, 1973; Swanston, 1980). Therefore it is expected that more N top-dressing at late stage will increase β-amylase activity.

This investigation showed that timing of N fertilizer application affected significantly most malt quality parameters. More application of N fertilizer at late growth stage significantly decreases malt extract due to more increase of grain protein concentration. Eagles *et al*.(1995) reported that malt extract would decrease with increased N application rate. It is implied that higher N level in soil and plants leads to more protein synthesis and accumulation in barley grains, as a result causing a reduced ability of grain components to be decomposed during malting and mashing, which may be demonstrated by the response of Kolbach index to timing of N application. Kolbach index is a value indicating protein solubility and is decreased with increased N application at late growth stage. It is interesting to note that the effect of timing of N fertilizer application on malt quality is also cultivar-dependent. For instance there was a significant

Table 4 Effects of timing of N application on some malt properties

N treatment	Malt extract (%)		Diastatic power (°WK)**		Viscosity (cP)		Kolbach index	
	92-11	Xiumai 3	92-11	Xiumai 3	92-11	Xiumai 3	92-11	Xiumai 3
N_1	74.6 a*	78.7 a	278.7 b	307.3 b	1.68 a	1.50 a	31.3 a	44.3 a
N_2	74.2 a	77.5 a	300.3 b	331.3 ab	1.74 a	1.57 a	30.3 a	42.0 a
N_3	72.3 b	76.0 b	345.3 a	347.7 a	1.68 a	1.49 a	29.7 a	38.0 b

* Different letters after data within a column represent significant difference at 95% probability; ** °WK: Windisch-Kolbach units

Table 5 Correlations among grain protein concentration, weight, β-amylase activity and malt properties

	β-amylase activity (U/g)	Protein concentration (%)	Grain weight (g)
Malt extract (%)	−0.80**	−0.71**	−0.55*
Diastatic power (°WK)	0.18	0.59**	−0.55*
Viscosity (cP)	0.55*	0.38	0.79**
Kolbach index	−0.73**	−0.61**	−0.66**
Grain weight (g)	0.53*	0.22	
Protein content (%)	0.74**		

* and ** mean significant at 95% and 99% probability, respectively

difference in Kolbach index among three N treatments for Xiumai 3, while there was no difference for 92-11.

It is well documented that grain protein concentration is negatively correlated with malt extract and positively correlated with diastatic power (Arends *et al*., 1995; Bishop and Day, 1993; Howard *et al*., 1996; Smith, 1990). Molina-Cano *et al*.(1995) reported that grain protein concentration was significantly and negatively correlated with malt viscosity and Kolbach index, while malt extract significantly and positively correlated with Kolbach index. This research revealed that β-amylase activity was positively correlated with grain protein concentration, but its association with diastatic power was not close. It was found that β-amylase activity was negatively correlated with malt extract and Kolbach index. The results indicated that the influence of increasing protein concentration in barley grains on malt quality is dual: a positive effect by increasing β-amylase activity as well as diastatic power and a negative effect by reducing malt extract. So it is important to optimize rate and timing of N fertilizer application in order to obtain high grain yield and malt quality. According to the current results, it seems better to top-dress N fertilizer in equally at the 2-leaf and booting stage, respectively.

An effective approach to balance the conflict between high yield and malt quality on N application is to develop cultivars with grain protein concentration that are less sensitive, in terms of grain protein accumulation, to variable N levels in soil (Arends *et al*., 1995). Bertholdsson (1999) described an ideotype for low and stable grain protein concentration, which was characterized by late heading, many tillers and many seeds per ear. Thus it may be possible to increase β-amylase activity and diastatic power while keeping grain protein concentration below the critical level of 11.5%. In this study, the two barley cultivars had distinct difference in the response of grain quality and malt quality to N application timing. It is suggested that it is also possible to alleviate the contradiction between low protein level and high diastatic power through developing cultivars with high β-amylase activity and low/stable protein concentration.

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