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Genotypic and environmental variation in barley beta-amylase activity and its relation to protein content

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Abstract

β-Amylase activities of barley cultivars collected from various areas of China, and as well as from Canada and Australia, were assayed. Meanwhile a multi-location trial was conducted to determine variation of β-amylase activity in eight barley cultivars and the relationship between β-amylase activity and protein content. For 56 cultivars in study, β-amylase activity ranged from 458 to 1024 U/g, with a mean of 738 U/g. There was significant variation in both β-amylase activity and protein content for eight barley cultivars grown in four locations. No significant correlation was found betweenβ-amylase activity and protein content surveyed in 56 cultivars.

Keywords: β-Amylase activity; Barley (*Hordeum vulgare* L.); Protein content; Variation

1. Introduction

Beta-amylase, (1→4)-α-glucan malto-hydrolase (EC 3.2.1.2), catalyses the release of beta-maltose from the non-reducing ends of (1→4)-α-glucans (Dunn, 1974). It is highly and consistently correlated with diastatic power (DP) (Aredns, Fox, Henry, Marschke, & Symons, 1995; Delcour & Verschaeve, 1987; Gibson, Solah, Glennie Holmes, & Taylor, 1995), which is an important quality trait of malting barley and often used as an indicator of the capacity of malt to degrade starch to fermentable sugars. Beta-amylase is synthesized and accumulates during grain development in two main forms: an insoluble protein complex (mainly associated with the periphery of starch granules; Lauriere, Lauriere, & Daussant, 1986), and a soluble or free form. In mature barley kernels, the bound form is predominant. Beta-amylase present in the mature, ungerminated barley grains is a measure of the suitability of the grain for brewing purposes, and screening for beta-amylase can serve to identify cultivars suitable for malting and to monitor breeding effects to this end (Gibson et al., 1995).

Barley used for malt should have a grain protein concentration (GPC) below 11.5%, as higher protein content will deteriorate malting produce and final beer quality. However, it is often difficult to keep it below this upper limit, since the GPC is influenced, to a large extent, by both genotype and environment (Bathgate, 1987; Smith, 1990). In general, high availability of nitrogen and stress situation caused by drought or heat in combination with drought may increase GPC (Coles, Jamieson, & Haslemore, 1991; Savin & Nicolas, 1996; Weston, Horsley, & Schwartz, 1993). Synthesis ofβ-amylase during barley grain development is regulated by nitrogen nutrition (Giese & Hopp, 1984), and high levels of β-amylase are generally correlated with increased grain protein content (Hayter & Riggs, 1973; Swanston, 1980). The positive association between protein content and β-amylase in barley grains implies a potential risk of causing the protein content to be above the upper limit for malting, while β-amylase activity can be improved by means of genetics and agronomy.

There have been studies on the synthesis and measurement of beta-amylase (Hara-Nishimura, Nishimura, & Daussant, 1986; McCleary & Codd, 1989; Santos & Riis, 1996), but little is know about its genetic and environmental variation. This paper reports the pre-

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liminary results of a comprehensive study investigating the effects of genotype and environment on beta-amylase in barley.

2. Materials and methods

Fifty-six malting barley cultivars, including those being planted currently in China and other countries (mainly from Canada and Australia) were grown at the farm of Zhejiang University (Huajiachi Campus) in 2000. Each cultivar was grown in a 10-row plot with 2 m in row length and 0.25 m between rows. Meanwhile, eight two-rowed cultivars, being widely planted in Zhejiang province, China were selected for a multi-location experiment. All cultivars were planted in early November of 2000 at four locations with different climates in Zhejiang, i.e. Hangzhou, Jiaxing, Dongyang and Yuyao. The experiment was arranged as a randomized complete block design with three replications. All agronomic managements, including fertilization, weed and disease control, were conducted in the same way as practised locally.

At maturity, about 30 spikes were randomly harvested. Sampled grains were dried in an oven at the temperature of 80 °C for 2 days, ground in a Tecator cyclotec sample mill, and then passed through a 0.5 mm screen. Beta-amylase activity was analyzed using the commercial kits (Megazyme Ltd. Ireland), according to McCleary and Codd (1989), and protein content was determined by near infrared reflectance spectroscopy (Model 5000, FOSS Co. Denmark). A working curve had been previously established through measuring protein content of 350 barley grain samples by both NIRA and Kjeldahl methods.

3. Results and discussion

There were marked differences among the 56 barley cultivars under study in both beta-amylase activity and protein content (see Fig. 1). Beta-amylase activity ranged from 458 U/g of Shanlong 6055 (a Chinese cultivar planted in the northern China) to 1024 U/g of Ganpi 2 (a Chinese cultivar planted in east-southern China), with a mean of 738 U/g. A greater range of variation in beta-amylase activity was observed in other studies (Aredns et al., 1995; Georg-kraemer, Mundstock, & Cavalli-Molina, 2001). Aredns et al. (1995) studied 11 cultivars at six locations in Australian and found beta-amylase levels ranging from 201 to 1550 U/g. Georg-kraemer et al. (1998) monitored beta-amylase activity of 10 varieties in Brazil during 7 days of germination, showing that the average beta-amylase activity ranged from 716 to 1470 U/g. Protein contents of all varieties varied from 8.71 to 12.7%, and the mean was 10.3%. It was reported that there was positive correlation between β-amylase activity and grain nitrogen content (Hayter & Riggs, 1973; Swanston, 1980). In this study we did not find any positive association between these two characters. In fact some genotypes with more than 950 U/g of beta-amylase activity, such as Gadna, 8814-20-2-1, Harrington, Ganpi 2, Inoe and Stein, had less than 11.5% of protein. The inconsistency between our results and previous reports could be explained by the difference in experimental methods. In our study, we planted 56 cultivars under the same N level, while other workers have used few cultivars growing under different N levels. Therefore it is possible to develop the cultivars with the desirable combination of high beta-amylase activity and low protein content.

The variation in beta-amylase activity of eight barley cultivars, growing at four locations (environments) was significant (Table 1). 92-11 had the highest beta-amylase activity (843 U/g) and ZAU6 was the lowest (608 U/g). Coefficient of variation (CV) of beta-amylase activity for eight barley cultivars in different locations ranged from 6.09% (ZAU 7) to 14.5%(ZAU6). It may be suggested that ZAU 6 was most susceptible and ZAU 7 was the most stable to the environment in terms of beta-amylase activity.

The means and CV of protein contents of eight cultivars are also presented in Table 1. There were significant dif-

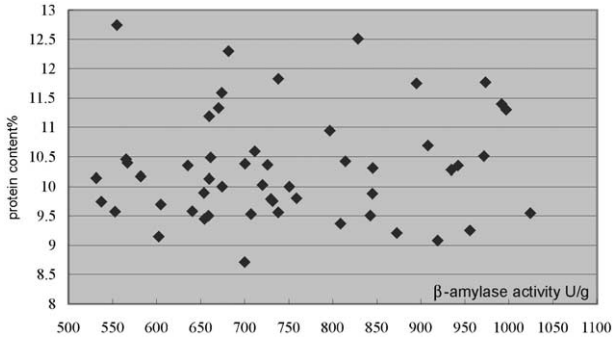

Fig. 1. The distribution of beta-amylase activity and protein content for 56 cultivars.

Table 1
Mean and coefficients of variation of beta-amylase activity and protein content of various barley cultivars grown at four locations

Cultivars	Beta-amylase activity		Protein content	
	Mean (U/g)	CV%	Mean%	CV%
Xiu92-47	771b	9.54	11.9b	17.1
ZAU 7	769b	6.09	11.7b	13.9
Xiumai 3	767b	6.83	11.8b	18.9
ZAU 6	608c	14.5	11.7b	17.2
92-11	843a	7.52	12.3a	14.8
ZAU 3	785ab	9.48	10.8d	13.7
Zhepi 4	776b	9.21	11.2c	15.9
Zheyuan 18	786ab	12.8	11.7b	17.4

Table 2
Mean and coefficients of variation of beta-amylase activity and protein content of barley cultivars grown at different locations

Locations	Beta-amylase activity		Protein content	
	Mean (U/g)	CV%	Mean%	CV%
Hangzhou	719b	12.1	10.2b	3.37
Jiaxing	710b	11.8	9.96c	4.81
Dongyang	811a	6.23	13.2a	5.73
Yuyao	813a	11.6	13.3a	5.71

Means within a column followed by a different letter are significantly different ($P < 0.05$).

ferences in protein contents among cultivars. The mean protein content of eight cultivars over four locations ranged from ZAU 3 of 10.8% to 92-11 of 12.3%. The coefficient of variation in protein content was much larger than that of beta-amylase activity, suggesting its greater variation caused by different environments.

There were differences in beta-amylase activity among locations (Table 2). The highest activity was 813 U/g in Yuyao, while the lowest was 710 U/g in Jiaxing. A great difference in CV of beta-amylase activity among locations was also noted. The lowest CV was 6.23% in Yuyao, and the highest CV was 12.1% in Hangzhou. Similarly, there were significant differences in protein contents among locations. Dongyang and Yuyao were significantly higher than the other two locations. The coefficient of variation in protein content for four locations ranged from 3.37 to 5.73%, much less than that of beta-amylase activity.

It may be concluded that there is a wide genetic variation among barley genotypes in both beta-amylase activity and protein content, which provides the opportunity, by breeding, to improve these traits for malting use. Meanwhile, the significant variations between locations suggests the possibility and importance of agronomic improvement for these malting qualities.

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