

Validating Quantitative Trait Loci for Diastatic Power in Populations of Malting Barley (*Hordeum vulgare* L.)

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Introduction

The combined and coordinated action of starch degrading enzymes is termed diastatic power, with α -amylase and β -amylase activity most highly correlated (Evans et al. 1995). Consequently enhanced expressions of these two enzymes are important malting quality barley breeding objectives. These quantitative traits typically exhibit low heritability, environmental influence, and continuous phenotypic variation (Tanksley, 1993) making detection of superior individuals difficult. QTL detection has contributed to an understanding of the genetic control of quantitative traits. Extensive QTL mapping information allows informed breeding decisions and efficient genome based selection strategies to be devised. A validation process is required to establish the usefulness of marker assisted selection (MAS) to improve quantitative traits. This involves estimating the weighted influence of selected QTL on the associated trait and measuring its relative expression when introgressed into alternative genetic backgrounds. The routine implementation of MAS in breeding programs has often been slow or failed because the validation step has not been completed.

Materials and Methods

Genetic material included 68 F₃ derived recombinant inbred lines (RILs) from Barque/Harrington and 45 F₃ derived RILs from Barque/Haruna Nijo. Barque is a South Australian feed quality barley with low diastatic power (Barr, 1998), Harrington is a North American malting barley with high diastatic power (Harvey and Rossnagel, 1984) and Haruna Nijo is a Japanese malting barley with high diastatic power (Aida, 1979). These populations were grown at the Charlick experimental station near Strathalbyn (South Australia) in 1996.

Each line was micro-malted with diastatic power, β -amylase and α -amylase activity measured spectrophotometrically using PAHBAH. Malt protein was measured using NIR. Harrington and Haruna Nijo have been used in four mapping populations, Chebec/Harrington and Galleon/Haruna Nijo (Langridge et al. 1995), Harrington/TR306 (Kasha et al. 1994) and Morex/Harrington (Hayes et al. 1996). Most QTL associated with hydrolytic enzyme expression are in the vicinity of genes known to encode key enzymes α -amylase and β -amylase (Hayes et al. 1997). The loci *Amy1*, *Amy2*, *Bmy1*, and *Bmy2* were therefore logical choices for QTL validation. A 5H locus (*Xabg463*) associated with putative α -amylase QTL from Harrington was also additionally studied.

No marker polymorphism was determined between Barque and Harrington or Barque and Haruna Nijo for *Xabg463* (5H) and *Amy2* (7H), so linked polymorphic markers *Xabg057* and *Xcdo358* were used as alternatives. Candidate gene clones *Amy1* (6H) and *Bmy1* (4H) were used as RFLP markers. The *Bmy1* cDNA (from Galleon) detected both the *Bmy1* (4H) and

Bmy2 (2H) loci. Polymorphism was found at the *Bmy1* locus only in the Barque/Haruna Nijo population, whilst the *Bmy2* locus was polymorphic in both populations. A PCR marker for the *Bmy1* gene (Erkkila et al. 1998) was polymorphic in both populations.

Each line of the segregating populations was separated into alternate marker allele classes for each locus. Heterozygous individuals were omitted from the analysis. Least squares means for all traits were estimated using ANOVA with marker allele class the single factor. Means for all allele classes were compared with contrasts. Locus effects were tested only on individual loci as the small population sizes prevented testing for locus interactions. Allele class differences were deemed significant at $p < 0.01$. All statistical analysis was conducted using JMP v1.2 (SAS Software, 1987).

Results and Discussion

Variation for α -amylase, β -amylase and diastatic power was identified in both populations. Significant differences between marker allele class means were detected for three marker loci *Xabg057*, *Bmy1*, and *Bmy2*. All other loci had no effect on the traits examined in both populations. Only the results for these three loci will be discussed.

5H locus effects α -amylase activity

Table 1 The effect of alternate alleles at five loci on α -amylase activity ($\mu\text{mol}/\text{min}/\text{g}$) in two populations of barley derived from crosses between the feed variety Barque and malting varieties Harrington or Haruna Nijo.

Loci	Allele	Barque/Haruna Nijo			Barque/Harrington		
		LSM	SE	Sig.	LSM	SE	Sig.
<i>Amy1</i> (<i>cDNA clone</i>)	Barque	62.4	2.4	ns	74.2	3.3	ns
	Alternative	55.3	3.6		76.4	6.5	
<i>Amy2</i> (<i>Xcdo358</i>)	Barque	56.1	2.3	ns	73.6	3.4	ns
	Alternative	62.6	3.5		76.8	6.8	
<i>abg463</i> (<i>Xabg057</i>)	Barque	51.3	3.1	21%	69.0	3.6	29%
	Alternative	62.0	2.3	*	88.9	5.0	*
<i>Bmy1</i> (PCR)	Barque	56.7	3.1	ns	78.7	3.8	ns
	Alternative	64.4	3.6		63.8	6.2	
<i>Bmy1</i> (<i>cDNA clone</i>)	Barque	52.1	2.6	29%			
	Alternative	67.1	3.0	**			
<i>Bmy2</i> (<i>Bmy1 cDNA</i>)	Barque	54.2	3.5	ns	77.0	4.2	ns
	Alternative	60.7	3.0		73.0	5.2	

LSM, Least Square Mean; SE, Standard Error; Sig, significance level ns, not significant; ** $p < 0.001$; * $p < 0.01$
Bracketed (), linked marker used (cDNA clone/RFLP or PCR)

Classes carrying malting parent marker alleles at the 5H locus had significantly greater α -amylase activity. Mean activity differences between alternative marker alleles were 21% ($p < 0.01$) for Barque/Haruna Nijo and 29% ($p < 0.01$) for Barque/Harrington (Table 1). In many mapping populations α -amylase QTL have been detected in the vicinity of the 5H locus. Additional validation gives good evidence that major gene(s) for α -amylase expression, and/or activity modification underlies it. Many other traits map to this locus and fine mapping will determine if contrasting QTL alleles exist at this locus. These coincident malting qualities QTL could indicate a multilocus cluster or a single regulatory gene responsible for the cascade of processes determining malting quality (Oziel et al. 1996).

Validation of β -amylase and diastatic power QTL associated with structural genes

Only two loci, *Bmy1* and *Bmy2*, were implicated with significant effects on β -amylase activity and diastatic power. Malting parent alleles at the *Bmy1* locus were associated with 29% ($p<0.01$) higher β -amylase activity in the Barque/Haruna Nijo population using the *Bmy1* RFLP, and 47% ($p<0.001$) in the Barque/Harrington population using the *Bmy1* PCR marker (Table 2).

Table 2 The effect of alternate alleles at five loci β -amylase activity ($\mu\text{mol}/\text{min}/\text{g}$) in two populations of barley derived from crosses between the feed variety Barque and malting varieties Harrington or Haruna Nijo.

Loci	Allele	Barque/Haruna Nijo			Barque/Harrington			Barque/Haruna Nijo Adjusted		
		LSM	SE	Sig.	LSM	SE	Sig.	LSM	SE	Sig.
<i>Amy1</i> (<i>cDNA clone</i>)	Barque	271.4	14.3	ns	214.0	8.6	ns	265.4	11.0	ns
	Alternative	241.1	21.8		250.6	17.2		249.2	16.7	
<i>Amy2</i> (<i>Xcdo358</i>)	Barque	268.4	13.4	ns	220.9	9.4	ns	261.9	9.8	ns
	Alternative	255.4	20.5		238.2	18.9		260.2	14.9	
<i>abg463</i> (<i>Xabg057</i>)	Barque	254.8	19.2	ns	222.3	11.5	ns	262.1	13.7	ns
	Alternative	252.8	14.1		214.0	16.0		256.7	10.4	
<i>Bmy1</i> (<i>PCR</i>)	Barque	245.9	17.5	ns	192.2	9.6	47%	240.7	13.8	ns
	Alternative	284.1	19.8		285.5	15.5	**	267.9	15.7	
<i>Bmy1</i> (<i>cDNA clone</i>)	Barque	228.3	15.0	29%				232.6	10.9	24%
	Alternative	292.3	17.2	*				288.4	12.5	*
<i>Bmy2</i> (<i>Bmy1 cDNA</i>)	Barque	212.0	19.1	37%	209.6	12.4	ns	232.5	15.9	ns
	Alternative	290.7	14.8	*	241.2	15.3		273.9	12.3	

LSM, Least Square Mean; SE, Standard Error; Sig, significance level ns, not significant; ** $p<0.001$; * $p<0.01$
Bracketed (), linked marker used (cDNA clone/RFLP or PCR)

Locus effects on diastatic power were reflected in higher mean β -amylase activity. Individuals in the Barque/Haruna Nijo population carrying the malting parent RFLP marker allele at the *Bmy1* locus were associated with 34% ($p<0.001$) higher diastatic power, while those carrying the *Bmy1* PCR marker showed a 29% ($p<0.001$) difference in the Barque/Harrington population (Table 3).

Table 3 The effect of alternate alleles at five loci on diastatic power ($\mu\text{mol}/\text{min}/\text{g}$) in two populations of barley derived from crosses between the feed variety Barque and malting varieties Harrington or Haruna Nijo.

Loci	Allele	Barque/Haruna Nijo			Barque/Harrington			Barque/Haruna Nijo Adjusted		
		LSM	SE	Sig.	LSM	SE	Sig.	LSM	SE	Sig.
<i>Amy1</i> (cDNA clone)	Barque	333.9	14.6	ns	284.6	8.90	ns	327.7	11.5	ns
	Alternative	296.5	22.3		325.0	18.0		304.8	17.6	
<i>Amy2</i> (<i>Xcdo358</i>)	Barque				294.6	9.60	ns	317.9	10.3	ns
	Alternative				315.2	19.2		322.9	15.7	
<i>abg463</i> (<i>Xabg057</i>)	Barque	306.2	19.8	ns	291.3	11.6	ns	316.2	14.5	ns
	Alternative	314.9	14.5		302.9	16.2		318.7	11.0	
<i>Bmy1</i> (PCR)	Barque	302.6	17.6	ns	270.9	10.3	29%	297.3	14.5	ns
	Alternative	348.9	19.9		349.5	16.6	**	332.4	16.4	
<i>Bmy1</i> (cDNA clone)	Barque	269.1	12.6	34%				278.5	9.90	28%
	Alternative	359.6	14.1	**				355.5	11.1	**
<i>Bmy2</i> (<i>Bmy1</i> cDNA)	Barque	266.3	17.9	29%	286.7	12.4	ns	287.3	16.2	ns
	Alternative	343.1	14.2	*	314.3	15.3		330.1	12.9	

LSM, Least Square Mean; SE, Standard Error; Sig, significance level ns, not significant; ** p<0.001; * p<0.01
Bracketed (), linked marker used (cDNA clone/RFLP or PCR)

The *Bmy1* locus encodes β -amylase that is expressed in the endosperm during grain development. QTL for diastatic power and β -amylase activity have been associated with this locus in many mapping studies. Three *Bmy1* alleles have been identified in cultivated barley. The corresponding enzyme Sd1 can be distinguished by its lower isoelectric point (Swanston, 1980) whilst the Sd2L and Sd2H enzymes are distinguished on the basis of their thermostability (Eglinton et al. 1998). The *Bmy1*-Sd2L cDNA was polymorphic between Barque (Sd2L) and Haruna Nijo (Sd2H) but not Barque and Harrington (Sd1), which is not unexpected due to few point mutations that exist between the Sd1 and Sd2L β -amylase allele sequences. The *Bmy1* PCR marker was polymorphic in both populations, but not between the malting quality parents. Thus, it can distinguish the Sd2L enzyme allele from the other two.

Using populations segregating for β -amylase enzyme IEF type (Sd1 and Sd2), the β -amylase allele was associated with variation in diastatic power (Swanston 1980; Evans et al. 1995). Pooling lines into *Bmy1* marker allele classes in this study revealed significant differences in diastatic power and β -amylase activity consistent with these former authors. In a survey of 14 barley cultivars a 126-bp insertion/deletion event in intron III detected by the PCR primers was associated with good malt quality cultivars (Erkkila et al. 1998). Analysis of the segregating populations in this study demonstrates the Sd2L allele contains the 126-bp insertion and is associated with lower activity. These results support the view that intron mediated gene expression modulates the level of β -amylase activity in barley (Erkkila et al. 1998; Simpson et al. 1999). Malting parent alleles detected using the *Bmy1* PCR marker were associated with higher β -amylase activity and diastatic power, although in the Barque/Haruna Nijo population this association was not significant. The *Bmy1* RFLP and PCR markers cosegregate. Six missing data points and the small population size of Barque/Haruna Nijo contributed to this lack of significance.

The *Bmy2* locus encodes ubiquitous β -amylase expressed at low levels, primarily in non-seed tissues (Kreis et al. 1988). Significant malt protein effect on β -amylase activity ($r^2=0.41$; $p<0.001$) and diastatic power ($r^2=0.39$; $p<0.01$) only in the Barque/Haruna Nijo population was detected by regression analysis. Adjustment for this effect showed Haruna Nijo alleles at

the *Bmy2* locus which were previously associated with 37% ($p<0.01$) higher β -amylase activity (Table 2) and 29% ($p<0.01$) higher diastatic power (Table 3) became non-significant. In contrast malt protein adjustment had no influence on the effect associated with the *Bmy1* locus. This indicates the locus may have a regulatory function on malt protein expression, influencing β -amylase activity and diastatic power indirectly. A linked GA response locus from Haruna Nijo (Li, 1997) may regulate its expression. Many agronomically related or inter-related traits map to this locus and the results suggest the *Bmy2* locus may influence key developmental stages that indirectly affect β -amylase activity and diastatic power.

Acknowledgments

Many thanks goes to the members of the Langridge Laboratory and the Waite Barley Quality Laboratory for their support and assistance during this project.

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