

Haplotype diversity in the endosperm specific β -amylase gene *Bmy1* of cultivated barley

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The amylolytic enzyme β -amylase catalyses the release of maltose from starch which is an important biochemical pathway during germination and the malting process. In cultivated barley two loci of β -amylase were mapped on the long arm of the chromosome 4H and the short arm of the chromosome 2H, however, only the first locus *Bmy1* was reported to be expressed in the seed tissue.

Five single nucleotide polymorphisms (SNPs) corresponding to substitutions in the protein sequence of β -amylase gene *Bmy1* at amino acid positions 115, 165, 233, 347 and 430 were genotyped in 493 cultivated barley accessions by Pyrosequencing and a CAPS assay. A total of 6 different haplotypes for the *Bmy1* gene were discovered of which 4 haplotypes were identified as previously described alleles *Bmy1*-Sd1, *Bmy1*-Sd2L, *Bmy1*-Sd2H, *Bmy1*-Sd3, while 2 haplotypes were new.

Linkage disequilibrium (LD) evaluated within the *Bmy1* gene as squared allelic frequency correlations r^2 with five SNPs revealed high r^2 values and was clearly dependent on the presence of the population structure. In the homogenous group of European 2-rowed spring barleys compared to the highly structured worldwide population the elevated values of r^2 were detected. The high LD within the gene between loci SNP115,

Table 1: Significant associations of individual SNPs and specific haplotypes with two quality traits performed with TASSEL. Associations are presented as exact *P*-values with *, **, * indicating $P < 0.001$, $P < 0.0001$ and $P < 0.00001$, respectively, „ns“ - not significant**

Locus	A set of 2-rowed winter and spring varieties		A set of 2-rowed spring varieties	
	Final attenuation	Malt extract	Final attenuation	Malt extract
snp115	*	*	**	ns
snp165	*	*	*	ns
snp347	*	ns	***	ns
snp430	*	*	**	ns
Haplotype				
Sd4	ns	**	ns	ns
Sd1a	*	*	*	ns
Sd2L	*		***	ns
Total R ² of the model	0.486	0.423	0.629	0.166

SNP165 and SNP430, allowed to suggest that SNP155, SNP233 and SNP347 could be considered as *Bmy1* haplotype tagging SNPs for plant breeding practice in Europe. These three SNPs allowed to capture 91.6% of the available diversity (452 out of 493 accessions were separated).

For the set of 36 German 2-rowed spring and winter varieties four SNPs and three observed haplotypes were tested for associations with the two traits, malt extract and final attenuation. Trait evaluation data were from the regular official publications (Bundessortenamt, 1994-2003). For the spring barleys no significant association was observed for the trait malt extract, but the associations for the trait final attenuation were highly significant (Table 1). Most significant

($P < 0.00001$) was the association with SNP347 and haplotype *Bmy1*-Sd2L; varieties carrying this β -amylase haplotype showed decreased values for final attenuation. Interestingly among 56 additional varieties from our set officially registered as „feeding“ cultivars according to descriptions of the Bundessortenamt, 50 carried allele *Bmy1*-Sd4.

The association analysis revealed that DNA sequence polymorphisms in the *Bmy1* gene were associated with the malting quality parameter final attenuation. The presented case study of the barley *Bmy1* gene shows that associations with agronomic traits are feasible for cereals with their extraordinary complex large genomes overloaded with different types of repetitive sequences.

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