Spatio-temporal profiling and degradation of alpha-amylase isozymes during barley seed germination

Ten genes from two multigene families encode barley alpha-amylases. To gain insight into the occurrence and fate of individual isozymes during seed germination, the alpha-amylase repertoire was mapped by using a proteomics approach consisting of 2D gel electrophoresis, western blotting, and mass spectrometry. Mass spectrometric analysis confirmed that the 29 alpha-amylase positive 2D gel spots contained products of one (GenBank accession gi|113765) and two (gi vertical bar 4699831 and gi vertical bar 166985) genes encoding alpha-amylase 1 and 2, respectively, but lacked products from seven other genes. Eleven spots were identified only by immunostaining. Mass spectrometry identified 12 full-length forms and 12 fragments from the cultivar Barke. Products of both alpha-amylase 2 entries co-migrated in five full-length and one fragment spot. The alpha-amylase abundance and the number of fragments increased during germination. Assessing the fragment minimum chain length by peptide mass fingerprinting suggested that alpha-amylase 2 (gi vertical bar 4699831) initially was cleaved just prior to domain B that protrudes from the (beta alpha)(8)-barrel between beta-strand 3 and alpha-helix 3, followed by cleavage on the C-terminal side of domain B and near the C-terminus. Only two shorter fragments were identified of the other alpha-amylase 2 (gi vertical bar 166985). The 2D gels of dissected tissues showed alpha-amylase degradation to be confined to endosperm. In contrast, the aleurone layer contained essentially only full-length alpha-amylase forms. While only products of the above three genes appeared by germination also of 15 other barley cultivars, the cultivars had distinct repertoires of charge and molecular mass variant forms. These patterns appeared not to be correlated with malt quality.

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