

Title Selectable marker-free transgenic barley producing a high level of cellulase (1,4- β -glucanase) in developing grains

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Abstract

The use of barley grains as bioreactors for high-level production of cellulase (1,4- β -glucanase) was investigated. A hybrid cellulase gene, *cel-hyb1*, driven by the rice *GluB-1* promoter was expressed specifically in developing endosperm. Codon usage optimisation of *cel-hyb1* increased its expression in barley grains 527-fold and led to cellulase production of up to 1.5% of total grain protein. CEL-HYB1 enzyme in barley grains was highly stable during post-harvest storage. Selectable marker gene (*hph*) was subsequently eliminated from transgenic lines through segregation of *hph* from synthetic *cel-hyb1* (*syn.cel-hyb1*) in T1 progeny, using a binary plasmid containing *hph* and *syn.cel-hyb1* in separate T-DNAs. These data suggest that barley grains can potentially be used for the commercial production of cellulase.