GENETIC TRANSFORMATION OF BARLEY (*Hordeum vulgare* L.) WITH A THERMOSTABLE (1→3,1→4)-β-GLUCANASE GENE

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Chapter Five

Transformation of barley with the gene encoding the mutated barley (1→3,1→4)-β-glucanase H300P enzyme

ABSTRACT

The work described in this thesis evaluated different transformation technologies for the genetic transformation of elite Australian barley cultivars with a mutated barley (1→3,1→4)-β-glucanase gene.

The initial aim of this study was to develop plant regeneration protocols for embryogenic cell suspension and protoplast cultures of barley and to use polyethylene glycol (PEG)-mediated DNA transfer into protoplasts to recover transgenic plants. Although plant regeneration was successfully achieved from the cell suspension cultures, it was accomplished at a much lower frequency for the cell suspension culture-derived protoplasts. This low frequency of plant regeneration from isolated protoplasts prevented the use of protoplasts as target cells for DNA transfer and indicated that alternative technologies were required to produce fertile transformants for the studied cultivars.

Microprojectile bombardment of the suspension-cultured cells demonstrated that DNA was successfully delivered into intact barley cells. The expression of the introduced genes in the callus recovered from selection and the detection of transgene sequences in the genomic DNA isolated from the same callus confirmed that transformed callus lines were produced. Although plants were not regenerated from the transformed callus lines, these results indicated that microprojectile bombardment represented a potentially useful technique for the production of transgenic barley, provided the target cells retained the capacity to regenerate plants following transformation.

The development of a copper sulphate-enhanced plant regeneration system for scutellum-derived embryogenic callus and the use of microprojectile bombardment conditions that combined efficient DNA delivery with minimal damage to the transformed cells subsequently led to the generation of fertile transgenic plants. Biochemical and molecular assays demonstrated the functional expression and nuclear integration of the transgenes in the primary transformants (T_0). The analyses of the first generation of progeny plants (T_1), derived from different primary transformants, confirmed the Mendelian segregation and inheritance of the introduced genes.

In the latter part of this study, microprojectile bombardment and *Agrobacterium tumefaciens*-mediated transformation were employed to transform barley with a mutated barley (1→3,1→4)-β-glucanase gene. In general, the transformed plants derived from *Agrobacterium tumefaciens*-mediated transformation had simpler transgene insertion patterns compared with the plants recovered from microprojectile bombardment. Reverse transcriptase (RT)-PCR was used to detect mRNA encoding the mutated (1→3,1→4)-β-glucanase enzyme in the germinated T_1 grains of four transformants obtained from *Agrobacterium tumefaciens*-mediated transformation. Molecular and biochemical assays indicated expression of the mutated (1→3,1→4)-β-glucanase gene at the mRNA and protein levels in the homozygous transgenic grain of one transformed plant line.