

Barley Transformation Breeding: Further Progress and Remaining Problems

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Introduction

Stable transformation of barley was first reported in 1994 when Wan and Lemaux (1994) used direct DNA delivery by microparticle bombardment to generate stable transformants from immature embryos, microspore-derived embryos, and callus from young embryos. Each year since then, there have been several reports of successful barley transformation. Most have used microparticle bombardment for DNA delivery, immature embryos or callus explants as targets, and a small number of barley cultivars (Lemaux et al. 1999). Golden Promise, a spring barley, and Igri, a winter barley, both form callus readily and regeneration of plantlets from callus is relatively easy. These cultivars have been transformed successfully in a number of cases. Other varieties of barley have been transformed (Cho *et al.*, 1999) albeit with greater difficulty and less efficiency. The short term goals in this field of research are to get efficient production of transformants from all varieties, to insert limited numbers of gene copies, to minimise genome disruption, to express transgenes stably, and to accomplish this in an environmentally responsible way with consumer, industry and scientific acceptability.

A procedure using *Agrobacterium* has allowed progress in some of these areas (Tingay et al. 1997). This method uses immature embryos and produces high transformation frequencies (>5%). The transgene copy numbers (usually 1-5) and the number of insertion sites (usually 1-3) are low and polyploidy (Cho et al 1999) occurs infrequently, if at all. Despite these advances, *Agrobacterium* transformation of immature embryos is still labor intensive and tedious. From embryo infection to seed takes 10 months. Successful transformation remains strongly dependent on genotype and sometimes appears to be accompanied by somaclonal mutations or other genetic damage. Selectable marker genes are still required. The following discussion describes progress in overcoming some of these difficulties and reviews remaining problems.

Overview

Progress

(1) *Transformation procedures.* Since development of the method, *Agrobacterium*-mediated transformation has been used to insert a number of genes into barley. With attention to consistency of procedures in growth and preparation of immature embryos and all downstream processes, average efficiency of more than 5 transformants per 100 embryos co-cultivated with *Agrobacterium* are obtained. Gene insertion loci and gene copy numbers continue to be low and non-Mendelian segregation ratios are infrequent. The most transformable cultivar is still Golden Promise but efforts are being made to transform other cultivars more adapted to Australian conditions. All transgenes have shown strong expression and there has been no transgene instability found in early generations.

(2) *Elimination of selectable marker genes.* Because of consumer and industry concerns about the undesirability of selectable marker genes in genetically modified crops, we have developed methods for elimination of these genes from genetically modified barley (see poster entitled “Transgene segregation to obtain selectable marker-free transformed barley” by P. Matthews et al.). Because of the efficiency of the transformation method, we have been able to produce selectable marker-free transgenic lines with an efficiency of 17 lines per 100 transgenic lines (ie about 1 per 100 embryos co-cultivated with *Agrobacterium*). This procedure is used routinely now for the production of transgenic barley in Canberra and in other places.

(3) *Transformation Breeding.* Many genes are being inserted into Golden Promise using *Agrobacterium*. The focus has been to develop transformation breeding technologies and procedures and where possible, to use genes which have the potential to improve quality and agronomic performance of barley. These include genes for:

(a) *Malting enzymes.* Additional copies of genes encoding both high- and low-pI α -amylase and α -glucosidase have been inserted into barley with the aim of increasing synthesis of these enzymes during germination, and thus perhaps diastatic power. A heat-stable β -glucanase has also been inserted with aim of increasing β -glucan hydrolysis during malting and mashing. In general, the transgenes are being expressed well and heritability is normal although the number of generations of transgenic lines is currently not high.

Insertion of extra copies of the GAMYB gene (Gubler et al. 1995; Gubler et al 1997) into rice has shown that this enhances production of several malting enzymes, and similar experiments are underway for barley with the aim of “supercharging” aleurone performance.

Feed enzymes. A heat-stable xylanase gene has been expressed in Golden Promise starchy endosperm with the aim of facilitating xylan breakdown in the guts of monogastric animals (Patel et al 1998). The enzyme accumulates during grain filling, it survives grain dehydration and is active in germinating grain.

(c) *Starch content of grain.* It is thought that the enzymes of the starch biosynthetic pathway may limit starch production in developing grain. Additional copies of the barley starch synthase gene (cDNA) are being introduced into barley with the aim of increasing production of this enzyme and testing the concept that it is limiting.

(d) *Disease resistance.* Synthetic genes designed to create resistance to BYDV have been inserted into Schooner and Golden Promise barley. These genes are based on RNA-dependent RNA polymerase sequences from BYDV and target both the RPV and PAV serotypes of the virus (Wang et al 1998). These approaches have the potential to create immunity to the virus.

Rust resistance genes have been cloned from flax (Lawrence et al 1995) and maize (Collins et al. 1999) and these genes are being transformed into barley to test the concept that foreign genes can create resistance when transformed into barley (M. Ayliffe, pers. com.).

Remaining Problems

Transformation Methods.

Transformation of barley with *Agrobacterium* is still not efficient enough and methods must be developed to increase it to levels obtained with rice or greater. Also, antibiotic and herbicide resistance genes are used as selectable markers and although it is possible to eliminate these genes as described above, these methods are costly in time and effort and it is desirable to develop methods which either do not require the use of selectable markers or which use genes which are “safe” and do not have to be removed.

Genotype Dependency.

Using *Agrobacterium*, it has been possible to transform Golden Promise with high efficiency and Schooner with lower efficiency. While we and others are attempting to transform other Australian cultivars, transgenes in Golden Promise appear to cross readily into more adapted genetic backgrounds.

Somaclonal Mutation.

A field trial of transgenic barley containing several transgenes indicates that somaclonal mutations may be a problem using *Agrobacterium*-mediated transformation as it is with biolistics. In our first field trial, although plant architecture and growth pattern was similar to untransformed plants, the yield of one of the two lines tested was reduced primarily because of small grain size. This may be due to somaclonal mutations introduced by the transformation process. Pilot AFLP analyses (R. Genger, pers. com.) produced evidence for this.

Intellectual Property.

Transformation of barley using *Agrobacterium*, or at least some aspects of it, may be patented and if so, licenses may have to be obtained to commercialise transgenics using this technology. While it may be possible to negotiate satisfactory terms, it is desirable to continue to develop transformation technology which is intellectual property-free. Also, IP surrounding use of transgenes continues to be an issue.

Availability of Useful Genes.

As efficient methods for barley transformation are being developed and pathways to commercialisation are being identified, limitations to the process of transformation assisted breeding are becoming less. Thus, our understanding of the processes of plant growth and development and the cloning of genes controlling those processes are becoming of greater significance as limitations to progress. The explosion of genomics programs and the identification of genes and their functions will probably address this limitation to a large degree.

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