

# **GENETIC TRANSFORMATION OF WHEAT**



***(Triticum aestivum L.)***

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## Abstract

Wheat (*Triticum aestivum* L.) was one of the earliest crops to be domesticated and is now becoming the world's most important food crop. The demand for this commodity has increased in parallel with the growth of world population. Thus, it is becoming increasingly important to secure the supply of wheat, and it is therefore crucial to continuously enhance worldwide wheat production. So far, the main strategy for improving wheat production has been through conventional breeding methods. However, it is becoming apparent that to maintain production targets it will be necessary to complement conventional breeding methods with genetic engineering technology.

The successful application of genetic engineering in wheat is dependent on the availability of suitable tissue culture and transformation methods, and the development of these technologies using elite Australian wheat varieties was the primary objective of experiments described here. The specific goals of this project were:

- to screen Australian wheat genotypes for *in vitro* culture responsiveness
- to transform the responsive wheat genotypes with selectable marker and reporter genes, and
- to study the inheritance and expression of transgenes in successive wheat generations.

In testing the responsiveness of wheat genotypes in culture, four Australian wheat genotypes (cvs. Hartog, Frame, Krichauff and Janz) were used. Immature scutella were cultured onto Murashige and Skoog (MS) basal medium containing different combinations and concentrations of 2,4-dichlorophenoxyacetic acid and

benzylaminopurine. Three genotypes (*cvs.* Hartog, Frame and Krichauff) grew well in culture and one genotype (*cv.* Janz) did not. Two genotypes, namely *cvs.* Hartog and Krichauff, responded well in a medium containing 2 mg/l 2,4-dichlorophenoxyacetic acid, while another genotype (*cv.* Frame) needed the same concentration of 2,4-dichlorophenoxyacetic acid with the addition of 0.1 mg/l benzylaminopurine. With these media, almost all immature scutella produced embryogenic callus, which was subsequently regenerated into mature, fertile plants.

Regeneration systems developed for the three responsive genotypes were coupled with a microprojectile bombardment-mediated transformation method. By bombarding immature scutella of *cvs.* Hartog, Frame and Krichauff, or freshly isolated immature embryos of *cv.* Frame with a construct carrying the *bar* gene, with or without another construct carrying the *GUS* gene, four transgenic plants (*cv.* Frame) were produced. Three of the four transgenic plants were shown to carry two or more copies of the *bar* gene, and another plant carried one copy of the *bar* gene and three or four copies of the *GUS* gene. The introduced transgenes were expressed in the transgenic plants; *bar* gene expression was indicated by the presence of PAT activity and herbicide tolerance, whilst the expression of the *GUS* gene was followed by the presence of GUS activity in histochemical assays that led to blue staining in both vegetative and reproductive organs.

The *bar* and *GUS* transgenes integrated into the genome of transgenic wheat were transmitted to successive generations. The transmission of the transgenes showed a Mendelian pattern of inheritance, and a homozygous genotype was achieved at T2 progeny. This suggests that the transgenic wheat



analysed here had a heterozygous genotype of integrated *bar* and *GUS* transgenes. It was observed that the expression of the *GUS* gene was stable over several generations, but that the expression of the *bar* gene was inactivated in some progeny, as indicated by the loss of PAT activity and herbicide tolerance.

The phenotypic characteristics of the primary transformants were, in most cases, slightly inferior to non-transformed, control plants, but showed some improvement in subsequent generations.

As a result of the work, transformation of elite Australian wheat varieties should be achievable on a routine basis, albeit at relatively low transformation frequency. This opens the way for the insertion of potentially useful genes into wheat, with the longer term aim of enhancing productivity and/or quality characteristics.