

## **Elimination of selectable marker genes via segregation of uncoupled T-DNAs in populations of doubled haploid barley**

Eszter Kapusi, Goetz Hensel, María-José Coronado, Sylvia Broeders, Cornelia Marthe, Ingrid Otto and Jochen Kumlehn

Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Plant Reproductive Biology,  
Corrensstr. 3, D-06466 Gatersleben, Germany

Barley represents one of the economically most important and most widely distributed crops worldwide, and genetic engineering is expected to play a crucial role in its further improvement. Once transgenic plants are obtained, the selectable marker gene is not any more necessary or even unwanted.

Here we present a fast and efficient method to produce selectable marker-free, homozygous transgenic lines. Primary co-transgenic barley plants ( $T_0$ ), containing both the selectable marker gene (*hygromycinphosphotransferase*, in this case coupled with a *green fluorescent protein* gene) and a 'gene of interest' ( *$\beta$ -glucuronidase*, without selectable marker) were generated via *Agrobacterium*-mediated gene transfer to immature embryos using separate T-DNAs. The binary vectors were introduced into the *Agrobacterium* strains AGL1 and LBA4404. The efficiency of the method was optimised by comparing more than ten different protocols to co-transfer independent T-DNAs to immature embryos. Uncoupled T-DNAs present at hemizygous state in the primary transformants are randomly and independently distributed during male meiosis to the pollen grains. Homozygous selectable marker-free transgenic regenerants of such segregating androgenetic cultures can be efficiently produced and identified among doubled haploid individuals of the resultant  $T_1$ . The method presented is not covered by complicated intellectual property rights as is the case in some alternative approaches. Moreover, only comparatively small populations are needed to find selectable marker-free, true-breeding transgenic  $T_1$ -plants, and further segregation analyses are unnecessary.