MINISTRY OF AGRICULTURE, FISHERIES AND FOOD

CSG 15

Research and Development

Final Project Report

(Not to be used for LINK projects)

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Research Policy and International Division, Final Reports Unit
MAFF, Area 6/01
1A Page Street, London SW1P 4PQ
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Project title	Genetic transformation of wheat using Agrobacterium tumifaciens.
MAFF project code	**AR 1002**
Contractor organisation and location	IACR-Rothamsted Harpenden Herts AL5 2JQ
Total MAFF project costs	£
Project start date	21/10/00
Project end date	20.10.01

Executive summary (maximum 2 sides A4)

We report on the successful completion of the scientific objectives set out in the CSG7, and the generation of *Agrobacterium*- mediated transgenic wheat plants expressing GUS and *bar.* We have established parameters that allow effective T-DNA delivery into immature embryos, and the successful regeneration of fertile adult wheat plants after *Agrobacterium* co-cultivation. These protocols have been applied to a large (>3000) number of embryos and stable transformations events have been recorded, both in terms of regenerating plant organs in tissue-culture, and of adult plants in soil.

Background.

Wheat was first genetically modified in the early 1990s (eg. Vasil *et al.* 1992). Microprojectile bombardment was the DNA-delivery method of choice because wheat, along with the other major graminaceous crop species, were widely considered to be recalcitrant to the *Agrobacterium*-mediated transformation methods being developed for dicotyledonous plants.

Wheat transformation methods based on particle bombardment continued to be refined (eg, Pastori *et al*. 2001, Rasco-Gaunt *et al.* 2001), and although these methods require time, skilled labour, and dedicated controlled-environment / laboratory facilities, are now relatively routine in a small number of laboratories world-wide.

Research to make wheat amenable to *Agrobacterium*-based transformation has continued as this method has several potential advantages over other forms of transformation including: the ability to

transfer large segments of DNA with minimal rearrangements, genetic insertions with fewer gene copies and lower cost with the potential for higher transformation efficiencies.

The first report of *Agrobacterium*-mediated wheat transformation came from Monsanto (Cheng *et al.* 1997), and has also been repeated by researchers at CSIRO Australia (Weir *et al.* 2001). However, prior to this project, no publically-funded UK laboratory had the capacity to genetically modify wheat using *Agrobacterium*. In addition, previous work focussed on model varieties such as Bobwhite, and we believe this is the first evidence that commercial winter wheat varieties can be transformed by *Agrobacterium.*

Summary of Research Activities and Main Findings

Effect of Silwet L-77 on embryo survival, callus induction and DNA delivery

The surfactant Silwet L-77 has been shown to improve *Agrobacterium*-mediated transformation efficiency of floral-dip methods in *Arabidopsis* (Clough and Bent 1998), and was also used by Cheng *et al* (1997). We compared its phytotoxicity and effect on T-DNA delivery at concentrations ranging from 0 – 0.1%. Our data showed increasing phytotoxicity above 0.0025%, but improved T-DNA-delivery to embryos at higher concentrations. It was used in subsequent experiments at 0.01%.

Effect of selection pressure on regeneration after Agrobacterium co-cultivation.

After confirming that it was possible to obtain GUS positive plant tissues and organs in culture, we applied selection pressure and allowed tissue-culture material to progress to plants. The application of phosphinothricin (PPT) at 3-4 mg/l in the rooting medium allowed effective selection of transformed plants. This was augmented by GUS assays on leaf fragments from regenerating plantlets. Wheat variety Cadenza was more tolerant to PPT than Florida.

Consolidation of protocols and scale-up of explant numbers for Agrobacterium co-cultivation and regeneration.

The basic parameters necessary for effective T-DNA delivery and regeneration of fertile wheat plants via embryogenic callus have been established. More than 3500 wheat embryos have been inoculated with *Agrobacterium* suspension, co-cultivated, induced to form callus and cultured to recover plants. Approximately 1300 of these were sacrificed before they developed into adult plants. From the remaining, a total of 25 plants that survived selection and that showed GUS expression in their leaves, have been regenerated to date. Of these, eight have been transferred to soil and confirmed to contain transgenes by PCR. Southern analysis is currently underway.

Project title	Genetic transformation of wheat using Agrobacterium tumifaciens.	MAFF project code	AR **1002**

Scientific report (maximum 20 sides A4)

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