GENE TRANSFER INTO EMBRYOGENIC DOUBLED HAPLOID CELL LINES OF WHEAT

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Introduction

Within the last decade or so, numerous exciting breakthroughs in genetic engineering of the major cereal crops have been obtained. For wheat, the world's second most important crop in terms of calories produced per hectare, several techniques including particle discharge of foreign DNA directly into cells and tissues have been used successfully. Encouraged by these developments in transgenesis, our interest lies in the incorporation and stable expression of foreign genetic information into doubled haploid lines of wheat.

As a first step, we have developed a reliable system for obtaining high frecuency embryogenesis and green plantlet regeneration from another culture-derived suspension cell aggregates in these wheat lines (1). In the current study, we have extended these results by evaluating the efficiency of transgenesis using three plasmids on transient reporter gene expression in various inocula via (i) microprojectile bombardment and (ii) silicon carbide-fibre mediation.

Experimental Procedures

Plant material and culture conditions: All transformation experiments were conducted using:

1. Callus derived from pollen embryos.

2. Friable callus visually selected from fast growing embryogenetic segments.

3. Suspension culture-derived embriogenic cell aggregates of two doubled haploid lines of spring-type wheat cultivars.

Induction and maintenance of the various types of culture as well as evaluation of embryogenesis were undertaken as described previously (1).

Plasmid DNA: Three plasmids (pAHC 25, pDM 803 and pORCE Hyg) which carry the *uidA* and *bar* genes were purified using a QIAGEN plasmid Maxi kit (QIAGEN Inc., Chatworth, CA, USA).

Mode of DNA delivery: Two methods of DNA delivery - particle bombardment and silicon carbide fibre (2) meditation were evaluated on the different types of callus/cell aggregates using published protocols.

Gus analysis of transformed callus/cell aggregates: Histochemical analysis of β-glucuronidase (GUS) expression in microprojectile bombarded and silicon fibre treated inocula (*calli*) were performed according to Jefferson *et al.* (3). Transient gene expression was identified by counting the number of blue spots (*foci*) per cell colony/aggregate.

Selection of transformed tissue: Transformed tissues were selected using Basta, Bialaphos and hygromycin at concentrations found optimal in our preliminary studies.

Results

The following results were obtained from several replicated experiments.

1. Microprojectile bombardment mediation produced more blue spots per cell aggregate than silicon fibre treatment.

2. On the average 1,6 μm gold particle size produced more blue spots than 1,0 μm.

3. Friable embryogenic callus was the most suitable for both methods of DNA delivery. Moreover, it also produced a higher frequency of embryogenesis than either of the other tissues used for transformation.

4. More embryos were regenerated on the tissues transformed with silicon fibre than those with particle bombardment.

Conclusions

On the basis of the results above it could be concluded that although particle bombardment mediation is a more efficient method of DNA delivery into these tissues; however, it produces a higher degree of tissue disturbance (consequently lower embryogenesis) than seen with silicon fibre mediation.

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2. Kaeppler HF, W Gu, DA Somers, HW Rines and AF Cockburn. Plant Cell Rep. 1990; 9:415-418.

3. Jefferson RA. Plant Molec. Biolog. Rep. 1987; 5:397-405.