A modified protocol for laser- mediated gene transfer in wheat

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ABSTRACT

A modified laser-mediated setup for introducing exogenous DNA (pAB_6 plasmid with GUS and bar genes) into cells of embryogenic calli of the Egyptian wheat (Triticum aestivum L.) cv. Giza 164 was done. The new setup secures the transformation as high as 400,000 embryo-derived cells in less than 35 min using a homemade UV excimer laser with two dimensional translation stages, a suitable computer program and a proper optical device. Immature embryos of wheat were grown for six days on TW medium. Osmotic treatment was done by using mannitol (0.4 M) mixed with the exogenous DNA, in which laser treatment was immediately conducted. The calli were irradiated by a focused laser microbeam to puncture holes ~ 0.5 μm in the cell wall and membrane to allow uptake of the exogenous DNA. Three regenerated putative transgenic events were evaluated for the presence and expression of both genes and results indicated that this modified procedure of laser-mediated transformation can be successfully used in transforming wheat with a very high efficiency.

Keywords: *Laser microbeam, wheat transformation, immature embryo, exogenous DNA*

INTRODUCTION

Wheat (*Triticum aestivum* L.) is considered as the most important field crop worldwide. Conventional plant breeding in wheat has offered a great deal towards the improvement of flour quality and resistance to biotic and abiotic stresses (Potrykus, 1990). The accessibility of tissue culture to improve cereal characteristics through genetic engineering in wheat is limited due to the low rate of regeneration of transformed calli (Bahieldin *et al.*, 2000). Attempts to develop a novel plant transformation system to secure the least damage to transformed cells developed by other mechanical transformation devices are needed to allow for the recovery of transformed cells and the stable transgene integration and expression. Therefore, we report an effective, less damaging system for introducing exogenous DNA into cells of embryogenic wheat calli using laser microbeam cell surgery (Guo *et al.*, 1995).

MATERIALS AND METHODS

UV excimer laser system setup

A modified laser microbeam setup to that of Guo *et al.* (1995) was used in which a Lambda Physics Excimer Laser device (193 nm wavelength, 6 ns pulse duration, 13 mJ energy and repetition rate up to 200 Hz) was constructed. The mechanical system was developed with two Oriel steper motors in the X-Y directions to allow a lateral motion of 8-