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Shoot apical meristem injection: A novel and efficient method to obtain transformed cucumber plants

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ABSTRACT

The objective of this study was to develop a simple and efficient system for the genetic transformation of cucumber (*Cucumis sativus* L.). This study investigated the factors influencing the efficiency of gene transfer by microinjection of the shoot apical meristem (SAM) with *Agrobacterium* shooter strain. The SAM microinjected with *Agrobacterium* cells containing binary plasmid was incubated in co-cultivation medium without plant growth regulators (PGRs) for 2 days in the dark and subsequently transferred to solid bacterial elimination shoot regeneration medium containing MS salts plus 20 g l^{-1} glucose, 8 g l^{-1} agar and 300 mg l^{-1} cefotaxime. Developed transgenic shoots were screened in selection medium containing the antibiotic kanamycin (Km). The efficiency of gene transfer using various germination media and infection conditions was analyzed by root growth in the presence of Km. The present investigations show that the maximum efficiency of gene transfer was sixty-five percent by Km-resistant plants. In the putative transgenic plants the presence of the transgene was confirmed by polymerase chain reaction (PCR). This transformation system was reproducible, suggesting that it could be adopted for other plant species.

1. Introduction

Cucumber (*Cucumis sativus* L.) is one of the world's most important vegetable crops and has received much attention as a model plant for research on Cucurbitaceae. Phytopathogenic fungi, bacteria and viruses severely affect and reduce the yield of cucumber worldwide. Breeding systems for disease resistance is one of the most crucial objectives in cucumber cultivation. Conventional breeding of cucumber to improve disease resistance and other horticultural traits is limited by its narrow genetic base and severe incompatibility barriers to related species (Kho et al., 1980). Recombinant DNA technology provides a novel and powerful way to minimize the loss by improving yield and supplementing traditional plant breeding. The transgenic approach provides a plethora of opportunities to genetically manipulate plants across the species barrier thus making it possible to transfer any gene, be it of bacterial, animal or plant origin to the desired crop (Birch, 1997). These efficient genetic engineering tools could be applied to improve the cucumber crop.

Agrobacterium tumefaciens-mediated gene transfer is far from routine in many recalcitrant plants, including *C. sativus* (Rajagopalan and Perl-Treves, 2005; Nanasato et al., 2012). Utilization of this method for gene transfer requires both a susceptibility to infection by *A. tumefaciens* and an ability to regenerate plants from individual transformed cells via tissue culture (Fang and Grumet, 1990). To establish a successful strategy for practical plant genetic engineering, it is important to develop systems for recovering large numbers of whole plants from primary explants (Vasudevan et al., 2007). The lack of an efficient transformation and regeneration system often limits the use of gene transfer technologies in vegetable crops (Wang et al., 2012). Therefore, the development of a simple and effective approach for gene transfer to tissues amenable for regeneration is of major interest.

A shoot meristem-based transformation system uses a strategy to multiply transgenic shoot apical meristem cells and/or germline cells *in vitro*, which can be reprogrammed in the developmental direction. Transient and/or stable gene expression in cereals has been reported after delivery of DNA into cells via *Agrobacterium*-mediated transformation (Sticklen and Oraby, 2005; Baskaran and Dasgupta, 2012). The key steps of shoot apical meristems (SAM) tissue culture involve separation of the tissues surrounding the meristem followed by dissection to expose the meristem for gene transfer (Walden et al., 1989). Overall, the SAM is an optimal explant for genetic manipulation of crops because it is easily cultured *in vitro*, quickly regenerable, competent to genetic transformation, produces plants genetically identical to the parent, and can be sustained *in vitro* for long periods of time.

Recently, SAM has been used for quick regeneration, genetic transformation, clonal propagation and *in vitro* sustainability in cereals and

Abbreviations: AS, acetosyringone; GM, germination medium; IAA, indole-3-acetic acid; IBA, indole-3-butyric acid; IFM1, infection medium 1; IFM2, infection medium 2; Km, kanamycin; LM, liquid medium; MS, Murashige and Skoog medium; NAA, α-naphthaleneacetic acid; PCR, polymerase chain reaction; PGR, plant growth regulator; PPF, photosynthetic photon flux; SAM, shoot apical meristem; SM, solid medium.

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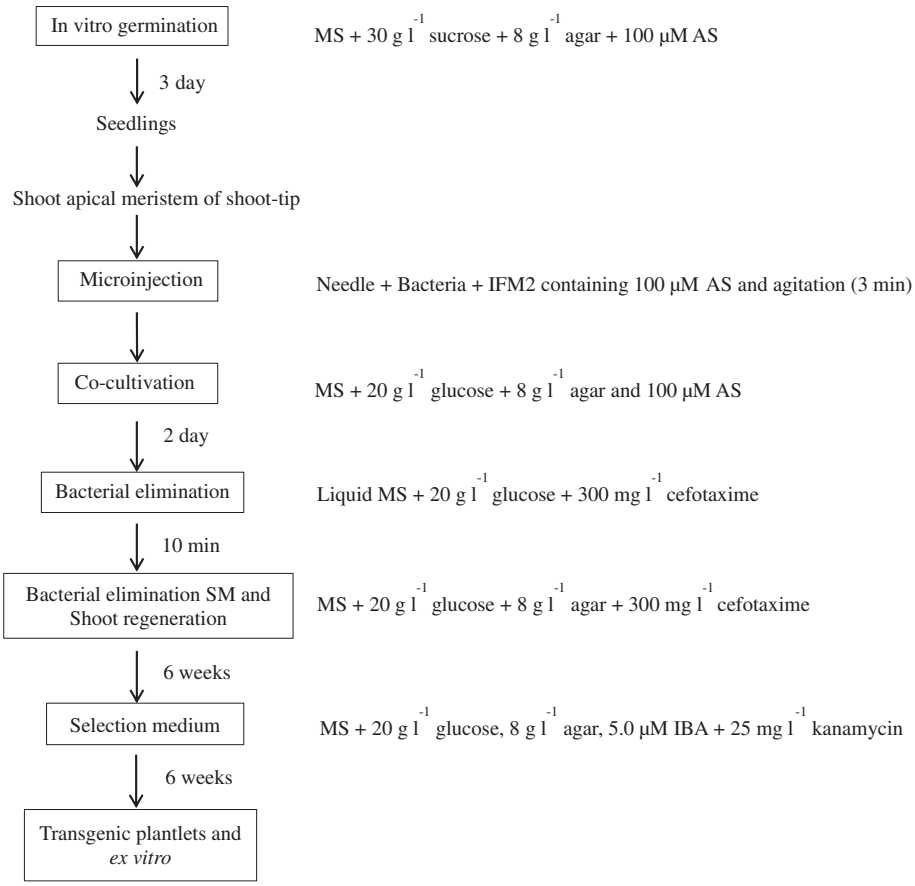

Fig. 1. Steps for transformation by microinjection protocols in *C. sativus* L. cv. Ashley.

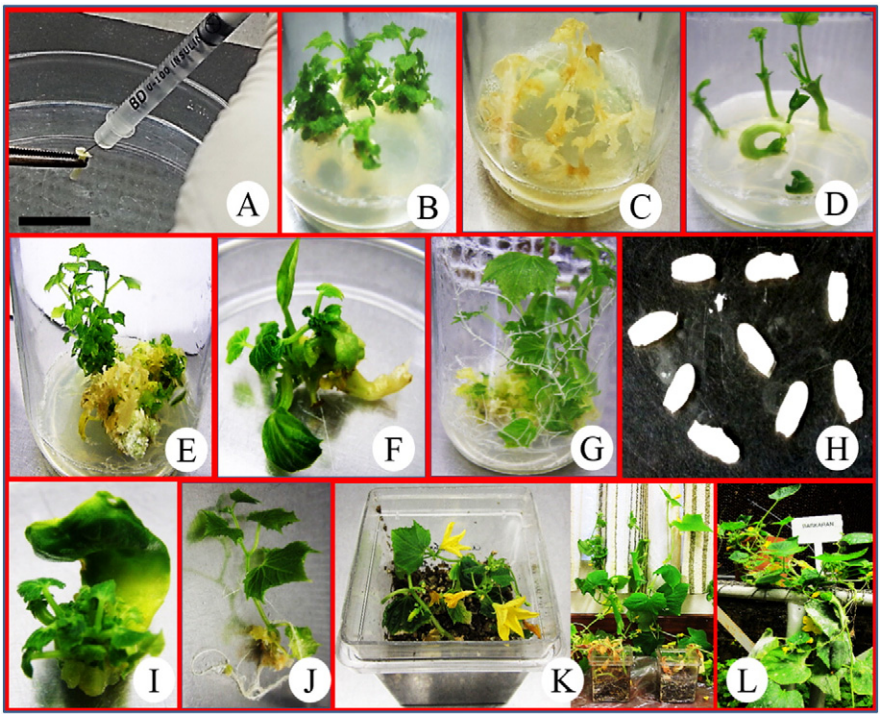

Fig. 2. Gene delivery and plant regeneration using microinjection and *Agrobacterium*-mediated transformation of *C. sativus* L. cv. Ashley: A Microinjection on shoot apical meristem (SAM) explant. B Shoot development after elimination of excess *Agrobacterium* growth during gene transfer by microinjection. C Necrosis of the shoots in 5.0 μM IBA and 25 mg l^{-1} kanamycin. D Single shoot induction from SAM after microinjection without bacteria. E Killing of the non-Km resistant shoots. F Development of shoots from SAM after bacterial microinjection. G Rooting of kanamycin-resistant shoots from SAM. H Cotyledon explants after bacterial infection. I Shoot regeneration from cotyledon explants after bacterial infection. J Rooting of kanamycin-resistant shoots from cotyledon. K The transgenic plants growing in a growth room and L in the greenhouse. Bar (A–J) = 5 mm.