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Transformation of Constituent Cells of Tomato Callus Aggregates by Intranuclear Microinjection

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One of the most prominent features of microinjection is that foreign macromolecules can be introduced directly into specific intracellular compartments of plant cells. Target sites for injection have been vacuole,[1] cytoplasm,[2] and nucleus[3] of plant protoplasts. In the previous paper,[4] the authors applied the technique to callus cells and established an intranuclear microinjection for transforming plant cells. Moreover, our previous works have presented the system for a cytoplasmic microinjection by which tobacco mosaic virus was introduced into single cells[5] or cell-aggregates[6,7] obtained from tomato callus tissues. These approaches enabled us to examine the mechanisms of virus multiplication or translocation and the expression of virus-disease resistance in the multi-cellular system of cultured plant cells. For further expanding the applicability of this technique to callus tissues, the present study describes the transformation of constituent cells of tomato callus aggregates by an intranuclear microinjection.

Friable callus tissues obtained from leaf-explants of tomato[8] (*Lycopersicon esculentum* cv. Fukuju No. 2) were used in the present experiment. Callus tissues were shake-cultured in liquid Murashige-Skoog[9] (MS) medium supplemented with 0. 1 μg/ml 2, 4-dichlorophenoxy acetic acid and 0. 05 μg/ml kinetin (pH 6. 5), and filtered with a stainless sieve (pore size, 250 μm diameter). Single and 2-to 10-cell-aggregates were involved in cell suspension which passed through a sieve. For microinjection, cell-aggregates were embedded in agar medium and cultured using the plate culture method previously described.[4] Transformation vector for higher plants, pBI 121[10] containing such two reporter genes as NPT II gene for kanamycin resistance and GUS gene for β-glucuronidase production was injected into the nuclei of constituent cells of the aggregate according to the conditions for microinjection previously reported.[4] After injection, cells were incubated at 26℃ for 2 days, subjected to 200 μg/ml kanamycin and vitally stained with fluorescein diacetate (FDA).[11] Alternatively, injected cells were incubated and fixed with formaldehyde for histochemical analysis of β-glucuronidase activity.[12]

In the culture method used in the present study, as described in the previous paper,[4] it was possible that injected cells of the aggregates were specified according to the grid lines scored onto the surface of a Petri dish. This method allowed us to critically distinguish between injected and non-injected cells of the aggregates and to analyze an expression of introduced genes in these cells. **Figure 1** shows light and fluorescent micrographs of pBI 121-injected cells which were stained with FDA. The data clearly indicated that only injected cells of the aggregate expressed a resistance against kanamycin. Moreover, **Fig. 2** shows an enzymatic activity of β-glucuronidase in injected cell of the aggregate. In the present study, each 500 aggregates was used for an assay of kanamycin resistance or β-glucuronidase production and about 10% of the injected cells showed kanamycin resistance or specific expression of the enzymatic activity. Thus, the present results show that specified target cells of callus cell-aggregates can be successfully transformed by the present technique.

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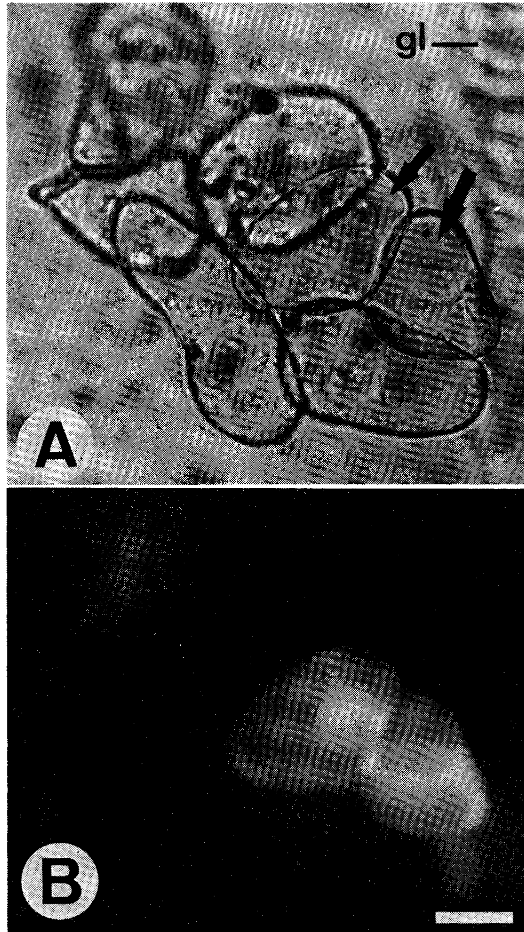

Fig. 1. Light (A) and fluorescent (B) micrographs of tomato callus cell-aggregate injected with pBI 121.

Two constituent cells (arrows) of the aggregate were injected with pBI 121, treated with 200 μg/ml kanamycin, and stained with FDA. Injected cells were specific from the location on the grid lines (gl) scored onto the surface of a Petri dish. Cells were injected with DNA solution (10 μg/ml) for 10 sec using a glass needle (tip diameter ; 0. 3 μm) under a constant pressure of 2. 5 kg/cm^2 by means of an Olympus Injectoscope.[4] Note the fluorescence appearance of FDA only in injected cells. Bar represents 20 μm.

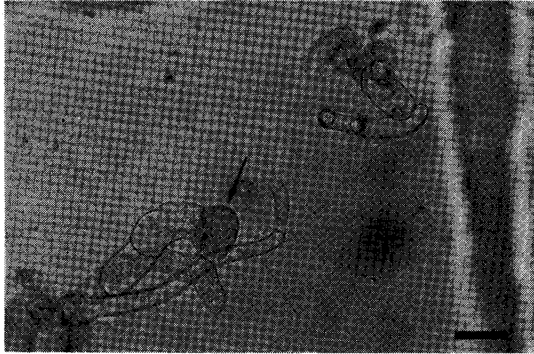

Fig. 2. Detection of β-glucuronidase activity in pBI 121-injected cell (arrow) of tomato callus aggregate.

Cell-aggregate was incubated for 2 days after injection, fixed with formaldehyde, and reacted with 5-bromo-4-chloro-3-indolyl glucuronide, a substrate for β-glucuronidase.[12] Bar represents 50 μm.

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≪和文要約≫

核へのマイクロインジェクションによるトマトカルス集塊構成細胞の形質転換

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トマト葉由来の friable カルスを使用し，カルス集塊を構成する細胞の形質転換を試みた．導入する外来遺伝子としては，植物形質転換ベクター，pBI 121 を使用し，マイクロインジェクション法で特定標的細胞の細胞核に注入した．形質転換細胞はカナマイシン添加培地で選抜するか，5-bromo-4-chloro-3 indolyl glucuronide を基質として，注入細胞における β-glucuronidase 活性を細胞化学的に検出した．その結果，約 10% の注入細胞がこれらの活性を示し，本法でカルス集塊の特定細胞が形質転換されることが明らかとなった．