PLANT GENE DELIVERY WITH A POLY(AMIDOAMINE) DENDRIMER

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Abstract

Plant gene delivery is considered far more challenging than mammalian gene delivery due to the thick plant cell wall. We have formed supramolecular complexes via an electrostatic interaction between poly(amidoamine) (PAMAM) dendrimer and green fluorescence protein (GFP)-encoding plasmids. The binding of the PAMAM-GFP gene complexes was confirmed by gel electrophoresis. Using confocal fluorescence microscopy we observed the penetration of the complexes through the cell walls of turf grass callus and the consequent GFP gene expression in the plant cells. Compared to conventional means such as viral vectors, biolistic particle bombardment, electroporation, or polyethylene glycol attachment, our current scheme utilizes the physiochemical properties especially the nanosize of the PAMAM dendrimer for direct and less invasive plant gene and drug delivery. The transfection efficiency may be much improved and optimized by changing the pH of the callus buffer and the ratio of positively charged primary (and tertiary) amines of the dendrimer to the negatively charged phosphates of the DNA.

Materials and Methods

Generation 4 dendrimers were purchased in aqueous solution from Dendritech. The primary amine groups of the G4-PAMAM molecules reacted with fluorophore TRITC (Anaspec, MW 443.52, λ_{ex} = 543 nm, λ_{em} = 571 nm) at a molar ratio of 1:1 and the labeled dendrimers were incubated at room temperature overnight. Unreacted TRITC molecules were filtered out from the solution using Amicon Centriplus YM-3 filters (Millipore, MWCO 3,000).

Callus cells of creeping bentgrass (*Agrostis stolonifera* L., cv. Penn-A-4) initiated from mature seeds were immersed in MOPS–minimal salts–tryptose medium with 0.8% glycerol (MMSTG medium, pH 7-8).

Plasmid DNA of 330 ng was incubated for 20 min at room temperature with unlabeled G4-PAMAM at an N/P (amino to phosphate) ratio of 0.025, 0.05, 0.1, 0.2, 0.5, 1, and 2, respectively. All samples were prepared in Tris-Cl buffer (pH 7.4) and mixed on a shaker. To confirm the binding of DNA to PAMAM, gel electrophoresis was performed on the samples (0.7% agarose, 1 hr, 80V) along with plasmid DNA and G4-PAMAM as two separate controls.

The plant cells after incubation were observed with an inverted confocal fluorescence microscope (Zeiss, LSM 510).

Figure 1

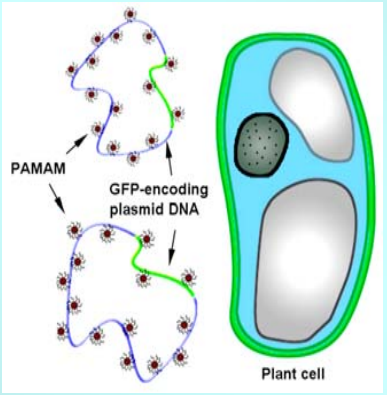

Figure 2

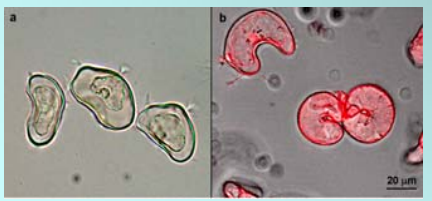

Figure 3

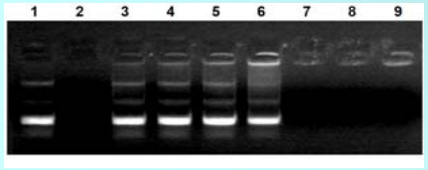

Figure 1. Experimental scheme. A plant expression vector, 35SC4PPDK-sGFP(S65T) containing a reporter gene encoding for GFP is bound with multiple G-4 PAMAM molecules. The length of the plasmid is 4230 bp (shown in blue) while the gene encoding for GFP is 739 bp (shown in green). The illustrated turfgrass cell has a cell wall (green), a nucleus (black), and two vacuoles (grey).

Figure 2. Confocal images of (a) turfgrass cells (bright field) and (b) turfgrass cells incubated overnight with TRITC-labeled PAMAM (fluorescence overlaid with bright field). The fluorescence of TRITC indicates significant uptake of the dendrimer by plant cells.

Figure 3. Gel electrophoresis on the binding of GFP-containing plasmid and PAMAM. Lanes 1 and 2 correspond to GFP-containing plasmid (230 ng) and G4-PAMAM (512 ng), respectively. Lanes 3-9 correspond to samples of an N/P ratio of 0.025, 0.05, 0.1, 0.2, 0.5, 1, and 2, respectively. At an N/P ratio of 0.5 and above, the binding of plasmid with PAMAM caused the significant trapping of the DNA in the top wells (lanes 7-9).

Summary

We have studied the feasibility of using poly(amidoamine) dendrimers as a new carrier for gene delivery into plants with intact cell walls. Our data demonstrated the formation of supramolecular complexes of poly(amidoamine) dendrimer-DNA via electrostatic interactions. These complexes were observed penetrating through the cell walls of turfgrass callus and expressing foreign genes within the cells. The use of the delivery system developed here with turfgrass cells as a target can be easily extended to virtually all plant species having successful regeneration systems in place. This demonstrated method for DNA delivery offers a number of advantages compared to currently existing protocols for gene transfer into plant cells, and could dramatically enhance the procedures for crop improvement using transgenic technology.

Figure 4

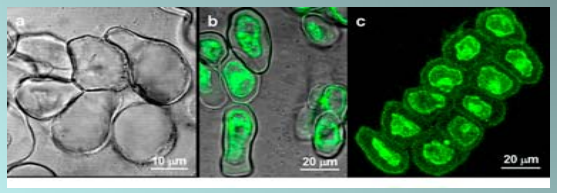

Figure 4. Confocal images of (a) turfgrass cells (control, bright field), (b) scattered turfgrass cells incubated with DNA-PAMAM at an N/P ratio of 0.2 for 3 days (fluorescence overlaid with bright field), and (c) grouped turfgrass cells incubated with DNA-PAMAM at an N/P ratio of 0.2 for 3 days (fluorescence). The green fluorescence in (b) and (c) indicates the expression of the GFP gene after their uptake by the plant cells.

Figure 5

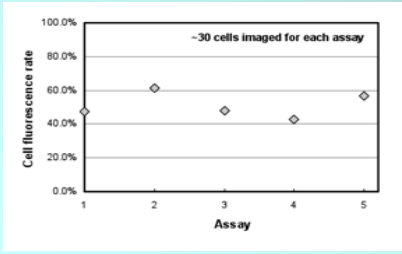

Figure 5. Rate of fluorescence in turfgrass cells incubated with DNA-PAMAM for 3 days. For the 5 independent assays performed, 48.5% of the randomly chosen cells displayed GPF expression. N/P ratio: 0.2.

Acknowledgement

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