Abstract

A system was developed for the isolation, culture and transformation of protoplasts derived from carrot (*Daucus carota* L.) roots (var. New Kuroda). Factors such as, protoplast stability, the effect of coconut water concentration on embryoid formation and the effect of PEG concentration on transformation efficiency were evaluated. Protoplasts isolated enzymatically from carrot roots developed into cell clusters by 10 days in liquid protoplast medium containing 1.0–5.0% (w/v) coconut water and naphthalene acetic acid (NAA). Cells of the resulting calluses differentiated into embryoids at a higher frequency (16%), on agar medium containing 3.0–5.0% (w/v) coconut water. Transient gene expression was observed in carrot root protoplasts, using PEG – mediated direct DNA transformation, with plasmid pCAMBIA 1303 containing the green fluorescent protein (*gfp*) reporter gene, driven by the constitutive 35S promoter from Cauliflower Mosaic Virus (CaMV). Transformation was confirmed by observing under UV light. DNA uptake into protoplasts under different PEG concentrations were evaluated, and a PEG concentration of 30.0% (w/v) proved to be considerably better (with a green fluorescent protein (GFP) expression average of over 30% of transformed protoplasts) than a PEG concentration of 20.0% (w/v) based on higher *gfp* gene expression frequency, indicating that root – derived protoplasts of carrot are suitable recipients for transformation.