Abstract

A high yield of isolated protoplast and reliable regeneration system are prerequisite for successful somatic hybridization and genome editing research. However, reproducible plant regeneration from protoplasts remains a bottleneck for many crops, including cassava. We evaluated several factors that influence isolation of viable protoplasts form leaf mesophyll, induction of embryogenic calli, and regeneration of plants in three cassava cultivars; Muchericheri, TMS60444 and Karibuni. A relatively higher protoplast yield was obtained with enzyme mixture containing 5 g/L Macerozyme and 10 g/L cellulase. Muchericheri recorded relatively higher protoplast yield of $20.50\pm0.50\times10^6$ whereas TMS60444 ($10.25\pm0.25\times10^6$) had the least protoplast yield in 10 g/L cellulase and 4 g/L cellulase. Freshly isolated protoplast cells were plated on callus induction medium (CIM) solid medium containing MS basal salt, 60 g/L D-glucose, 30 g/L sucrose, B5 vitamins, 100 mg/L myo-inositol, 0.5 mg/L copper sulphate, 100 mg/L casein hydrolysate, 4.55 g/L mannitol, 0.1 g/L MES, 10 mg/L picloram and 3 g/L gelrite to induce protoplast growth and development. The three cultivars reached colony formation but no further development was observed in this culture method. Protoplast growth and development was further evaluated in suspension culture using varying cell densities (1, 2 and 3×10^5 p/mL). Development with highest number of minicalli was observed in cell density of 3×10^5 p/mL. Minicalli obtained were cultured on CIM supplemented with 10mg/L picloram. Callus induction was observed in all cell densities with the cultivars. Highest somatic embryogenesis was observed in 2×10^5 p/ml while no somatic embryogenesis was observed in cell density of 1×10^5 p/mL. Somatic embryos were matured in EMM medium supplemented with 1 mg/L BAP, 0.02 mg/L NAA and 1.5 mg/L GA_3 then germinated in hormone free medium for plant regeneration. This protocol which used simple mixture of commercial enzymes is highly reproducible and can be applied in biotechnology research on cassava.