

Efficient protocol improved the yield and viability of oil palm protoplasts isolated from in vitro leaf and mesocarp

ABSTRACT

The absence of a cell wall on the protoplast contributes to its versatility. Its flexibility for DNA manipulation and the possibility of rapid cell-based assay is desirable in the plant biotechnology field. This study was carried out to improve protoplast isolation from oil palm in vitro leaf and mesocarp tissues. The factors affecting protoplast isolation efficiency were optimized, including the protocols and enzyme composition involved, focusing on the oil palm in vitro leaf first. Incubation of oil palm leaf sample with an enzyme mixture of cellulase R-10, macerozyme R-10, driselase, and pectolyase Y-23, for 14 h has successfully produced up to 2.5×10^6 protoplasts g⁻¹ fresh weight (FW)-1 with 95% viability. Incubation of oil palm mesocarp tissue with the optimized enzyme mixture for 2 h at static condition has also successfully produced 3.98×10^6 protoplasts g⁻¹ FW-1 with 85% viability. Besides, it was found that increasing the sample's surface area in contact with enzyme solution by slicing the samples into narrow strips and thin layers has improved the penetration of enzymes into the tissues and enhanced the isolation efficiency. In addition, a plasmolysis step before enzymatic treatment has also improved the protoplast viability by minimizing the damage incurred during isolation. The successful isolation of protoplast from oil palm leaf and mesocarp has enabled the study of gene function and the characterization of endogenous tissue-specific promoter being carried out in vivo.

Keyword: Protoplast isolation; Oil palm in vitro leaf; Oil palm mesocarp; Cellulase; Macerozyme; Driselase; Pectolyase; Ficoll; Viability