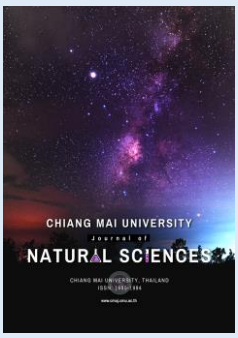

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Research article

Effect of Enzyme Treatments on Protoplast Isolation from Leaves of Vetiver (*Vetiveria spp.*)

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Abstract Protoplast isolation is a first and important step for establishing a new plant with desired traits through protoplast fusion technology. This experiments were conducted to evaluate various concentration of enzymes and incubation time on protoplast yield and viability in two vetiver ecotypes, Kamphaeng Phet 2 (*Vetiveria zizanioides* Nash) and Prachuap Khiri Khan (*V. nemoralis* A.Camus). The results revealed that protoplast yields were significantly affected by different enzyme treatments. The highest protoplast yield ($6.12x10^5$ protoplasts/ml) and high viability (98.61%) in Kamphaeng Phet 2 was obtained through the process of cell wall digestion when treated with enzyme solution containing 0.5% (w/v) cellulase onozuka R-10 and 0.5% (w/v) macerozyme R-10 in combination. While, the optimal enzyme solution for protoplast isolation from leaves of Prachuap Khiri Khan was the combination of 1.0% (w/v) cellulase onozuka R-10 and 0.4% (w/v) macerozyme R-10, resulting in the highest yield ($6.80x10^5$ protoplasts/ml) and viability (96.56%) of protoplasts. Meanwhile, incubation time of 24 h with the optimal enzyme solution resulted in the highest protoplast yields of both ecotypes. Our findings have the potential to generate an efficient protocol to isolate the protoplast from leaves of vetiver which can be used for further research studies in protoplast culture and fusion for vetiver improvement.

Keywords: Cellulase onozuka R-10, Macerozyme R-10, Protoplast isolation, Vetiver

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