Mitragyna speciosa: Hairy Root Culture for Triterpenoid Production and High Yield of Mitragynine by Regenerated Plants

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Hairy root cultures of *Mitragyna speciosa* were established by infection of *Agrobacterium rhizogenes* ATCC 15834 and maintained in McCown woody plant medium (WPM) supplemented with 0.5 mg/l naphthaleneacetic acid. The hairy roots were identified for the rooting genes loci of *rol*A and *rol*B by polymerase chain reaction. For studying the secondary metabolite production, the *n*-hexane extract of the hairy roots was prepared and the compounds were isolated by silica gel column chromatography, affording triterpenoids (ursolic acid and oleanolic acid) and phytosterols (β-sitosterol and stigmasterol). The shoots from the hairy root cultures were regenerated and differentiated to the plantlets. For micropropagation, shoot multiplication was successfully induced from the axillary buds of the regenerated plantlets in WPM supplemented with 0.1 mg/l thidiazuron. The mitragynine contents of 5-month-old regenerated plants and *in vitro* plantlets (germinated from seeds) were determined using the TLC-densitometric method. The regenerated plants contained (14.25 ± 0.25) mg/g dry wt mitragynine, whereas the *in vitro* plantlets contained (4.45 ± 0.09) mg/g dry wt.

Key words: Mitragyna speciosa, Ursolic Acid, Mitragynine, Hairy Root Culture