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High frequency plant regeneration and enhanced production of shikonin from hairy root cultures in *Arnebia hispidissima*

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Arnebia belonging to family *Boraginaceae*, a group of hispid herbs, is a source of shikonin, a naphthoquinone red pigment. A rapid and efficient protocol for micro propagation from such as shoot tip, nodal, leaf and internodal tissue has been developed. Highest plant regeneration was observed from shoot tip (93±0.00%) and nodal segment (60.0±0.13%) cultured on MS media supplemented with plant growth regulators (KIN 0.5mgl^{-1}, BAP 0.25mgl^{-1}, IAA 0.1mgl^{-1}) and CH (100mgl^{-1}). Proliferating shoots were efficiently rooted on MS medium supplemented with 2.0mgl^{-1} IBA upon transfer to soil. Highest response for induction of callus for shikonin production of 80.0% from nodal segments was observed on media combination MS+mgl^{-1} BAP 0.25^{+1}.0mgl^{-1} IAA. UV-visible spectroscopic analysis of fresh callus showed induction and accumulation of 0.50mgg^{-1} of shikonin content at the end of the 50 days of culture. Additionally, a method for *Agrobacterium rhizogenes*-mediated genetic transformation of *Arnebia hispidissima* for hairy root cultures was also optimized for enhancing the shikonin production to meet its ever-increasing demand in pharmaceutical industry. Among the various tissues employed, leaf explants showed maximum (70.7%) response followed by shoot tips (52%), nodal segments (38.7%) and internodal segments (9.3%). The presence of Ri plasmid rolB gene in the transformed hairy root cultures was confirmed by PCR analysis using forward (*FrolB*) and reverse (*Rrol*B) primers of *rolB* gene resulting in the amplification of ~0.8kb fragments. Medium composition has been optimized for *in vitro* induction of shikonin in hairy root cultures of *Arnebia hispidissima*. The shikonin content in transformed hairy root was estimated spectrophotometrically using authentic sample of shikonin. The total content of shikonin was 0.85mgg-1 fresh weight of tissue at the end of day 50 of the culture period. These results will help to design strategies for bridging the gap between ever increasing demand and supply of raw products necessary for obtaining shikonin for cosmetic, dyeing, food, medicinal, and pharmaceutical industries.

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