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Enhanced Bioproduction of Moscatilin in Dendrobium ovatum through Hairy Root Culture

Authors : Ipsita Pujari, Abitha Thomas, Vidhu S. Babu, K. Satyamoorthy

Abstract : Orchids are esteemed as celebrities in cut flower industry globally, due to their long-lasting fragrance and freshness. Apart from splendor, the unique metabolites endowed with pharmaceutical potency have made them one of the most hunted in plant kingdom. This had led to their trafficking, resulting in habitat loss, subsequently making them occupiers of IUCN red list as RET species. Many of the orchids especially wild varieties still remain undiscovered. In view to protect and conserve the wild germplasm, researchers have been inventing novel micropropagation protocols; thereby conserving Orchids. India is overflowing with exclusive wild cultivars of Orchids, whose pharmaceutical properties remain untapped and are not marketed owing to relatively small flowers. However, their germplasm is quite pertinent to be preserved for making unusual hybrids. Dendrobium genus is the second largest among Orchids exists in India and has highest demand attributable to enduring cut flowers and significant therapeutic uses in traditional medicinal system. Though the genus is quite endemic in Western Ghat regions of the country, many species are still anonymous with their unknown curative properties. A standard breeding cycle in Orchids usually takes five to seven years (Dendrobium hybrids taking a long juvenile phase of two to five years reaching maturity and flowering stage) and this extensive life cycle has always hindered the development of Dendrobium breeding. Dendrobium is reported with essential therapeutic plant bio-chemicals and 'Moscatilin' is one, found exclusive to this famous Dendrobium genus. Moscatilin is reported to have anti-mutagenic and anti-cancer properties, whose positive action has very recently been demonstrated against a range of cancers. Our preliminary study here established a simple and economic small-scale propagation protocol of Dendrobium ovatum describing in vitro production of Moscatilin. Subsequently for enhancing the content of Moscatilin, an efficient experimental related to the organization of transgenic (hairy) D. ovatum root cultures through infection of Agrobacterium rhizogenes 2364 strain on MS basal medium is being reported in the present study. Hairy roots generated on almost half of the explants used (spherules, in vitro plantlets and calli) maintained through suspension cultures, after 8 weeks of co-cultivation with Agrobacterium rhizogenes. GFP assay performed with isolated hairy roots has confirmed the integrative transformation which was further positively confirmed by PCR using rolB gene specific primers. Reverse phase-high performance liquid chromatography and mass spectrometry techniques were used for quantification and accurate identification of Moscatilin respectively from transgenic systems. A noticeable ~3 fold increase in contents were observed in transformed D. ovatum root cultures as compared to the simple in vitro culture, callus culture and callus regeneration plantlets. Role of elicitors e.g., Methyl jasmonate, Salicylic acid, Yeast extract and Chitosan were tested for elevating the Moscatilin content to obtain a comprehensive optimized protocol facilitating the in vitro production of valuable Moscatilin with larger yield. This study would provide evidence towards the in vitro assembly of Moscatilin within a short time-period through not a so-expensive technology for the first time. It also serves as an appropriate basis for bioreactor scale-up resulting in commercial bioproduction of Moscatilin.

Keywords : bioproduction, Dendrobium ovatum, hairy root culture, moscatilin
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