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(57) Abstract :

The present invention discloses a method of enhancing rebaudioside-A concentration in liquid in vitro cultures of Stevia rebaudiana (Bert.) Bertoni using OH-functionalised multi-walled carbon nanotubes (OH-MWCNT). The method comprises collecting nodal segments (nodal explants or explants) of S. rebaudiana for in vitro propagation of S. rebaudiana (1), washing the explants under running tap water followed by surface sterilization of washed explants with 0.1% HgCl₂ (2), culturing the sterilized explants on shoot proliferation medium (SPM) containing 1X MS (Murashige and Skoog) medium, 2.5 mgL⁻¹ IAA (Indole Acetic Acid), 2.5 mgL⁻¹ BAP (Benzyl Amino Purine), and 10X CuSO₄.5H₂O (Copper Sulphate Pentahydrate) (3), maintaining the explant culture in a plant growth chamber at 25 ± 2°C, 85% relative humidity, 16h photoperiod, and 25µmol m⁻² s⁻² light intensity (4), preparing OH-MWCNT suspensions (0, 5, 10, 25, 50, 75, 100 and 125 ppm) in sterile deionized water using a probe sonicator and adding to the nodal explant culture in liquid SPM, with SPM without OH-MWCNTs serving as the control to obtain OH-MWCNT-treated nodal explant cultures (5), and maintaining the OH-MWCNT-treated nodal explant cultures in liquid shoot proliferation medium (SPM) for 2 weeks on a rotary shaker at 100 rpm in a growth chamber under controlled conditions to obtain plantlet (6,7). The method enhances the reb-A content, photosynthetic activity and regeneration frequency of the explants at specific concentration of OH-MWCNTs.

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