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(71)Name of Applicant :

**1)Prof. Sumita Kachhwaha**

Address of Applicant :Department of Botany, University of Rajasthan, JLN Marg, Jaipur 302004, Rajasthan, India Jaipur -----

**2)Shilpa Sharma**

**3)Prof. Shanker Lal Kothari**

**4)Dr. Rohit Jain**

Name of Applicant : NA

Address of Applicant : NA

(72)Name of Inventor :

**1)Prof. Sumita Kachhwaha**

Address of Applicant :Department of Botany, University of Rajasthan, JLN Marg, Jaipur 302004, Rajasthan, India Jaipur -----

**2)Shilpa Sharma**

Address of Applicant :Department of Botany, University of Rajasthan, JLN Marg, Jaipur 302004, Rajasthan, India Jaipur -----

**3)Prof. Shanker Lal Kothari**

Address of Applicant :Amity Institute of Biotechnology, Amity University Rajasthan, Jaipur, Rajasthan, India Jaipur -----

**4)Dr. Rohit Jain**

Address of Applicant :Department of Biosciences, Manipal University Jaipur, Jaipur, Rajasthan, India Jaipur -----

(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% HgCl<sub>2</sub> (2), culturing the sterilized explants on shoot proliferation medium (SPM) containing 1X MS (Murashige and Skoog) medium, 2.5 mgL<sup>-1</sup> IAA (Indole Acetic Acid), 2.5 mgL<sup>-1</sup> BAP (Benzyl Amino Purine), and 10X CuSO<sub>4</sub>.5H<sub>2</sub>O (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<sup>-2</sup> s<sup>-2</sup> 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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