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Optimized micropropagation protocol to establish high-yielding true-to-type plantations of elite genotypes of *Tinospora cordifolia* for consistent production of therapeutic compounds

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Abstract

***Tinospora cordifolia* (family Menispermaceae) is an ancient medicinal plant and is commonly known as amrita, giloy, and guduchi. This woody liana is a large, deciduous, climbing shrub with heart-shaped, membranous, cordate leaves. The ayurvedic charisma of the plant is by virtue of its succulent stem and aerial roots. It is widely distributed in the Asian and African subcontinents and grows to an altitude of 300 m. The plant is characterized by being a therapeutic amalgamation of secondary metabolites including alkaloids, terpenoids, glycosides, steroids, and other classes of secondary products. Therefore, it is known as a natural immune-modulator against jaundice, skin diseases, constipation, tuberculosis, leprosy, cancer, malaria, dengue, and diabetes. Tremendous usage of this plant has made it a threatened species and a need for its conservation is focused on plant tissue culture technology, allowing micropropagation of the plant throughout the year and providing elementary material for pharmaceutical research.**

The present investigation presents a successful method for large-scale clonal propagation of the plant using nodal segment explants taken from field-grown parent plants and initiated on MS medium (Murashige and Skoog, 1962) supplemented with 6-benzylaminopurine (BAP). An average of 8-fold shoot multiplication of *T. cordifolia* was obtained within 5 to 6 weeks of culture for those explants exhibiting multiple shoot proliferation. The in vitro leaves from established elite clonal lines were utilized to generate high-yielding callus cultures of *T. cordifolia* for enhanced production of protoberberine alkaloids. In vitro callus cultures were obtained on MS medium supplemented with 6-benzylaminopurine (BAP) and 1-naphthaleneacetic acid (NAA) at varied concentrations. The identification and purification of alkaloids was performed via thin layer chromatography, high performance liquid chromatography, and mass spectrometry from in vitro-raised cell cultures of *T. cordifolia*. Therefore, present research highlights the suitable strategies for conserving the parental characters of the plant and superior production of medicinally important alkaloids, devoid of any seasonal and regional variations, in minimal space and time.

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