

# PLANT GENOMICS AND PLANT SCIENCE

November 23-24, 2018 | Bangkok, Thailand

Manju Anand, J Agric Sci Bot 2018, Volume 2 | DOI: 10.4066/2591-7897-C2-005

## IN VITRO MASS CLONING OF STEVIA REBAUDIANA AND EXTRACTION AND QUANTIFICATION OF STEVIOSIDE BY HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

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*Stevia rebaudiana* Bertoni (family Asteraceae) popularly known as “Sweet leaf” is an important medicinal plant used for obesity, heart disease, dental caries, as contraceptive and anticancerous agent. The leaves of *Stevia* are the source of diterpene glycosides and among these stevioside is a high intensity, non-caloric, high potency sweetener being 300 times sweeter than sucrose. An efficient and reproducible *in vitro* protocol was established for the mass cloning of this valuable plant followed by extraction and purification of stevioside--the major secondary metabolite from micropropagated plants and cell cultures using High performance thin layer chromatography (HPTLC). It exhibited a high propensity of *de novo* adventitious shoot formation both directly from the leaf explants and indirectly through leaf callus on variously augmented Murashige and Skoog's medium. Individual shoots were rooted on half strength basal MS medium and plantlets were acclimatized and successfully established in the field. Extraction of stevioside from leaves of micropropagated plants collected at different time intervals (3, 4, 5, 18, 30 months), callus and suspension cultures were achieved following solvent extraction with petroleum ether, methanol, diethyl ether and butanol. The crude extract was initially purified on glass TLC followed by its fine purification on pre-coated silica gel 60 F254 plates by using High performance thin layer chromatography scanned at 210 nm. The highest amount of stevioside was obtained from thirty months old plants which yielded 94.9 µg/ml of stevioside followed by 69.40 µg/ml and 44.37 µg/ml in suspension cultures harvested at stationary phase and callus respectively.

## BIOGRAPHY

Manju Anand has completed her PhD from Panjab University, Chandigarh, India. Presently she is a Professor in Amity Institute of Biotechnology, Amity Institute of Biotechnology, Haryana, India. A professional in Plant Biotechnology specializing in Plant Tissue Culture, she has rich experience of working on the micropropagation of some economically important hardwood and softwood trees and edible bamboos and ascertaining their clonal fidelity through different molecular markers. Presently she is working on the mass propagation of some valuable and endangered medicinal plants and extraction of secondary metabolites from *in vitro* cultures and their evaluation as therapeutic agents. She has nearly 40 publications in peer reviewed journals that have been cited over 192 times and her publication H-index is 9.

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