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Title: Evaluation of phytochemicals in Agrobacterium rhizogenes transformed hairy roots of Phlogacanthus thyrsoiflorus Nees and Stevia rebaudiana L. Bertoni

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**Abstract:** The present work has been conceived to establish root to root culture of *Phlogacanthus thyrsoiflorus* and *S. rebaudiana* in solid and liquid MS medium with and without auxin and phytochemical evaluation of the hairy roots of *Stevia rebaudiana* L. Bertoni. The work focused on evaluation of phytochemicals in *Agrobacterium rhizogenes* (ATCC 15834 and MTCC 532) transformed hairy roots of *Stevia rebaudiana* L. Bertoni. To establish root to root culture from transformed roots of *Phlogacanthus thyrsoiflorus* and *Stevia rebaudiana*, roots were inoculated onto the MS solid medium with and without auxins for further proliferation. Hairy roots provide an efficient way of biomass production due to fast growth and display high biosynthetic capabilities. Roots were cultured on MS solid basal and supplemented with different concentration of IAA, IBA and NAA. After three weeks well established roots was found to be best on MS solid without hormone medium. One mg/l IAA and 1.0 mg/l IBA found effective accelerating growth of transformed roots. After three weeks well established roots from MS solid medium, were subcultured in MS liquid, and incubated at 100 rpm at 28°C under dark condition. The developed root culture exhibited fast growth and high lateral branching on growth regulator free MS liquid medium. Complete dark culture was more effective for the production of roots than light illumination culture. The present study was designed to identify secondary metabolites in root extracts induced by *A. rhizogenes* strain 532 and 15834 of *Stevia rebaudiana* through standard compound Stevioside, Steviol and Rebaudioside-A by High Performance Thin Layer Chromatography. HPTLC was performed with silica gel 60F254 plates with solvent system ethyl acetate:methanol:water (75:15:10 v/v/v) as mobile phase. Detection of compound Stevioside, Steviol and Rebaudioside-A was performed by scanning the developed plate at 254 nm and 366 nm. The results indicated that at 254 nm Peak of in vivo root extract and peak of transformed root (532) was found similar to peak of Stevioside and Steviol with Rf value 0.38. Peak of transformed root (15834) was found to match with peak of Rebaudioside A with Rf value 0.79 and 0.75 respectively. At 366 nm, peak of in vivo root extract and transformed root extract (532) was found similar to Stevioside with Rf value 0.09. While 0.08 for peak of transformed root extract (15834), was found similar to Steviol and Rebaudioside A with Rf value 0.08 and Peak of Rebaudioside A with 0.75 Rf almost matched with peak of transformed root (532) and in vivo root having 0.81 Rf.

**Description:** Evaluation of phytochemicals in *Agrobacterium rhizogenes* transformed hairy roots of *Phlogacanthus thyrsoiflorus* Nees and *Stevia rebaudiana* L. Bertoni

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