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Abstract: Hairy root is a plant disease caused by *Agrobacterium rhizogenes* Conn, a gram-negative soil bacterium. Hairy root cultures have several properties that have promoted their use for plant biotechnological applications. Their fast growth and genetic and biosynthetic stability offer an additional advantage for their use as an alternative to plant cell suspension cultures, for production of secondary metabolites of interest. *Stevia rebaudiana* L. Bertoni is a herbaceous perennial plant of the Asteraceae family. It is an important plant known for its zero-calorie diterpene glycosides (stevioside and rebaudiosides), a non-nutritive, high-potency sweetener, and substitute to sucrose. The leaf extract of this plant has been used traditionally in the treatment of diabetes. In the present research work, an efficient in vitro method for hairy root induction on shoot tips, nodes and leaves of *Stevia rebaudiana* L. Bertoni with *A. rhizogenes* strain ATCC 15834 was developed. To get aseptic explants for in vitro induction of hairy roots, micropropagation was carried out. Contamination free culture was established by treating shoot tip explants with 0.05% HgCl₂ for 15 minutes. Shoot multiplication was induced on MS media supplemented with BAP 1.0 mg/l, AdSO₄ 25.0 mg/l and citric acid 1.0 mg/l. Bacterial growth on semisolid YEB medium was achieved after 48h incubation at 28 o C in dark. The optimum growth of *A. rhizogenes* (OD 0.6) was observed after 48h of incubation at 28 o C in rotary shaker in dark on YEB broth supplemented with 50.0 mg/l rifampicin. For inducing hairy root, different period of co-culture were tested. Explants were inoculated into MS supplemented with and without 250.0 mg/l cefotaxime sodium salt. Hairy root emergence was observed after 8 days of infection on all explants. Among different periods of co-culture, 48 h was found to be most effective for root induction in shoot tips and nodal explants, while it was 24h for the leaves. Among different explants, nodes showed best induction percentage (95.83%) while leaves showed maximum numbers of hairy root per explant (32.50). Of the two media tested, MS without cefotaxime was found effective for better root growth while MS supplemented with cefotaxime results in reduction in induction percentage. The level of carbohydrate in hairy roots were quantified and compared with leaves from mother plant as well as leaves from the plant parts treated with *A. rhizogenes*. The individual carbohydrate content of different plant extract was analyzed in spectrophotometer and expressed in percentage (w/w). The concentration of carbohydrate was estimated 11.5 mg percent and 12.8 mg percent in the leaves from mother plant and from plant parts induced hairy roots respectively (Table:4.9; Graph:4.9). The highest carbohydrate content i.e. 14.8 percent was observed in the induced hairy roots.

Description: *Agrobacterium* mediated transformation in *Stevia rebaudiana* L. Bertoni for quality parameters

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