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Title: Influence of different strains of Agrobacterium rhizogenes on induction of hairy roots in Eclipta alba (L.) Hassk.

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Abstract: The present work has been carried out with a view to establish the protocol for *Agrobacterium rhizogenes* mediated hairy root induction on *Eclipta alba* (L.) Hassk, an important medicinal plant, using three different strains of *Agrobacterium rhizogenes* MTCC 2364, MTCC 532 and MTCC 15834. Aseptic explants for transformation were obtained by inoculating shoot tips of *E.alba* on MS media supplemented with BAP- 2.0 mg/l, AdSO₄ - 50mg/l and Citric acid- 1.0 mg/l. All three strains were evaluated for their transformation efficiency in shoot tips, nodes and leaves of *E.alba*. MTCC 2364 was maintained at 28°C for 48 hours in YEP medium. MTCC 532 strain was grown in YEN medium at 25°C for 48 hours, whereas ATCC 15834 strain was cultured in YEB medium for 48 hours at 28°C. Acclimatization of bacterial culture in MS medium for 12 hours was found effective for transformation. The explants were treated for different co-culture periods. The percentage of hairy root induction varied with infection period. The strain MTCC 2364 exhibited the highest transformation frequency with 87.5± 0.77% in shoot tips, 83.33 ± 0.66 % in nodes and 45.83 ± 0.21% in leaves. The highest number of hairy roots, 10.25 ± 0.43 in shoot tips, 8.70 ± 0.27 in nodes and 3.1 ± 0.19 was found at 225 minutes of co-culture period after 24 days at co-culture period of 225 mins. In case of ATCC 15834 strain highest transformation efficiency of 83.33 ± 0.64 % in shoot tips, 75 ± 0.53 % in nodes and 33.33 ± 0.21 % in leaves was achieved at 180 minutes of co-culture period with the highest number of hairy roots, 8.95± 0.13 in shoot tips, 7.41 ± 0.31 in nodes and 2.625 ± 0.11 in leaves after 24 days. For MTCC 532 strain, the highest transformation frequency of 79.16 ± 0.61% in shoot tips, 70.83 ± 0.62% in nodes and 25 ± 0.16% in leaves at co culture period of 225 minutes with highest number of hairy roots, 7.47 ± 0.26 in shoot tips, 6.75 ± 0.22 in nodes and 1.94 ± 0.11 in leaves. MTCC 2364 was found to be most effective for transformation frequency as well as number of hairy roots/ explant. Root to root cultures derived from hairy roots of shoot tips infected with each strain were established in MS basal liquid medium. The highest dry weight of hairy roots obtained after 30 days of culture were 746.25 ± 0.63 mg in MTCC 2364, 709.89 ± 0.65 mg in ATCC 15834 and 697.52 ± 0.44 mg for MTCC 532. Regeneration of entire plant from root cultures of each strain was also achieved. Molecular characterisation of hairy roots was done through PCR using rolC gene specific primer. Presence of rolC gene was confirmed in the transformed explants of MTCC 532 at 540 bp. Amplification of rolC gene at 540 bp was not found in transformants of MTCC 2364 and ATCC 15834. Phytochemical analysis of induced hairy roots from shoot tips through 3 different strains and in vitro grown leaves of *E.alba* was done through GC-MS. Four different phytochemicals, Trimethyl -2-pentadecanone, Decanoic acid 10, methyl ester, Pentadecane and naphthalene were identified based on similar retention time and molecular weight, enlisted in NIST 0.5 L.

Description: Influence of different strains of *Agrobacterium rhizogenes* on induction of hairy roots in *Eclipta alba* (L.) Hassk.

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