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Agrobacterium rhizogenes mediated genetic transformation of *Spilanthes paniculata* (DC.) Jansen

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SUMMARY

An efficient method for the production of hairy roots of *Spilanthes paniculata* (DC.) Jansen through *Agrobacterium rhizogenes* infection was developed. Surface-sterilized cotyledons and hypocotyl segments were inoculated and co-cultured with *A. rhizogenes* strains MTCC 2364 and MTCC 532. In case of *A. rhizogenes* MTCC 532, the best frequency explant infection perc ent for hypocotyl and cotyledon explants were 75±2.88% and 76.66±4.40% at 10^8 cells/ml and 10^7 cells/ml, respectively. The values for *A. rhizogenes* MTCC 2364 were 78.33±4.40% and 76.66±4.40% at 10^8 cells/ml and 10^7 cells/ml, respectively. Per cent frequency explants infection with *A. rhizogenes* MTCC 532 was studied with respect to age of explants, it was highest (85±2.88%) after 2 days. Rooting performance of *A. rhizogenes* transformed explants improved in presence of IAA.

Key words : *Agrobacterium rhizogenes*, IAA, Hairy roots, T-DNA, Ri Plasmid.

Spilanthes paniculata (DC.) Jansen plants are generally called as Toothache plants. Toothache plants are annual herbs or short-lived perennials, approximately half meter tall with prostrate or ascending branched cylindrical hairy stems and simple ovate opposite leaves with stipules (Nakatani and Nagashima, 1992). They belong to family Asteraceae, the tribe Helianthae, and the sub-tribe Ecliptinae. Phytochemically, flowers of *Spilanthes acmella* are reported to contain amino acids (Mondal *et al*., 1998;Peiris *et al.*, 2001), alkaloids (Peiris *et al*., 2001) and *N*-isobutylamides (including spilanthol; undeca–2*E*,7*Z*,9*E*–trienoic acid isobutylamide; undeca–2*E*–en-8,10-diyonic acid isobutylomide; 2*E*–*N*-(2-methylbutyl) –2–undecene–8,10–diynamide; 2*E*,7*Z*–*N*-isobutyl–2,7-tridecadiene–10,12–diynamide and 7*Z*-*N*-isobutyl-7-tridecene-10,12-diynamide) (Nakatani and Nagashima, 1992; Ramsewak *et al*.,1999).

The larvicidal potential of *Spilanthes acmella* against *Culex quinquefasciatus* has been shown by Pitasawat *et al*. (1998). *S. mauritiana* contains alkaloids that were found to be able to control *Aedes aegypti* in Kenya (Jondiko, 1986). Ratansooriya *et al.*, 2004 reported about strong diuretic activity and renoprotective effect of cold water extract (CWE) of *Spilanthes acmella* flowers. Hexanic extracts of *Spilanthes acmella* plants is reported to induce full tonic-clonic convulsions in rats (Moreica *et al.*, 1989).

Tumour inducing plasmid (Ti) of *Agrobacterium tumefaciens* causes crown gall tumour whereas root inducing plasmid (Ri) of *A. rhizogenes* causes production of hairy roots on susceptible dicotyledonous plants (De Cleene and De Ley, 1981). *A. rhizogenes*, the causative agent of hairy root syndrome is a common soil microorganism, capable of entering a plant through a wound and causing a proliferation of secondary roots (McAfee *et al*., 1993; Heijden *et al.*, 1994). This bacterium transfers its transfer-DNA (T-DNA) which is a portion of the large plasmid called the root-inducing plasmid (pRi) to susceptible plant cells where the T-DNA, if integrated into the nuclear genome of the plant cell, will encode genes that direct the synthesis of auxin (indole-3-acetic acid) and/ or increase the sensitivity of the transformed plant cells to auxin (McAfee *et al*., 1993; Hatta *et al*., 1996). The endogenous production of auxin and / or an increase in auxin sensitivity can lead to the formation of hairy roots at the site of infection (White *et al*.,1985). Root induction is due to stable integration of the Ri T-DNA (transferred DNA) into the plant genome and its subsequent expression (Tepfer, 1984).

Ri transformed roots can be cultivated *in vitro* on hormone free media and in some species regenerated into plants. The symptoms observed with *A. rhizogenes* are suggestive of auxin effects resulting from an increase in cellular auxin sensitivity rather than auxin production (Heijden *et al*., 1994). Hairy root culture has been reported from a wide host range of plants. *Agrobacterium rhizogenes* has been used to generate hairy roots in dicotyledonous plants (De Cleene and De Ley, 1981; Strobel and Nachmias, 1988), monocotyledonous (Porter, 1991) and polycotyledonous plants (McAfee *et al.*, 1993). This paper reports for the first time the production of hairy roots of *Spilanthes paniculata* (DC.) Jansen.

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