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Direct Organogenesis in *Geophila reniformis* D. Don., an Important Medicinal Herb

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Abstract

Adventitious multiple shoots were developed from leaf, petiole and internode explants of *Geophila reniformis* D. Don. on MS medium supplemented with various concentrations of N^6-benzylaminopurine (BAP) or Kinetin (KIN) alone or in combination with indole-3-acetic acid (IAA). Leaf showed maximum organogenetic potential, followed by petiole and internode. Murashige and Skoog (MS) medium supplemented with 22.22 μM BAP and 4.57 μM IAA induced maximum shoot buds from leaf explants. Internodal segments showed low potential of direct organogenesis. The regenerated shoots rooted the best in presence of 10.75 - 13.44 μM α-naphthalene acetic acid (NAA) along with 2.22 μM BAP, and were successfully established in the field with a survival rate of 89.11%.

Key words: Direct organogenesis, *Geophila reniformis*, Rubiaceae

Introduction

Geophila reniformis D. Don. (Rubiaceae), a small creeping perennial prostrate pubescent herb with long stems and rooting at nodes, is found in India, S. China, Tropical Africa and Malay Archipelago (Hooker 1882). The herb possesses properties somewhat similar to those of *Cephalis ipecacuanha* and is given for curing diarrhoea and poulticing sore legs (Nadkarni 1954). This medicinal plant also promotes or improves voice and semen; cures oedema, leprosy, piles, fever, inflammatory swellings; and enlargement of spleen (Sivarajan and Balachandran 1994). When boiled with whey of milk it stops diarrhoea and when cooked in oil it removes ophthalmic diseases and inflammations of the eye (Manilal 2003). This is also one of the species selected for development of phytomedicine for liver diseases, because of its ability to protect liver against toxic chemical induced liver damage (Subramoniam and Pushpangadan 1999). Owing to the medicinal importance, *in vitro* conservation methods and multiplication are vital. In this communication, we describe a protocol of direct regeneration from leaf blade, internode and petiole explants.

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Materials and Methods

Stock plants of *Geophila reniformis* were procured from the Regional Research Institute of Ayurveda, Thiruvananthapuram, Kerala, India and were established in the green house of the Department of Botany, University of Kerala. The leaf, internode and petiole segments, excised from the stock plant, which served as the experimental material for the present study, were thoroughly washed with running tap water for two h. They were treated for 12 min in a 10% (v/v) solution of Labolene, a neutral detergent and further rinsed with double distilled water several times to remove the traces of detergent. They were sterilized in 0.1% (w/v) solution of mercuric chloride for 8 min and washed three times with sterile double distilled water. Murashige and Skoog medium (Murashige and Skoog 1962) containing 3% sucrose as the carbon source and various concentrations and combinations of cytokinins

- BAP, KIN and auxins - IAA, Indole-3-butyric acid (IBA) and NAA were used. The pH of the medium was adjusted to 5.8 and gelled with 0.8% (w/v) agar. Sterilisation was done at 121℃ for 15 min. All the cultures were incubated under a photoperiod of 12 h (100 μ molm^{-2}s^{-1}) at a temperature of 27±1℃.

For rooting of the *in vitro* shoots, various concentrations of IBA, IAA or NAA were added to the MS basal media. Rooted plants were washed well with sterile water and transferred to paper cups containing sterile sand and garden soil (1:1), and covered with plastic bags to maintain humidity. The plantlets were nourished with liquid half strength MS basal medium for five days and then with tap water. After maintaining the plantlets in greenhouse for 75 days, they were transferred to field and the survival rate recorded. Experiments were done in 15 replications and the data subjected to analysis of variance and the means were compared by Duncan's multiple range test (α = 0.05).

Results and Discussion

Multiple shoot buds were initiated within 20 days from proximal and distal ends of midrib and then towards the lateral vein endings (Figure 1a). In the case of petiole and internode explants, shoot organogenesis was observed from the cut ends (Figure 1b and 1c). Of the cytokinins, BAP at a concentration of 22.22 μM induced the maximum regeneration from leaf and petiole segments, while for internodal segments, 31.11 μM BAP was the best. Presence of IAA (3.43 - 4.57 μM) significantly enhanced initiation of shoot buds along with 22.22 μM BAP, except in the internodal explants (Table 1). IAA (4.57 μM) along with 22.22 μM BAP induced maximum shoots from leaf (Figure 1d) (Table 1) after 40 days of incubation. Presence of IAA along with KIN did not evoke much response (Table 1). Leaf segments showed maximum organogenetic potential followed by petiole and internode. Superiority of leaf explants for greater regeneration potential was also reported in *Cuphea ericoides* (Rita and Floh 1995) and in red pepper (Christopher and Rajam 1996).

In all the explants used, the ability of KIN in inducing shoot organogenesis was inferior in comparison with BAP (Table 1). Shoot length was between 10 mm and 12.3 mm without any significant difference (Table 1).

Three different auxins were tested for rooting of excised shoots in presence of a lower concentration of BAP (2.22 μM). The best rooting was observed at 10.75 - 13.44 μM NAA in presence of 2.22 μM BAP (Figure 1e). IAA was also capable of inducing roots while the ability of IBA was comparatively lesser than the other two auxins (Table 2).

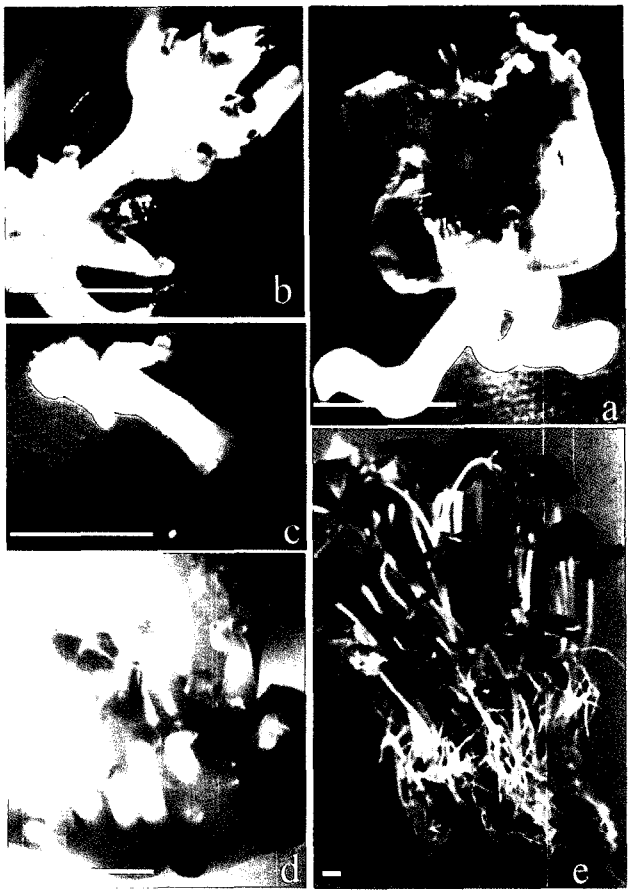

Figure 1. Direct regeneration from leaf explant of *Geophila reniformis* (a), petiole (b), internode (c), leaf explant showing maximum regeneration (d) and complete plantlets (e). (Bar = 5 mm).

Similar results were also obtained in direct regeneration of *Garcinia mangostana* (Goh et al. 1994), Black cherry (Chin-Wei and Reed 1998) and Soyabean (Dan et al. 1998). The rooting ability of shoots and survival rate of hardened plants did not differ significantly among the three explants. Out of the 150 plants each developed from leaf, petiole and internode; 138, 130 and 133 respectively were successfully established in the field.

In conclusion, leaf explants showed maximum organogenetic potentiality followed by petiole and internodal segments. BAP was found to be the best in inducing shoots in combination with IAA. These results demonstrate the vigorous organogenetic potential of the aerial photosynthetic parts of *G. reniformis*, particularly leaf. The protocol described not only provides an efficient method of shoot regeneration from leaf explants for clonal multiplication, but will also be useful for genetic transformation of this medicinal creeper.

Table 1. Effects of BAP and KIN alone and in combination with IAA on direct shoot organogenesis from different explants of *G. reniformis* after 40 days of incubation. Mean ± SE, n =15. Mean followed by same letters are statistically not different within the column (α = 0.05).

Growth regulators (μM)	Number of shoots (explant^{-1})			Shoot length (explant^{-1})		
	Leaf	Petiole	Internode	Leaf	Petiole	Internode
MS basal (Control)	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
BAP 4.44	$7.60^{d}\pm0.44$	$6.47^{c}\pm0.44$	$0.80^{ab}\pm0.18$	$11.6^{bcd}\pm0.30$	$12.3^{c}\pm0.55$	$13.1^{c}\pm0.60$
BAP 13.33	$14.53^{f}\pm0.61$	$12.33^{d}\pm0.44$	$2.93^{ef}\pm0.40$	$11.5^{bcd}\pm0.31$	$11.5^{bc}\pm0.45$	$11.9^{bc}\pm0.67$
BAP 22.22	$21.27^{gh}\pm0.90$	$15.27^{e}\pm1.12$	$3.40^{fg}\pm0.39$	$11.3^{bcd}\pm0.35$	$11.7^{bc}\pm0.34$	$12.1^{bc}\pm0.55$
BAP 31.11	$13.27^{f}\pm0.69$	$12.73^{d}\pm0.61$	$3.80^{g}\pm0.33$	$11.1^{bcd}\pm0.60$	$11.1^{bc}\pm0.54$	$11.0^{b}\pm0.54$
BAP 40	$10.20^{e}\pm0.63$	$6.00^{c}\pm0.37$	$3.00^{efg}\pm0.31$	$11.1^{bcd}\pm0.40$	$11.1^{bc}\pm0.50$	$10.8^{b}\pm0.61$
KIN 18.61	$1.87^{b}\pm0.27$	$1.27^{a}\pm0.21$	0^{a}	$10.3^{bc}\pm4.81$	$10.1^{bc}\pm0.33$	0^{a}
KIN 27.91	$6.53^{c}\pm0.44$	$3.80^{b}\pm0.39$	$2.33^{de}\pm0.35$	$11.0^{bcd}\pm0.53$	$11.0^{bc}\pm0.54$	$11.0^{b}\pm0.54$
KIN 37.21	$5.33^{cd}\pm0.37$	$3.60^{b}\pm0.26$	$1.47^{bcd}\pm0.30$	$10.1^{b}\pm0.34$	$10.6^{bc}\pm0.50$	$10.8^{b}\pm0.35$
BAP 22.22, IAA 3.43	$22.60^{h}\pm0.76$	$17.13^{f}\pm0.60$	$4.60^{h}\pm0.34$	$11.7^{cd}\pm0.64$	$11.7^{bc}\pm0.63$	$11.6^{bc}\pm0.69$
BAP 22.22, IAA 4.57	$26.20^{i}\pm1.02$	$20.33^{g}\pm0.72$	$2.20^{de}\pm0.31$	$12.3^{d}\pm0.47$	$11.3^{bc}\pm0.45$	$10.6^{b}\pm0.55$
BAP 22.22, IAA 5.71	$20.20^{j}\pm0.71$	$16.93^{f}\pm0.52$	$2.07^{d}\pm0.25$	$11.8^{cd}\pm0.49$	$10.4^{bc}\pm0.23$	$11.2^{bc}\pm0.76$
KIN 27.91, IAA 3.43	$6.93^{cd}\pm0.44$	$3.87^{b}\pm0.32$	$1.20^{bc}\pm0.18$	$11.4^{bcd}\pm0.62$	$11.2^{bc}\pm0.22$	$10.7^{b}\pm0.68$
KIN 27.91, IAA 4.57	$9.87^{e}\pm0.44$	$4.07^{b}\pm0.38$	$1.73^{cd}\pm0.21$	$11.5^{bcd}\pm0.56$	$10.8^{bc}\pm0.69$	$10.5^{b}\pm0.81$
KIN 27.91, IAA 5.71	$6.46^{cd}\pm0.39$	$3.47^{b}\pm0.32$	$1.60^{bcd}\pm0.24$	$11.0^{bcd}\pm0.53$	$11.1^{bc}\pm0.51$	$10.8^{b}\pm0.64$

Table 2. Effects of IAA, IBA and NAA in presence of 2.22 μM BAP on root induction in the shoots regenerated from various explants of *G. reniformis* after 30 days of incubation. Mean ± SE, n = 15. Mean followed by same letters are statistically not different within the column (α = 0.05).

Growth regulators (μM)	Number of roots (explant^{-1})		
	Leaf derived shoots	Petiole derived shoots	Internode derived shoots
MS basal (Control)	$1.80^{a}\pm0.48$	$1.06^{a}\pm0.37$	$1.27^{a}\pm0.33$
IAA 8.57	$14.53^{d}\pm0.40$	$14.53^{c}\pm0.48$	$11.73^{c}\pm0.52$
IAA 11.43	$20.60^{f}\pm0.84$	$20.67^{e}\pm0.86$	$13.07^{c}\pm0.42$
IAA 14.26	$18.47^{e}\pm0.58$	$18.13^{d}\pm0.56$	$16.53^{d}\pm0.68$
IBA 7.39	$9.87^{b}\pm0.64$	$10.40^{b}\pm0.62$	$9.53^{b}\pm0.65$
IBA 9.85	$14.40^{d}\pm0.52$	$14.33^{c}\pm0.55$	$12.93^{c}\pm0.67$
IBA 12.32	$11.87^{c}\pm0.42$	$10.87^{b}\pm0.89$	$12.60^{c}\pm0.49$
NAA 8.07	$20.47^{f}\pm0.63$	$20.47^{e}\pm0.61$	$17.60^{d}\pm0.86$
NAA 10.75	$21.80^{f}\pm0.45$	$21.33^{e}\pm0.58$	$21.80^{e}\pm0.48$
NAA 13.44	$23.13^{g}\pm1.08$	$23.53^{f}\pm1.90$	$20.80^{e}\pm0.62$

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