Micropropagation of *Andrographis producta* through axillary and adventitious shoot regeneration

Sathish Shekhappa Kadapatti and Hosakatte Niranjana Murthy*

**Abstract**

**Background:** *Andrographis producta* (C. B. Clarke) Gamble is a valuable medicinal plant that yields several therapeutic compounds. In addition, this species is endemic to the Western Ghats regions of South India. Natural populations of *Andrographis producta* have dwindled due to the overexploitation of this species. The objective of the present study was to develop a reliable *in vitro* propagation protocol for this plant species.

**Results:** *In vitro* plant regeneration protocol has been developed in *Andrographis producta* using nodal and shoot tip explants. The highest axillary shoots (14.60) were regenerated from nodal explants on MS medium amended with 10 μM 2-iP. Similarly, on MS media amended with 5 μM BAP, 17.50 shoots were regenerated from shoot tip explants. Optimal of 27.66 adventitious shoots were regenerated from the cut end of shoot tip explants on MS medium amended with 10 μM 2-iP. Medium amended with 10 μM 2-iP was optimum for regeneration of multiple axillary shoots from nodal explants and for the adventitious shoots regeneration from shoot tip explants. Shoot tips were ideal explants for the micropropagation of *A. producta*. Quarter strength MS media supplemented with 10 μM IBA has resulted in maximum rooting of the shoots.

**Conclusions:** A reliable *in vitro* micropropagation method was developed in *Andrographis producta* through direct organogenesis, and this method is helpful for the multiplication, conservation, and utilization of this plant.

**Keywords:** Adventitious shoots, *Andrographis producta*, Axillary shoots, *In vitro* regeneration
treat skin diseases by the local tribes of Nilgiris Biosphere Reserve [8]. Endemism, habitat loss, forest fires, and overexploitation are significant threats to the survival of Andrographis species [9]. Therefore, plant regeneration protocols have been developed for A. paniculata [10], A. alata [11], A. macrobotrys [12], A. echioides [13], and A. lineata [14]. In addition to the facts mentioned above, conventional propagation of Andrographis producta through seeds is hampered by poor seed germination and short seed viability [15, 16], and there are no regeneration protocols for the micropropagation of Andrographis producta. Given the above, the In vitro propagation method was adopted for the large-scale production of Andrographis producta plants. Here, we report successful methods for large-scale propagation using nodal and shoot tip explants.

**Methods**

**Plant material**

Plants of Andrographis producta (C.B. Clarke) Gamble were collected from Bababudan Hill ranges, Chikmagalur district, Karnataka, India (lat: 13° 25’ 10.2108″; long: 75° 44’ 37.0026″; MSL 1467.30 m) and were maintained in a botanical garden. Identification of plant species was confirmed by Prof. S. R. Yadav, Shivaji University, Kolhapur, India, and voucher specimens were maintained at herbarium, Shivaji University, Kolhapur, India.

**In vitro seed germination**

Seeds were sterilized with 5% (v/v) sodium hypochlorite solution for 15 min, cultured on 1/10th-strength Murashige and Skoog [17] (MS) medium supplemented with 3% sucrose, and solidified with 0.8% agar and incubated in culture room at 25 ± 2 °C with a photoperiod of 16/8 h (light and dark). All processes were carried out under sterile conditions using a laminar air-flow hood. In addition, the pH of the medium was set at 5.8 and sterilized by autoclaving at 121 °C for 15 min.

**Shoot tip and nodal cultures**

Shoot tips (1–3 mm) and nodal explants (5 mm in length) were obtained from 6-week-old seedlings. They were cultured on MS nutrient medium supplemented with 3% (w/v) sucrose and 2.5, 5.0, 7.5, and 10.0 µM BAP, KN, 2-iP, and TDZ (HiMedia, India) individually. The cultures were maintained in culture room wherein temperature, light, and relative humidity were set at 25 ± 2 °C, 16 h light (50 μmol m⁻² s⁻¹)/8 h dark, and 60% relative humidity, respectively.

**In vitro root formation**

Regenerated shoots were individually cultured onto ¼ strength MS nutrient medium containing 3% (w/v) sugar, supplemented with 1.0, 2.0, 5.0, and 10 µM IAA, IBA, and NAA (HiMedia, India) for induction of roots.

**Acclimatization of plants**

Micropropagated plants (5 cm in height) were transplanted to pots containing equal volumes of cocopeat and vermiculite and plants were reared in growth chambers wherein temperature, light, and relative humidity were set at 25 ± 2 °C, 16 h light (50 μmol m⁻² s⁻¹)/8 h dark, and 60%, respectively. After 2 weeks, plants were transferred to potting mix containing soil and cocopeat and maintained in the greenhouse.

**Histological analysis**

For histological studies, cultured nodal and shoot tip explants were fixed in FAA (10 ml of formalin, 85 ml of 70% ethyl alcohol, and 5 ml of glacial acetic acid) for 12 h at room temperature and dehydrated by ethanol-butyl alcohol series and embedded in paraffin as recommended by Johansen [18]. The material was sectioned (thickness of 6 µm) and stained with 0.05% toluidine blue (HiMedia, Mumbai, India) and examined under a compound microscope (Nikon, Tokyo, Japan).

**Data analysis**

A randomized block method was followed for the establishment of experiments. Data were statistically analyzed with the help of one-way analysis of variance (ANOVA) followed by Duncan’s multiple range test was applied using SPSS statistical software (version 20).

**Results**

**Axillary shoot regeneration from nodal explants**

The nodal explants cultured on MS medium containing cytokinin involved in axillary shoot induction within 2 weeks of culture (Table 1). Axillary shoots were regenerated on all cytokinin-supplemented media; however, optimum regeneration was observed on MS medium supplemented with 2-iP. The highest percentage of response, and a greater number of shoots, and mean shoot length were optimum with 2-iP containing medium (Table 1). On 2-iP containing media, initially, few shoots were emerged from nodal regions after 2 weeks in culture (Fig. 1A). Shoot proliferation was evident with the advancement of time (after 4 and 6 weeks) in culture (Table 1, Fig. 1B–C); at the end of 8 weeks in culture, highest shoot proliferation was recorded (Fig. 1D). On MS media amended with 10 µM 2-iP, the highest number of 14.60 shoots
were regenerated from nodal explants (Table 1). Nodal explants involved in direct shoot regeneration without callus phase or callus mediation were examined histologically (Fig. 2A).

**Multiple and adventitious shoot regeneration from shoot tip explants**

Seeds were germinated on 1/10th strength MS basal medium (Fig. 1E), and shoot tips harvested from young seedlings were used for plant regeneration. Shoot tip explants cultured on MS medium amended with KN and TDZ (2.5, 5.0, 7.5, and 10 μM) developed a single shoot. However, the shoot tip explants cultured on BAP and 2-iP media showed a differential response. On media supplemented with BAP, shoot tip explants developed multiple shoots from shoot meristem (Table 1). Shoot tip explants initially produced a few shoots (Fig. 1F), and shoot proliferation was observed after 4 and 8 weeks in culture (Table 1; Fig. 1G–H). On MS media amended with 5 μM BAP, 100% shoot tips were responded and developed 17.50 shoots per explant (Table 1; Fig. 1H). Shoot tip explants which were cultured on 2-iP containing media showed adventitious shoot regeneration from the cut end of shoot tips. In contrast, a single shoot was regenerated from the shoot meristem on basal medium. Shoot buds sprouted from the cut end of the shoot tip on MS medium containing 10 μM 2-iP after 2 weeks in culture (Fig. 1I); such shoot buds were involved in proliferation in subsequent weeks (Fig. 1J–K). Optimal of 27.66 adventitious shoots were regenerated from the cut end of shoot tip explants on MS medium amended with 10 μM 2-iP (Table 1). Preexisting meristem of shoot tip explants cultured on 5 μM BAP divided and differentiated into multiple shoots, and shoot meristems directly generated multiple shoots without callus tissue’s mediation, according to histological preparations of shoot tip explants (Fig. 2B). Similar to this, histological studies revealed direct adventitious shoot regeneration (without callus intervention) from the cut end of shoot tip explants as a result of mitotic activity of epidermal cells in response to the media supplemented with 10 μM 2-iP (Fig. 2C).

**In vitro root formation**

For root induction, *In vitro* raised shoots (2-5 cm in length) were cultured on quarter strength MS media
amended with 1, 2, 5, and 10 μM IAA, NAA, or IBA, and the results are presented in Table 2. Roots were sprouted from the shoots on all the auxin-supplemented media (Table 2); however, optimal root induction was recorded on MS medium containing IBA. There was a linear increase in the number of roots with the increasing concentration of IBA in the medium (Table 2, Fig. 3A). The highest percent of root induction (100%) and optimum roots per shoot (18.66 per shoot) were observed on MS medium supplemented 10 μM IBA (Table 2, Fig. 3A).

Acclimatization

In vitro regenerated plantlets were removed from culture vessels and media adhering to the roots of the plantlets was carefully washed with distilled water. 

Fig. 1 In vitro shoot regeneration from nodal and shoot tip explants of *Andrographis producta* on Murashige and Skoog medium supplemented with cytokinins. A Induction of axillary shoots from the nodal explant on MS medium supplemented with 10 μM 2-iP after 2 weeks of culture (bar-, 0.20 cm). B Shoot multiplication from nodal explant on MS medium supplemented with 10 μM 2-iP after 4 weeks (bar-, 0.23 cm). C Shoot proliferation from nodal explant on MS medium containing 10 μM 2-iP after 6 weeks (bar-, 0.35 cm). D Multiple shoots regeneration from nodal explant after 8 weeks of culture on medium supplemented with 10 μM 2-iP (bar-, 0.30 cm). E In vitro seed germination on 1/10th strength MS basal medium (bar-, 0.7 cm). F Initiation of multiple shoots from shoot tip explant on MS medium supplemented with 5 μM BAP after 2 weeks (bar-, 0.75 cm). G Proliferation of shoots from shoot tip explant on medium supplemented with 5 μM BAP after 4 weeks (bar-, 0.9 cm). H Multiple shoots regeneration from shoot tip explant after 8 weeks of culture on MS medium supplemented with 5 μM BAP (bar-, 0.98 cm). I Initiation of direct shoot buds from cut end region (basal region) of shoot tip explant after 2 weeks of culture on MS medium containing 10 μM 2-iP (bar-, 0.60 cm). J and K Proliferation of shoots from cut end region (basal region) on shoot tip explant on MS medium containing 10 μM 2-iP after 4 and 6 weeks, respectively (bar-, 0.55 cm and 1.15 cm for J and K, respectively). L Multiple shoots regenerated from a cut end region of shoot tip explant after 8 weeks of culture on MS medium supplemented with 10 μM 2-iP (bar, -1.03 cm)
The plantlets were transplanted to poly-cups containing cocopeat and vermiculite (1:1 ratio) and reared in controlled conditions for 2 weeks (Fig. 3B). Later, they were transferred to bigger pots containing potting mix (Fig. 3C), and the survival percentage was 95%.

**Discussion**

The present *In vitro* propagation studies reveal that multiple axillary shoots could be regenerated by using nodal explants of *A. producta* on MS medium supplemented with 2-iP. In contrast, media supplemented with KN, BAP, and TDZ were less efficient in multiple axillary shoot regeneration. On MS media amended with 10 μM 2-iP, the highest number of multiple shoots were regenerated from nodal explants. Additionally, histological examination showed that nodal explants directly produced shoots without the need for callus mediation (Fig. 2A), and the similar reports on use of histological evidences to trace the mode of regeneration from nodal explants have been reported in *Vitex trifolia* [23], and *Andrographis paniculata* [10], where meristematic cells at outer protoderm layer of axillary bud divided and differentiated into axillary shoots. Multiple axillary shoot regeneration from nodal explant on cytokinin supplemented medium was also reported in *Andrographis paniculata, Artemisia nilagirica* var. *nilagirica, Artemisia japonica, Feronia limonia, Nothapodytes nimmoniana, Spilanthes oleracea, and Vitex trifolia* [10, 19–24]. Among various cytokinins used in the present study, 2-iP supplemented medium was superior in axillary shoot induction. In contrast to the present results, BAP was reported to be potent cytokinin in axillary shoot induction in *Andrographis alata* and *A. macrobotrys* [11, 12].

Shoot tip explants of *Andrographis producta* developed multiple shoots from shoot meristem on MS media amended with 5 μM BAP. In contrast, the development of adventitious shoots was recorded from the cut end of the shoot tip explants (the basal portion of the shoot tips) on media amended with 2-iP. Optimal of 27.66 adventitious shoots were regenerated from the
cut end of shoot tip explants on MS medium amended with 10 μM 2-iP. Histological preparations again showed direct regeneration of multiple shoots from the shoot meristem and basal portion of the shoot tips, and this is in consistent with the reports on *Clementis* cultivar where meristems of shoot tip are involved in

| Growth regulator | Concentration of hormone (μM) | Percentage of response | Mean number of roots per shoot | Mean root length (cm) |
|------------------|--------------------------------|------------------------|-------------------------------|----------------------|
| Control          | 0                              | 66.66                  | 3.25 ± 0.47ij                | 2.47 ± 0.12c         |
| IAA              | 1                              | 50                     | 2.33 ± 0.33j                 | 1.96 ± 0.08d         |
|                  | 2                              | 83.33                  | 5.20 ± 0.37gh                | 3.70 ± 0.15a         |
|                  | 5                              | 100                    | 6.33 ± 0.42fgh               | 2.95 ± 0.08b         |
|                  | 10                             | 100                    | 8.50 ± 0.42de                | 2.60 ± 0.11c         |
| NAA              | 1                              | 100                    | 13.00 ± 0.85b                | 2.01 ± 0.15d         |
|                  | 2                              | 100                    | 10.00 ± 0.51cd               | 1.31 ± 0.12e         |
|                  | 5                              | 83.33                  | 10.40 ± 0.50c                | 1.24 ± 0.08e         |
|                  | 10                             | 66.66                  | 6.75 ± 0.62efg               | 1.87 ± 0.11d         |
| IBA              | 1                              | 83.33                  | 4.80 ± 0.37hi                | 1.88 ± 0.11d         |
|                  | 2                              | 100                    | 7.50 ± 0.34ef                | 1.98 ± 0.10d         |
|                  | 5                              | 100                    | 9.83 ± 0.47cd                | 1.30 ± 0.05e         |
|                  | 10                             | 100                    | 18.66 ± 1.02a                | 2.03 ± 0.09d         |

Mean data was recorded after 4 weeks of culture. Mean values followed by the same letter are not significantly different according to Duncan’s multiple range test \((p = 0.05)\).
division and differentiation to form direct shoots [25] and Neolamarckia cadamba [26], where epidermal and subepidermal cells regained mitotic activity and differentiated directly into new shoots. Among the four individual cytokinins tested, i.e., KN, BAP, TDZ, and 2-iP, the highest multiple shoot regeneration was achieved on 2-iP supplemented medium. Similar to the current results, an efficient In vitro plant regeneration was achieved from shoot tips explants of Cursculigo latifolia [27] and Enicostema axillare [28] on TDZ and BAP supplemented medium, respectively. Adventitious shoot regeneration from shoot tip explants was described in Vanda coerulea by Jitsopakul et al. [29] which was the efficient mode of In vitro regeneration.

An essential stage for plantlet regeneration and adaptation is the rooting of an In vitro regenerated shoot. A single shoot of Andrographis producta cultivated on quarter strength MS media supplemented with 10 μM IBA resulted in the optimum root induction. After 2 weeks of root initiation, there was a rapid root elongation. Andrographis paniculata [10], Andrographis alata [11], and Andrographis macrobotrys [12] all showed comparable results.

Conclusion
The MS media with 10 μM 2-iP was proved as most effective for the adventitious shoot and axillary shoot induction from shoot tip and nodal explants, respectively. For rooting, quarter strength MS media amended with 10 μM IBA has resulted in maximum rooting. This study showed an efficient direct shoot regeneration of Andrographis producta using nodal and shoot tip explants. The In vitro regeneration protocol developed for Andrographis producta is useful for the multiplication and conservation of this plant.

Abbreviations
BAP: 6-Benzylaminopurine; IAA: Indole-3-acetic acid; IBA: Indole-3-butyric acid; NAA: α-Naphthalene acetic acid; KN: Kinetin; 2-iP: 2-isopentenyl adenine; MS: Murashige and Skoog medium; TDZ: Thidiazuron.

Authors’ contributions
HNIM planned and designed the experiments; SSK conducted the experiments. Both authors together wrote and approved the manuscript.

Funding
No specific funding was received for this paper.

Availability of data and materials
Not applicable.

Declarations
Ethics approval and consent participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Received: 1 June 2022 Accepted: 23 October 2022 Published online: 01 November 2022

References
1. Gunasekaran G, Murthy GVS (2015) Andrographis producta (Acanthaceae), an endemic species from the Western Ghats: its taxonomy, lectotypification, and distribution. Telopea 18:209–206
2. Khiirisagar RD, Singh NP (2001) Some less known ethnomedicinal uses from Mysore and Coorg districts, Karnataka state India. J Ethnopharmacol 75:231–238
3. Alagesaboopathi C (2012) Ethnobotany of Andrographis lineata Walli ex Nees - an endemic medicinal plant of India. Int J Recent Sci Res 3:71–74
4. Ignacimuthu S, Ayyanan M, Sharasivaraman K (2006) Ethnobotanical investigations among the tribes in Madurai district of Tamilnadu (India). J Ethnopharmacol 21:1–20
5. Abu-Ghefeh AA, Canatan H, Ezeamuzie CI (2009) In vitro and in vivo anti-inflammatory effects of andrographolide. Int Immunopharmacol 9:313–318
6. Hossain MS, Urbi Z, Sule A, Hafizur Rahman KM (2014) Andrographis paniculata (Burm. f.) Wall. ex Nees: a review of ethnobotany, phytochemistry, and pharmacology. Sci World J Article ID 274905. https://doi.org/10.1155/2014/274905
7. Dalawai D, Murthy HN (2021) Chemical profile and antioxidant properties of Andrographis producta (C.B. Clarke) Gamble. Pharmcoojn J 13:475–485
8. Ponnusamy S, Arumugam R, Aryan S, Chinnaiyan R (2017) Ethnobotanical knowledge of threatened plant species Andrographis in Nilgiris biosphere reserve, Tamil Nadu. India Int J Herb Med 5(6):103–107
9. Neeraja C, Krishna Pri, Reddy CS, Giri CC, Rao KV, reddy VD (2015) Distribution of Andrographis species in different districts of Andhra pradesh. Proc Natl Acad Sci India Sect B Biol Sci 85:601–606
10. Dandin VS, Murthy HN (2012) Regeneration of Andrographis paniculata nees: analysis of genetic fidelity and andrographolide content in micropropagated plants. Afr J Biotechnol 11:12464–12471
11. Kadappati SS, Murthy HN (2021) Rapid plant regeneration, analysis of genetic fidelity, and neoandrographolide content of micropropagated plants of Andrographis alata (Vahli) nees. JGEB 19:20
12. Kadappati SS, Murthy HN (2021) In vitro micropropagation of Andrographis macrobotrys. J Herbs Spices Med Plants 28:89–98
13. Savitikadi P, Jogan P, Rohela GK, Ellendula R, Sandha D, Allini VR et al (2020) Direct regeneration and genetic fidelity analysis of regenerated plant of Andrographis echiodes (L) - an important medicinal plant. Ind Crops Prod 155:112766
14. Mohammed A, Chiruvella KK, Ghanta RG (2016) In vitro plant regeneration, flowering and fruiting from nodal explants of Andrographis lineata Nees (Acanthaceae). J Crop Sci Biotechnol 19:195–202
15. Dalawai D, Aware C, Jadhav JP, Murthy HN (2021) RP-HPLC analysis of diterpene lactones in leaves and stem of different species Andrographis. Nat Prod Res 35:2239–2242
16. Dalawai D, Murthy HN (2021) Pollen and seed morphology of selected species of Andrographis (Acanthaceae) from India. Grana 60:459–476
17. Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497
18. Johansen DA (1940) Plant microtechnique. McGraw-Hill, New York
19. Shinde S, Joseph KS, Jain JR, Manohar SH, Murthy HN (2016) Efficient In vitro propagation of Artemisia nilagirica var. nilagirica (Indian wormwood) and assessment of genetic fidelity of micropropagated plants. Physiol Mol Biol Plants 22:595–603
20. Shinde S, Karewal PR, Shanbhag DD, Joseph KS, Murthy HN (2017) In vitro propagation of Artemisia japonica. J Herbs Spices Med Plants 23:36–43
21. Hiregoudar LV, Ashok Kumar HG, Murthy HN (2005) In vitro culture of Feronia limonia (L.) Swingle from hypocotyl and internodal explants. Biol Plant 49:41–45
22. Dandin VS, Naik PM, Murthy HN, Park SY, Lee EJ, Paek KY (2014) Rapid regeneration and analysis of genetic fidelity and scopoletin contents of micropropagated plants of Spilanthes oleracea. J Hortic Sci Biotech 89:79–85
23. Hiregoudar LV, Murthy, HN, Bhat JG, Nayeem A, Hema BP, Hahn EJ, Paek KY (2006) Rapid clonal propagation of Vitex trifolia. Biol Plant 50:291–294
24. Dandin VS, Murthy HN (2012) Enhanced in vitro multiplication of Nothapodytes nimmoniana Graham using semisolid and liquid cultures and estimation of camptothecin in the regenerated plants. Acta Physiol Plant 24:1381–1386
25. Mitrofanova I, Ivanova N, Kuzmina T, Mitrofanova O, Zubkova N (2021) In vitro regeneration of Clematis plants in the Nikita Botanical Garden via somatic embryogenesis and organogenesis. Front Plant Sci 12:541171
26. Huang H, Li JC, Ou’Yang KX, Zhao XH, Li P, Liao BY, Chen XY (2014) Direct adventitious shoot organogenesis and plant regeneration from cotyledon explants in Neolamarckia cadamba. Plant Biotechnol 31(2):115–121
27. Babaei N, Abdullah AP, Saleh G, Abdullah TL (2014) An efficient in vitro plantlet regeneration from shoot tip cultures of Curculigo latifolia, a medicinal plant. Sci World J Article ID 275028. https://doi.org/10.1155/2014/275028
28. Sasidharan P, Jayachitra A (2017) Direct shoot bud regeneration from shoot tip explants of Enicostema axillare: an important medicinal plant. Agroforest Syst 91:471–477
29. Jitsopakul N, Thammaviri K, Ishikawa K (2013) Efficient adventitious shoot regeneration from shoot tip culture of Vanda coerulea, a Thai orchid. Sci Asia 39:449–455

Publisher's Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.