INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 3, No. 1, p. 49-57, 2013

RESEARCH PAPER OPEN ACCESS

Effect of growth regulators on rooting of *Andrographis neesiana* Wight. - a valuable endemic medicinal plant of India

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Key words: *Andrographis neesiana*, endemic plant species, stem cuttings, vegetative propagation, growth regulators.

Article published on January 20, 2013

Abstract

The present study deals with the vegetative propagation prospects of an endemic medicinal plant *Andrographis neesiana* Wight, which is familiarly used in herbal medicine for the treatment of several diseases. Due to over exploitation this plant is disappearing from original habitat hence its cultivation on commercial scale is suggested. Stem cuttings of *Andrographis neesiana* are advantageous to root. Treatment with Indole-3-Butyric Acid (IBA) and α-Napthalene Acetic Acid (NAA) raised rooting and increased shoot development in greenhouse under intermittent misting. IBA and NAA treated cuttings performed improve in all development parameters compared to control. The highest percentage of rooting was observed in IBA 1500 ppm (68.10%). The maximum root length was noted in IBA 2000 ppm (11.20 cm). The roots were profuse and branched in characteristic. Besides this, the survival rate of IBA treated plantlets was 86.45% and NAA treated plantlets was 72.61% in comparison to control where it was 7.32%. The percentage of rooting and root length upgraded by using plant growth hormones, either separate or together. The present investigation concludes that clonal multiplication of these an endemic medicinal plant is feasible through application of plant growth regulators. The significance of these findings on the propagation and conservation of *A. neesiana* is discussed.

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Introduction

Medicinal plants contain importances that can be used for biotherapeutic applications or which are used as precursors for the synthesis of beneficial (Sofowora 1993). The medicinal characteristics of plants lies in their phytochemicals namely alkaloids, flavonoids in addition to other phenolic compounds that co-opt as to producing particular physiological properties to the body of man and animals (Che Man, 2010). However, analysis of extract of *Andrographis neesiana* was found to be rich in flavones to the expensive phytoconstituent potentialities of the plant. *Andrograhis neesiana* is therefore grown as a medicinal species for the curing of many ailments particularly in India. (Alagesaboopathi and Balu 1999; Alagesaboopathi and Sivkumar 2011; Ponvinobala *et al.,* 2012a). *Andrographis neesiana* Wight belongs to the family Acanthaceae and has been widely used in healthcare traditions. The rooted plants by cottage have various advantages such as faster growth rate (Ooyamma and Toyoshima, 1965), greater stock stand uniformity, improve site matching and true-to-type planting material production (Fielding, 1969). Cuttings can be categorized into 3 groups as comfortable to root, difficult to root and inflexible to root (Nanda, 1970).

Species of *Andrographis* Wallich ex Nees (Acanthaceae) are used in the Indian systems of medicine namely, Siddha, Ayurveda, Unani, Naturopathy, Homeopathy, Amachi and Modern (Alagesaboopathi and Balu, 1999). The genus *Andrographis* as a whole is of potentialities significance to India. The genus exhibits antipyretic characteristics (Kirtikar and Basu, 1975). This genus consists 40 species distributed in Tropical Asia (Anonymous, 1948). About 21 species are distributed in India (Gamble, 1982) and all of them available in Tamilnadu. (Henry *et al.,* 1987). Among the 21 species 18 species are reported to be endemic to India (Ahmedullah and Nayar, 1986). *Andrographis neesiana* Wight (Fig. 1) is an endemic medicinal species (Ahmedullah and Nayar, 1986) found in wild in Shevaroy Hills of Salem district of Tamilnadu (11°45′ and 11°55′ N and 78°11 to 78°20′E) upto 1500 m.

The pharmaceutical industries is widely dependent upon the wild populations for providing these plant species for extraction of their intrinsic phytochemicals. Moreover, herbal medicine practitioners, local medicine men, village dwellers, vaidyas, tribals (Malayalis), forest dwellers, and other traditional healers often use these collection of the plant materials from forests and lacking experiments either to allow the replenishment or propagation, these critical flora are rapidly vanishing. As a importance, *A. neesiana* declared as an endemic plant in India (Ahmedullah and Nayar, 1986). Therefore, there is an immediate need to enlarge serviceable cultivation techniques for propagation of these noteworthy medicinal floras which will excessively commercial application. *Andrographis neesiana* has been used in the treatment of aphrodisiac and antifungal property (Alagesaboopathi and Balu, 1999; Alagesaboopathi and Balu, 2000). Several medicinal properties such as cough, edema, laxative, bitter and overcomes trouble in breathing, worms, liver complaints, acidity, burning sensation, syphilitic ulcers, skin disorders and also veterinary medicines have been attributed to this plant in the traditional systems of Indian medicine (Sivarajan and Balachandran, 2001). It is used in the treatment of antimicrobial, antioxidant and anticancer activity (Alagesaboopathi and Sivakumar, 2011; Ponvinobala *et al.,* 2012 a, b).

Two new flavonoids, 2′, 4′, 6′, 2′, 3′, 4-hexamethoxychalcone and 5-hydroxy -7′, 2′, 5′ -trimethoxyflavone together with a known flavone glycoside, echioidinin 5-O-beta-D-glucopyranoside were isolated from the whole plant extract of *Andrographis neesiana* (Muntha Kesava Reddy *et al.,* 2003). There is no previous report on use of growth hormones in vegetative propagation of this useful plant. The work was undertaken to result rooting response of *A. neesiana* under greenhouse using growth regulators and outcomes reported. Auxin is one of the factors for stimulating root

productions in cuttings (Hartman *et al.,* 1990). Thakur and Gupta (1998) reported that cutting of *Alnus nitida* with various concentration of IBA and obtained the maximum root percentage at IBA 800 ppm.

Rooting of stem cutting through action of growth hormones has been undertaken as a protocol for broad scale propagation of this plant following the approach of Senthilkumar *et al.,* 2009; Akwatulira *et al.,* 2011; Saradha and Paulsamy, 2012). Auxins, a category of plant evolution substances are often called as plant growth hormones and performance an important role in coordination of many growth and behavioral technique in the plant life cycle (Tiwari and Kuntal Das, 2010). Indole-3-Acetic Acid (IAA), α-Naphthalene Acetic Acid (NAA) and Indole -3-Butyric Acid (IBA) are conventionally the leading auxins which are usable commercially and can be applied with liquid (liquid formulation) or in talc (powder formulation) for rooting and sprouting of stem cuttings (Hopkins, 1999). The present study therefore aimed at ascertaining the most suitable propagation *A. neeisana* using stem cuttings. In the present study, the stem cuttings of *A. neesiana* were treated by plant growth regulators (PGRs), IBA and NAA. The aim of this investigation was to test the potential outcome of plant growth regulators on the stem cuttings and to prefer an optimal concentration of plant growth regulators for stem cuttings of *A. neesiana*.

Materials and methods

ndrographis neesiana Wight was used as experiment plant in the present study. All the research was conducted at Department of Botany, Government Arts College (Autonomous), Salem, Tamilnadu, India. Ailment free evenly matured *A. neesiana* were collected from plants growing in Shevaroy Hills in July 2012. The matured stem cuttings of varying length (10-20 cm) and thickness (0.5-1.2 cm) having at least 2-3 nodes were taken from the well-established *Andrographis* plant. The cutting were surface sterilized with 0.1% mercuric chloride solution for 3-5 minutes followed by rinsing in water. Ten cuttings were taken for each treatment. These cuttings and treated with Indole-3-Butyric Acid (IBA) (500, 1000, 1500, 2000 and 2500 ppm) and α-Naphthalene Acetic Acid (NAA) (500, 1000, 1500, 2000 and 2500 ppm). The basal cut ends (upto 3.0 cm) of cuttings were soaked in Indole-3-Butyric Acid and α-Naphthalene Acetic Acid solution for 24 hrs. The top cut ends were sealed with inert paraffin wax to elude desciccation through surface loss of water. Cuttings soaked in sterile distilled water and without any treatment (control) were included in the experiments for comparison. After 24 hours the cuttings were planted in polybags (15.5 cm x 24.5 cm) filled with garden soil and sand mixture (1:2) and kept in greenhouse. The cuttings were watered regularly. No fertilizer was applied and no pesticide was sprayed. Water logging was avoided in all the stages. After 45 days of planting the number of sprouted cuttings were counted whereas, their survival was recorded at 65 days of plantation. The data on root number, root length (cm), shoot length, percentage of rooting, percentage of sprouting and classified in randomized complete block design with triplicates for each treatment and the data was analysed for the variance.

Results and discussion

Various sprouting rates were obtained for the cuttings treated with different plant growth auxins (Fig. 2,3 and 4) (Table 1). The outcome of treatments on rooting capability of stem cuttings of *A. neesiana* has been observed and results on adventitious root formation are presented in Table 1. All the treatments of IBA and NAA significantly enhanced percentage of rooting and were compared with the control treatment. Results denoted a wide spectrum of efficiency of growth regulators on percent sprouted, percent of rooting, root number, root length and shoot length of stem cuttings of *A. neesiana*. The result of IBA and NAA on rooting of stem cuttings in *A. neesiana* growth hormones of different concentrations of IBA 500, 1000, 1500, 2000 and 2500 and NAA 500, 1000, 1500, 2000 and 2500 ppm were used. Interpretation *A. neesiana* as valuable medicinal species, particularly as a attribute

for andrographolide, chalcone and flavones is known to plant studies for a extended time. As propagation through seeds is rather difficult, cuttings have been noted for the application. The growth hormones IBA and NAA had deep root inducing ability. The results on response of rooting attributes to plant growth regulators were recorded 65 days after planting (Table 1).

Table 1. The effect of auxins on rooting of Andrographis neesiana stem cuttings 65 days after treatment and planting under green house condition.

Hormone	Concentrations	Percentage of survival	Percentage of rooting	Root numbers	Root length (cm)	Shoot length (cm)
IBA	500 ppm	42.15	34.62 ± 0.13	7.30 ± 0.27	8.40 ± 0.17	8.62 ± 0.30
	1000 ppm	50.16	40.17 ± 0.34	7.92 ± 0.36	8.11 ± 0.75	9.41 ± 0.63
	1500 ppm	86.45	68.10 ± 0.20	8.95 ± 0.55	10.93 ± 0.16	9.21 ± 0.18
	2000 ppm	62.70	65.38 ± 0.16	8.71 ± 0.42	11.20± 0.43	11.48 ± 0.28
	2500 ppm	56.33	42.60 ± 0.47	9.49 ± 0.63	9.48± 0.14	9.84 ± 0.11
NAA	500 ppm	40.25	32.17 ± 0.12	6.70 ± 0.35	8.75 ± 0.13	8.41 ± 0.41
	1000 ppm	46.54	38.27 ± 0.43	7.11 ± 0.23	9.20 ± 0.35	8.75 ± 0.19
	1500 ppm	57.10	47.13 ± 0.17	8.60 ± 0.46	10.15 ± 0.73	10.50 ± 0.54
	2000 ppm	72.61	62.74 ± 0.76	8.73 ± 0.11	9.49 ± 0.12	9.05 ± 0.18
	2500 ppm	49.85	44.19 ± 0.51	7.80 ± 0.68	8.10 ± 0.58	8.79 ± 0.91
Control	No treatment	7.32	14.10 ± 0.25	1.92 ± 0.20	2.35 ± 0.36	2.80 ± 0.14

ppm = Concentration in parts per million; Data represents the mean ± standard error of two independent; each with 10 cuttings per treatment.

The various treatments showed various response in rooting percentage and its characters. Highest number of leaves per cuttings was found for IBA 1500 ppm in *A. neesiana* (Table 1). All the treatment of IBA and NAA indicatively the percentage of rooting in comparison to the control. Among the IBA treatments highest rooting percentage was recorded with 1500 ppm (68.10%) which was followed by 2000 ppm (65.38%), 2500 ppm (42.60%), 1000 ppm (40.17%) and 500 ppm (34.62%) (Fig 3). The

NAA treatments highest rooting percentage was recorded with 2000 ppm (62.74%), which was followed by 1500 ppm (47.13%), 2500 ppm (44.19%), 1000 ppm (38.27%) and 500 ppm (32.17%) (Fig 4). Rooting was also observed in control (14.10%) but the response was very poor as the values for number of roots and length of roots were very reduced in comparison to other treatments (Table 1).

Fig. 1. *Andrographis neesiana* in natural habitat.

Fig. 2. *Rooted stem cuttings of Andrographis* neesiana in polybags after PGR treatments.

Fig. 3. Rooted stem cuttings *Andrographis neesiana* with IBA (2000 ppm) treatments.

Fig. 4. Rooted stem cuttings *Andrographis neesiana* with NAA (1500 ppm) treatments.

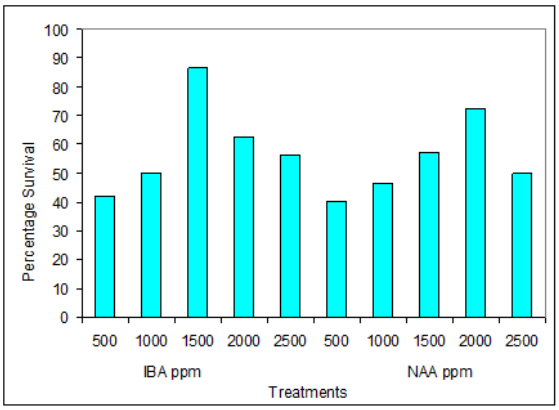

Fig. 5. Effects of IBA and NAA concentration on percentage survival of stem cutting in *Andrographis neesiana* Wight.

Significantly more number of roots per cutting was recorded in the treatment IBA 2500 ppm (9.49) followed by the treatment IBA 1500 ppm (8.95), IBA 2000 ppm (8.71), IBA 1000 ppm (7.92) and IBA 500 ppm (7.30). IBA treatment significantly increased the number of roots / cuttings as compared to NAA 2000 ppm (8.73), NAA 1500 ppm (8.60), NAA 2500 ppm (7.80), NAA 1000 ppm (7.11) and NAA 500 ppm (6.7) treatments respectively. The control observed the smallest number of roots per cutting (1.92). Highest survival (86.45%) was recorded when the cuttings were treated with 1500 ppm IBA against least survival (7.32%) in control cuttings. Maximum survival (72.61%) was noted when the cuttings were treated with 2000 ppm NAA against minimum survival (7.32%) in untreated cuttings (Fig 5). Root length raised highest (11.20 cm) with 2000 ppm IBA followed by 1500 ppm IBA (10.93 cm) and 1500 ppm

NAA (10.15 cm) treatments respectively. The IBA and NAA treatments showed significantly lengthy shoots than the control (2.80 cm). The higher shoot lengths in 2000 ppm IBA (11.48 cm) and 1500 ppm NAA (10.50 cm) treatments could be due to the upgrade effects of the treatments. No difference was noticed in the total lengths and internodal lengths of the cuttings after the period of culture. But most of the cuttings produced vegetative shoots profusely. Number of vegetative shoots per cuttings ranged between 2 and 4. Lengths of vegetative shoots varied between 8.41 and 11.48 cm. Roots were produced from the underground nodal region and also from cut ends. Number of roots from the cuttings ranged between 5 and 16. Maximum root length between 8.10 and 11.20 cm.

The differential effects of many auxins on rooting of stem cuttings of several plant species have been ascribed to the chemical constitution of auxin, the approach of treatment and morpho-physiological attribute of the cuttings (Tiwari and Kuntal Das, 2010; Anyasim 2011). Utilize of vegetative stem cuttings for the propagation of plants have been found very effective technique in number of plants such as *Warburgia ugandensis, Hildegardia populifolia, Thunbergia grandiflora, Jatropha curcus* (Vinaykumar *et al.,* 2008; Dhillon *et al.,* 2009; Akwatulira *et al.,* 2011; Saradha and Paulsamy, 2012). The earlier work by Alagesaboopathi (2011) and Chinappan Alagesaboopathi (2012) convince the important of IBA and IAA to root stem cuttings of endemic medicinal species of *Andrographis lineata* and *Andrographis elongata*. Shamet *et al.,* (1989) reported that obtain 80% rooting rate of *Celtis australis* cuttings treated with 3000 ppm IBA. A previous investigation by Sharma and Pandey (1999) showed that application with 5000 ppm of IBA had a very highest rooting rate of 89.30 percentage in *Dalbergia sisso* and 76.43 percentage in *Dalbergia latifolia*. Majeed *et al.* (2009) notable the maximum rooting rate (50%) for *Aesculus indica* cuttings treated with IBA at 2000 ppm. Baul *et al.,* (2008) noted a comparable trend in the vegetative propagation of *Stereospermum suaveolens*. Sun and Chen (1998) reported the highest effects of plant growth regulators (NAA, IBA and IAA) on sprouting of rose buds. Growth hormones effects increased of plant cells, cell division, laterals branching of roots and shoots, vascular differentiation and early embrayonic evolution (Hobbie *et al.,* 2000). Utilize of vegetative stem cuttings for the propagation of plants have been found very efficient technique in many plants such as *Ficus carica, Nerium olender, Aesculus indica, Jatropha* species (Nambison *et al.,* 1977; Majeed *et al.,* 2009; Gaikwad, 2011; Torkashvand and Shadparvar, 2012). Medicinal plants familiarly develop in unpropagated in natural habitat, but scientific technique would be advantages to extend their biomass supply as well as andrographolide contents.

Thus proliferation by means of stem cuttings is the maximum saving suitable and successful technique of clonal multiplication. Moreover to upgrade sprouting, rooting and survival of stem cuttings, plant growth regulators can be widely and broadly used. It is noted that *A. neesiana* has got different uses. Due to over exploitation this plant is disappearing from natural habitat hence its clonal multiplication on commercial scale is recommended.

Conclusion

The present study provides preliminary results concerning vegetative propagation of *A. neesiana* by stem cuttings. The study produces a further research scope on *in vitro* and *ex vitro* propagation of the species. The research laid a potent foundation for the conservation of this valuable endemic medicinal plant.

Acknowledgements

The author is thankful to Prof Dr.A.R. Rajamani, Principal, Governement Arts College (Autonomous), Salem, Tamilnadu for his support and to Prof. C.S. Suriyanarayanan, Head, Department of Botany, Governement Arts College (Autonomous), Salem, Tamilnadu for providing all necessary facilities to to conduct the experiment.

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