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Evaluation of *in vitro* seed germination and micropropagation techniques in *Andrographis echioides* (L.) Nees

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ABSTRACT

***Andrographis echioides* (L.) Nees (Gopuram thanki) is one of the important medicinal plants which is given importance recently for its excellent medicinal properties. A study was carried out to evaluate the seed germination under open and *in vitro* conditions, *in vitro* response of different explants / media for regeneration and also to standardize the direct regeneration procedure in *Andrographis echioides*. The earliest seed germination (8.67 days) was recorded in the treatment comprised of MS medium supplemented with BAP (1 mgl^{-1}) under *in vitro* conditions. The germination percentage (67.10 %) of seeds and survival percentage (79.93 %) of seedlings were also recorded high in the same treatment. For direct regeneration, among the various explants, shoot tips responded positively for shoot induction. MS medium fortified with BAP (2.5 mgl^{-1}) was found highly responsive for shoot induction. The multiple shoot induction was achieved in MS medium + BAP (3.0 mgl^{-1}) and for shoot elongation, BAP (2.0 mgl^{-1}) + GA_3 (1.0 mgl^{-1}) was found better. Rooting was best (94.85 %) in ½ MS + IAA 0.5 mgl^{-1} + IBA 1.0 mgl^{-1}. Pot mixture containing vermiculite + red earth + sand (1:1:1) was found optimum for hardening.**

Key words : *Andrographis echioides*, Seed germination, Micropropagation, MS medium

INTRODUCTION

Andrographis echioides (L.) Nees (Gopuram thanki) is one of the important medicinal plant species belonging to the family Acanthaceae. *Justicia echioides* L. and *Indoneesiella echioides* (L.) Sreemadh. are the synonyms of this plant. There are many species in the genus *Andrographis* of which *Andrographis alata*, *Andrographis echioides*, *Andrographis paniculata*, *Andrographis serpyllifolia* and *Andrographis wightiana* are gaining importance. In the Indian Systems of Medicine, predominantly *Andrographis echioides* is used against blood cancer. The leaf extract is recommended for oral consumption. The plant is known by various vernacular names *viz.*, Kalu kariyatu (Gujarathi), Birbhubat (Hindi), Banchimani (Marathi), Gopuramthanki (Malayalam and Tamil) and False water willow (English). The plant is common in all the dry districts of Tamil Nadu. Traditionally, the plant has been used as febrifuge, bitter tonic, astringent, anodyne and also for dysentery, cholera and diabetes. The ethanol extract of this plant used as diuretic and in sluggishness of liver and jaundice has been reported as the modern use of this plant. The chemical constituents of this plant are echioidin and echioidinin (Guhabakshi *et al*., 1999). The availability of seeds of this Andrographis species is very limited. The erratic germination behaviour of these seeds is also another factor which limits the multiplication of such species under field conditions. The research works on direct regeneration are also very meagre in this plant. Hence, the present research works on evaluating the seed germination under open and *in vitro* conditions and also to standardize the techniques for *in vitro* culture of *Andrographis echioides* (L.) Nees has been conducted.

MATERIALS AND METHODS

The present investigation was carried out during 2005 – 2007 at Medicinal Plants unit, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore.

Seed germination :

The seeds of local type were used for the experiment. The experiment was laid out in Completely Randomized Design with six treatments with four replications comprising of open and *in vitro* conditions. For evaluating under open condition, a raised nursery bed of 2 x 1 m size was made with a fine tilth of soil. Matured seeds of *Andrographis echioides* were collected, cleaned and mixed with 10 parts of sand. After line sowing the seeds, a fine layer of sand was spread over the seed and mulched with paddy straw or dried grass. Water was sprinkled over the mulch using a rose can until seed germination.

For evaluating under *in vitro* condition, seeds were sterilized with ethyl alcohol (70%) for 25 seconds and were rinsed with 0.1 per cent mercuric chloride for 3 minutes. The treated seeds were then taken to laminar airflow chamber and washed for four to five times with

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sterile distilled water to make them free from sterilants (George and Sherrington, 1984). Before inoculation, the seeds were placed on sterilized filter paper in a sterilized Petridish to remove the excess moisture present on the surface of the seeds. The surface sterilized seeds were inoculated on test tubes containing full strength semisolid MS media alone or with growth regulator BAP (1 or 2 mg l^{-1}) or NAA (1 or 2 mg l^{-1}). After covering with kiln film, they were maintained under light conditions until seedling emergence. The observations on germination percentage, number of days taken for germination and survival percentage were recorded.

Micropropagation :

For micropropagation, the explants of shoot tips (1.5 – 2 cm), nodal segments (2 – 2.5 cm), leaf bits (0.5 – 1.0 cm^2), root bits (1.0 – 2.0 cm) and stem bits (1 – 1.5 cm) were collected from healthy mother plants and trimmed off to required sizes with a sterilized knife before inoculation. The explants were rinsed with liquid detergent for five minutes and then rinsed with distilled water for three to four times. Prior to inoculation, explants were sterilized with ethyl alcohol (70%) for 25 seconds and were rinsed with 0.1 per cent mercuric chloride for different durations (2-6 minutes), depending upon the type and physiological status of the explants.

The nutrient media chosen for the study was MS medium (Murashige and Skoog, 1962). For shoot formation, the explants were cultured in MS basal medium alone and in combination with BAP (0.5 to 4.0 mgl^{-1}). For multiple shoot production, the explants were inoculated in MS basal medium supplemented with BAP (1.0 – 5.0 mgl^{-1}). The shoot elongation was tried in MS basal medium alone and with BAP (1.0 – 4.0 mgl^{-1}) and GA_3 (0.5 – 1.0 mgl^{-1}) combinations. The individual microshoots obtained from the shoot induction media were transferred to ½ MS basal medium (Control), IAA (0.5 to 1.0 mgl^{-1}), IBA (0.5 to 1.0 mgl^{-1}) and their combinations for root induction. Activated charcoal (200 mgl^{-1}) was added to all the treatment combinations. The plantlets with well developed shoots and roots were transferred to hardening media containing Vermiculite, Red earth, Sand and their combination.

RESULTS AND DISCUSSION

The results obtained from the present investigation are summarized below :

Seed germination :

The seeds sown under open condition were found to exhibit lowest germination percentage of 13.33 per cent (Table 1). The germination percentage was recorded maximum (67.10 %) in the treatment T_3 containing MS medium + BAP (1 mgl^{-1}). The same treatment also recorded the earliest seed germination (8.67 days) and maximum survival percentage of seedlings (79.93 %). The seedlings raised under open condition recorded 70 per cent survival percentage.

The enhanced seed germination through *in vitro* culture was found useful in securing seedlings when seeds are limited and the germinated seeds are a good source of explant material for subsequent mass micropropagation. Gubisov and Klcov (2000) stated that seed germination ability was not only affected by the plant species, but the physiological state of seeds also plays a significant role in this process. Controlled germination regimes and *in vitro* culture methods often improve germination activity. According to Nikolic *et al*. (2006), culture of seeds on cytokinin-containing media would help for rapid production of a large number of uniform regenerants.

Explant standardization and shoot induction:

The explants like shoot tips, nodal segments, stem bits, leaf bits and root bits were sourced from the stock plant that were maintained for the purpose of micropropagation. Among the various explants used, shoot tips gave significantly highest response (85.72 %) followed

Table 1 : Effect of culture environments on seed germination in *Andrographis echioides* (L.) Nees

Treatments	Germination (%)	Days taken for germination	Survival (%)
T_1 – Open condition	13.33	15.00	70.00
T_2 – MS basal	41.27	14.67	57.20
T_3 – MS + BAP 1 mgl^{-1}	67.10	8.67	79.93
T_4 – MS + BAP 2 mgl^{-1}	60.47	10.33	66.36
T_5 – MS + NAA 1 mgl^{-1}	54.35	10.67	63.63
T_6 – MS + NAA 2 mgl^{-1}	53.51	11.55	67.34
Mean	48.34	11.82	67.41
S.E. $\pm$	0.840	0.589	1.107
C.D. (P=0.05)	1.831	1.284	2.411

by nodal segment (70.63 %). Explants that have a rudimentary or organized structure (shoot tip, nodal segment) are the most responsive and pose the least problems in respect of an organized structure which it will suffice to reveal in an appropriate medium (Haripriya, 2003). This response might be also due to the higher meristematic activity (Suryanarmada, 2000). The endogenous auxin content in both these explants was high, which promotes cell division and thereby good regeneration.

In the present study, the best response to direct shoot regeneration from shoot tips was observed on MS medium supplemented with BAP (2.5 mgl^{-1}) (Table 2). The response to shoot induction decreased as the concentration of BAP increased. The decrease in shoot production at higher concentration of BAP may be due to the inhibition of shoot initiation or induction of calli. The advantage of direct organogenesis helps to retain clonal fidelity (Broertjes and Keen, 1980) than that of shoot production through callus.

Multiple shoot induction :

The multiple shoots were observed more in media composition containing BAP (3.0 mgl^{-1}) (Table 2). Days taken for multiple shoot induction were lower and number of multiple shoots was higher at higher concentration of BAP. However, shoot length was not correlated with shoot proliferation at higher concentration of BAP.

Table 2 : Effect of BAP on shoot induction and proliferation from shoot tip explants in *Andrographis echioides* (L.) Nees

Treatments	BAP (mgl^{-1})	Shoot induction (%)	Multiple shoot induction (%)
T_1	MS basal	0.00 (0.64)	0.00 (0.64)
T_2	0.5	20.21 (26.70)	-
T_3	1.0	37.45 (37.73)	0.00 (0.64)
T_4	1.5	61.65 (51.76)	-
T_5	2.0	63.67 (52.96)	65.12 (53.82)
T_6	2.5	83.22 (66.10)	-
T_7	3.0	74.56 (59.81)	78.62 (62.52)
T_8	3.5	67.18 (55.10)	-
T_9	4.0	43.33 (41.16)	67.64 (55.35)
T_{10}	4.5	-	-
T_{11}	5.0	-	60.33 (50.97)
Mean		50.14 (43.55)	45.29 (37.32)
S.E. $\pm$		2.431	1.448
C.D. (P=0.05)		5.107	3.155
C.D. (P=0.01)		6.998	4.423

Values in parentheses are arcsine-transformed

Shoot elongation :

The effect of GA_3 in combination with BAP was studied for shoot elongation in which longest shoots were produced in BAP (2.0 mgl^{-1}) + GA_3 (1.0 mgl^{-1}) treatment in 8.97 days. GA_3 stimulates cell elongation and cell wall plasticity. Cell division was stimulated in the shoot apex especially in the more basal meristematic cells, from which develops the long files of cortex and pith cells. GA_3 treatment helps in both the transport of potassium ions and increase in the number of mitotic figures throughout the meristematic zone (Sachs, 1965). They also promote cell growth because they increase hydrolysis of starch and sucrose into glucose and fructose molecules (Salisbury and Ross, 1986).

Rooting :

The media composition containing ½ MS + IAA 0.5 mgl^{-1} + IBA 1.0 mgl^{-1} gave highest (94.85 %) and earliest (11.45 days) rooting. The same treatment composition was found better for producing longer (3.73 cm) and more number of roots (10.75) (Table 3). A mixture of more than one auxin can particularly be effective for root induction, since auxin was implicated in vascular differentiation (George and Sherrington, 1984).

Hardening :

The treatment combination of vermiculite + red earth + sand performed better in hardening of *in vitro* derived plantlets with least mortality and highest survival percentages (29.72 % and 60.38 %, respectively) (Table 4). Low survival percentage in hardening media containing vermiculite alone might be due to high water holding capacity of the media (Saraswathi, 2006). The natural habitat of *Andrographis echioides* was observed in the dry tracts. Hence, a potting mixture with high water holding capacity was found unsuitable for survival of the *in vitro* derived plantlet.

Hence, it may be concluded that *Andrographis echioides* seeds can be effectively germinated under *in vitro* condition in MS medium supplemented with BAP (1.0 mgl^{-1}) and can be used as a source material for further propagation and research trials. Among the various explants, shoot tips responded positively for shoot induction. MS medium fortified with BAP (2.5 mgl^{-1}) was found highly responsive for shoot induction. The multiple shoot induction was achieved in MS medium + BAP (3.0 mgl^{-1}) and for shoot elongation, BAP (2.0 mgl^{-1}) + GA_3 (1.0 mgl^{-1}) was found better. Rooting was best (94.85 %) in ½ MS + IAA 0.5 mgl^{-1} + IBA 1.0 mgl^{-1}. Pot mixture containing vermiculite + red earth + sand (1:1:1) was found optimum for hardening.

Table 3 : Effect of growth regulators on rooting percentage and days taken for rooting in *Andrographis echioides* (L.) Nees

Treatments	Growth regulators (mgl^{-1})		Rooting (%)	Days taken for rooting	Number of roots / plant	Root length (cm)
	IAA	IBA				
T_1	½ MS basal	-	0.00 (0.64)	0.00 (0.64)	0.00 (0.64)	0.00 (0.64)
T_2	0.5	-	20.50 (26.92)	3.85 (11.31)	3.85 (11.31)	1.50 (7.03)
T_3	1.0	-	46.43 (42.95)	4.45 (12.17)	4.45 (12.17)	1.98 (8.09)
T_4	-	0.5	70.37 (57.04)	6.45 (14.71)	6.45 (14.71)	2.10 (8.33)
T_5	-	1.0	88.55 (70.37)	6.90 (15.22)	6.90 (15.22)	2.70 (9.45)
T_6	0.5	0.5	90.50 (72.26)	8.02 (16.44)	8.02 (16.44)	3.07 (10.08)
T_7	0.5	1.0	94.85 (77.60)	10.75 (19.13)	10.75 (19.13)	3.73 (11.13)
T_8	1.0	0.5	88.52 (70.34)	7.05 (15.39)	7.05 (15.39)	2.70 (9.45)
T_9	1.0	1.0	90.83 (72.60)	13.05	8.43 (16.87)	3.45 (10.70)
Mean			65.62 (54.52)	16.05	6.21 (13.54)	2.36 (8.32)
S.E. ±			2.448	0.551	0.425	0.292
C.D. (P=0.05)			5.144	1.157	0.893	0.613
C.D. (P=0.01)			7.049	1.586	1.223	0.840

Values in parentheses are arcsine-transformed.

Table 4 : Effect of pot mixtures on hardening of *in vitro* derived plantlets in *Andrographis echioides* (L.)Nees

Treatments	Pot mixture	Survival (%)	Mortality (%)
T_1	Vermiculite	41.67	48.37
T_2	Red earth	50.50	42.00
T_3	Sand	52.01	38.06
T_4	Vermiculite + red earth + sand	60.38	29.72
Mean		51.14	39.54
S.E. ±		0.298	0.463
C.D. (P=0.05)		0.648	1.009
C.D. (P=0.01)		0.909	1.414

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