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Evaluation of seed germination in *Andrographis echinoides* (L.) under open and *in vitro* conditions

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Abstract

Andrographis echinoides (L.), Nees (Gopuram thanki) is one of the important medicinal plant which is given importance recently for its excellent medicinal properties. The erratic germination behaviour of *Andrographis* seeds is a great barrier in multiplication of such species under field conditions. Hence an experiment was laid out in Completely Randomized Design comprising of six treatments. Among these treatments, the earliest seed germination (8.67 days) was recorded in the treatment comprised of MS medium supplemented with BAP (1 mg l⁻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. Hence *Andrographis echinoides* seeds can be uniformly germinated for large scale cultivation and also can be used as a base for various research trials.

Keywords: Gopuram thanki – seed germination - MS medium - *in vitro* condition

Introduction

Andrographis echinoides (L.) Nees (Gopuram thanki) is one of the important medicinal plant species belonging to the family Acanthaceae. *Justicia echinoides* L. and *Indoneesiella echinoides* (L.) Sreemadh. are the synonyms of this plant. The plant is known by various vernacular names viz., Kalu kariyatu (Gujarathi), Birbhubat (Hindi), Banchimani (Marathi), Gopuramthangi (Malayalam and Tamil) and False water willow (English). The plant is common in all the dry districts of Tamil Nadu (Tadulingam *et al.*, 1985) [6]. The plant is an erect, annual herb, simple or slightly branched, growing up to a height of 20 to 60 cm. 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 echinoidin and echinoidinin (Guhabakshi *et al.*, 1999) [4]. It is also used as remedy for fever. The new compound of this plant is echinoidinin, characterized as 5, 2'-dihydroxy-7-methoxyflavone and its glucoside, echinoidin with melting point of about 261 °C (Govindachari *et al.*, 1965) [2]. 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. Hence, the field cultivation of this crop is not yet been undertaken. The research work on seed germination aspects under open and *in vitro* conditions is also very meager in this plant. Hence, the present experiment on evaluating the seed germination under open and *in vitro* conditions was carried out in *Andrographis echinoides* seeds.

Materials and Method

The present investigation was carried out during 2005 – 2006 at Medicinal Plants unit, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore. 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. The *in vitro* treatments were carried out with two different growth regulators at two different concentrations as follows.

T₁ – Open condition, T₂ – MS basal, T₃ – MS + BAP 1 mg l⁻¹, T₄ – MS + BAP 2 mg l⁻¹, T₅ – MS + NAA 1 mg l⁻¹ and T₆ – MS + NAA 2 mg l⁻¹

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For evaluating under open condition, a raised nursery bed of 2x1 m size was made with a fine tilth of soil. Matured seeds of *Andrographis echinoides* 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, the seeds were washed in running tap water followed by washing with Tween 20 emulsifier (two-three drops in 100 ml distilled water) and distilled water for 2-3 times. 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 sterile distilled water to make them free from sterilants (George and Sherrington, 1984) [1]. 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 with different growth regulators. 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.

Results and Discussion

Germination percentage

The germination percentage was recorded maximum (67.10%) in the treatment T₃ containing MS medium + BAP (1 mg l⁻¹) followed by T₄ fortified with 2 mg l⁻¹ of BAP (60.47%). The seeds sown under open condition were found to exhibit lowest germination percentage of 13.33 per cent (Table 1).

Days taken for germination

The earliest seed germination was found in the medium containing 1 mg l⁻¹ of BAP (8.67 days) (Table 1). Seeds under open condition and MS basal medium recorded delayed germination of seeds (15.00 and 14.67 days respectively).

Per cent Survival

Among all the treatments, survival percentage was highest in the treatment T₃ containing 1 mg l⁻¹ of BAP (79.93%) followed by seeds under open condition (70.00%). The lowest survival percentage of 57.20 per cent was recorded in MS basal medium (Table 1).

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. In this experiment, the performance of seeds inoculated in solid MS medium with BAP (1.0 mg l⁻¹) under *in vitro* condition for germination was higher when compared to the open condition. Gubisov and Klčov (2000) [3] 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. Nikolic *et al.* (2006) [5] concluded that culture of seeds on cytokinin-containing media would help for rapid production of a large number of uniform regenerants. Similar *in vitro* seed germination was attempted in *Acampe longifolia* (Kalita and Sharma, 2001) and *Trachyspermum ammi* (Seghal and Abbas, 1994) for initiation of tissue culture.

Table 1: Effect of culture environments on seed germination in *Andrographis echinoides* (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 mg l ⁻¹	67.10	8.67	79.93
T ₄ – MS + BAP 2 mg l ⁻¹	60.47	10.33	66.36
T ₅ – MS + NAA 1 mg l ⁻¹	54.35	10.67	63.63
T ₆ – MS + NAA 2 mg l ⁻¹	53.51	11.55	67.34
Mean	48.34	11.82	67.41
SEd	0.840	0.589	1.107
CD (0.05)	1.831	1.284	2.411

Fig 1: Effect of culture environments on seed germination in *Andrographis echinoides* (L.)

Fig 1: *In vitro* seed germination (BAP 1.0 mg l⁻¹) in *Andrographis echinoides* (L.) Nees.

Fig 2: Hardened plants of *Andrographis echinoides* (L.) Nees.

Conclusion

The *in vitro* seed germination proved better than open condition. The highest germination percentage (67.10%), earliest germination (8.67 days) and highest survival (79.93%) were recorded under *in vitro* condition in MS medium supplemented with BAP (1.0 mg l⁻¹). Hence, it may be concluded that *Andrographis echinoides* seeds can be effectively germinated under *in vitro* condition in MS medium supplemented with BAP (1.0 mg l⁻¹) and can be used as a source material for further propagation and research trials.

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