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IN VITRO GERMINATION AND DEVELOPMENT OF AESCHYNOMENE INDICA L.

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ABSTRACT

A procedure for *in vitro* culture of the economic important plant *Aeschynomene indica* L. is described. A factorial experiment evaluated the effects of media Murashige and Skoog's medium (MS) and Gamberg *et al*., (B_5) medium, temperatures (15° C and 20° C) presence or absence of light and plant growth regulators. Seed explants germinated in less than one week in culture and produced radicles. Optimal conditions for radical elongation were B_5 at 20° C in the presence of light and without plant growth regulators. Plants were elongated on the same medium without plant growth regulators and acclimation to green house conditions.

KEY WORDS : ***Aeschynomene indica*** **L., seed germination, radicle, Papilionaceae, *in vitro***

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INTRODUCTION

Aeschynomene indica L. is an erect slender herb with pithy stems in swamps and belongs to the family Papilionaceae. The plant is distributed in all districts and popularly known as 'nettithakkai'. The genus contains two species. The plant stem is used as fishing floats for making hats[1]. In traditional Cambodian medicine the young leaves and flowers are consumed in salad and used topically as poultice. The crushed young shoot and leaves are taken walls as an anti-haemorrhagic during labour. The potential use of it as a fast growing nitrogen source for wet-rice fields has only recently been noted. Since the late 1980s it has been widely used as a pre-rice mature crop on experimental stations and in extension demonstration trails[2].

Most of the farm forestry species are reported to exhibit some amount of dormancy which lead to delayed or staggered germination. Rapid uniform early and complete germination are pre-requisites for achieving good plants from the seeds. The seeds which are viable but in the state of some dormancy can be induced to germinate by various artificial treatments that break dormancy finally resulting in early and better germination[3].

In temperate regions, growth of the radicle ceases during periods of unfavorable conditions and resumes when favorable conditions which include warmer temperature (15° C and 20° C) [4,5] presence of light[6] and an uninterrupted period of high humidity[7] return.

Several members of Papilionaceae (Fabaceae) have been cultured in vitro and production of shoots, roots callus seedlings and seedlings establishment has been reported[8, 9,10,11,12]. Since *Aeschynomene indica* L. has not previously been grown in tissue culture the objectives of this study were to

- investigate whether growth *in vitro* was possible
- determine the optimal conditions for growth, germination and production of the early seedling stage and
- establishment of seedlings

The goal of this research is to produce callus and germinated seeds for *in vitro* screening of potential inhibit development of in nature population.

MATERIALS AND METHODS

Seed collection

Aeschynomene indica L. branches containing clusters of young fruit were collected by junior scientist Dyaku (UKG student) (**Fig. 1 a**) in December 2010 from J J Botanic Garden, Pudukkottai placed in plastic bags and stored in 4° C prior to seed extraction. Fruit were squeezed and seeds were collected onto a large piece of cotton cloth. Seeds were then transferred to petri dishes and left to air dry for 48 hours before storage at 4° C.

Figure – 1a

Preliminary investigations were done to examine why some seeds turned yellow when placed on tissue culture media (**Fig. 1 b**). The endosperm and embryo were checked for the presence of a red precipitate indicating viability. This showed that green seeds were viable and yellow seeds were not. After one month's storage seeds were pre-screened for viability as follows. Seeds were surface sterilized by washing for 30 minutes in running tape water

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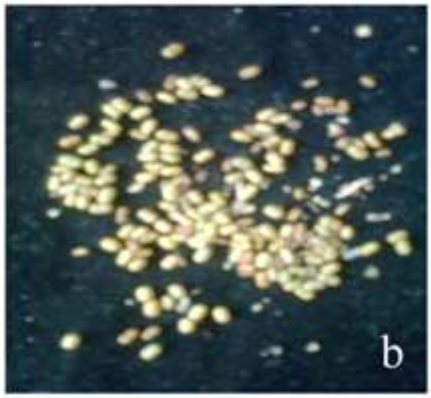

Figure – 1b

In a laminar flow hood seeds were rinsed three times in a sterile sieve with distilled de-ionized water and then transferred to sterile petri plates containing moistened What man # 1 filter paper and incubated at 4° C with 12 h photoperiod. Green seeds were left to air-dry in the laminar flow hood (about 2 h) and then sealed within petri plates and stored in the dark at 4° C until needed for tissue culture experiments.

Media

Preliminary experiments using different media Murashige and Skoog[13] (Murashige and Skoog's, 1962) and B_5 medium[14] (Gamberg *et al*., 1985) were superior for growth of callus and seed germination activity.

Experimental design

Viable seeds were aseptically transferred to the tissue culture media. Cultures were maintained at 15° C to 20° C under both light and dark conditions in a factorial experiment. There were 30 seeds per treatment for MS (6 replicates with 15 seeds per plate) and 10 seeds per treatment for B_5 (3 replicates with 15 seeds per plate).

Statistical Analysis

Quantitative data were analyzed with three General Linear Model procedure[15] to test for effects of media, temperature, light, 2 4-D and BAP on radical length.

RESULTS AND DISCUSSION

Aeschynomene indica L. seeds germinates within the first week after plating and slow growing radicles reached a length of 0.5 -12 cm after 20 days (**Fig. 1 c & d**) on both types of media all culture conditions and all plant growth regulator combinations and continued to elongate beyond seven weeks. Germination in nature requires the presence of water[16] which is readily available in tissue culture media[17]. *A. indica* germinated more quickly one week compared to *A. aspera* (**Fig. 1 e**).

Radicle length was significantly affected ($p = 0.001$) by medium temperature light 2 4-D and BAP during the first 2 months significantly ($p = 0.02$) longer radicles were produced on B_5 medium (10.5 cm) compared to MS medium (7.2 cm). Temperature also had significant $p = 0.02$ effect with 20° C being better than 15° C for production of longest

radicles (8.6 to 10.7 cm respectively). Light increased radicle length in both media and all combinations of plant growth regulators in 2 months (**Fig. 1 f**). The highest 2 4-D and BAP concentrations reduced radical growth (**Table 1**). In plates without 2 4-D radicles were significantly longer than with 1 mg/l 2 4-D. This is consistent with the function of cytokinin as a root inhibitor[18].

Table 1
Influence of plant growth regulators on average radicle length after 4 months

BAP (mg/l)	24-D (mg/l)			
	0	**0.5**	**1.0**	**2.0**
0	7.6 ± 0.02	4.6 ± 0.02	3.2 ± 0.02	0.92 ± 0.05
0.5	5.9 ± 0.04	4.1 ± 0.03	4.5 ± 0.12	1.7 ± 0.04
1.0	6.9 ± 0.01	**10.7** ± 0.02	6.9 ± 0.0.5	3.4 ± 0.02
2.0	5.5 ± 0.0	2.3 ± 0.02	5.2 ± 0.02	2.8 ± 0.03
3.0	3.2 ± 0.01	3.5 ± 0.01	4.2 ± 0.01	1.5 ± 0.04

Mean ± standard error Significant differences at p = 0.05 by Dunn's method

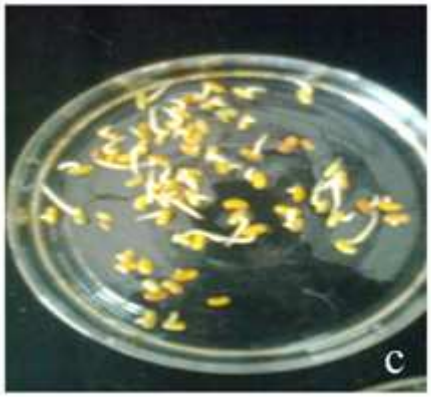

Figure – 1c

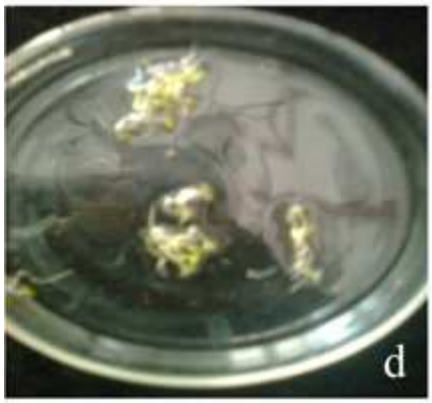

Figure – 1d

Figure – 1e

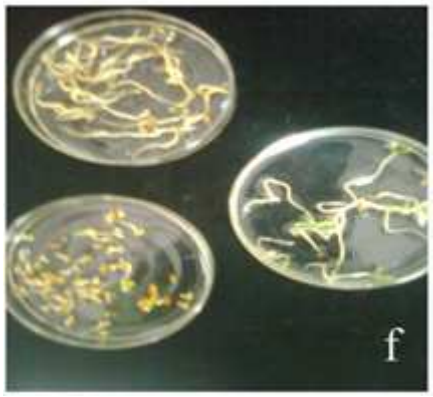

Figure – 1f

After emerging from the seed during germination, the radicles either grew horizontally along the surface of the medium (91% of the seeds). About 5% of the radicles formed tight or loose curls which curled over or around the seed (**Fig. 1 g**).

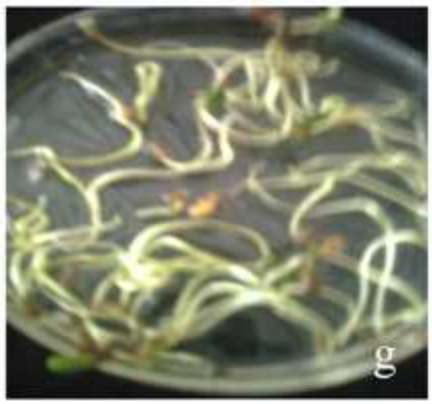

Figure – 1g

Callus development

Callus started to develop after about 2 weeks in culture from radicles (**Fig. 1 d)** and continued to be produced for the duration of the experiment. The quantity of Callus production was less and this was influenced by the difference in media, light and plant growth hormones. Callus growth was very slow after 4-6 weeks in culture on B_5 medium

at 20˚C in the dark, there were a few seeds in which callus arose from endosperm tissue.

Acclimatization and field culture

Plantlets with fully expanded leaflets and well developed roots were successfully transferred to soilrite and hardened off (**Fig. 1 h**) in a growth chamber for 4 weeks. All the germinated seedlings survived and grew normally following transfer to soil during monsoon rains. There was no detectable variation among potted plants with respect to morphological and growth characteristics.

The protocol reported could be used for conservation and large scale propagation of this medicinal economic plant which is restricted to tropical climates.

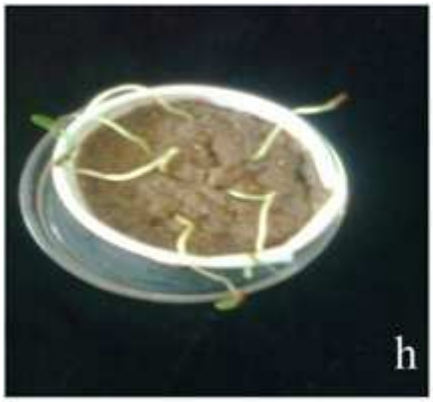

Figure – 1h

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