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In vitro seed germination of *Adansonia digitata* L.: An endangered medicinal tree

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ABSTRACT

Adansonia digitata is an important arboreal species which is being threatened of going into extinction. In order to preserve this genetic resource of great economic and medicinal value, studies on germination were carried out and *in vitro* regeneration of this multipurpose tree species through Tissue culture techniques is adopted. The germination of seeds under natural conditions is a limiting factor for plant regeneration. To mitigate this problem, an efficient protocol was developed for *in vitro* micropropagation via seed germination of *Adansonia digitata.* Mature and immature seed explants from the mother tree were used for the plant regeneration.

Key words: *Adansonia digitata,* germination, genetic resource, extinction, Tissue culture.

INTRODUCTION

Adansonia digitata L. belongs to the Bombacaceae family and is generally known as the African Baobab. It is a massive, deciduous tree upto 25m in height and may live for hundreds of years. It has thick, angular, wide spreading branches and a short, stout trunk which attains 10 – 14m or more in girth and often becomes deeply fluted. The form of the trunk varies. In young trees it is conical, in mature trees, it may be cylindrical, bottle shaped or tapering with branching near the base[13]. Almost all parts of *A. digitata* are used as medicines and also possess high nutritional value. Leaves, bark and fruits of this plant are traditionally employed as food stuffs and for medicinal purposes, and for that reason baobab is also named "the small pharmacy or chemist

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tree"[4,7]. Despite its potential, which is well recognized, very little is known about its genetic diversity[5]. The baobabs have not been subjected to well known horticultural propagation techniques[8]. Currently the tree is suffering from drought and desertifica-tion and fear has been expressed about its rege-neration[11]. *Adansonia digitata* absorbs huge quantities of carbon dioxide from the atmosphere and is resistant to forest fires. The tree at present is facing a crisis of survival and is enlisted as an endangered species in the Red data book with only 30 to 40 trees available in India[6].

Baobab seeds have very hard seed coats and germination[2] is usually less than 20%. Dormancy in seeds of *Adansonia digitata* can be attributed partly to the testa and partly to the pulp. Several methods, such as wet heat treatment, total or partial seed decoating and scarification of seeds with concentrated acids, herbicides, fungicides and growth regulators, were tested in 1988 and it was found that seeds treated with herbicides and fungicides did not germinate[3]. Natural regeneration of Baobab is poor because of browsing animals and uncontrolled bush fires. Therefore, vegetative propagation is reported to be advantageous[10]. Both the depth of planting and soil type affected seed germination and seedling performance of *Adansonia digitata in vivo*[1]. The only effective pretreatment is to crack the seed coat, but this can damage the seed[12].

It is further reported that germination is poor and the seed coat can be easily damaged. The germination period is extremely variable, between 3 weeks and 6 months. Seeds may take up to a year to germinate in the pot, but should germinate well in the nursery where adequate moisture can be provided regularly. In the wild, seeds are thought to germinate only in exceptionally good rainy seasons. Attempts to propagate it vegetatively are reported to have failed, and planting by seed may be the only means of propagation. Seeds apparently keep their viability for years if stored in a cool dry place[9]. Despite the immense importance of this plant, not much is known about its *in vitro* propagation which is very crucial for seedling establishment and subsequent development of the plants.

Propagating plants through tissue culture techniques is an established area of research with huge scope. There is a substantial pau city of available data especially in case of perennial plants, where flowering and fruiting takes a very long time and propagation through seeds involves extended dormang period. In such cases, tissue culture gives a ray of hope for quick propagation of species. Also, there is an increased felt need to alter economically important plants to make them survive under changed environmental conditions. If this is delayed, there is a risk of losing

Fig. 1. *In vitro* seed germination of *Adansonia digitata:* A: Seed coat removal and establishment on MS medium B: Seed germination with plumule formation C: Shoot induction and root formation D: *In vitro* regenerated seedling

few commercially viable plant species which will become extinct due to unfavourable climate and unconducive conditions. The study was undertaken to develop a efficient *in vitro* seed germination protocol and examine the seedling development in mature and immature seeds of this endangered plant species.

MATERIAL AND METHODS

Plant material:

The immature and mature oblong, pendulous fruits of *Adansonia digitata* were harvested from the mother tree located in the centre of the city after every 15, 30,45,60,75 days from the beginning of the fruiting season. The pulp was manually separated from the seeds using a knife.

Seed pretreatments:

The seeds were isolated and immediately washed with tap water, divided into 5 groups (5 seeds each) *i.e* S1, S2, S3, S4 and S5.

S1 = 1 day old seeds, no pre-treatment
S2 = 30 day old seed, no pre-treatment
S3 = 45 day old seed, no pre-treatment
S4 = 60 day old seed, no pre-treatment
S5 = 75 day old seed, soaked in conc. HNO_3 for 24 hrs, Soaked in tap water for 1-10 days.

Media and culture conditions:

The basal nutrient medium consisted of Murashige and Skoog, 1962 medium with vitamins, 3% sucrose and 0.8% agar. Murashige and Skoog half strength medium was also used.The medium was adjusted to the desired pH 5.8 using HCl or NaOH, was heated until the solution was clear and then dispensed into culture vessels before autoclaving. The medium was sterilized in an autoclave at 121°C for 20 min.

The cultures were maintained in a culture room at 25 ± 2°C under 16 hour cool white fluorescent light at 40 μmol m-2s-1.

Surface disinfection and sterilization:

The seeds were washed with tween-20 for 10 minutes. These were rinsed with double distilled water 3-4 times. The seeds were surface sterilized with 0.1% $HgCl_2$ for 1-10 min, finally rinsed with sterilized double distilled water 3-4 times to remove the traces of sterilizing agent.

Inoculation and incubation:

After the seed pre-treatment and surface sterilization, the seeds were aseptically grown in presterilized jam bottles and test tubes for germination. These were incubated in dark for 1 week at 25 ± 2°C and light of 2000 flux intensity was provded.

Culture:

Two month old seedlings obtained *in vitro* were directly used for other tissue culture experiments without any sterilization. The seedlings were cut into several parts (cotyledonary node, epicotyl, hypocotyl, cotyledonary leaf and roots) and these were aseptically transferred in MS medium with different BAP concentrations.

RESULTS AND DISCUSSION

Seed germination :

In the 15 day old seeds, only swelling of the seeds was observed and no germination occurred. Thirty to Forty five days old seeds showed remarkable sign of growth in 10 days. In these, 80-90% seed germination occurred in both media (MS Half and MS Full). When 60 day old seeds were inoculated, no seed germination was observed even after 1 month. Therefore, S5 group (75 day old seeds) were given conc. HNO_3 treatment for 24 hrs. followed by soaking in tap water for 1-10 days, for the softening of their seed coat. But, the seeds failed to germinate on both MS Half and MS Full medium (Table-1).

The seed germination was significantly affected by the age of fruits/seeds collected during the study as well as the seed pre-treatments. The immature and mature seeds were treated with 0.1%$HgCl_2$ for 1-10 minues and were subcultured after 20 days on a fresh medium. These were maintained for 2 months but did not show any sign of growth. Hence, it was concluded that mature seeds failed to germinate.

Table-1. Showing of seed germination and ludling height of seeds collected from various triut ages and under MS full & MS half culture media

S.No.	Age of frui (days)	Medium	%Seed germination	heght of Seedlings (cm)
1	S1	MS Full	Nil	_
		MS half	Nil	_
2	S2	MS Full	85%	4.5_
		MS Half	90%	3.5_
3.	S3	MS Full	80%	4.0_
		MS Half	85%	3.0_
4.	S4	MS Full	Nil	_
		MS Half	Nil	_
5.	S5	MS Full	Nil	_
		MS Half	Nil	_

Adansonia digitata endosperm cultures showed best response when immature seeds from 30-45 days fruits were used as explants. After 15 days of germination, germinated seeds showed cotyledonary growth and long hypocotyl area having thick roots. After 1 month, seedlings were observed to have grown 4-5 cm in height with big green cotyledonary leaf. Formation of pointed apical leaf was also observed. Fully grown 2 month seedlings showed apical and axillary nodes and were used for further experiments for the initiation and multiplication of shoots (Fig. 1).

This technique might be the first step towards the biotechnological application of this endangered species.

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