IN VITRO SEED GERMINATION OF AN ENDANGERED PLANT SPECIES OF JHARKHAND:-*ADANSONIA DIGITATA* L.

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ABSTRACT

Adansonia digita L. (African Baobab) is a rare species, highly endangered medicinal tree found in the land of Jharkhand. To mitigate the problem of its survivability and to conserve its rich genetic resources tissue culture technique was adopted. For it mature and immature seeds from gourd like fruits were collected from the site and were soaked in water for three to four days, then first scarification and total and partial decoating methods were employed, explants were sterilized and inoculated to freshly prepared Murashige and Skoog Medium (MS 1962) with no hormonal supplementation. The seeds were categorized under six groups based on the age of seeds used for the inoculation. The S1 (one day old seeds), S2 (10days old seeds), S3 (20 days old seeds) and S6 (50 days old seeds) did not respond and failed to germinate. In group S2 and S3 swelling of seeds were observed. The result obtained in S4 (30 days old seeds) and in S5 (40 days old seeds) showed sign of seed germination. Here within two weeks seedlings achieved the height of 5 to 6cm.

KEYWORDS: *Adansonia digitata*, endangered, genetic resources, tissue culture.

INTRODUCTION

Adansonia digitata L. (African Baobab) belongs to the Bombacaceae family is a rare and highly endangered medicinal tree present near the busy road of Doranda College, Ranchi, Jharkhand. The generic name of it is to commemorate Michel Adanson (1726-1806), the French naturalist and explorer and the species name '*digitata*' means hand-like which represent to the shape of leaves. The common name of it are 'dead rat tree', 'monkey bread tree', 'upside-down tree' and 'cream of tartar tree'. Locally it is known by several names 'kalpvriksha','kalptaru' and 'lumkhatai' (http:en.m.wikipedia.org). It was introduced here by Muslim traders from tropical Africa (Nitish Priyadarshi, 2008). At present three rare plant species are of over 300years old (The Telegraph, 2008). Due to heavy traffics, heavy vehicles and other anthropogenic activities made *Adansonia digitata* to the borderline of extinction. It grows up to 25 meters tall (Priyadarshi, N.
2008 and Singh et al, 2010). The girth of trunk is 10-14m (Singh et al, 2010) or more; looks massive in size and appears to be large bottle shaped. In young tree it is conical and in mature tree it is cylindrical, bottle shaped or tapering with branching near the base (Yusha et al. 2010; Singh et al. 2010). As stated by Von Breitenbach (1985) the development of *Adansonia digitata* can be distinguished under four growth phases; the sapling phase (10-15 years), cone phase (up to 60-70 years), bottle phase (up to 200 -300 years) and last is an old age phase (up to

500- 800 years). The study assures the age of *Adansonia digitata* possessing bottle shaped trunk near to the busy road of Doranda to be over 300 years old. It store water about 400 gallons in medium sized tree and over 2000 gallons water in large size trees (Orwa et al., 2009). It is a deciduous tree and when the leaves are shed in dry season it looks strange and weird as some devil is standing with arms open. The leaves are palamately compound; an important protein sources (Nordeide et al., 1996); rich in Vitamin C; young leaves are used as soup vegetable in West Africa (Orwa et al, 2009) or cooked and eaten as spinach (Venter et al., 1996). When dry season gets over the tree blooms with pendulous, showy and white flowers (www.flowersofindia.net).The baobab flowers are pollinated by bats, insects and wind as stated by FAO, 1988. The fruits are pendant and looks like gourd hanging downwards; the capsulated fruit contains numerous hard and brownish seeds embedded in a yellowish white, floury acidic pulp (Gebaurer et al.,2002).The white pulp of it is edible which is powdered and used as a refreshing drink. It is very rich in Vitamin C and B2; used in milk to make fermented flavoured porridge (Orwa et al, 2009). It is used effectively in treating bronchial asthma, dermatitis, sickle cell anemia, as a diuretic, anti-diabetic, antioxidant, antipyretic, analgesics, antiviral and antimicrobial, anti-trypanosoma, as a laxative properties (Donaties et al.,2011;Singh et al,.2013).. All parts of it are used as foodstuff and in medicinal purposes hence also named as "the small pharmacy or chemist tree" (Kerharo et al.,(1974); Etkin et al.,(1982);Singh et al.,(2010). Currently this rare species needs conservation as it is enlisted endangered species in the Red data book with only 30 to 40 trees available in India (Johri et al., 2008; Singh et al., 2010). The seeds of baobab possesses very hard seed coat and if we talk about germination is generally less than 20% (Danthu et al., 1995). Dormancy of Baobab seeds can be attributed partly to testa and partly to the pulp and in nature the dormancy of seed is broken by the passage through the digestive tract of large mammals and in cultivation by immersing the seeds in hot water or by cutting the seed coat (Esenowo,1991).The optimal pretreatment methods of keeping seeds for scarification in acid for 6-12 hours overcome inhibitory action of seed coat (Danthu et al.,1995).Several methods were employed and tested in 1988 to break the dormancy of seeds by wet heat treatment, total or partial seed decoating and scarification with concentrated acids, herbicides and growth regulators, and found that treatment with herbicides and fungicides did not germinate as stated by Esenowo et al., (1991). Natural regeneration of *Adansonia digitata* seeds found to be poor and through vegetative propagation reported to be advantageous (Sidibe et al., 2002).Both depth of sowing and type of soil affected seed germination (Chia et al., 2008).The inhibitory factor in germination is impermeability of seed coat to water (Falemara et al.,2013).To facilitate the uptake of water and oxygen into seeds before planting is to go for the pretreatment as done by (Teel et al., 1984) cracking the seed coat but can damage the seed. *Adansonia digitata* gifted with both numerous medicinal and non-medicinal uses in Africa (Von Maydell, 1990; Wyk et al.,2000).Every part of the Baobab tree is reported to be useful(Owen, 1970). Despite its immense importance not much is known about its in vitro propagation. The only known are in vitro regeneration via seed culture carried out by Katsuaki and Sie (2007), Singh et al.,(2010) and from different explants of seedling by N' Doye et al, (2012). Due to prolong dormancy observed and being economically, rare and endangered important plants there is an immediate need to propagate through the involvement of tissue culture techniques. The present study was undertaken is to break the dormancy of seeds by manual scarification and by total and partial decoating pretreatment methods and to check the development of seedlings obtained from immature and mature seeds of the three rare plant species in Jharkhand.

MATERIAL AND METHODS

Plant material and explants sterilization:

The immature and mature seeds of Adansonia digitata L. were harvested from gourd-like fruits from the busy road near to the Doranda College, Ranchi, Jharkhand, India. The seeds were separated from fruit pulp. As all normal farmers do before sowing the seeds check the viability of it by floatation method. The sound seeds sink down into the water and dead and defective seeds floats on the water. It is now categorized among six groups (5 seeds each) that is S1, S2, S3, S4, S5 and S6 with differences of 10 days each used for inoculation.

S1= 1 day old seeds, S2= 10 days old seeds, S3= 20 days old seeds, S4= 30 days old seeds, S5= 40 days old seeds and S6= 50 days old seeds. The methods adopted for in vitro propagation of *Adansonia digitata* seeds were similar to (Singh et al.,2010;Aziza M. Taj Aldin.,2015).

The seeds were manually scarified with sandpaper to make water permeable. Now the seeds were soaked in water for three to four days. To enhance the germination of seeds *in vitro* total and partial decoating method was employed. The seeds were washed thoroughly with running tap water for 10 minutes. Thereafter, the explants washed with 1% (v/v) solution of Savlon for 5minutes followed by Tween 20 detergent (2% v/v) for 10minutes and then soaked in 70% (v/v) ethanol for 30 seconds after each treatment the explants were rinsed three times with distilled water. Further, sterilization treatments were done under laminar air flow chamber. Here explants were sterilized with freshly prepared 0.1 (w/v) of mercuric chloride for 2-5minutes.These explants were then thoroughly washed three to four times with sterilized double distilled water to remove any traces of sterilizing agent(Murugan et al.,(2018).

Culture Media:

The Murashige and Skoog basal medium containing 3% (w/v) sucrose, 0.8% (w/v) agar without any hormonal supplementation used for in vitro seed germination. The pH was adjusted to 5.6 to 5.8 using hydrochloric acid and sodium hydroxide. It was heated until the solution was clear and transparent and then finally dispensed into pre sterilized culture tubes before autoclaving. The medium was sterilized in an autoclave at 121ºC for 30minutes.

Inoculation of explants:

After the explants pre treatment and surface sterilization were aspetically inoculated into culture tubes and kept under the light intensity of 100 lux, photoperiod 12hours and relative humidity 70-75%.

RESULT AND DISCUSSION

Seed germination:

In S1 group (one day old seeds) there was no sign of seed germination observed. In both S2 (10 days old seeds) and S3 (20 days old seeds) only swelling of seeds were observed which later on failed to germinate and started to decay. In both S4 (30 days old seeds) and S5 (40 days old

seeds) remarkably respond to culture medium and started to germinate within two weeks. In S6 (50 days old seeds) not even swelling of seeds were observed. In S4 and S5 seed groups *in vitro* plantlets attained the height of 5-7 cm. It showed cotyledonary growth with hypoctyl formation. The apical leaves and the roots hairs were observed in 30 days old seedlings. Later some seedlings parts were sub cultured to MS medium with different grades of auxins and cytokinin ratio for morphological responses and rest for hardening process.

Table 1:- Showing percentage of seed germination and height of seedling under different groups of seeds in MS culture medium

S. No.	Age of fruits (days)	Medium	Percentage of Seed germination	Height of Seedling (cm)
1.	S1	MS medium	Nil	-
2.	S2	MS medium	Nil	-
3.	S3	MS medium	Nil	-
4.	S4	MS medium	90%	5.0
5.	S5	MS medium	90%	5.3
6.	S6	MS medium	Nil	-

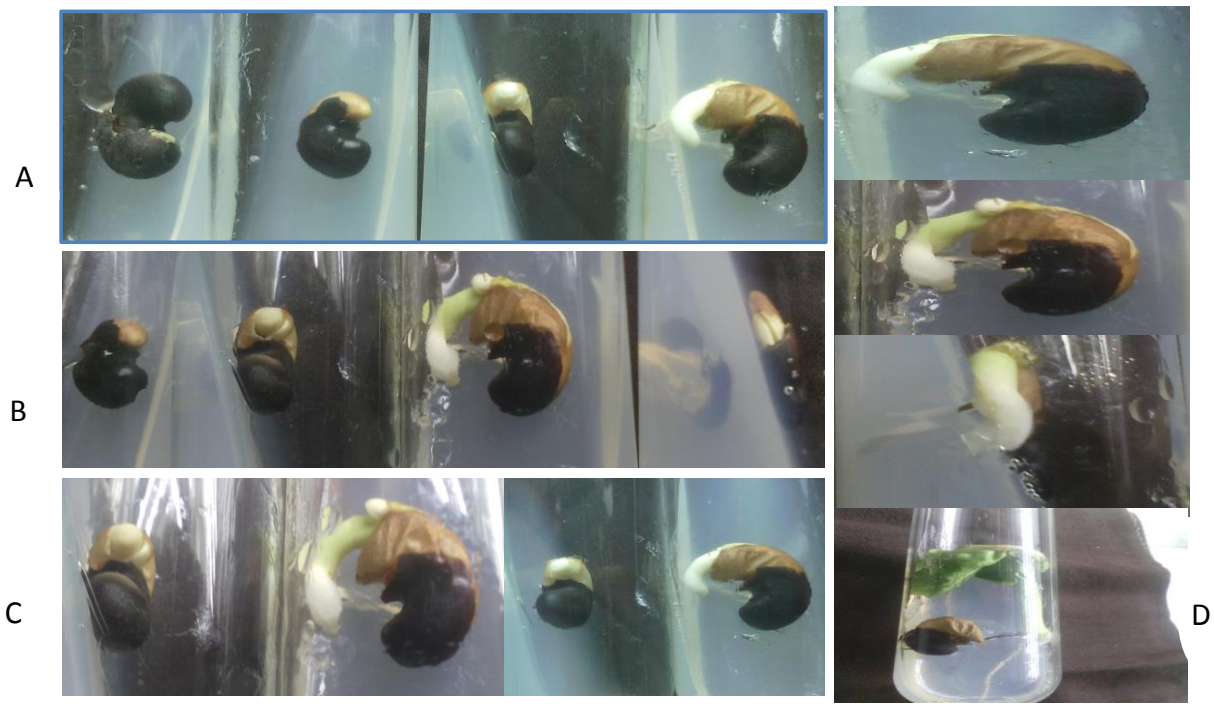

Fig 1- *In vitro* seed germination of *Adansonia digitata (MS media with no hormone) :* **A**:Response of seeds on 3th day. **B**: Response of seeds on 5th day **C**: Response of seeds on 7th day. **D**: Stages of germination of seed

CONCLUSION:

The present investigation reports an efficient protocol for in vitro seed germination by grading the seeds in accordance with the age of seeds and response of seeds in culture medium. The seeds of thirty to forty days old responded well. The seedlings germinated to a height of 5-7cm within two weeks. This rare and endangered species of *Adansonia digitata* possess immense biological properties could be conserved through biotechnological approaches.

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