IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 3, Issue 5 (Sep.- Oct. 2017), PP 45-52
www.iosrjournals.org

Effect of pre-treatment on dormancy and *in vitro* seed germination in globally endangered forest tree *Adansonia digitata L.*

Shama Nazrin, SamathaTalari, Mahitha Banala, Srikanth Kagithoju and Rama Swamy Nanna*

Plant Biotechnology Research Laboratory, Department of Biotechnology,
Kakatiya University, Warangal – 506009 (TS), India
*Corresponding Author: Rama Swamy Nanna**

***Abstract**: The study assessed the effect of three pre-treatment techniques and three different growth media for breaking seed dormancy and in vitro germination in Adansonia digitata L., a multi-purpose and globally endangered forest tree. The seeds were pretreated with three different techniques (mechanical, thermal and chemical treatments). After pre-treatment, the seeds were allowed to germinate on three different types of growth media (Potting soil-PS, Murashige and Skoog's -MS medium and Paper-boat - PB). It was observed that hot water (100^{o}C) treatment showed 88.33% of germination in potting soil. Whereas, the seeds treated with 98% H_2SO_4 for 24 hours showed 94.33% of germination with a reduced period of emergence on Paper-Boat method. The species, A. digitata seeds showed the least percentage of germination with mechanical nicking in two (PS, MS) types used. Seed germination was absent on PB method similar to control. Data analysis revealed the correlation between germination percentage, number of days for germination and seedling length were significant and positive. Thus, as a cost efficient method,treatment of seeds with conc. H_2SO_4 (98%) for 24hrs pre-treatment and germinating on paper-boat technique was recorded the best, followed by thermal treatment with 5min on PS medium.*

***Keywords:** Pre-treatment, Growth media, Seed dormancy, in vitro germination, Adansonia digitata.*

Date of Submission: 28-09-2017 Date of acceptance: 23-10-2017

I. Introduction

Many forest tree species face difficulty in seed germination due to seed dormancy or the presence of hard seed coat. This results in poor germination and poor seedling growth. There is an urgent need to overcome such problem and domesticate it. The species, *Adansonia digitata* L. Is one of such species which face the same problem, hence recorded as endangered.

The species *A. digitata* belongs to family, Bombacaceae. It is a deciduous tropical forest fruit tree originated from the African Savannah (woodland grassland ecosystem). It is commonly called as *Baobab* and has many common names like *dead-rat tree*, *monkey-bread tree*, *up-side-down tree*, *HathiyanJaad* (elephant tree - Urdu), *GorakhImbli* (monkey's tamarind – Marathi). It can grow up to a height of 5-23 meters and a circumference of about 25m.

Baobab tree has many medicinal properties and various plant parts are used for food (Sidibe and Williams, 2002; Christine Buchmann et al., 2010). Every part of the tree is used as a medicine as they are screened and found secondary metabolites(Samatha et al., 2015) Hence, it is also named as "*the small pharmacy or chemist tree*" (Kerharo and Adam, 1974; Etkin and Ross, 1982). Research findings show that the dried leaves contain 13-15% protein, 60-70% carbohydrate, 4-10% fats, around 11% fibre, and 16 % ash. The energy value varies from 1180-1900KJ/100g of which 80% is metabolisable energy (Gebauer et al.,2002).

Seeds are reported to be rich in amino acids and oils. The oils of baobab seeds are useful as alimentary oil. The parts of *Adansonia digitata* are used as immune-stimulant, analgesic, anti-inflammatory, insect repellent and pesticides. It is also used for treatment of diarrhea and dysentery (Ramadan, 1994;El-Rawy,1997). The leaves and bark are reported to possess free radical scavenging, cytotoxic, thrombolytic, anti-diarrheal, membrane stabilizing properties.

Fruits are used in normal human resistance against liver damage due to endemic causes (Al-Qarawi, A.A. et al., 2003). Besides this, traditionally they are used in Africa as a treatment against fevers, dysentery, bleeding wounds (Bosch et al., 2004), semi-fluid gum extracted fromthe bark is used in treating sores(Yushau et al., 2010), the leaves and dried powdered roots is given to malarial patients as a tonic, root bark contains beta-sitosterol and two glycosides. Hence, used in traditional medicine.

The seeds of *A. digitata* have very hard and impermeable seed coat that prevents the entry of water, nutrients and oxygen into the seed for its natural propagation. The seed dormancy is allocated partly by seed coat, zygotic embryo and partly by its pulp. Because of these barriers, natural germination and regeneration is very poor and the species has become critically endangered. In view of its medicinal value and it's on the verge of extinct, the present work is aimed to standardize a protocol for breaking dormancy and *in vitro* seed germination in *A. digitata.*

II. Material And Methods

Germplasm collection

The fruits were collected from the mother plant located at DodlaKousalyamma Women's (DKW) College, Nellore, Andhra Pradesh, India and the species was identified by Prof. Nanna Rama Swamy, Department of Biotechnology, Kakatiya University, Warangal, Telangana state. The seeds were isolated from the fruit and washed with tap water to remove the white pulp surrounding them. They were checked for viability using the flotation method. The floating ones were discarded as non-viable and the viable seeds were left to dry. These viable seeds were stored at ambient temperature (28^{o}C) until their use.

Seed pre-treatment experiments

The isolated seeds were washed with tap water (twice), tween-20(once) for 5 minutes, surface sterilized with 0.1% $HgCl_2$ for 7minutes followed by rinsing in sterile distilled water for 3-4 times to remove the traces of sterilizing agent. All these were carried out under laminar-air-flow chamber.

Mechanical nicking

The sterilized seeds were soaked for 24hrs in distilled water. Later the soaked seeds were nicked with a surgical blade before inoculation/planting on media.

Thermal treatment

100 seeds were immersed in boiling tap water at 100^{o}C and were allowed to boil at three different timings i.e., 2min, 5min and 7minutes. Later, they were immediately transferred into normal water, followed by transfer on to respective medium.

Chemical treatment

100 seeds were soaked in 98% conc. H_2SO_4 with utmost care at three different timings i.e., 2hrs, 6hrs and 24hrs. After H_2SO_4 soaking, the seeds were rinsed with tap water for 3-4 times to remove the traces of remnant of acid. Simultaneously, controls were also maintained.

Germination media

Potting soil (PS)

This organic potting soil supplied by Casa De Amor, Amazon is a combination of peat moss, compost, dolomite lime and vermiculite. After the pre-treatments, the seeds were planted in the sterilized potting soil.

MS medium

MS (Murashige and Skoog, 1962) medium without plant growth regulators (MSO) was used for *in vitro* seed germination. The medium was adjusted to pH 5.8 with either 0.1N HCl or 0.1N NaOH and solidified with 8gm/L Agar-agar. The treated seeds were blot dried on sterile tissue paper under laminar-air-flow cabinet and inoculated on MSO culture medium. The culture medium was sterilized at 121°C under 15lbs for 15-20 minutes. All the cultures were incubated at ± 25°C under 16/8 hr light/dark periods provided with light intensity of 40-50 µmol $m^{-2}s^{-1}$.

Paper-boat (PB) method

The culture tubes were filled with distilled water (07ml) and sterilized. These culture tubes containing sterilized water were inserted with sterilized paper-boat (made with Whatmann No.1 filter paper) under laminar-air-flow cabinet. Treated seeds were blot dried and were placed on the paper - boat containing sterilized distilled water.

Experimental design

A fully random design was set up with 3x3x3 factorial experiment which involves three seed pre-treatment techniques (mechanical nicking, thermal and chemical treatments); three growth media (PS, MS, PB) and three time intervals (2, 5, 7min-hot water treatment; 2, 6, 24hrs – acid treatment).

Data analysis

The data were collected on percentage of *in vitro* seed germination, number of days for germination and average length of seedling. The data were analyzed by using IBM SPSS statistics 20 software. The collected data were computed and subjected to Analysis of Variance (ANOVA). Duncan's Multiple Range Test ($P \leq 0.05$) is used for Mean Separation in order to determine the most suitable pre-treatment technique and growth media for *in vitro* seed germination in *A. digitata*.

III. Results And Discussion

Effect of pre-treatment techniques

As shown in Table-1, seeds without pre-treatment (control) didn't germinate on PB and showed least germination on MS and PS growth media (19.33% – MS, 24.33% – PS). This was followed by mechanical nicking.

The low germination percentage of hot water (thermal) treatment on MS medium and Paper boat gave an opposite result when compared with the same treatment on Potting soil, which yielded a good germination percentage (88.33±1.66). This implicated that hot water treatment of MS and Paper-boat growth media were not found suitable for *in vitro* seed germination in *A. digitata* (Fig-1). Seeds exposed to prolonged contact with hot water will kill the embryotic embryo (Amusa, 2011). Saikou et al (2008) opined that seeds exposed to hot water treatment for 10min will increase its growth potential in *Acacia Senegal* which is in contrast to our results.

Sterilized PS supported good seed germination in thermal treatment (5min) than MS medium and PB methods. According to Chia et al (2008), seeds were prone to decay if they are subjected to excess water as in the case of growth media or soils due to high water retention capacity; therefore they are not unlikely to germinate. This could explain why hot water treatment of MS and PB methods had lower performance in percentage of germination in *A. digitata*.

The seeds which underwent chemical (98% conc. H_2SO_4) treatment had a reduced dormancy period when compared to other pre-treatment techniques. It was also found that chemically treated seeds for 24hrs emerged earlier (7days) in paper-boat method compared to on MS medium which was 10days (Fig-2). The risk of contamination was also non-existent. To our findings, chemically treated seeds for more than 24hrs have damaged the embryo.

Table-1: Effect of different pre-treatment techniques and growth media on *in vitro* seed germination in *A. digitata.*

Pre-treatment techniques and Growth media			Percentage of germination (±SE)[a]	No. of days for germination (±SE)[a]	Average Length (cms) of seedling (±SE) [a]
Control		**PS**	19.66±0.33^{ef}	32.66±0.33^{a}	7.16±0.16^{bc}
		MS	19.33±0.66^{c}	31.00±1.15^{a}	7.53±0.29^{b}
		PB	0.00±0.00^{d}	0.00±0.00^{e}	0.00±0.00^{d}
Mechanical nicking		**PS**	24.33±0.66d^{e}	19.33±0.66^{c}	6.33±0.33^{c}
		MS	19.33±1.20^{c}	23.33±0.88^{b}	8.23±0.23^{b}
		PB	0.00±0.00^{d}	0.00±0.00^{e}	0.00±0.00^{d}
Thermal treatment (100°C)	**2min**	**PS**	18.66±0.66^{f}	22.33±0.33^{b}	8.23±0.23^{ab}
		MS	4.66±0.33^{e}	23.33±0.33^{b}	5.76±0.23^{d}
		PB	4.66±0.33^{c}	28.00±1.00^{a}	5.76±0.23^{b}
	5min	**PS**	**88.33±1.66^{a}**	**9.00±1.00^{f}**	**9.10±0.10^{a}**
		MS	28.66±0.66^{b}	23.00±1.52^{b}	5.26±0.37^{d}
		PB	14.33±0.66^{b}	22.66±1.45^{b}	7.00±0.57^{a}
	7min	**PS**	28.66±1.33^{d}	12.66±1.45^{e}	8.00±4.04^{ab}
		MS	9.33±0.66^{d}	23.33±0.88^{b}	6.66±0.33^{c}
		PB	0.00±0.00^{d}	0.00±0.00^{e}	0.00±0.00^{c}
Chemical treatment (acid)	**1hr**	**PS**	9.66±0.33^{g}	22.00±1.15^{bc}	8.70±1.52^{a}
		MS	0.00±0.00^{f}	0.00±0.00^{f}	0.00±0.00^{f}
		PB	0.00±0.00^{d}	0.00±0.00^{e}	0.00±0.00^{c}
	6hrs	**PS**	56.66±3.33^{c}	15.66±0.66^{d}	8.66±0.66^{a}
		MS	19.33±0.66^{c}	16.33±0.66^{c}	7.93±1.76^{b}
		PB	13.33±3.33^{b}	18.33±0.88^{c}	7.30±0.35^{a}
	24hrs	**PS**	67.66±2.33^{b}	12.00±1.00^{e}	8.83±0.60^{a}
		MS	**78.66±2.02^{a}**	**10.66±0.66^{d}**	**9.10±0.26^{a}**
		PB	**94.33±0.66^{a}**	**9.00±1.00^{d}**	**7.86±0.46^{a}**

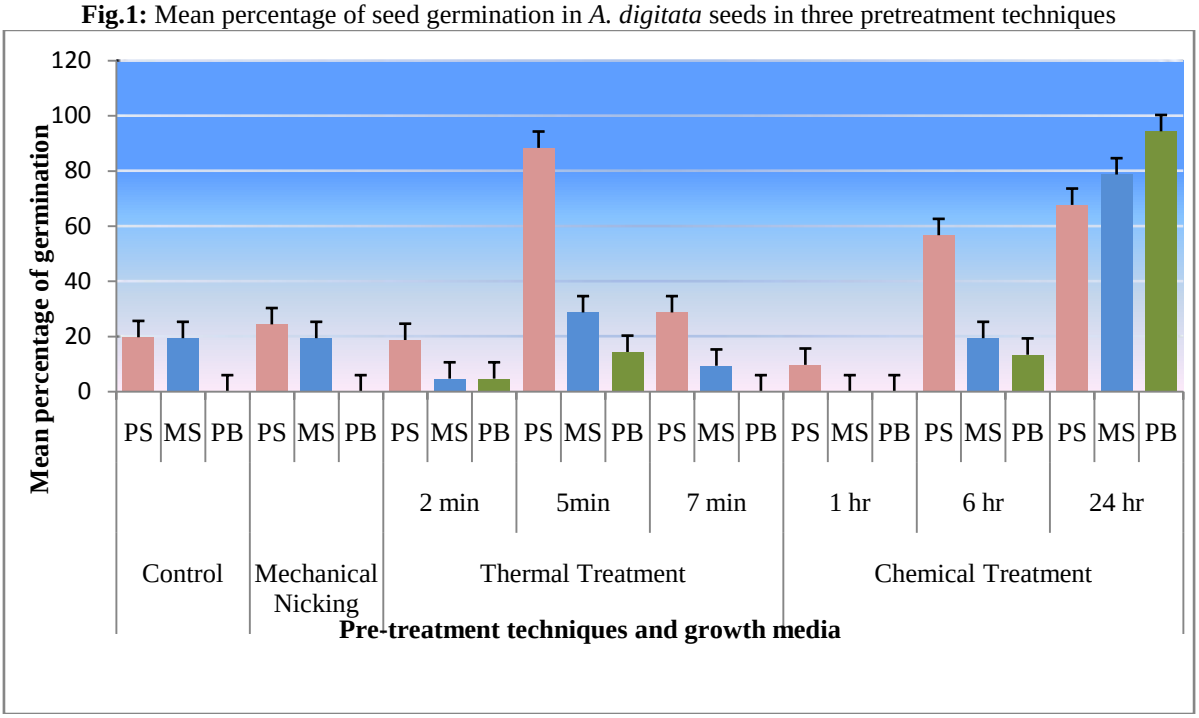
[a]Mean±Standard Error; PS- Potting Soil; MS- Murashige and Skoog's medium; PB- Paper boat

The highest percentage of germination accorded to chemical treatment. This is an indication that the more rapidly the seed coat is ruptured, due to that the rate of germination was found early. Maximum percentage

of *in vitro* seed germination (94.33%) was also observed in acid treatment for 24hrs which was significantly different at 1 hour and 6 hours period of soaking in *A. digitata*. Similarly, it was also recorded by Amusa (2011). Sulphuric acid disrupts the seedcoat and exposes the macrosclereids cells' lumen distributed in seed and permits diffusion of water, which triggers germination (Nikoleave, 1977). It is observed that exposing the seeds to high concentration of acids for a long period of time will terribly affect seed germination.

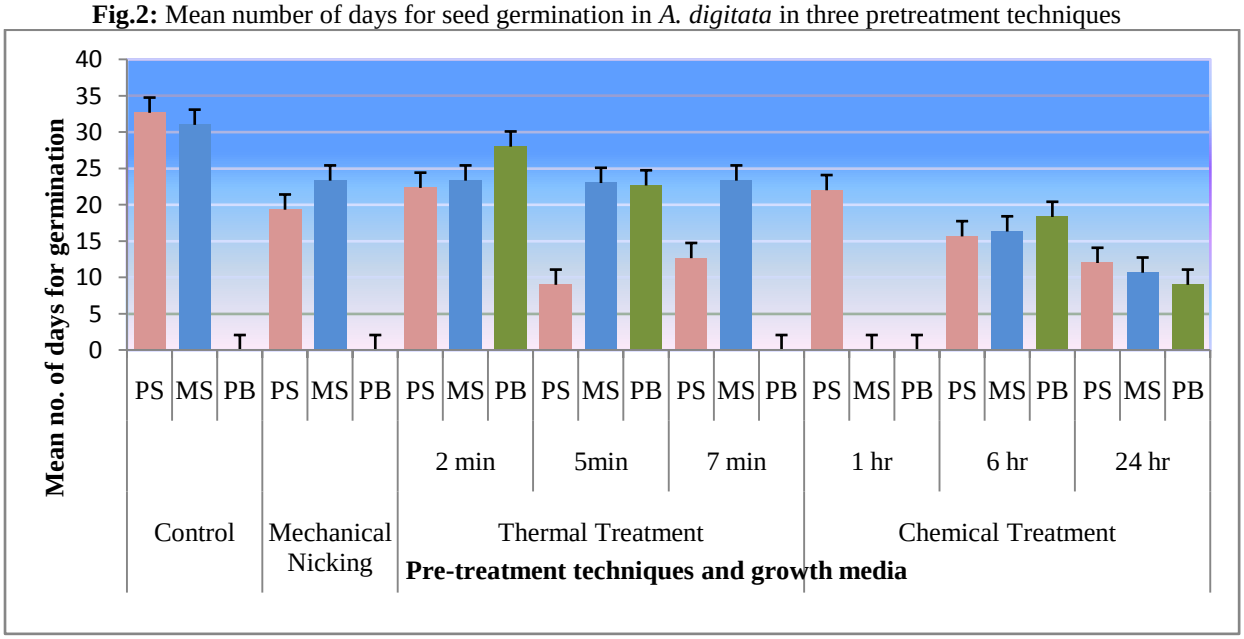
Various techniques have been used for breaking seed dormancy and seed germination in Baobab (Esenowo, 1991; Danthu et al., 1995; Sidibe& Williams 2002; Chia et al., 2008; Falemara et al., 2013). According to these, the acid pre-treatment was found more effective (Fig-5).

Fig.1: Mean percentage of seed germination in *A. digitata* seeds in three pretreatment techniques



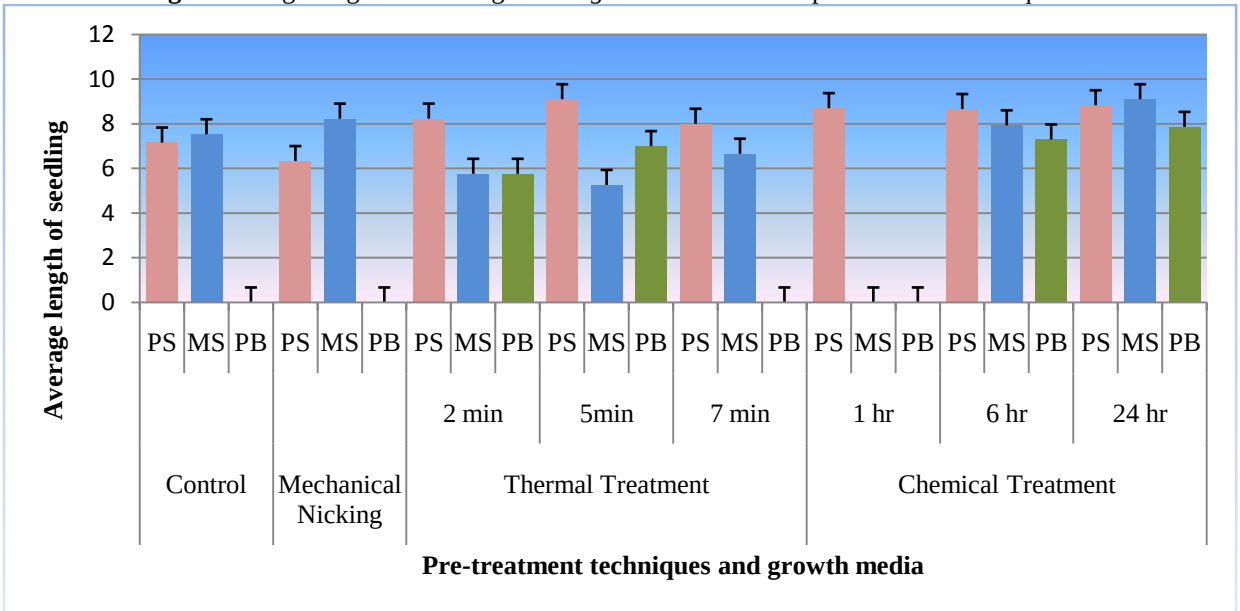
PS- Potting soil, MS- MS (Murashige and Skoog, 1962) medium, PB- Paper-Boat

Fig.2: Mean number of days for seed germination in *A. digitata* in three pretreatment techniques



PS- Potting soil, MS- MS (Murashige and Skoog, 1962) medium, PB- Paper-Boat

Fig.3: Average length of seedling of *A. digitata* seeds in three pretreatment techniques



PS- Potting soil, MS- MS (Murashige and Skoog, 1962) medium, PB- Paper-Boat

Phenotypic correlation coefficient

The phenotypic correlation coefficient with different pre-treatment techniques using PS shows that the correlation between percentage of germination - length of seedling is significant and positive, whereas on the same the correlation between percentage of germination - number of days for germination is negatively significant. The correlation between number of days for germination – length of seedling is observed as significant and negative (Table-2).

Table-2: Phenotypic correlation coefficient of measured characteristics with different pre-treatment techniques using Potting Soil as growth medium in *A. digitata*

	Percentage of Germination	**No. of days for germination**	**Length of seedling**
Percentage of germination	1		
No. of days for germination	-0.729**	1	
Length of Seedling	0.487*	-0.454*	1

**correlation is significant at the 0.01 level (2- tailed).
*correlation is significant at the 0.05 level (2- tailed).

The phenotypic correlation coefficient with different pre-treatment techniques using MS medium shows that the correlation between percentage of germination – no. of days for germination – length of seedling is significant and positive (Table-3).

Table-3: Phenotypic correlation coefficient of measured characteristics with different pre-treatment techniques using MS as growth media in *A. digitata*.

	Percentage of Germination	**No. of days for germination**	**Length of seedling**
Percentage of germination	1		
No.of days for germination	-0.116	1	
Length of Seedling	0.570**	0.552**	1

**correlation is significant at the 0.01 level (2- tailed).

The phenotypic correlation coefficient with different pre-treatment techniques using PB shows that the correlation between percentage of germination – no. of days for germination – length of seedling is significant and positive (Table-4).

Table-4: Phenotypic correlation coefficient of measured characteristics with different pre-treatment techniques using PB as growth medium in *A. digitata*

	Percentage of Germination	**No. of days for germination**	**Length of seedling**
Percentage of germination	1		
No. of days for germination	0.117	1	
Length of Seedling	0.608**	0.803**	1

**correlation is significant at the 0.01 level (2- tailed).

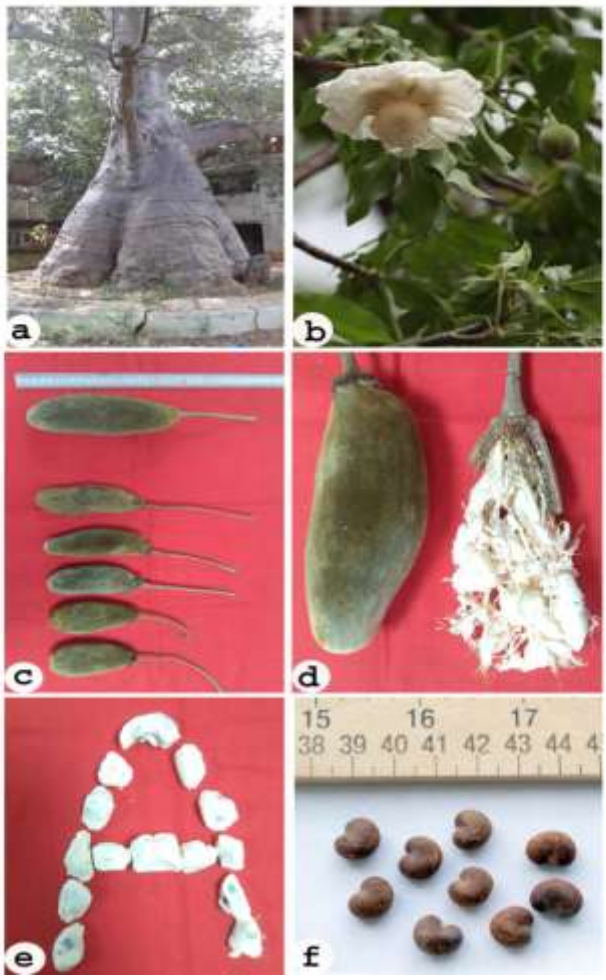
IV. Conclusion

This study examined the effect of three pre-treatment techniques on three types of growth media for breaking seed dormancy and to enhance *in vitro* seed germination response. Maximum percentage (94.33%) of seed germination was found with chemical (98% conc.H2SO4) treatment for 24hrs treatment in comparison to all other pre-treatments and growth media used. The seedling emergence was also recorded early in the same pre-treatment.

These processes of pre-treatment techniques for breaking seed dormancy and seed germination help in enhancement of conservation of this critically endangered medicinal forest tree species and to conserve it from becoming extinct.

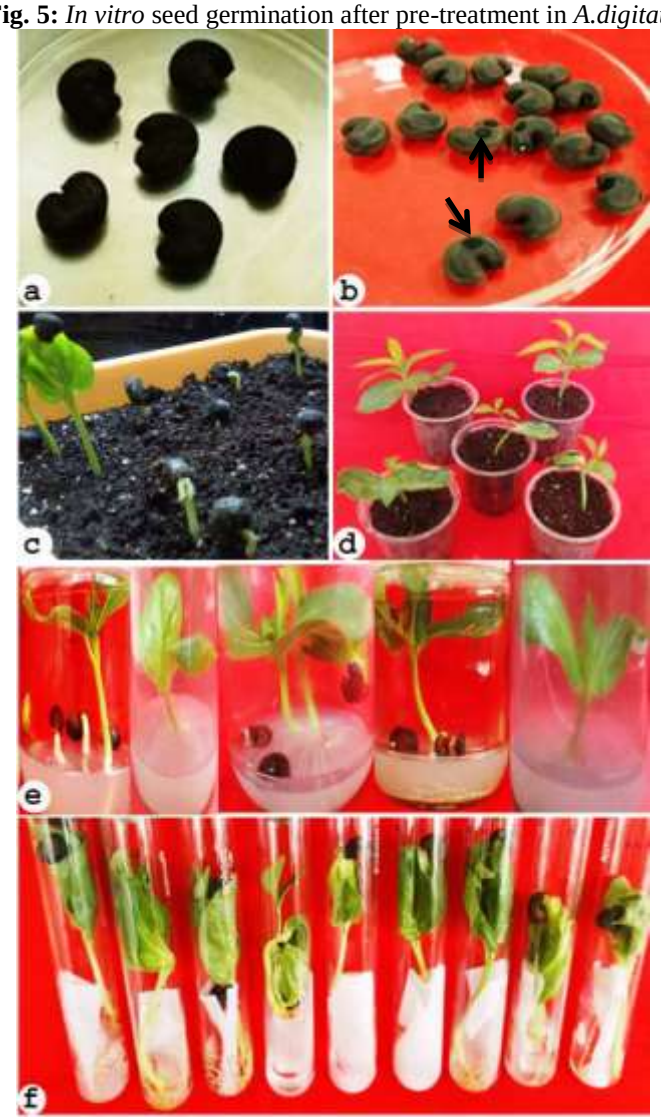
Based on the findings of our research work, soaking of seeds in 98% H2SO4 for 24hrs and inoculating on PB with sterilized distilled water is therefore recommended for breaking of seed dormancy and enhancing seed germination in *A. digitata*.

Fig.4: Showing habit, flower, fruit and seeds of *A. digitata*



(a)Treegrowing at DKW College premises, Nellore, AP (Note with huge trunk); (b) Flower and fruit (hanging from the tree); (c) Different sizes of fruits; (d) Fruit and also an opened fruit showing pulp respectively; (e) Seeds surrounded by pulp; (f) Reniform seeds.

Fig. 5: *In vitro* seed germination after pre-treatment in *A.digitata*



(a) Seeds treated with hot water (turned into black); (b) Seeds treated with 98% H_2SO_4 (some of the seed coat dissolved and note the pores); (c) Hot water treated seeds germinating on potting soil (4 days old); (d) 15 days old seedlings of the same; (e) Germinated on MS medium (10 days old) after acid treatment for 24hrs; (f) Germinated on paper-boat (7 days old) after acid treatment for 24hrs.

Acknowledgments

The authors are grateful to the Head, Department of Biotechnology, Kakatiya University for providing necessary facilities, Dodla Kousalyamma Women's (DKW) College, Nellore, Andhra Pradesh, India for providing germplasm of *Adansonia digitata* and UGC- MANF (F1-17.1/2014-15/MANF-2014-15-MUS-TEL-40612) for financial assistance.

Declaration of interest

Authors have declared that no actual or potential conflicts of interest exist.

References

[1]. Al-Qarawi, A.A., Al-Damegh, M.A. and El-Mougy, S.A., 2003. Hepatoprotective influence of *Adansonia digitata* pulp. Journal of Herbs, Spices & medicinal plants, 10(3), pp.1-6.

[2]. Amusa, T.O., 2011. Effects of three pre-treatment techniques on dormancy and germination of seeds of Afzeliaafricana (Sm. Ex pers). Journal of Horticulture and forestry, 3(4), pp.96-103.

[3]. Bosch, C.H., Sié, K. and Asafa, B.A., 2004. *Adansonia digitata* L. PROTA (Plant Resources of Tropical Africa/Ressourcesvégétales de l'Afriquetropicale), Wageningen, Netherlands.

[4]. Buchmann, C., Prehsler, S., Hartl, A. and Vogl, C.R., 2010. The importance of baobab (*Adansonia digitata* L.) in rural West African subsistence—suggestion of a cautionary approach to international market export of baobab fruits. Ecology of Food and Nutrition, 49(3), pp.145-172.

[5]. Chia, A.M., Iortsuun, D.N. and Carthage, B.A., 2008. Studies on the seedling growth of *Adansonia digitata* AL. Science World Journal, 3(1).
[6]. Danthu, P., Roussel, J., Gaye, A. and El-Mazzoudi, E.H., 1995. Baobab (*Adansonia digitata* L.) seed pretreatments for germination improvement. Seed Science and Technology, 23(2), pp.469-475.
[7]. El-Rawy, M.E., Gergis, S.M., Bazaid, S. and ElMougy, S.A., 1997. The immunostimulant effect of *Adansonia digitata* on the immune response of chicken vaccinated with avian cholera vaccine. Journal of Egyptian Veterinary Medical Association, 57, pp.959-970.
[8]. Esenowo, G.J., 1991. Studies on germination of *Adansonia digitata* seeds. The Journal of Agricultural Science, 117(1), pp.81-84.
[9]. Etkin, N.L. and Ross, P.J., 1982. Food as medicine and medicine as food: an adaptive framework for the interpretation of plant utilization among the Hausa of northern Nigeria. Social Science & Medicine, 16(17), pp.1559-1573.
[10]. Falemara, B.C., Nwadike, C. and Obashola, E.O., 2013. Germination response of baobab seeds (*Adansonia digitata* L) as influenced by three pre-treatment techniques. In Forest industry in a dynamic global environment: Proceedings of the 35th Annual Conference of Forestry Association of Nigeria, Sokoto, Sokoto state (pp. 44-55).
[11]. Gebauer, J., El-Siddig, K. and Ebert, G., 2002. Baobab (*Adansonia digitata* L.): a Review on a Multipurpose Tree with Promising Future in the Sudan/Baobab (*Adansonia digitata* L.): EinÜberblickübereinevielseitigverwendbareBaumartmitgutenZukunftsaussichtenfür den Sudan. Gartenbauwissenschaft, pp.155-160.
[12]. Kerharo, J. and Adam, J.G., 1974. La pharmacopéesénégalaisetraditionnelle: plantesmédicinalesettoxiques.
[13]. Nikolaeva, M.G., 1977. Factors controlling the seed dormancy pattern. The physiology and biochemistry of seed dormancy and germination, pp.51-74.
[14]. Ramadan, A., Harraz, F.M. and El-Mougy, S.A., 1994. Anti-inflammatory, analgesic and antipyretic effects of the fruit pulp of *Adansonia digitata*. FITOTERAPIA-MILANO-, 65, pp.418-418.
[15]. Saikou, E.S., Kabura, B.H. and Wen-chi, H., 2008. Effect of some seed pre-treatments on emergence of Acacia senegal. World journal of Agricultural Science, 4(2), pp.213-219.
[16]. Sidibe, M., 2002. Baobab, *Adansonia digitata* L (Vol. 4). Crops for the Future.
[17]. Talari, S. and Nanna, R.S., 2016. *In vitro* preliminary screening of bioactive compounds of different parts of *Adansonia digitata* L. a globally endangered tree. International Journal of Phytomedicine, 7(4), pp.402-410.
[18]. Yusha'u, M., Hamza, M.M. and Abdullahi, N., 2010. Antibacterial activity of *Adansonia digitata* stem bark extracts on some clinical bacterial isolates. Int. J. Biomed. &Hlth. Sci. Vol, 6(3).

ShamaNazrin. "Effect of pre-Treatment on dormancy and in vitro seed germination in globally endangered forest tree Adansonia digitata L." IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB) , vol. 3, no. 5, 2017, pp. 45–52.