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In vitro Seed Germination and Seedling Development of *Mimusops laurifolia* (Forssk.) Friis: An Endangered Plant Species

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Key words: Mimusops laurifolia, Endangered plant, Woody Plant Medium, *In vitro* seed germination, Regeneration.

Abstract

Mimusops laurifolia (Forssk.) Friis is an endangered medicinal plant and natural propagation of this plant through seeds is found to be very difficult due to its hard seed coat. To conquer this problem, an efficient *in vitro* seed germination as well as subsequent *in vitro* regeneration technique has been developed in conserving this endangered plant species. Seed germination was achieved on half strength of MS (HMS), full strength MS (FMS) and MS medium supplemented with different concentration and combination of Plant Growth Regulators (PGR) like BA, Kn and GA. The highest seed germination rate (18%) was obtained from water soaking de-coated seeds inoculated on full strength of MS (FMS), on the other hand, 12% germination of seeds was recorded on HMS medium, but both the conditions required more than 90 days to germinate. A similar rate of germination was also found on MS with PGRs, but it required 55-60 days. Best responses towards seed germination with elongated shoots (2.8 cm and 3.0 cm) were obtained when seeds were inoculated on MS with 3.0 mg/l BA, 0.5 mg/l Kn and 0.2 mg/l GA. After a long period of period of 8 months following germination, seedlings produced elongated tap roots only. Rooted seedlings were successfully transferred to soil for their further growth and development. Apart from this, for *in vitro* regeneration of shoots, explants of nodal segments and shoot tips from the natural plant were cultured on woody plant medium (WPM) supplemented with various combination and concentration of BAP, Kn and TDZ. In these cases, there was no positive response recorded towards the initiation of shoots from nodal segment and shoot tips.

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Introduction

Mimusops laurifolia (Forssk.) Friis is a large evergreen tall (15-25 m), broad-canopied tree belonging to the Sapotaceae family. It has small white fragrant flowers and small, rounded, or ovoid, green, sweet edible, hard seeded fruits. Leaves usually densely clustered and the plants look like rounded crowns from the upper side. Locally it is called 'Dudh phol' or 'Phirni gach' due to the presence of milky latex. It is native to Yemen, Saudi Arabia, Ethiopia, and Somalia.

It has been cultivated in Egypt since Pharos times as it is frequently found in their tombs (Friis 1980, Friis 1992). *M. laurifolia* the persea tree was known to the ancient Egyptians as the "Tree of Life". In Bangladesh only two plants are located at the Institute of Leather Technology of University of Dhaka.

The genus *Mimusops* is considered as an important medicinal plant in the Indian traditional medicine as febrifuges, purgatives, astringents, and stimulants (Shahu et al. 1995, Shah et al. 2003). Recently, *M. laurifolia* regained popularity since the extract from bark of this plant was patented for leather tanning, skin-conditioning and moisturizing effects as part of preparations used in cosmetics, bath formulations and detergents (Ohara et al. 2001). Seeds of this plant are known to produce nine kinds of saponins and their ulcer protective and anti-inflammatory activities (Eskander et al. 2006). The leaves of *M. laurifolia* contain saponins with wide range of antimicrobial and antivirulence activities reported by Mostafa et al. (2023). Leaves of *M. laurifolia* can be considered as the natural medicinal plant with a potential anticancer and hepatoprotection due to its bioactive ingredients in both HF (n-haxen) and EAF (ethyl acetate) (Hifnaway et al. 2012).

Propagation of this plant through seeds is not very efficient as the germination frequency of the seeds is very poor because of hard seed coat and very slow growing properties. Thus, conventional propagation through seeds and vegetative cuttings are not adequate to meet the demand of this important medicinal as well as endangered plant. *M. laurifolia* is a characteristic species of the threatened southwest Arabian valley forest habitat. The main threats to this species are the loss of valley forest habitat through overgrazing and road construction, overexploitation of *M. laurifolia* for wood as well as climate change. Many of those were extremely old trees with little or no regeneration. Attempts to multiply this slow growing tree have so far failed (Kilian et al. 2004). *M. laurifolia* was classified as endangered in the region on the IUCN red list (Hall et al. 2010). It is also a poorly studied plant species in Bangladesh. Therefore, it is essential to take proper strategy for the propagation as well as conservation of *M. laurifolia*.

In tree species seed germination is difficult task due to hard seed coat and dormant embryos (Jaiswal and Chaudhary 2005) and they often fail to germinate even under favorable conditions like optimum availability of moisture, oxygen, and soil conditions (Urgenc and Cepel 2001). As *M. laurifolia* is a naturally slow growing tree, its flowering and fruiting takes a very long time, and the seed coat is too hard. 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 a few commercially viable plant species which will become extinct due to unfavorable climate and unconducive conditions.

To develop conservation strategies, seed germination studies of this medicinal endangered plant, has been useful (Kandari et al. 2007). Moreover, *in vitro* propagation is another possible way to conserve this plant species by which many genetically uniform plants can be propagated. But there are no reports for *in vitro* seed germination and propagation of *M. laurifolia.* A very few reports are available on the in vivo germination and growth of *Mimusops laurifolia* (Alshehddi 2020) and *M. elengi* (Gami et al. 2010). Under these circumstances, this study was undertaken to develop an efficient *in vitro* seed germination protocol, *in vitro* regeneration system as well as the nature of seedling development under controlled environment for *Mimusops laurifolia.*

Materials and Methods

The immature and mature fruits of *M. laurifolia* were collected from the mother tree located at the Institute of Leather Technology, University of Dhaka (Fig. 1a). The seeds were collected twice during the fruiting season in the month of April and at the end of July-August. To reduce the level of surface organisms the seeds were washed first with running tap water. Then some seeds were sun dried for testing of viability (Fig. 1b). The pulp was manually separated from the seeds using a knife. The seed viability test was carried out by using 2, 3, 5-tri-phenyltetrazolium chloride (TTC). Tri-phenyltetrazolium chloride (TTC) is a clear, water-soluble compound which is reduced by respiring tissues to yield tri-phenylformazan, a water insoluble red pigment. Before the test, seeds were soaked in distilled water for 1 hour and then de-coated the seeds by using a surgical blade. After removing the hard seed coat, they were placed in TTC solution containing Petri dish and were incubated at 30°C for 2 - 3 hours in a dark chamber. After incubation, stained seeds were then rinsed several times with distilled water until the water became clear. The seed coat of *Mimusops laurifolia* was so hard and it was visibly difficult to germinate them (Fig. 1c). Therefore, hot water treatments were done to test the capability of seed germination. Mature seeds without green pulp were soaked in a beaker containing 100 mL distilled water which was maintained at 100°C for 20-, 40- and 60-min. Seeds were then washed with cold distilled water and germinated on a petri dish containing Whatman filter paper. The observations for seed germination were recorded regularly up to 30 days.

On the other hand, surface sterilization must be needed to perform *in vitro* seed germination and seedling development. For this, seeds with green pulp were washed thoroughly in running tap water followed by treating with detergent for 2-3 mins and then 2-3 drops Tween 20 for 5-10 mins. Then again washed with distilled water 3-5 times by shaking to remove sterilizing agents completely. Green pulp was removed by using a

surgical blade and these seeds were washed with distilled water several times to remove milky latex. After that, they were soaked in distilled water for 24 hrs and placed in dark. Next day, Seeds were washed several times by using sterile distilled water in a Laminar Air Flow cabinet. Subsequently they were sterilized on the surface with 0.1% $HgCl_2$ solution by shaking gently for 8 minutes and again washed well in sterilized distilled water to remove the traces of $HgCl_2$. Then the seeds were immersed in 70% (v/v) ethanol for 3-5 minutes followed by washing thoroughly with sterilized distilled water for 2-3 times. They were kept in a petri dish containing sterile filter paper to remove excess water. Then the sterilized seeds were inoculated on two strengths of MS medium without any growth regulators, namely, full strength of MS media (FMS) and half strength of MS media (HMS). After 28-30 days, the black color hard seed coat was cracked on the media. The cracked seed coat was discarded by using a sterilized scalpel and forceps in the Laminar Air Flow cabinet. In another experiment seeds without seed coat were cultured in a fresh MS containing PGR (Plant Growth Regulators). MS with different combination and concentration of PGR were used for *in vitro* seed germination and seedling development. For this purpose, MS medium supplemented with BA (6-Benzyle adenine), Kn (6-furfuryl amino purine) and GA (Gibberellic acid) were used. The MS medium consisted of 3% sucrose and 0.8% agar and adjusted to the desired pH 5.8 using HCl or NaOH. The medium was sterilized in an autoclave at 121°C for 15 min.

Fresh explants namely nodal segment and shoot tip were collected from the mother plant which were used for *in vitro* regeneration. Explants were prepared by washing thoroughly under running tap water for 20 min to clean the dust and surface contaminants. They were surface sterilized with detergent for 2 - 3 min and then with 2 - 3 drops of Tween-20 in water for 3 - 5 min by shaking. Again, explants were washed with distilled water several times for removing the sterilizing agents. The materials were then dipped into 0.1% $HgCl_2$ solution with shaking for 5-6 mins in a laminar air flow cabinet. $HgCl_2$ solution was removed by washing them thoroughly 3 - 5 times with sterilized distilled water. After that the explants were sterilized by rinsing with 70% ethanol for 1-2 min and washed with autoclaved distilled water 2 - 3 times. After sterilization, nodal segments and shoot tips were cultured on Woody Plant Medium (WPM) supplemented with various combinations of BAP, Kn and TDZ (thidiazuron). All the *in vitro* cultures were maintained under a 16h and 8h light and darkness, respectively, at 25 ± 2°C with a photosynthetic light intensity of 3000 lux.

Results and Discussion

The viability test of seeds is necessary to know the germination capability. *Mimusops laurifolia* plant produces many seeds in a season but their natural germination rate is very poor because of hard seed coat (Figs 1b and c). Seed viability indicates the capability of seeds to germinate and produce normal seedlings under suitable germination conditions (Copeland and McDonald 2001). It has been known that three factors; temperature, seed

moisture content and oxygen pressure are most important for viability. Because of this 2, 3, 5-tri-phenyltetrazolium chloride (TTC) solution was used for testing the viability of seeds. For testing the seed viability, seed endosperm and embryos were isolated from the seeds. (Figs 1d and e). Viable and non-viable seeds can be differentiated by this TTC solution. Highly viable seeds were uniformly red, whereas non-viable seeds were white and/or speckled white and some seeds were found light pink in color (Fig. 1f). The viability of *M. laurifolia* seeds was observed to be 35.46% (Table 1). A similar type of experiment was reported by Hoque et al. (2020).

Table 1. Viability of *M. laurifolia* seeds as determined by 0.1% TTC solution.

Lot no.	No. of seed tested	No. of viable seeds	% of viable seeds	Viability (%)
1.	70	25	35.71	
2.	65	22	36.84	35.46
3.	57	21	33.84	

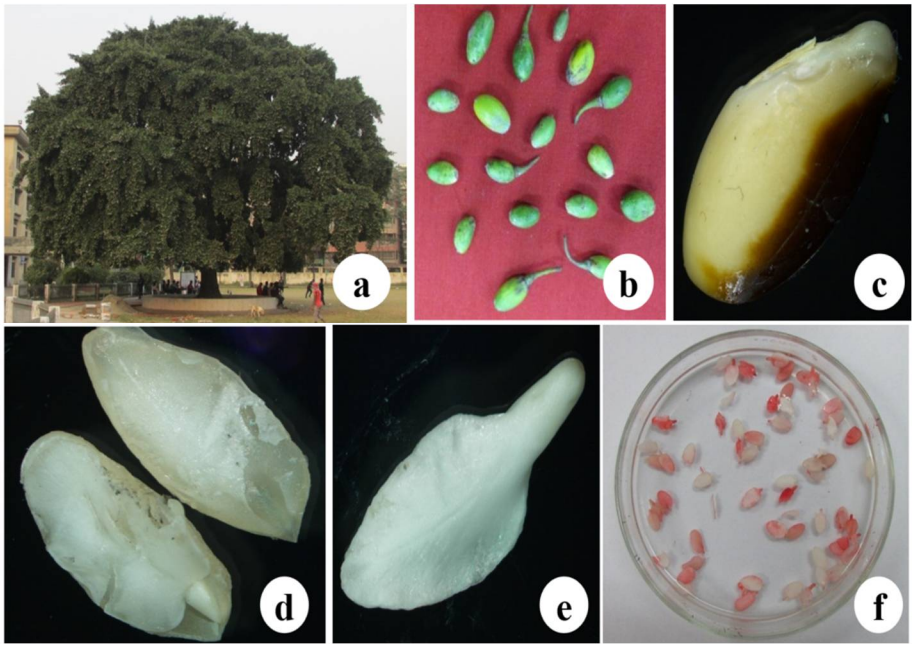

Fig. 1. (a) *Mimusops laurifolia*; Representative plants at Institute of Lather Technology, University of Dhaka. (b) Mature and immature fruits from the mother plant. (c) Stereomicroscope view of mature seed showing the hard seed coat 12x. (d) Seed showing endosperm and embryo chamber with the embryo under stereomicroscope 12x. (e) Magnified view of embryo under stereomicroscope before dipping into TTC solution. (f) Viable embryos became red in color after incubation in TTC solution used for viability test.

To our knowledge, there are no reports on *in vitro* seed germination of this endangered plant species. However, one report has been published on *in vivo* seed germination and growth of *Mimusops laurifolia* from Saudi Arabia. The result was completely negative and there was no germination after the treatment of nanoparticles and Sulfuric acid (Alshehddi 2020). The seed coat of this plant is very hard and maybe that is the main reason for the low rate of germination of seeds in nature. As a result, this plant is almost lost from our nature. The hard seed coat renders the seeds impermeable to water and oxygen needed for germination process (Baskin and Baskin 1998). So, the main objectives of this study were to develop a suitable protocol for *in vitro* seed germination as well as development of plantlets so that the protocol can be utilized in raising the required number of plants of this valuable and endangered species.

Different pretreatments are essential for effective germination. Depending on the plant species and type of dormancy, various methods like scarification, stratification, removal of inhibitors and treatment with growth regulators are used to break dormancy (Baskin and Baskin 1998, Hidayati et al. 2012). For this, hot water treatment was carried out in this investigation, and it had no positive effect on seed germination. Similar kind of results observed on germination of *Mimusops elengi* reported by (Gami et al. 2010). Seed did not germinate in hot water; maybe high temperature damages the embryo. Apart from this, full and half- strength of MS medium without any PGR were used for *in vitro* seed germination. This kind of medium was also used for *in vitro* seed germination of *Adansonia digitata* L. an endangered medicinal tree which was reported by Singh et al. (2010). Water soaking (24 hrs.) seeds showed better response for the softening of their seed coat than normal seeds. The normal seeds showed delayed and reduced percent germination than the water soaking seeds.

After 20-30 days of inoculation of water soaking seeds, swelling, and cracking of the seeds was observed, but no germination occurred on full strength of MS medium. Then seeds were de-coated by using sterile forceps and scalpel. These de-coated seeds are again inoculated on the same media for germination. Radical and plumule were emerged out after 80 days of inoculation. But in case of half- strength of MS medium, more time required for swelling and cracking. The normal seeds failed to germinate on both HMS and FMS medium after 90 days of inoculation. 18% germination was observed when water soaking seeds were placed on full strength of MS (FMS) medium and 12% was observed on half strength of MS in agar solidified medium (HMS). However normal seeds on different germination medium as referred to above showed delayed germination (15-20 days) and reduced germination when placed on FMS (11%) and HMS (4%). Soaking in water prior to sowing is also known to enhance germination percent and rate in different tree species (Bedell 1998).

On the other hand, MS medium with different PGR had less significant effect on the percentage of germination compared to without PGR medium. But PGR has been found to play a significant role in seedling development. Only GA and Kn did not show any positive effect on seed germination as well as seedling development. Different

combination and concentration of BA with Kn and GA was effective towards seedling development. Among all the concentrations 3.0 mg/l BA with 0.5 mg/l Kn showed the best response to increase the length of seedlings (Table 2). It took less time (55-58 days) to germinate than was observed in other experiments. After a few months, 3.0 cm long seedlings were found (Fig. 2a). Initially cotyledonary leaves were a little green in color but in the later stages turned into dark green in color. 3.0 mg/l BA with 0.2 mg/l GA also showed similar results. One important observation was that PGR had no significant effect on root morphology. Although seeds of *Mimusops laurifolia* germinated on both MS medium (with and without PGR), formation of lateral root was not observed (Figs 2b and c). These observations with PGR supplements were partially like that of Gami et al. (2010). Sarika and Mahendra (2005) also employed almost the same *in vitro* technique for *Buchanania lanzan* (Spreng.) to overcome hard seed coats that led to low germinating capacity.

Due to slow growth, the seedlings of *M. laurifolia* were subsequently sub-cultured for proper elongation. After 8 months of germination, well-developed plantlets were transferred aseptically to the small pot containing autoclaved soil covered with polythene bag for hardening in the growth room (Fig. 2d). Following proper acclimatization plantlets were transferred to larger pots into the green house (Figs 2e and f). Among them only one plant survived and transferred to the Botanical Garden of Dhaka University. Currently the endangered plant species is adorning the Botanical Garden of University of Dhaka with proper care and maintenance (Fig. 2 g).

Table 2. Effect of different PGR on *in vitro* seed germination and seedling development of *Mimusops laurifolia*

MS Medium with	Concentration (mg/l)	Shoot Length (cm)	Days of germination
BA + Kn	1.0 + 0.5	2.1	65 - 75
	2.0 + 0.5	2.6	60 - 70
	3.0 + 0.5	**3.0**	**55 - 58**
	4.0 + 0.5	1.8	80 - 85
BA + GA	1.0 + 0.2	2.0	60 - 65
	2.0 + 0.2	2.4	58 - 60
	3.0 + 0.2	**2.8**	**58 - 60**
	4.0 + 0.2	1.78	75 - 80

Afterwards, some experiments were carried out for *in vitro* regeneration of *M. laurifolia*. Micropropagation of woody plants by *in vitro* techniques is a challenging task, as most of the tree species are recalcitrant. To our knowledge there are no published reports on *in vitro* regeneration system of this endangered plant. In regeneration system, meristem is one of the most promising ways. In that sense nodal segment and shoot tip were used as explants for shoot regeneration.

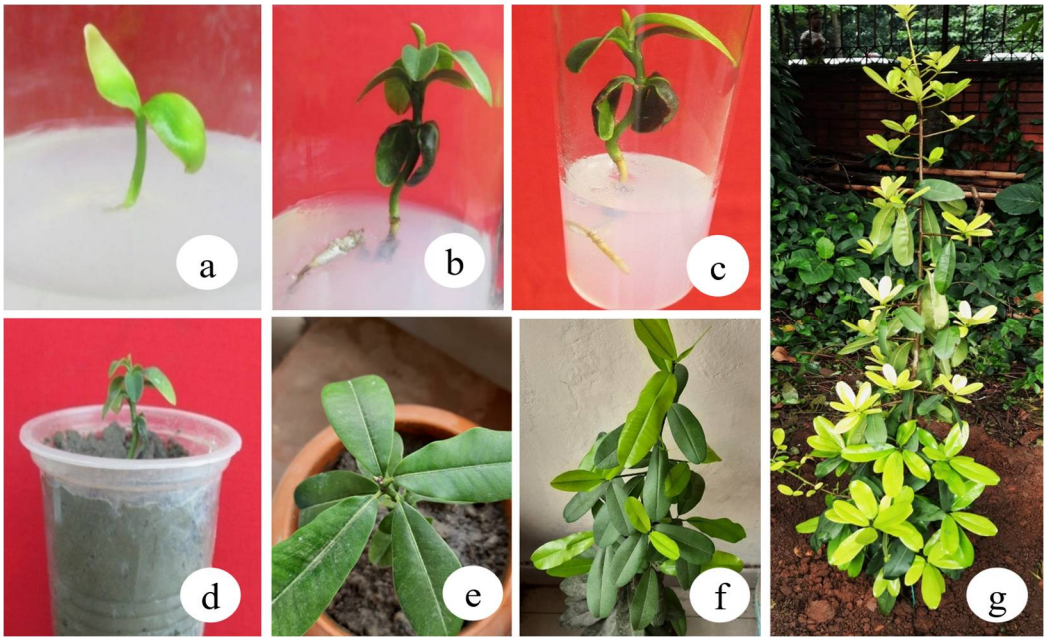

Fig. 2. Various stages of seedling development and establishment of *in vitro* raised plants of *Mimusops laurifolia*: (a) Germination of seed with cotyledonary leaves coat on MS media. (b) and (c) Elongation of shoots on MS with 3.0 mg/l BA and 0.5 mg/l Kn where no lateral root formation was noticed. (d) Hardening of *in vitro* raised seedlings on soil containing small pot. (e and f) Developing plants following hardening. (g) Mature plant transferred to the Botanical Garden of University of Dhaka.

Cytokinins (BAP, Kn, TDZ and Zeatin) are commonly used PGR in different woody plant species for regeneration. Further, in many woody species, BAP was reported to be more effective than Kn for shoot induction and multiplication (George 1993, Sharma and Vashistha 2010, Sharma et al. 2015). Bunn (2005) and Asthana et al. (2011) reported that BAP resulted in the highest shoot multiplication rates when compared to Kn and Zeatin on the micropropagation of tree species. Micropropagation of *Madhuca latifolia* (Sapotaceae) was achieved by culturing excised nodes on WPM supplemented with different PGR. The best shoot elongation was obtained on WPM supplemented with kinetin (1.66-2.32 µM) which was reported by Yogendra et al. (2000). Multiple shoots were induced from shoot tips and nodal segments of *Cinnamomum camphora* on Woody Plant Medium (WPM) supplemented with BAP and kinetin reported by Babu et al. (2003). Besides these growth regulators, TDZ plays a promising role in the entire regeneration processes. Many workers used TDZ in their culture medium and found positive effects on shoot induction and multiplication.

For the above reasons we also tried WPM with BAP, Kn and TDZ on micropropagation of *Mimusops laurifolia*. Different combinations of BAP (1.0 - 4.0 mg/l) and Kn (0.5 - 2.0 mg/l) were used with WPM to look at their effects on regeneration of shoots of *M. laurifolia*. In this case, after four weeks of inoculation, no significant

difference was observed among the culture media. Apart from this, WPM supplemented with (0.5 - 2.0 mg/l) TDZ were also used for proliferation of shoots. All explants failed to initiate shoots after 8 weeks of inoculation (Fig. 3a). However, the explants were turned black in color (Fig. 3b). It was also noticed that most cultures were found to be contaminated by fungus. The nodal segment and shoot tip of *M. laurifolia* contain latex and this latex could not be taken out completely from these explants. Similar results were observed in Micropropagation of *Mimusops elengi* reported by Bhore and Preveena (2011). Few contaminations free explants were sub-cultured to fresh media in every 15 days and 20 days. It is very important that subculture interval should not exceed more than 20 days. Even after 3 months we had no positive results towards *in vitro* regeneration of *Mimusops laurifolia*.

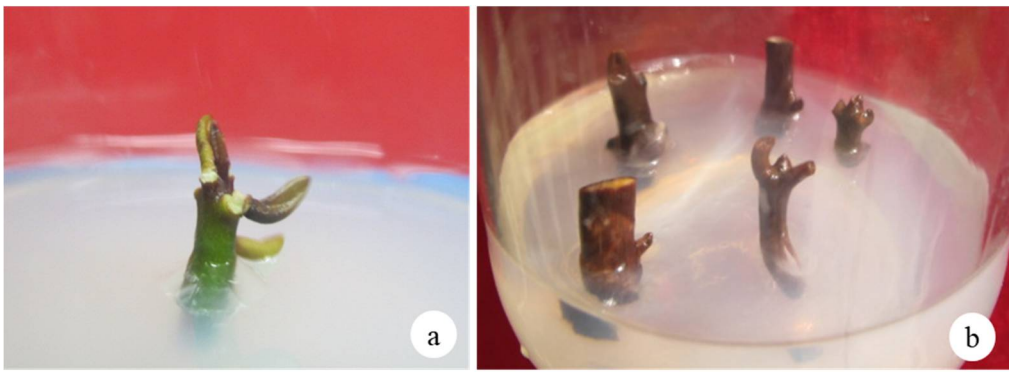

Fig. 3: (a) Shoot tip explants on WPM medium supplemented with 3.0 mg/l BAP and 0.5 mg/l Kn after eight weeks where no responses were noticed. (b) Explants of nodal segment cultured on WPM medium supplemented with 2.0 mg/l TDZ after eight weeks the explants were deep brown in color.

From the present investigation, it can be concluded that the hard seed coat of *Mimusops laurifolia* was the main obstacle for seed germination. In this endangered plant the germination of seeds can be achieved by seed pretreatments and removing the hard seed coat followed by their germination on plant growth supplemented media. This technique might be the first step towards the *in vitro* seed germination and subsequent plantlet development of this endangered species. The outcome of this study could be very useful in future research on the propagation as well as conservation of *Mimusops laurifolia*.

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References

Alshehddi AAL and **Bokhari N** (2020) Influence of gold and silver nanoparticles on the germination and growth of *Mimusops laurifolia* seeds in the South-Western regions in Saudi Arabia. Saudi J. Biol. Sci. Jan. **27**(1): 574-580.

Asthana P, Jaiswal VS and **Jaiswal U** (2011) Micropropagation of *Sapindus trifoliatus* L. and assessment of genetic fidelity of micropropagated plants using RAPD analysis. Acta Physiol. Plant. **33**: 1821-1829.

Babu KN, Sajina A, Minoo D, John CZ, Mini PM, Tushar KV, Rema J and **Ravindran PN** (2003) Micropropagation of camphor tree (*Cinnamomum camphora*), Plant Cell, Tissue Org. Cult. **74**: 179-183,

Baskin CC and **Baskin JM (1998)** Non-deep complex morphophysiological dormancy in seeds of Osmorhiza claytonia (Apiaceae). Am. J. Bot. **78**: 588-593.

Bedell PE (1998) Seed Testing. Seed Science and Technology (Indian Forestry Species), pp. 218-222.

Bhore SJ and **Preveena J** (2011) Micropropagation of *Mimusops elengi* Linn.: Identification of Suitable Explant and Comparative Analysis of Immature Zygotic Embryos Response on Three Basal Media. Am-Euras. J. Agric. & Environ. Sci. **10**(2): 216-222.

Bunn E (2005) Development of *in vitro* methods for ex situ conservation of *Eucalyptus impensa*, an endangered mallee from southwest Western Australia. Plant Cell Tissue Org. Cult. **83**: 97-102.

Copeland LO and **McDonald MB** (2001) Seed Viability and Viability Testing. *In:* Principles of Seed Science and Technology. Springer, Boston, MA. pp 124-139.

Eskander J, Lavaud C, Pouny I, Soliman HS, Khalik SMA and **Mahmoud II** (2006) Saponins from the seeds of *Mimusops laurifolia*. Phytochem. **67**: 1793-1799.

Eskander JY (2005) Pharmacognostical and biological study on some plants belonging to the family sapotaceae. Ph.D. Thesis, Faculty of Pharmaceutical Sciences, Helwan University, Cario, Egypt.

Friis I (1980) The taxonomy and distribution of *Mimusops laurifolia* (Sapotaceae). Kew Bulletin. **35**: 785-795.

Friis I (1992) Forests and forest trees of Northeast tropical Africa. Kew Bull. Add. Series 15.

Gami B, Parabia M and **Kothari IL** (2010) *In vitro* Development of Callus from Node of *Mimusops elengi* - As Substitute of Natural Bark. Inter. J. Pharma. Sci. Drug Res. **2**(4): 281-285.

Gami B, Parabia M and **Kothari IL** (2010) Pretreatment Effects on Germination of *Mimusops elengi*, Seed Technol. **32**: 138-144.

George EF (1993) Plant propagation by tissue culture (part I: the technology). Exegetics LTD, UK.

Hall M, Neale S, Al-Abbasi TM and **Miller AG** (2010) Arabia's tallest trees: ecology, distribution and conservation status of the regionally endangered tree species *Mimusops laurifolia*. Nordic J. Bot. **28**: 240-245.

Hidayati SN, Walck JL, Merritt DJ, Turner SR, Turner DW and **Dixon KW** (2012) Sympatric species of *Hibbertia* (Dilleniaceae) vary in dormancy break and germination requirements: Implication for classifying morphophysiological dormancy in Mediterranean Biomes. Ann. Bot. **109**: 1111-1123.

Hifnawy M, sokkar N, Ezzat S, Raslan M, Salib JY and **Sleem A** (2012) Cytotoxicity and Suppressive effect of leaves of *Mimusops laurifolia* on Carbon Tetrachloride-induced Liver injury in Rats and its Bioactive Constituents. Asian J. Plant Sci. **11**(3): 124-130.

Hoque R, Borna RS, Hoque MI and **Sarker RH** (2020) *In vitro* Plant Regeneration of *Rauvolfia tetraphylla* L.: A Threatened Medicinal Plant, Plant Tissue Cult. & Biotech. **30**(1): 33-45.

Jaiswal P and **Chaudhary S** (2005) Germination behavior of some trees and grasses of arid lands. Bull Nat Inst Ecol. **15**: 201-205.

Kandari LS, Rao KS, Chauhan K, Maikhuri RK, Purohit VK, Phondani PC and **Saxena KG** (2007) Effect of presowing treatments on the seed germination of two endangered medicinal herbs of the Himalaya (Angelica glauca Edgew and Pleurospermum angelicoides Wall. Ex DC. Benth. Ex C B Clarke). Proc. Indian Nat. Sci. Acad. **73**: 11-16.

Kilian, Norbert, Hein P and **Hubaishan MA** (2004) Further Notes on the Flora of the Southern Coastal Mountains of Yemen. *Willdenowia* Bd. **34**: 159-182.

Mostafa HMA, Taha M, El-Gendy AO, Khairalla AS, Abd El Fattah M and **Raslan M** (2023) Antimicrobial and antivirulence saponins of *Mimusops laurifolia* leaves. Lett Appl Microbiol. **76**(7).

Ohara M, Doi M and **Kondo M** (2001) Cosmetics, bath preparations and detergents containing moisturizing extracts of Sapotaceae plants. Japanese Kokai Tokkyo Koho JP. 200122732.

Sarika S and **R Mahendra** (2005) Multiple shoot formation and plant regeneration of a commercially useful tropical plant, *Buchanania lanzan* (Spreng). Plant Biotechnol. **22**(1): 59-61

Shah PJ, Gandhi MS, Shah MB, Goswami SS and **Santami D** (2003) Study of *Mimusops elengi* Bark in experimental gastric ulcers. J. Ethnopharmacol. **89**: 305-311.

Shahu NP, Koike K, Jia Z and **Nikaido T** (1995) Novel triterpenoid saponins from *Mimusops elengi.* Tetrahedron. **51**: 148-151.

Sharma H and **Vashistha BD** (2010) *In vitro* propagation of *Cinnamomum camphora* (L.) Nees & Eberm using Shoot Tip Explants. Ann. Biol. **26**(2): 109-114.

Sharma H, Vashistha BD, Singh N and **Kumar R** (2015) *Tinospora cordifolia* (Willd.) Miers ex Hook. f & Thoms. (Menispermaceae): Rapid *in vitro* propagation through shoot tip explants. Int. J. Recent Sci. Res. **6**(2): 2714-2718.

Singh S, Rai S and **Khan S** (2010) *In vitro* seed germination of *Adansonia digitata* L.: An endangered medicinal tree. Nanobiotechnica Universale Vol. **1**(2): 107-112

Urgenc S and **Cepel N** (2001) Species Selection for affrestations: Practical Fundamentals of Sowing and Planting. TEMA foundation. Publication no.33, Istanbul.

Yogendra K, Bansal and **Chibber T** (2000) Micropropagation of *Madhuca latifolia* Macb. Through nodal culture, Plant Biotechnol. **17**(1): 17-20.

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