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Effects of Storage Periods and Pre-Treatment Applications on Germination of *Morinda Lucida* Benth Seeds

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ABSTRACT

The dormant nature of *Morinda lucida* embryo is a vital concern for seed germination. However, there are limited information on its seeds storage. This study was designed to determine the effects of different storage periods and pre-treatment applications on germination of *Morinda lucida* seeds. Mature *Morinda lucida* seeds were collected, cleaned, stored in airtight vials and maintained under traditional seeds storage technique. The seeds were stored at different periods of 2, 4, 6, 8, 10 and 12 weeks respectively while freshly collected seeds were used as control. Disinfection of seeds was achieved, using 1, 2 and 3% (w/v) sodium hypochlorite for 20 minutes respectively. Afterwards, each category of stored seeds was subjected to pre-treatment applications, using the protocol of Ajongbolo *et al.,* (2018). Data were analyzed, using ANOVA at P≤ 0.05. This study showed that seeds disinfected with 2% (w/v) sodium hypochlorite for 20 minutes, gave optimum response with no contamination; seeds kept in storage for 2-6 weeks and control (fresh), responded to germination while seeds stored for 8-12 weeks showed no response to germination. Observations on morphological characteristics showed that seeds kept in storage for 4 weeks had significant growth performance. This study's result confirms that *M. lucida* seeds are recalcitrant seeds and their storage can only be achieved within short period of time after collection, because prolong storage showed inhibition of germination response. Therefore, storage of *M. lucida* mature seeds within short post-collection time, coupled with pre-treatment applications, enhanced good response to proper germination and growth performances for future utilization.

Keywords: Storage, In-vitro, Morinda lucida, Pre-treatment, Seeds, Germination.

Introduction

Morinda lucida Benth is a tropical tree found in the Africa rainforest and commonly known as Brimstone tree, belonging to the family of Rubiaceae (Adewole *et al*., 2021). In the South and Central Regions of Cameroon, *Morinda lucida* is commonly known as "akeng", in Yoruba: "Oruwo", Hausa; ''Marga", Igbo: "Injisi". It is one of the most widely used plants in this region for medicinal purposes (Zapfack and Ngobo, 2002). It grows in grassland, exposed hillsides, thickets, forest, often on termite mounds, sometimes in areas which are regularly flooded, from sea level up to 1300m altitude (Ken, 2023). Propagation is possible by seeds and cuttings, but no details are known (Jansen and Cardon, 2005). Several benefits and medicinal usefulness are associated with the plant (Christian and Newman (2013), Nwobodo *et al. (*2021).

The plant has been over exploited (Oladeji *et al.,*

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2022), hence there is need for appropriate seeds storage conditions and pre-treatment applications to enhance mass production. The dormant nature of its seeds embryo also limits the natural and commercial propagation of the plant (Zimudzi & Cardon, 2005). Storage of *M. lucida* seeds by drying will stimulate germination of seeds (Eggers *et al*., 2005), with pre-treatment applications which can enhance good germination performances for full domestication and future utilization (Ajongbolo *et al*., 2018).

This study was carried out to determine the standard protocols for seeds storage condition and germination of *Morinda lucida*, on growth media using Conventional Tissue Culture (CTC) for mass production, conservation and all year round availability.

Materials and Methods

Study Area

The study was carried out in the Tissue Culture Laboratory of National Centre for Genetic Resources and Biotechnology (NACGRAB), Moor Plantation, Ibadan. The Herbarium voucher number of the plant was acquired at Herbarium Unit, Botany Department, University of Ibadan, with Herbarium Voucher number UIH-23264.

Seeds Collection and Storage Condition

Matured seeds of *M. lucida* plant were harvested from the mother plant. Pods were detached and thoroughly washed under running tap water using sieve technique.

Afterwards, the Seeds were divided into different foil papers for proper dehydration and reduction in the moisture content which can embolden the growth of microbial organisms, at room temperature (between 20^0C to 25^0C) and stored in air tight container, using the standard protocol described by Yoshinaga (2010), modified by Kevin (2020). Using air drying condition and not refrigerator to prevent negative issues from erratic power supply and maintain proper accessibility by the populace.

The seeds were subjected to different periods of storage prior excision and inoculation on prepared media via in-vitro technique. Seven different treatments (periods) were utilized with five replicates each, namely:

Period of seeds storage prior inoculation (in-vitro):

- 0 week (Freshly harvested)
- 2 weeks
- 4 weeks
- 6 weeks
- 8 weeks
- 10 weeks
- 12 weeks

Media Preparation and Aseptic Condition

Murashige and Skoog Media (1962), was prepared and used for the *in-vitro* propagation of *M. lucida* stored seeds.

Major basic constituents in MS media are; macronutrients (50mls/L), micronutrients (5mls/L), inositol (10mg/L), vitamin (5ml /L), sucrose (30g/L), Iron (37.3mg/L), EDTA (27.8mg/L), gelling agent (5% /L), pH (5.7)

The media were prepared under aseptic condition without adding any plant growth regulators (PGRs') and sterilization was achieved under 121^0C of temperature and 1.05kg/cm^2 of pressure for 20minutes, via autoclave (wet sterilization), using the described methods by Gbadamosi and Egunyomi (2010) and Balogun *et al*. (2020).

Disinfection of Explants and Inoculation

Each stored packed seeds were washed thoroughly with liquid wash, under running tap water to remove dirt that were attached to the tissues of the explants. Thereafter, 70% ethanol was prepared for surface sterilization of the seeds for 5 minutes and 2%w/v Clorox was also used as disinfectant for 20minutes. Then thorough rinsing of the disinfected seeds was carried out thrice, using sterile water. This was done under the Laminar Flow Cabinet.

After proper disinfection of the seeds, pre-treatment application for breaking of seed dormancy was attained by first soaking the seeds for five minutes inside 30mg/L concentration of GA_3 and scarified (nicked).

This was applied, according to the modification method by Ajongbolo *et al*. (2018).

Excised embryo with cotyledon from seeds cultures were inoculated on the prepared media, using sterile forceps, surgical blade and petri-dishes with germinator under the Laminar flow cabinet. The above techniques were achieved for each packed of stored seeds prior excision, inoculation and arrangement on the growth room shelves for proper germination and development.

Growth Room Condition

This was a controllable environment with photoperiods of 16hrs day light and 8hrs darkness. Temperature is $25^0C \pm 2^0C$, relative humidity of 50-55% and illumination was between 3000 to 4000 lux. The cultured seeds were subjected to this condition for proper growth and development.

Data Collection and Statistical Analysis

The stored seeds of *M. lucida* cultured and excised via *in-vitro* propagation technique were monitored on daily basis and the data were recorded fortnightly (every two weeks). The experiment was a Completely Randomized Design (CRD) with five replicates.

Data collected were subjected to Analysis of Variance (ANOVA), using STAR statistical package, version 2022. Means were separated by Duncan Multiple Range Test (DMRT), at 5% significant level.

Results and Discussion

Effects of Disinfection on Stored Seeds of *M. lucida*

Stored seeds of *M. lucida* disinfected showed different responses to the disinfection protocols (Plate 1). The seeds disinfected with 2%(w/v) Sodium hypochlorite protocol gave the best disinfection response with no contamination, optimum germination and growth rate, while other disinfection protocols had negative effects on *M. lucida* seeds germination (Table 1).

Effects of Different Storage Periods on Germination of *M. lucida* Seeds, via *In-Vitro* Technique

Seeds of *M. lucida* not stored– 0 week (freshly harvested) and seeds stored for 2 to 6 weeks responded to germination (Figure 1), while significant germination rate and growth performances were recorded in seeds stored for 4weeks (Plate 2). Seeds stored for 8 to 12 weeks showed no response to germination. This indicated that storage periods had major effects on the germination response of *M. lucida* seeds.

The disinfection of *M. lucida* seeds was achieved using sodium hypochlorite and ethanol respectively. This was in agreement with the study of Jaime *et al*. (2015), which stated that disinfection procedures, can be determined by species and the type of explant used for establishing the *in-vitro* culture. Seeds stored for

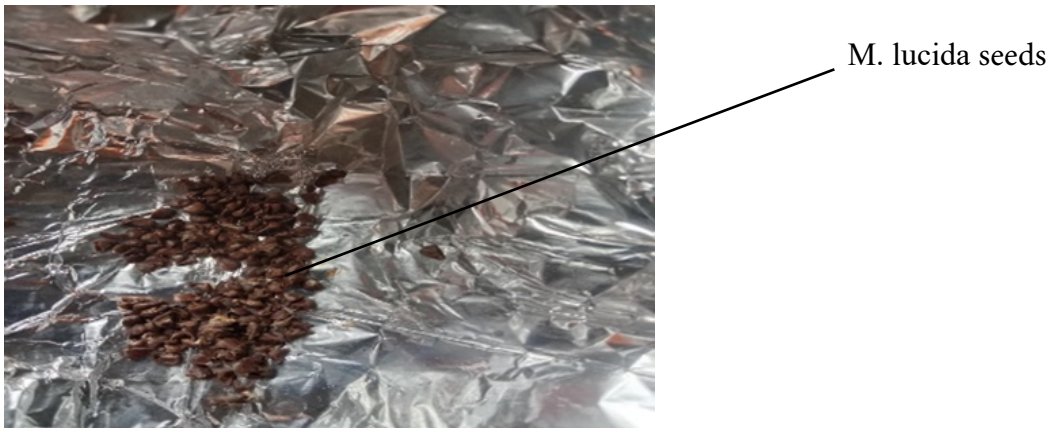

Plate 1: Seeds of M. lucida air dried on foil paper and stored at different periods prior propagation.

Table 1: Protocol development for *Morinda lucida* seed disinfection.

Disinfectant Concentration (sodium hypochlorite) (w/v)%	Number of seed propagated	Response to germination	Contamination rate	Observation
1	5	No growth	Four contaminated	Reagent concentration was not active on seed cultures there were growth of microorganisms.
2	5	Four germinated	None	The concentration was effective for proper disinfection and growth of *M. lucida* seed cultures via *in-vitro*.
3	5	No growth	None	Reagent concentration was too active and this prevented the germination of excised embryos without any contamination.
Control	5	No growth	All contaminated	No growth of seed cultures was recorded and contamination was excessive which caused nutrients depletion for seeds germination via *in-vitro*.

0 to 6 weeks responded positively to germination percentage, while seeds stored for 8-12 weeks showed no response to germination. The result supported the early study on seeds by Edwards (2005), which stated that long periods of seeds storage stimulate greater decline in seed quality.

Edward (2005) stated that some major factors played vital roles in the quality of seeds storage. These are the periods of seeds storage, temperature condition for storage and moisture content of seeds for storage. In this study, *M. lucida* seeds stored beyond 6weeks showed no response to germination after inoculated under favorable conditions with necessary pre-treatment applications for breaking dormancy. *Morinda lucida* seeds stored for 2 to 6 weeks with control showed response to germination coupled with pre-treatment application for breaking seed dormancy. This result corroborated the study of Kildisheva (2020) on dormant nature of seeds and growth traits. The inability of the seeds stored for 8 to 12 weeks coupled with pre-treatment application to germinate

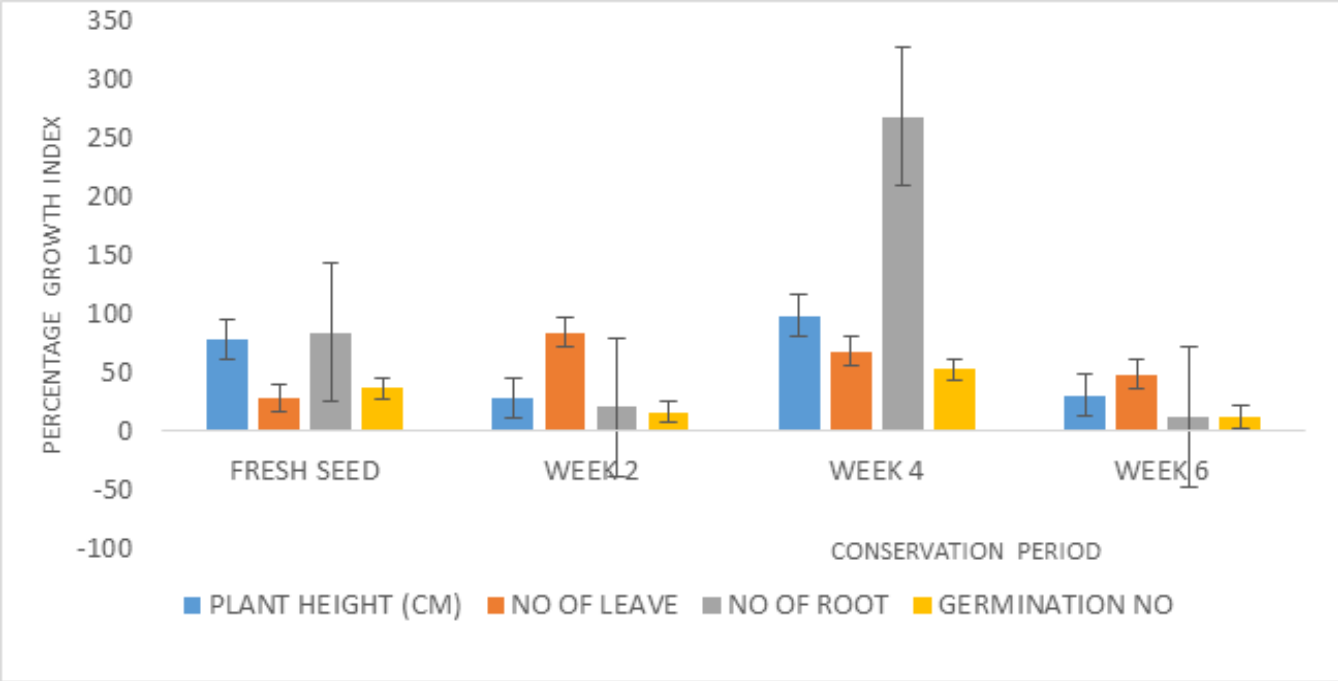

Figure 1: Effects of different storage periods on germination of M. lucida seeds, via in-vitro propagation.

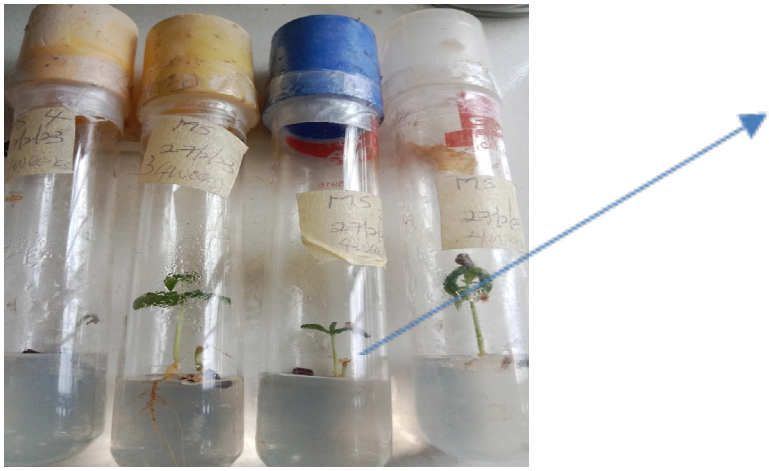

Plate 2: Plantlets emerged from seed cultures of *M. lucida* stored for 4 weeks.

showed that the seeds are recalcitrant and from a tree plant. This study confirmed the report of De *et al*. (2020) which stated that recalcitrant storage traits are more dominant in tree plant species. Freshly harvested seeds and seeds stored within short period of time (2 to 6weeks) coupled with pre-treatment applications were able to germinate within two weeks of propagation, which was different from the report of Francis (2003) on *Morinda citrifolia.*

Conclusion and Recommendations

The results from this study suggest that seeds of *Morinda lucida* can only be stored for short period

of time (2 to 6 weeks), for effective germination response and growth performances. Aside storage periods, pre-treatment applications and disinfection protocol also played significant roles in germination response of *M. lucida* seeds via *in-vitro* technique. These factors improved germination and growth performances in *M. lucida* seeds. Nevertheless, *in-vitro* propagation technique can also be used as an alternative method for conservation (semi- term), to enhance mass production and all year round availability of *M. lucida* for future utilization.

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