### POOR SEED GERMINATION OF *MELIA DUBIA*— UNRAVELLING THE BIOLOGICAL CAUSES AND DESIGNING AN APPROPRIATE SEED TREATMENT PROTOCOL

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*Melia dubia* produces indehiscent drupes which are sown for seed germination. Studies revealed that the germination is highly protracted, sporadic and the final germination is very low (< 10 %). The exocarp and mesocarp of *M. dubia* fruits are impermeable to water (physical dormancy), while the endocarp causes mechanical resistance to germinating embryo (mechanical dormancy). The kernels extracted from the drupes responded well to  $GA_3$  (250 mg L<sup>-1</sup>) treatment, when applied through seed soaking (24 hours) + humid priming (2 days), compared to seed soaking (24 hours) in  $GA_3$  (250 mg L<sup>-1</sup>) alone. Excavation of ungerminated seeds from nursery beds revealed that the remaining kernels were either fresh ungerminated (FUG) or decayed. This might be due to presence of a large proportion of low vigour seeds within a seed lot. Exposing the kernels to dry heat (40 °C for 4 hours) followed by seed soaking in  $GA_3$  (250 mg L<sup>-1</sup>) for 24 hours + humid priming with  $GA_3$  (250 mg L<sup>-1</sup>) for two days, and sowing in a polyhouse with high relative humidity ( $80 \pm 5$  % RH) resulted in highest improvement in synchrony of germination, with corresponding decrease in FUG and seed decay. The protocol enabled to increase the seed germination of *M. dubia* is attributed to multiple factors such as physical dormancy imposed by exocarp and mesocarp, mechanical dormancy caused by endocarp and higher proportion of low vigour seeds in a seed lot.

Keywords: Fruit structure, physical dormancy, mechanical dormancy, seed vigour, seed treatment, relative humidity

#### **INTRODUCTION**

Melia dubia is a fast-growing tree species which grows in a wide range of soils. Its commercial cultivation is getting popularised among farmers due to its characteristics such as fast growth, stem straightness without many branches, less shade effect and being less susceptible to pest and insect attacks (Goswami et al. 2020). The tree can grow to a height of 20 m with a cylindrical straight bole of 9 m length, 1.2-1.5 m girth and accumulates a biomass of 300 tonnes ha-1, within 6 years. With a life cycle of 8 to 12 years, the tree is highly suitable for agroforestry or farm forestry and is gaining economic importance both in domestic and global markets. Sharma et al. (2021) observed that the particle board made using the lops and tops of M. dubia confirmed to the requirements of IS 3087: 2005, grade II particle board of wood and other lignocelluloses materials for general purpose. Owing to this, the seedling demand of M. dubia is rapidly

increasing in recent years. However, supply of seedlings is highly constrained due to problems in seed germination. The tree species records only less than 10% seed germination, that too after six months of sowing, demonstrating highly protracted, sporadic and erratic germination behaviour (Tilakaratna 1991, Nasayao et al. 1993, Nair et al. 2002 & 2005). Many of the reports made on seed germination of *M. dubia*, have suggested that the indehiscent hard stony drupe may be the cause for poor germination (Nair et al. 2005, Manjunatha 2007, Anand et al. 2012).

The indehiscent fruit of *M. dubia* is an oval drupe with leathery excocarp, sticky and fleshy mesocarp and hard woody endocarp. As the drupes dry, the leathery exocarp and fleshy mesocarp inseparably adheres to the endocarp and eventually form a thick layer that is impermeable to water. The hard and woody endocarp contains minute pores on the distal end, covered by warty, pith like

dry tissue which is water permeable. These pores are directly connected with the micropyle of seeds which are tightly enclosed within the endocarp. It is perceived that seed germination of *M. dubia* might be inhibited due to exclusion of water from the embryo due to the impermeable exocarp and mesocarp, resulting in physical dormancy, as described by Hartmann et al. (2002). Even after the exocarp and mesocarp are removed by natural or artificial means to facilitate water imbibition by seeds, the hard and woody endocarp might mechanically restrain the embryo enlargement and radicle emergence of the germinating seed, resulting in mechanical dormancy, as described by Baskin and Baskin (2001).

Many studies have been conducted to improve the seed germination potential of M. dubia. It has been observed that, despite water impermeability caused by fruit structure, in most of the germination studies in M. dubia, soaking of intact drupes/whole fruits in hot water, cold water, cow dung slurry, sulphuric acid, hydrogen peroxide, potassium nitrate, gibberellic acid and exposure of M. dubia drupes to microwave energy followed by pelletising with microbial consortia were included in various experiments (Anand et al. 2012, Geetha et al. 2018, Ravi et al. 2012). Rekha (2011) tried to improve seed germination by sowing the drupes which were cut open. However, in most of these studies, efforts had not been taken to address the issue of physical or mechanical dormancy arising due to fruit structure of M. dubia. Therefore, in the present study, efforts were taken to extract the kernels from the indehiscent drupes and expose them to various treatments, and to identify the cause of poor seed germination as well as to standardise the treatment procedure to improve seed germination potential.

Apart from overcoming the impeding fruit characteristics, seed vigour enhancement treatments also hold significant potential for improving seed germination percentage. Gibberellic acid (GA3) can increase seed germination due to its impact on mobilisation of reserves, since it exerts control over hydrolysis of storage reserve and supply of energy to the embryo and breaking of seed dormancy (Martin 1983, Taiz & Zeiger 2002). Higher temperatures known to facilitate 'after ripening' are process, thereby enhancing seed germination. Dry-heat treatment is a useful tool to control external and internal seed borne pathogens such as fungi, bacteria, viruses and nematodes, and also to break seed dormancy (Nakagawa and Yamaguchi 1989, Zhang 1990, Bewley & Black 1994). Seed priming is a useful technique which involves mobilisation of mitochondria and proteins by synthesis of new mRNA and enzymes, enabling repair of organelles and eventually facilitating improvement in germination percentage as well as speed of germination (Bray et al. 1989, Girolamo & Barbanti 2012, Venkatasubramanian & Umarani 2007).

Against this background, separate experiments were conducted to ascertain the influence of fruit parts (exocarp, mesocarp and endocarp) on seed germination as well as to explore the effect of seed treatments such as dry heating, soaking in  $GA_3$  and seed priming on seed germination potential of *M. dubia*. The objective of the experiments was to unravel the underlying biological causes for poor germination and to develop a suitable consortium of pre-sowing seed treatments to improve the seed germination potential of *M. dubia*.

#### MATERIALS AND METHODS

#### Experimental site

М. dubia fruits were collected from a phenotypically superior tree in a natural population existing in Nellithurai (11° 29' N latitude and 76° 88' E longitude), located at 37 Km north west to Coimbatore in Tamil Nadu, India. Fruits were harvested at physiological maturity stage, when they turned to yellowish green colour, during the month of February. The fruits were size graded manually to obtain uniform sized drupes. A series of experiments were conducted at Forest College and Research Institute, Mettupalayam, Tamil Nadu. The climate of the region is mostly of semi-arid type with an average annual rainfall of 922 mm, maximum temperature of 32 °C and minimum temperature of 21 °C with slight variations. The average relative humidity in the morning (7.22 hours) and evening (14.22 hours) was around 78.22 and 53.54% respectively during the research period.

## Experiment 1: Role of fruit structure in retarding seed germination process of *M. dubia*

*M. dubia* fruits were fed into the mechanical scarifier machine in two stages to remove

leathery exocarp and sticky fleshy mesocarp. Two kilograms of fresh fruits were fed into the scarifier and the machine was operated for 10 minutes. By this time the exocarp was fully removed and the mesocarp was partially removed. After collecting the partially scarified fruits from the mechanical scarifier, the drum of the machine was cleaned to remove the pulpy debris. Later, the partially scarified fruits were once again fed into the scarifier and run for another 5 minutes to remove the remaining mesocarp and to obtain clean intact endocarps. A part of the endocarps collected were cracked using a mechanical device called 'vice', and split endocarps with exposed kernels were collected. Later, the kernels were carefully pricked out from the split endocarp using a needle (Plate 1). Then the seed germination study was conducted for different treatments in nursery beds maintained inside a shade house with 70% shade (Table 1). The experiment was carried out in a completely randomised design with five replications of 100 seeds each. The nursery beds were maintained for three months with regular watering, and observations were made on the number of days required for initiation of germination and completion of germination, and germination percentage (Mauromicale & Cavallaro 1995).



Mature fruits



Mechanical scarifier



Endocarp

Halh endocarp with kernels exposed





**Extracted** kernels

**Plate 1** Scarification of mature fruits of *M. dubia* in a mechanical scarifier helps to remove the exocarp and mesocarp and intact endocarps are collected; cracking of endocarps using tool called 'vice' helps to split the endocarp and expose the kernels and the kernels can be pricked out from the endocarps by using a needle

| Treatment No.  | Treatment details  |  |  |  |  |  |  |
|--|--|--|--|--|--|--|--|
|  | Experiment No. 1   |  |  |  |  |  |  |
| T1   | Whole fruit (control)  |  |  |  |  |  |  |
| T2   | Intact endocarp  |  |  |  |  |  |  |
| T3   | Endocarp splits with exposed kernels   |  |  |  |  |  |  |
| T4   | Extracted kernels  |  |  |  |  |  |  |
|  | Experiment No. 2   |  |  |  |  |  |  |
| T1 Soaking in GA <sub>3</sub> (250 mg L <sup>-1</sup> ) for 24 hours |  |  |  |  |  |  |  |
| T2   | Soaking in BAP (250 mg L <sup>-1</sup> ) for 24 hours  |  |  |  |  |  |  |
| T3   | Soaking in $H_2O_2$ (7%) for 24 hours  |  |  |  |  |  |  |
| T4   | Soaking in water for 24 hours (control)  |  |  |  |  |  |  |
| T5   | Soaking in $GA_3$ (250 mg L <sup>-1</sup> ) for 24 hours + humid priming for 2 days  |  |  |  |  |  |  |
| <b>T</b> 6   | Soaking in BAP (250 mg $L^{-1}$ ) for 24 hours + humid priming for 2 days  |  |  |  |  |  |  |
| Τ7   | Soaking in $H_2O_2$ (7%) for 24 hours + humid priming for 2 days   |  |  |  |  |  |  |
| T8   | Soaking in water for 24 hours + humid priming for 2 days   |  |  |  |  |  |  |
|  | Experiment No. 3   |  |  |  |  |  |  |
| T1   | Seed soaking $(GA_3 250 \text{ mg L}^{-1})$ for 12 hours + humid priming for 2 days  |  |  |  |  |  |  |
| T2   | Dry heating (40 °C) for 4 hours + seed soaking (GA <sub>3</sub> 250 mg L <sup>-1</sup> ) for 12 hours + hum priming for 2 days     |  |  |  |  |  |  |
| T3   | Dry heating (40 °C) for 8 hours + seed so<br>aking (GA <sub>3</sub> 250 mg L <sup>-1</sup> ) for 12 hours + hum priming for 2 days |  |  |  |  |  |  |
| T4   | Dry heating (40 °C) for 4 hours + alternate soaking and drying in cow dung solution for 5 days                                     |  |  |  |  |  |  |
| T5   | Dry heating (40 °C) for 4 hours  |  |  |  |  |  |  |
| <b>T</b> 6   | Untreated kernels (control)  |  |  |  |  |  |  |

Table 1 Treatment details for the experiments conducted in the study

#### Experiment 2: Effect of seed soaking and humid priming with growth promoters on seed germination

Kernels were obtained by subjecting the fruits to mechanical scarification as described in Experiment 1. The kernels were subjected to soaking for 24 hours to facilitate imbibition of respective growth promoters like gibberellic acid (GA<sub>3</sub>), benzyl amino purine (BAP) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and humid priming involving soaking in the above growth promoters for 24 hours followed by incubation in a dark and humid environment for 2 days. In the process of humid priming, the growth promoter-soaked kernels were rolled firmly inside a wet cloth and placed inside an opaque container over a raised platform. Small quantity of water was maintained inside the container to a level just below the platform to provide high relative humidity. The container was closed with an airtight lid to ensure dark and humid condition inside the container (Plate 2). The experiment was set up in a two

factor completely randomised design in nursery beds maintained inside a shade net. A 100 seeds each of four replications under eight treatments were sown and maintained for 30 days with regular watering. The treatment details are given in Table 1. The observations were made on the number of days required for initiation of germination and completion of germination, and germination percentage (Mauromicale Cavallaro 1995). After completing the & evaluation of seedling, the nursery beds were excavated and observations were also made on the number of fresh un-germinated seed (FUG) and decayed kernels present in the soil, and expressed in percentage.

#### Experiment 3: Effect of dry heating, seed soaking and humid priming treatments on seed germination of *M. dubia* under high relative humidity conditions

The kernels were extracted as described in Experiment 1 and subjected to various treatment



**Plate 2** The steps involved in humid priming, Step 1: soaking in GA<sub>3</sub> (250 mg L<sup>-1</sup>) for 24 hours, Step 2: decanting of the solution and spreading the endocarps/kernels on a wet cloth, Step 3: firmly rolling the endocarps/kernels with wet cloth, Step 4: placing it in an opaque container that is air tight to provide humid atmosphere, and leaving it undisturbed for 2 days, and later shade drying the endocarps/kernels to original moisture content

combinations involving dry heating, seed soaking and humid priming, as detailed in Table 1, and were sown in a completely randomised design with four replications of 100 kernels each in nursery beds inside poly house which was maintained at high relative humidity conditions of  $80 \pm 5\%$  RH. The nursery beds were watered regularly for 30 days and observations were made on germination percentage, percentage of FUG and decayed kernels, mean germination time (days) and synchrony of the germination (Ranal et al. 2009).

The mean germination time was calculated as follows:

$$\bar{t} = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i}$$

where  $t_i$  is the time from the start of experiment to the i<sup>th</sup> day,  $n_i$  refers to number of seeds germinated in the i<sup>th</sup> day (not the accumulated number, but the number correspondent to the i<sup>th</sup> day) and k is the last day of germination. Synchrony of the germination process can be calculated by the expression:

$$Z = \frac{\sum_{i=1}^{k} C_{n_i,2}}{C \sum n_i,2}, \text{ being } C_{n_i,2} = n_i (n_i-1)/2,$$

where,  $C_{n_i,2}$  refers to the combination of the seeds germinated in the i<sup>th</sup> day, two by two and  $n_i$  is the number of seeds germinated in the i<sup>th</sup> day. Observations were also made on root length (cm), shoot length (cm) and dry matter production (mg seedlings<sup>-1</sup>).

#### Statistical analysis

The data (in %) were transformed to arcsine values before statistical analysis in order to unify the variance of the data (Ansari et al. 2012). Similarly, if result data contained certain treatments without any values, the entire set was log transformed (log base 10). The data were then analysed by the F test for significance, as described by Panse & Sukhatme (1978), and treatment means were compared using LSD test at 0.05 level of probability.

#### **RESULTS AND DISCUSSION**

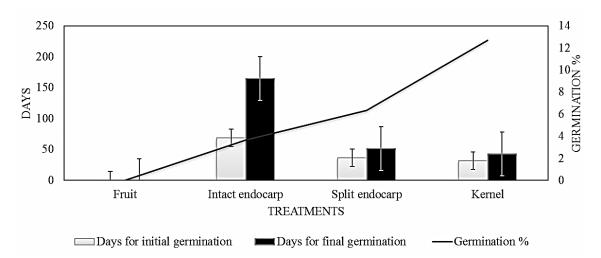
## Experiment 1: Role of fruit structure in retarding the seed germination process of *M. dubia*

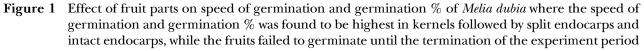
In the nursery experiment conducted with four treatments, first seedling was found to emerge from the kernels (T4) on  $32 \pm 1.53$  days after sowing (DAS), and the germination continued for the next 10 days with a highest germination of 12.67% by the end of  $42.67 \pm 0.88$  DAS. Seed germination of split endocarps (T3) initiated on  $36.33 \pm 1.45$  DAS and continued up to  $51.33 \pm 1.86$  DAS, and recorded only 6.33%germination. The intact endocarps (T2) started to germinate only after  $69.00 \pm 1.53$  days and continued up to  $165 \pm 5.69$  days, and registered the lowest germination of 3.67% (Figure 1). The fruits (T1), however, failed to register germination within the experimental period of three months. Thus, speed of germination and germination percentage were found to be the highest in kernels (T4), followed by split endocarps (T3) and intact endocarps (T2), in that order (Figure 1), while fruits (T1) could not initiate germination even up to three months of sowing. The germination of the fruits could have been prevented due to physical dormancy caused by leathery exocarp and sticky, fleshy mesocarp that forms a water impermeable layer.

When these layers were removed and the intact endocarp (T2) were sown, it could register a germination of 3.67%, although by taking a

longest period of  $165 \pm 5.69$  days to complete the germination. This result envisages that water could penetrate the intact endocarp, probably through the pores present in the distal end and connected to the micropyle (Plate 3), so as to initiate the seed germination process. However, the pores which are too small for the radicle to emerge out without loosening or cracking of the endocarp could have resulted hard-woody mechanical dormancy, and eventually inhibited the seed germination. A significantly higher germination  $(6.33 \pm 0.47\%)$ , registered by the split endocarp (T3) compared to intact endocarp (T2)  $(3.67 \pm 0.33 \%)$ , endorses the role of endocarp in causing mechanical dormancy. With respect to the low level of germination registered in kernels (T4), it is inferred that other than physical and mechanical dormancy caused by fruit structure, kernels of *M. dubia* might be inflicted by other physiological impediments that inhibit seed germination.

The effect of fruit structure on seed germination observed in *M. dubia*, have been found to be concomitant with other indehiscent fruits. In teak, Slator et al. (2013) stated that physical dormancy is not the cause for poor germination since water was observed in the locule of fruits after 12–24 hours of water immersion. It was reported that endocarp causes mechanical dormancy in teak seeds and germination couldn't happen unless the valve-like structures on the endocarp opens to allow emergence of radicle. McIntyre (1969) and Mullins et al. (2002) proposed that a combination of hard woody endocarp and dormant embryo prevented seed germination in *Persoonia pinifolia* 





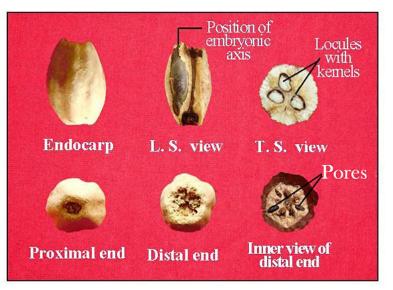


Plate 3 The endocarp of the *Melia dubia* fruits contain elongated kernels inside the locules, and the embryonic axis of the kernel is present in the distal end of the endocarp which are connected with minute pores, allowing entry of water into the locules, favouring seed imbibition

and *P. longifolia*, since germination was achieved after endocarp removal followed by gibberellic acid (GA<sub>3</sub>) treatment. Mayer & Poljakoff (1982) suggested that the dormancy of *P. sericea* and *P. virgata* seeds might be due to the endocarp, since removal of half of the endocarp allowed germination.

Therefore it is inferred that, in *M. dubia*, physical and mechanical dormancy caused by fruit structure played a dominant role in reducing the seed germination potential. However, since the kernels which were fully extracted from the endocarps could record only 12.67 % germination, it is speculated that other biological impediments could also be involved in inhibition of seed germination in this species.

# Experiment 2: Effect of seed soaking and humid priming with growth promoters on seed germination of *M. dubia*

The experimental results revealed that soaking for 24 hours alone was less effective in improving the seed germination as compared to seed soaking for 24 hours followed by humid priming for 2 days, irrespective of the growth promoters involved. Highest improvement in germination of 20.0% was obtained in treatments involving soaking in GA<sub>3</sub> (250 mg L<sup>-1</sup>) for 24 hours with humid priming for 2 days (T5), followed by T1 which registered germination of 11.67%. The number of days taken for initial and final germination were also found to be the lowest in T5 (Figure 2).

Observations on nursery bed excavation after completion of the experiment period revealed that, the kernels which remained ungerminated in the soil was either fresh ungerminated (FUG) or decayed. The kernels subjected to soaking in water for 24 hours (T4) recorded 23.67% of FUG but the treatment of soaking in water (24 hours) + humid priming for 2 days (T8)helped to decrease FUG to 19.67%, and the corresponding values for seed decay were 76.33 and 77.67%, respectively. When kernels were subjected to soaking in GA<sub>3</sub> (250 mg L<sup>-1</sup>) for 24 hours (T1), the FUG was found to be the lowest (17.33%) with high decay percentage of 71%. The FUG and kernel decay percentage further decreased to 15 and 65% respectively due to soaking in GA<sub>3</sub> 250 mg L<sup>-1</sup> for 24 hours + humid priming for 2 days (T5) (Table 2).

Humid priming is an effective seed invigouration technique which has been found to be significantly superior to conventional seed soaking treatments for improving seed germination and vigour. In *Thespesia populnea*, the seeds soaked in water for 24 hours followed by humid priming for 3 days recorded the highest seed germination (54%) whereas the untreated control seeds recorded only 14% (Jawahar & Umarani 2020). Seed humid priming (invigouration) for 2 days enables the seeds to accomplish Phase I (slow imbibition) as well as Phase II (rapid imbibition + reserve mobilisation)

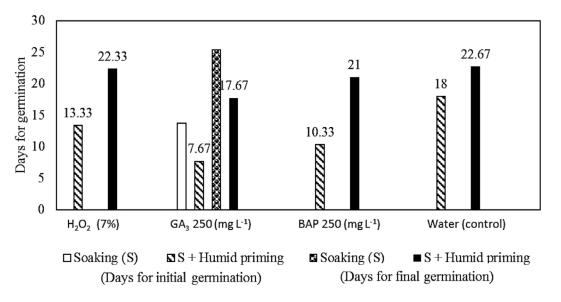


Figure 2 Effect of seed soaking and humid priming with growth promoters on the number of days taken for initial and final germination of *Melia dubia* where among the soaking treatments,  $GA_3$  (250 mg L<sup>-1</sup>) alone induced seed germination, however, the number of days taken for initial and final germination was lowest when seeds were subjected to seed soaking (S) (24 hours) + humid priming (2 days) with  $GA_3$  (250 mg L<sup>-1</sup>)

of seed germination process, thereby effectively improving the vigour level of seed and can culminate in improvement of seed germination. In the present experiment, since the FUG and kernel decay levels were greatly reduced due to invigouration with humid priming of kernels, it is inferred that prevalence of low vigour seeds could also be one of the important reasons for poor germination of *M. dubia*. Gbikpi & Grookston (1981) elucidated that late formed seeds are comparatively lower in vigour, since seed filling and development period are very short when compared to early formed seeds, thus manifesting in lower seed germination (Savage & Bassel 2015).

Humid priming with gibberellic acid (GA<sub>3</sub>) can increase seed germination due to its impact on mobilisation of reserves, since it exerts control over the hydrolysis of reserve tissues to supply energy for the embryo (Taiz & Zeiger 2002). Gibberellic acid (GA<sub>3</sub>) is also known to increase seed germination by alleviating morphological dormancy. However, *M. dubia* has a well-developed embryo (Faisal and Umarani, 2014), and seed soaking in GA<sub>3</sub> for 24 hours (T1) alone was less effective when compared to humid priming (T5), and it can be inferred that *M. dubia* seeds may not possess morphological dormancy as per the classification of Baskin and Baskin (2007). Thus, apart from the physical and mechanical dormancy caused by fruit structure and its characteristics, presence of large proportion of low vigour seeds in an inherently heterogeneous seed lot could have also contributed to the poor germination potential of *M. dubia*.

#### Experiment 3: Effect of dry heating and humid priming treatments on seed germination of *M. dubia* under high relative humidity condition

The seed vigour and seed bed environment are particularly crucial for seed germination and seedling establishment, especially in plant species with epigeal germination (Savage and Bassel 2015). Germination is a seed development process that might be influenced by environmental factors such as temperature and relative humidity (Pramanik et al. 2018, Zhang et al. 2012). Dry heat treatment has been reported to promote seed germination and seedling emergence in many crops (Lee et al. 1972, Basra et al. 2004, Farooq et al. 2004). Therefore, in the present experiment, the effect of seed drying on M. dubia was studied by subjecting the seeds to dry heating (4 and 8 hours) at 40 °C, with or without the combination of previously standardised treatment, *i.e.*, seed soaking in  $GA_3$  (250 mg L<sup>-1</sup>)

for 24 hours and humid priming for 2 days (T5). Further, the experiment was conducted in a poly-house maintained with high relative humidity (>  $80 \pm 5\%$  RH), in order to explore the possibilities of improved manifestation of the seed treatments.

The experimental revealed results significantly higher seed germination (42.33%), lower FUG (13.3%) as well as kernel decay (44.3%) for the treatment combination of dry heating (40 °C) for 4 hours + seed soaking in  $GA_3$  $(250 \text{ mg } \text{L}^{-1})$  for 24 hours + humid priming for 2 days (T2). The corresponding values recorded by untreated kernels (T6) were 32.0, 25.6 and 42.3%, respectively. The treatment (T2) also registered lower mean germination time (15.39 days), and synchrony of germination process (0.072)compared to all other treatments. The shoot length, root length and dry matter production were also found to be higher for T2 by 22.50 cm, 8.52 cm and 0.82 mg seedling<sup>-1</sup> respectively over the control (Table 2).

The positive correlation of temperature and germination release is in agreement with the metabolic theory, which postulates that most biological reaction rates are temperature dependent, demonstrating an Arrhenius relationship (Gillooly et al. 2001, Brown et al. 2004). Bazin et al. (2011) studied the sorption curves obtained with sunflower axes and cotyledons. It was reported that dry after-ripening (at 20 °C and 70% RH for 12 weeks) is associated with changes in water status within the seed tissues, especially in embryonic axes which bind more water in the intermediate zone (20-80% RH) of the isotherms when they are non-dormant. The higher relative humidity conditions of the poly house could have augmented the effectiveness of the seed treatments due to the synthesis and mobilisation of various plant enzymes and hormones involved in seed germination as stated by Limwiwattana et al. (2016) and Chhun et al. (2007). Limwiwattana, et. al. (2016) observed that increase in RH had significantly increased the seed germination as well as germination speed in black gram. After 24 hours of sowing, the seed germination was only 65% at 40%RH, while it was 84 and 89 % at RH of 60 and 80 % respectively. Since the kernels of M. dubia responded well to dry heat, humid priming and sowing in high relative humidity conditions by recording higher germination, speed of germination and lower FUG thus, it is inferred that, presence of low vigour seeds in the seed lot could be one of major biological impediment to germination of M. dubia kernels, besides the combinational seed dormancy (physical + mechanical dormancy) caused by fruit structure.

 Table 2
 Effect of seed soaking and humid priming with growth promoter on seed germination of Melia dubia seeds

| Treatments  | Germination (%)             |  |         |                             | FUG (%)                              |         | Decay (%)                   |                                      |         |
|---|-----------------------------|--|---------|-----------------------------|--------------------------------------|---------|-----------------------------|--------------------------------------|---------|
|   | Soaking                     | Humid<br>priming   | Mean    | Soaking                     | Humid<br>priming                     | Mean    | Soaking                     | Humid<br>priming                     | Mean    |
| $H_2O_2$ 7%                                       | 0.00                        | 6.00   | 3.00    | 13.67                       | 11.67                                | 12.67   | 86.33                       | 82.33                                | 84.33   |
|   | (0.29)                      | (14.15)  | (7.22)  | (21.69)                     | (19.97)                              | (20.83) | (68.31)                     | (65.15)                              | (66.73) |
| $GA_{3}250\;mg\;L^{\text{-}1}$                    | 11.67                       | 20.00  | 15.84   | 17.33                       | 15.00                                | 16.17   | 71.00                       | 65.00                                | 68.00   |
|   | (19.97)                     | (26.56)  | (23.27) | (24.60)                     | (22.78)                              | (23.69) | (57.42)                     | (53.73)                              | (55.58) |
| BAP 250 mg L <sup>-1</sup>                        | 0.00                        | 6.33   | 3.17    | 18.33                       | 18.33                                | 18.33   | 81.67                       | 75.33                                | 78.50   |
|   | (0.29)                      | (14.57)  | (7.43)  | (25.35)                     | (25.35)                              | (25.35) | (64.65)                     | (60.23)                              | (62.44) |
| Control (water)                                   | 0.00                        | 2.67   | 1.34    | 23.67                       | 19.67                                | 21.67   | 76.33                       | 77.67                                | 77.00   |
|   | (0.29)                      | (9.36)   | (4.83)  | (29.11)                     | (26.32)                              | (27.71) | (60.89)                     | (61.80)                              | (61.34) |
| Mean  | 2.92                        | 8.75   | 5.84    | 18.25                       | 16.17                                | 17.21   | 78.83                       | 75.08                                | 78.83   |
|   | (5.21)                      | (10.68)  | (7.95)  | (25.19)                     | (23.61)                              | (24.40) | (62.82)                     | (60.23)                              | (61.53) |
| Humid priming (H)<br>Growth promoter (G)<br>H x G | SED<br>0.28<br>0.40<br>0.57 | $\begin{array}{c} \text{CD} \\ (0.05) \\ 0.60 \\ 0.85 \\ 1.20 \end{array}$ |         | SED<br>0.21<br>0.29<br>0.41 | CD<br>(0.05)<br>0.44<br>0.62<br>0.87 |         | SED<br>0.23<br>0.32<br>0.46 | CD<br>(0.05)<br>0.49<br>0.69<br>0.97 |         |

FUG - fresh ungerminated seed, SED - standard error of difference, CD (0.05) - confidence distribution at 95 % level; figures in the parentheses are arcsine transformation values

| Treatments | Mean<br>germination<br>time | , ,    | Germination<br>% | FUG<br>%        | Seed<br>decay<br>% | Root<br>length<br>(cm) | Shoot<br>length<br>(cm) | Dry matter<br>production<br>(mg seedlings <sup>-1</sup> ) |
|------------|-----------------------------|--------|------------------|-----------------|--------------------|------------------------|-------------------------|---|
| T1         | 19.19                       | 0.048  | 34.6<br>(36.03)  | 16.6<br>(24.04) | 48.67<br>(44.23)   | 7.81                   | 17.31                   | 0.85  |
| T2         | 15.39                       | 0.072  | 42.3<br>(40.57)  | 13.3<br>(21.38) | 44.3<br>(41.72)    | 8.52                   | 22.50                   | 0.82  |
| T3         | 16.30                       | 0.060  | 35.6<br>(36.6)   | 19.0<br>(25.84) | 45.3<br>(42.30)    | 8.28                   | 20.41                   | 0.79  |
| T4         | 17.12                       | 0.066  | 34.3<br>(35.8)   | 17.6<br>(24.08) | 48.0<br>(43.85)    | 8.09                   | 18.81                   | 0.76  |
| T5         | 19.49                       | 0.054  | 34.6<br>(36.03)  | 19.3<br>(26.06) | 46.0<br>(42.70)    | 7.56                   | 16.54                   | 0.68  |
| T6         | 21.21                       | 0.049  | 32.0<br>(34.49)  | 25.6<br>(30.39) | 42.3<br>(40.57)    | 7.03                   | 15.13                   | 0.76  |
| Mean       | 18.14                       | 0.058  | 35.6<br>(25.75)  | 18.6<br>(25.42) | 45.7<br>(42.56)    | 7.88                   | 18.45                   | 0.76  |
| SED        | 0.372                       | 0.0018 | 0.71             | 0.38            | 0.93               | 0.18                   | 0.38                    | 0.015   |
| CD (0.05)  | 0.173                       | 0.0037 | 1.49             | 0.81            | 1.95               | 0.38                   | 0.80                    | 0.033   |

**Table 3**Effect of dry heating, seed soaking, humid priming and high relative humidity conditions (>80 % RH)on seed germination and seedling growth of *M. dubia* 

FUG - fresh ungerminated seed, SED - standard error of difference, CD (0.05) - confidence distribution at 95% level figures in the parentheses are arcsine transformation values

#### CONCLUSION

The fruits of M. dubia are indehiscent, hence, fruits are usually sown as such in the forest nurseries to raise the seedlings. The germination potential of the fruits is affected by both fruit and kernel characteristics. The leathery exocarp and sticky, fleshy mesocarp which dries after harvest of fruits fuses and adhere with hard woody endocarp to form an inseparable layer and becomes impermeable to water, resulting in physical dormancy. The pores present in the hard-woody endocarp are too small to allow radicle to emerge from germinating seed, thus imparting mechanical dormancy. With respect to kernels, the presence of a large proportion of low vigour seeds in the inherently heterogenous seed lot is also an important cause for low seed germination. In order to improve the seed germination percentage of M. dubia, kernels should be extracted and subjected to a combination of treatments, viz., dry heating  $(40 \text{ °C for 4 hours}) + \text{soaking in GA}_3 (250 \text{ mg L}^{-1})$ for 24 hours + humid priming for 2 days, and then sown in high relative humidity conditions (>  $80 \pm 5\%$  RH). This could improve the seed germination up to 42.33%, within 21 days after sowing. It is a significant improvement over

the < 5 % germination obtained after 3 months period, when the intact fruits were sown.

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