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Research Article

Effect of ageing on *in vitro* true seed and *in vivo* drupe germination and its dormancy mechanism in teak (*Tectona grandis* Linn.f)

Poomaruthai Masilamani*
Anbil Dharmalingam Agricultural College and Research Institute, Tamil Nadu Agricultural University, Tiruchirappalli - 27 (Tamil Nadu), India
Venugopal Rajanbabu
Anbil Dharmalingam Agricultural College and Research Institute, Tamil Nadu Agricultural University, Tiruchirappalli - 27 (Tamil Nadu), India
Subramanian Venkatesan
Anbil Dharmalingam Agricultural College and Research Institute, Tamil Nadu Agricultural University, Tiruchirappalli - 27 (Tamil Nadu), India
Arumugam Alagesan
Agricultural College and Research Institute, Tamil Nadu Agricultural University, Keezhvelur-05 (Tamil Nadu), India
Velanganni Alex Albert
Krishi Vigyan Kendra, Tamil Nadu Agricultural University, Sirugamani - 15, Tiruchirappalli district (Tamil Nadu), India
K. Nelson Navamaniraj
Krishi Vigyan Kendra, Tamil Nadu Agricultural University, Krishi Vigyan Kendra, Vamban - 03,Pudukkotai (Tamil Nadu), India

*Corresponding author E-mail: masil_mahesh@yahoo.com

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Abstract
The germination percentage of teak seed is generally very poor due to its higher percentage of empty seed and poor seed viability. The viable seeds exhibit protracted germination behaviour due to their inherent seed dormancy and other physiochemical characteristics. Hence establishing a teak nursery for largescale plantation activities is a challenging task. This study was undertaken to study the effect of ageing on *in vitro* true seed and *in vivo* drupe germination and its dormancy mechanism in teak. Fresh, one-year and two-year stored drupes were used to represent different levels of ageing. Under *in vivo* conditions, poor drupe germination was observed in fresh drupes (3%) and germination percentage was increased when the drupes were subjected to ageing for one year (17%) or two years (32%). When true seeds separated from fresh drupes and germinated under *in vitro* conditions, enhanced germination (58.3%) was observed. Biochemical analysis showed that indole-3- acetic acid, indole butyric acid, abscisic acid and coumarin are not present in fresh, one year and two-year-old true seeds. The gibberellic acid was increased with an increase in ageing, but the GA_3 did not influence the germination percent under *in vitro* conditions. Scanning electron microscope (SEM) image of fresh teak true seed showed that embryo tip was shrivelled, whereas one and two-year-old true seed embryo tip bulged; this was confirmed that one and two-year-old true seed embryos were matured and satisfied the after-ripening requirement. Nursery studies revealed that one and two-year-old drupes recorded the highest germination compared to fresh drupes.

Keywords: Teak, drupes, True seed, SEM, Growth regulators, Germination, Seedling vigour

INTRODUCTION

Teak (*Tectona grandis Linn.f*) grows naturally in South and Southeast Asia, Primarily in India, Indonesia, Malaysia and Burma. It is considered a valuable timber because of its adaptability, durability, rapid growth and wood (Yahya *et al.,* 2020). Teak is one of the top five tropical hardwood species in terms of Plantation areas established worldwide (Dah and Baw, 2001). On a larger scale, teak is primarily propagated through seeds (Tiwari *et al.,* 2002; Masilamani *et al.,* 2020). Teak drupes (fruit with seed) with combined dormancy, con-

sisting of two or more dormancy mechanisms. The different dormancy mechanisms involved in teak are physical dormancy (Keiding, 1985), Mechanical dormancy (Slator *et al.,* 2013), chemical dormancy (Gupta and Pattanath, 1975; Masilamani *et al.*, 2002) and embryo dormancy (Hartmann *et al.,* 1997). Seed dormancy is the putative cause for delayed and sporadic germination of teak seed but specific dormancy mechanisms have not been proven (Slator *et al.,* 2013; Venkatesan *et al.,* 2022). There is a very low rate of germination in fresh seed lots compared to those of one year and two-year-old (Keiding, 1993; Joshi and Kelkar, 1971; Suangtho, 1980; Masilamani *et al.,* 2002; Akram and Aftab, 2016; Dhaka and Jha, 2017; Ravichand and Gunaga, 2021). In practice, the previous year's seed collection is used for nursery stock production after superimposing various pre-treatments. The available literature provides different opinions on the effect of the drupe storage period on germination (Troup, 1921; Emmanual & Dharmasamy, 1991; Masilamani *et al.,* 2002). But definite inference has not been drawn so far, probably due to the discontinuity of the research work on seed dormancy.

Against the stalemate, a study was conducted to determine the influence of ageing treatments on germination, *in vitro* germination, SEM analysis and biochemical attributes of teak drupes collected from top slip seed production areas of Tamil Nadu, India.

MATERIALS AND METHODS

Seed collection

Teak drupes were collected from a twenty-year-old teak plantation at Agricultural Engineering College and Research Institute, Kumulur, Tiruchirappalli, Tamil Nadu (10º4' N; 78º5 E; 70 msl). Matured drupes fallen from the trees were collected, dried, and cleaned. After collection, insect-infected and shrivelled drupes were removed and stored in the gunny bag for ageing at ambient temperature. Fresh, one-year and two-year drupes were used for the present study.

Drupe germination

Fresh, one-year and two-year stored drupes were preconditioned by alternative soaking and drying at 12-hour intervals for six days. On the seventh day, the preconditioned drupes were sown in sand media in earthen pots along with non-preconditioned control and kept in direct sunlight for germination (Masilamani *et al*., 2020). The experiment was carried out using a Completely Randomized Block Design with ten replications of 30 drupes each. After 28 days, germination (%), number of seedlings/100 drupes, time taken for emergence, root length (cm), shoot length (cm), dry matter production (g/10seedlings) and vigour index were recorded by following standard procedures (International Seed Testing Association, 1985). The vigour index was calculated as per Abdul Baki and Anderson, (1973).

VI=Percent germination X Total seedling length (cm) …Eq.1

Nursery studies

One kg of Fresh, one-year and two-year-old drupes was placed for field emergence study in the nursery bed with a size of 1.6 x 4.1 m^2. This experiment was conducted with four replications. The number of seedlings/m^2 was recorded for 1, 2, 3, 4, 5 and 6 months after sowing (Masilamani *et al*., 2018).

True seed extraction

The true seeds were extracted from the different age groups of teak drupes with a wooden mallet; the seeds located inside the locules of the fruit were removed carefully without any damage to the cotyledon and seed coat. Those seeds were only used for *in vitro* germination studies.

Media preparation for *in vitro* germination

To perform *in vitro* germination, sterilized 0.8% Agar medium(M_1) and half-strength MS medium containing vitamins, 0.8% agar, 3 % Sucrose (M_2) as mentioned by Murashige and Skoog, (1962). M_2 medium with 0.5 ppm IAA (M_3), 1 ppm IAA (M_4), 0.5 ppm IBA (M_5), 1 ppm IBA (M_6), 0.5 ppm GA_3 (M_7), 1 ppm GA_3(M_8), 0.5 ppm BAP (M_9) and 1 ppm BAP (M_{10}) were prepared to assess the influence of plant growth regulators in true seed germination. The media with agar along with additional components was melted and evenly distributed in to culture bottles up to 50 ml per vessel. For sterilization the culture vessels with media were autoclaved at 121°C plus 15 psi pressure for 20 minutes.

Surface sterilization and inoculation of true seeds

True seeds separated from the whole drupes of different age groups were dried for 1 hour in sunlight prior to sterilization. The seeds were placed in distilled water containing 0.1 % Bavistin and 0.1 % Tween 20 for five minutes with constant shaking. After Bavistin treatment, the seeds were washed in tap water for one minute and washed in 70 % ethanol for 30 seconds. After ethanol wash, the true seeds were washed with sterile distilled water. Now the seeds were sterilized in 0.1 % mercuric chloride ($HgCl_2$) solution for five minutes with constant shaking. After $HgCl_2$ sterilization the true seeds were washed three times with sterile distilled water.

In vitro true seed germination

The surface-sterilized seeds were carefully inoculated into sterilized media bottle under a laminar airflow chamber by following the ascetic techniques. Six seeds were inoculated per bottle and eight replicates were made for each treatment. Then the culture vessels

were placed in a primary growth room maintaining 25°C temperature, 16 hours light and 8 hours dark conditions. Once a day, the *in vitro* seed inoculates were observed for the time taken for initial emergence and germination per cent were taken 14 and 28 days after sowing (International Seed Testing Association, 1985). Three seedlings were selected randomly for the estimation of dry matter production and kept in a hot air oven maintained at 85°C for 24 hours after measuring their root and shoot length. The vigour index was derived from the formula (Abdul Baki and Anderson, 1973).

VI=Percent germination X Total seedling length (cm) ….Eq.2

Scanning electron microscope (SEM) analysis

SEM captures the image of the sample's surface through a bombarding beam of high-energy of electrons over the sample in a raster scan pattern. Randomly selected seeds from fresh, one-year and two-year-old teak true seeds were used for imaging, where seeds are applied with an ultra-thin coating of gold onto seeds using a sputter coater (EMITECH SC7620). Sputtered seeds of teak were mounted rigidly on specimen stubs for characterization. Scanning Electron Microscope (Quanta 250, FEI, Netherlands) was used for imaging with an acceleration voltage of 10 kV to study the surface features and morphology (Barrera *et al*., 2013).

Biochemical studies

Chemicals and reagents

Coumarin (≥99%), gibberellic acid (≥98%), abscisic acid (≥98%), indole-3-butyric acid (≥99%) and indole-acetic acid (≥99%) and glacial acetic acid (99.7%) purchased from Sigma Aldrich (Germany) were used. Methanol (HPLC grade) was purchased from Fisher Scientific (United Kingdom). Disposable syringe filters with a pore width of 0.2 µm were purchased from Thermo Scientific (USA). Water for HPLC analysis was purified using Milli-Q water system (Millipore, St. Louis, MO). HPLC mobile phase was prepared freshly at the time of analysis and filtered through 0.45 µm membrane filter.

Seed extract for biochemical analysis

Fresh, one-year and two-year true seeds samples (0.5 g) were weighed and pulverized with a mortar and pestle with 2.5 ml methanol, followed by centrifugation for 10 min at 3000 rpm. The supernatant was transferred to a 2 ml Eppendorf tube and kept for drying. The final volume was adjusted to 1 ml with methanol and mixed thoroughly. Prior to injection, an adequate volume (1 ml) was passed through a 0.2-µm nylon membrane filter. 1 mL was collected in a LC sample vial and injected in Ultra-High-Performance Liquid Chromatography (HPLC) (Solaiman & Al-Zehouri, 2017).

HPLC instrumentation and chromatographic conditions

Biochemical compounds were separated using a C18 column (UHPLC) (Thermo Scientific Ultimate 3000). The instrumental conditions during the analysis: Using mobile phase A (Methanol) and mobile phase B (water, 1% (v/v) acetic acid) in an isocratic programme (50 per cent A: 50 per cent B) with a flow rate of 1mL/min, the column temperature was set to 30°C. For quantitative analysis, the injection volume was 10 L, and the wavelength with the highest intensity was 270 nm for coumarin and gibberellic acid (Solaiman & Al-Zehouri, 2017). Gibberellic acid, IAA, IBA, ABA and coumarin had standard peaks with retention times of 4.4, 6.8, 16.1, 10.5 and 7.8 minutes, respectively.

Statistical analysis

The results were subjected to analysis of variance and tested (t-test) for significant difference (p=0.05) as suggested (Panse & Sukhatme, 1995). Percentage values were transformed into arc sine values prior to statistical analysis.

RESULTS AND DISCUSSION

Drupe germination

The study findings revealed that there was considerable variation between the treatments. Two-year-old treated drupes recorded the highest germination of 32 %, followed by two-year-old control 17 % and fresh drupes both control and treated drupes recorded minimum germination of 2 % and 3 %, respectively (Table 1). These results indicated increases in germination in teak drupes with increases in storage period. The improvement in germination was a clear reflection of the after-ripening effect on germination when other conditions remain equal. This is conforming with the report of (Dharmalingam & Masilamani, 1997; Masilamani *et al*., 2002; Anandalakshmi *et al*., 2005; Akram & Aftab, 2016; Dhaka & Jha, 2017; Ravichand & Gunaga, 2021) in teak. Vichien *et al*. (1974) reported that the germination capacity of teak drupes was retained for more than 7 years.

Gunaga *et al*. (2008) found that teak drupes stored for one year had higher germination of 43.1% compared to fresh drupes (26.2%). Several factors influence seed longevity during storage: temperature, nature of the seeds, seed moisture content and relative humidity (Onyekwelua & Fayose, 2007; Pradhan & Badola, 2008; Zinsmeister *et al*., 2020). Proper storage conditions may effectively retain viability in seeds over a storage period (Pradhan & Badola, 2008; Butola & Badola, 2004; Chen *et al*., 2007; Solberg *et al*., 2020).

Poor embryo maturation and increased mechanical dormancy due to tightly bound mesocarp and compact endocarp in fresh drupes hinder the germination in

Table 1. Effect of fresh, one-year and two-year stored drupes on *in vivo* germination of teak

Treatments	Germination (%)	Number of seedlings/100 drupes	Root length (cm)	Shoot length (cm)	Dry matter production (mg/10 seedlings)	Vigour index
T_1-Fresh-drupes	2.0 (8.13)	3.0	2.0	3.5	40	9
T_2-Fresh drupes - S-D for 6 days	3.0 (9.97)	4.0	2.2	3.4	42	12
T_3- One year-old drupes	6.0 (14.17)	10.0	2.5	1.8	36	13
T_4- One year-old drupes -S-D for 6 days	14.0 (21.97)	24.0	3.0	3.4	38	50
T_5-Two year-old drupes	17.0 (24.35)	30.0	2.7	3.5	36	62
T_6-Two year-old drupes -S-D for 6 days	32.0 (34.45)	38.0	2.9	4.0	37	130
Mean	12.3 (20.26)	18.1	2.5	3.2	38.1	46.0
SEd	0.119	0.464	0.075	0.069	1.099	1.294
CD (P=0.05)	0.255	0.990	0.168	0.154	2.450	2.884

S-D – Soaking and Drying (Values in parentheses are arc sine transformed values)

fresh drupes. This mechanical dormancy is not present *in vitro* germinated true seeds. Ageing of drupes promotes embryo maturation and causes defibrization of mesocarp and cellulose degradation of endocarp loosens the valve, gives way for radicle emergence and increases germination percentage when drupes are stored for one year or two years. During the ageing process it hastens the after-ripening process, which results at least in part from rapid and extensive water loss because of the conversion of soluble nutrients to their stored forms. The mature and bulged embryo was observed under a scanning electron microscope in one-year and two-year-aged true seeds, but it was shrivelled and immature in fresh true seeds. The true seeds separated from one-year and two-year ageing drupes showed a decreased germination percentage. Separation of true seeds by applying mechanical force affecting the mature bulged embryo may be a reason for the decrease in germination per cent of true seeds from the ageing of drupes.

The nursery studies results revealed that two-year-old treated drupes recorded a maximum number of seedlings (30) up to six months under nursery conditions followed by two-year-old untreated control (28) and a minimum number of seedlings were recorded in fresh drupes of untreated control and treated (16 and 20) (Table 2).

The inability of the seed to swell and form radicle to begin growth is the main reason for low germination in fresh drupes (Hartmann *et al*., 1997). The endocarp is the main mechanical hindrance in fresh drupe germination. In fresh drupes, the endocarp is compact and tightly covers the true seed may produce a strong mechanical barrier against germination. When the seeds are stored for one or two years, the cellulose material in the cell wall weakens, and gradually the intactness and integrity of endocarp around the true seed loosen. This leads to the opening of valve structures and facilitates the emergence of radicles (Rajput &Tiwari, 2001).

Hence the teak drupes' poor germination is associated with dormancy mechanisms. The impact of mechanical dormancy was higher than physical dormancy (Slater, 2013). Over the period of ageing and pre-treatments, this dormancy was released. At least one-year of storage of drupes is necessary to eliminate the dormancy mechanisms. The pot culture experiment revealed that the germination was very low in freshly collected drupes and increased in one year of stored drupes.

In vitro true seed germination

The true seeds extracted from fresh teak drupes had significant (0.05 %) variation between the treatments. Agar medium (M_1) recorded maximum germination per cent of 41.6 and 58.3 14 and 28 days after sowing with a vigour index of 159.9. Extracted true seeds from one year old drupes significantly varied between treatments. IAA 1ppm recorded maximum germination per cent of 25.0 and 33.3 and 14 and 28 days after sowing with vigour index of 125.2 followed by all other treatments. Extracted true seeds from two-year-old had significant variation between the treatments. IAA 1ppm, ½ MS medium, GA3 1ppm and BAP 1ppm recorded maximum germination per cent of 8.3 and 28 days after sowing, followed by all other treatments (Table 3,4,5).

True seeds inoculated in different nutrient medium results indicated that true seeds extracted in fresh drupes recorded more germination in all nutrient media than in one- and two-year-old seeds. These results clearly indicated that true seeds extracted from drupes viability were lost when storage was increased. Dhaka and Jha (2017) also found that teak drupes collected from all five different provenances showed that only 13.55 per-

Table 2. Effect of fresh, one-year and two-year stored drupes on number of seedlings/m^2 under nursery conditions

Treatments	Number of seedlings/m^2					
	1 MAS	2MAS	3MAS	4MAS	5MAS	6MAS
T_1-Fresh-drupes	5	11	15	15	15	16
T_2-Fresh drupes -S-D for 6 days	9	16	18	18	20	20
T_3- One year-old drupes	11	15	16	18	16	17
T_4- One year-old drupes -S-D for 6 days	16	19	20	21	24	24
T_5-Two year-old drupes	16	17	22	26	28	28
T_6-Two year-old drupes -S-D for 6 days	24	26	27	29	30	30
Mean	13.5	17.3	19.6	21.1	22.1	22.5
SEd	0.242	0.318	0.293	0.313	0.362	0.364
CD (P=0.05)	0.515	0.679	0.626	0.667	0.772	0.776

S-D - Soaking and drying; MAS - Month after sowing

cent germination, but in the case of true seeds, 54 percent germination was observed, which was four times higher than drupes germination but the remaining 46 percent of true seeds were not germinated. These results clearly indicate that teak propagating material had physical, mechanical dormancy and also morphological dormancy. Yasodha *et al.* (2005) also reported that *in vitro* method of seedling production from teak true seed was a promising method for producing high-quality teak seedlings. True seed germination was only 40 per cent in this study, and the remaining ungerminated true seeds may possess morphological dormancy. The seed with morphological dormancy requires conducive conditions after harvest to make the embryo attain full growth (De Sousa Soares *et al.*, 2017).

The increasing demand for teak plantation purposes by forest departments and private companies has necessitated research on unconventional methods for improving productivity. The potential benefits of using clonal planting stock in reforestation programs have long been recognized. However, to achieve the maximum possible genetic gain for teak improvement, both sexual reproduction and vegetative multiplication must be followed. This can be accomplished through micropropagation using seeds as explants by germinating them under in vitro conditions. The present result showed

Table 3. Effect of different growth medium on *in vitro* germination and seedling vigour of fresh true seeds

Treatments	Days taken for initial emergence	14 DAS germination (%)	28 DAS				
			Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter production (g/10 seedlings)	Vigour index
M_1 - (Agar)	8	41.6 (39.8)	58.3 (49.6)	2.5	2.7	0.05	160
M_2 - (½ MS)	8	25.0 (30.0)	25.0 (30.0)	1.8	2.0	0.03	52
M_3- (M_2+IAA 0.5 ppm)	9	8.3 (16.4)	25.0 (30.0)	1.6	2.0	0.06	52
M_4 - (M_2+IAA 1 ppm)	10	25.0 (30.0)	25.0 (30.0)	1.8	3.7	0.07	94
M_5 - (M_2+IBA 0.5 ppm)	8	33.3 (35.0)	33.3 (35.0)	3.8	2.0	0.05	70
M_6 - (M_2+ IBA 1 ppm)	15	0.0 (0.28)	8.3 (16.4)	1.7	1.5	0.03	14
M_7 - (M_2 +GA3 0.5 ppm)	8	16.6 (23.5)	16.6 (23.5)	2.2	2.4	0.03	42
M_8 - (M_2 +GA3 1 ppm)	12	16.6 (23.5)	16.6 (23.5)	2.0	2.5	0.02	75
M_9 - (M_2 +BAP 0.5 ppm)	9	16.6 (23.5)	16.6 (23.5)	2.5	1.7	0.03	31
M_{10} - (M_2 +BAP 1ppm)	14	16.6 (23.5)	16.6 (23.5)	1.7	1.5	0.02	27
Mean	10.1	19.96 (25.8)	24.13 (29.3)	2.16	2.2	0.039	61.622
SEd	0.197	0.397	0.377	0.065	0.053	0.0003	1.299
CD (P=0.05)	0.412	0.828	0.786	0.136	0.112	0.0005	2.710

DAS – Days after sowing (Values in parentheses are arc sine transformed values)

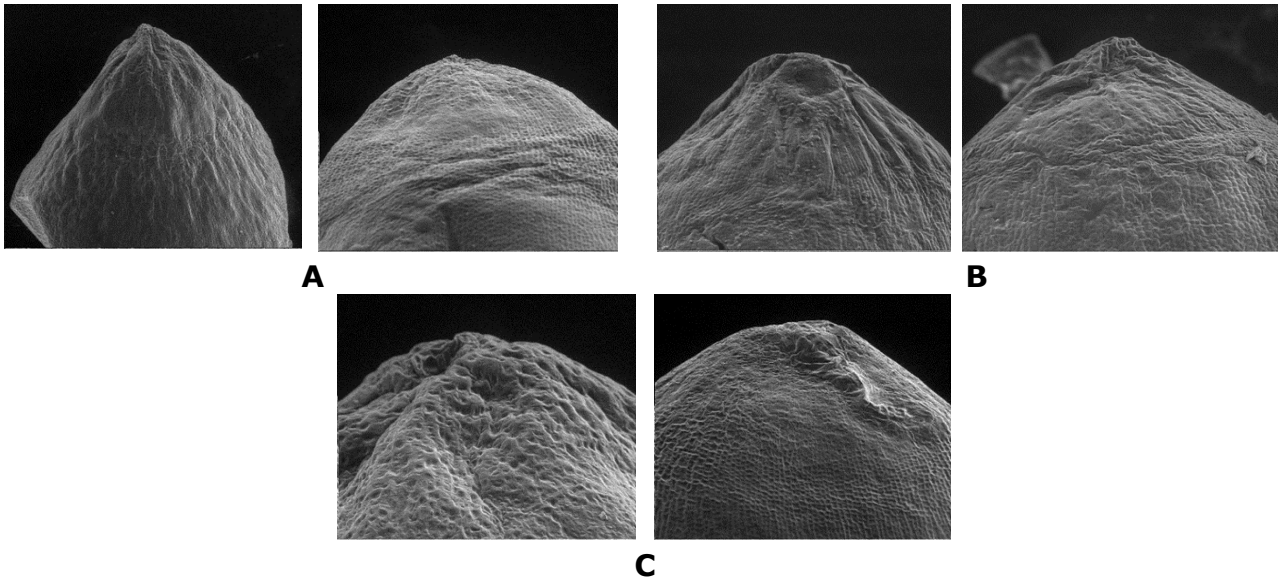

Fig.1. *Embryo growth observed by Scanning Electron Microscope: A- Teak fresh true seeds, B- Teak one-year old true seeds and C-Teak two-year old true seeds*

that agar medium is best for producing more seedlings than nutrient medium. In teak, after extraction of true seeds from fresh teak drupes, it maintains high germination percentage when compared to one and two-year -olds. *In vitro* germination can be successfully applied in teak and it has become an alternative tool to overcome problems occurring in mechanical dormancy.

Testing under SEM

SEM image of teak true seeds showed that embryo tip is shrivelled. It needs after ripening for embryo maturation in fresh true seeds. Compared to one and two-year -old true seeds embryo tip was bulged embryo was fully matured. It does not need after-ripening requirements (Fig. 1).

SEM is one of the most versatile instruments available for examining and analysing the microstructure morphology (Zhou *et al*., 2006). SEM benefits from a large depth of field so most of the specimen surface is simultaneously in focus whatever the surface roughness. Optical microscopes operating at high magnification have a very small depth of field so image quality is very dependent on the surface being smooth. Much higher magnifications can be achieved (up to 1,000,000x), with an ultimate resolution of 1 nm. The maximum useful magnification in an optical microscope is around 1000x (Vernon-parry, 2000). It needs an in-depth study for interpreting SEM image of the embryonic axis on embryo dormancy of teak.

The bulged and well-developed appearance of the embryo in aged true seeds showed that the embryo of fresh drupe needs time for maturation (Fig. 1). The poor embryo maturation leads to poor germination of fresh drupes. When the drupes were stored for one year/two years, the embryo matured enough and the germination per cent also increased. The germination per cent was increased when the true seeds of freshly collected drupes were cultured under *in vitro* conditions. This might be due to the reliving of mechanical damage prevailed by the endocarp. Hence the germination of fresh true seeds was high under *in vitro* conditions. This report evidenced that the storage of drupes may be avoided by germinating true seeds under in vitro conditions (Table 1, 2, 3).

Biochemical parameters

In the biochemical analysis of methanolic extracts of teak true seeds from fresh, one year and two-year-old drupes, gibberellic acid was found in fresh, one year and two-year-old true seeds. Two-year-old true seeds had the highest gibberellic acid level of 0.65 mg/kg, while teak fresh true seeds had the lowest gibberellic acid content of 0.22 mg/kg (Fig. 2, 3; Table 6). The growth regulators IAA, IBA, ABA and Coumarin, are not present in fresh, one year and two-year-old true seeds.

Gibberellins (GA) are essential plant regulators for multiple plant development processes, including seed germination, stem elongation, leaf extension, pollen maturation, and flowering induction (Cornea-Cipcigan *et al*., 2020). In the present study, one and two-year-old drupes had the highest germination and it had highest GA3 content of 0.65mg/kg. Hence, GA3 play a major role in germinating old teak drupes (Table 6). But, GA3 does not play a role in in vitro germination (Table 3, 4, 5). Where, *in vitro* germination test had the highest germination recorded fresh and one-year-old true seed. Studies on in vitro germination with different growth regulators showed that sterile agar medium itself is sufficient for true seed germination under in vitro conditions (Table 3).

Table 4. Effect of different growth medium on *in vitro* germination and seedling vigour of one-year old true seeds

Treatments	Days taken for initial emergence	14 DAS germination (%)	28 DAS				
			Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter production (g/10 seedlings)	Vigour index
M_1 (Agar)	11	8.3 (16.4)	8.3 (16.4)	1.5	1.0	0.06	10
M_2 (½ MS)	11	8.3 (16.4)	16.6 (23.5)	1.5	2.5	0.02	43
M_3 (M_2 +IAA 0.5 ppm)	12	8.3 (16.4)	16.6 (23.5)	2.1	1.5	0.04	27
M_4(M_2+IAA 1 ppm)	11	25.0 (30.0)	33.3 (35.0)	2.0	3.7	0.03	125
M_5(M_2+IBA 0.5 ppm)	0	0 (0.28)	0 (0.28)	0	0	0	0
M_6(M_2+ IBA 1 ppm)	11	16.6 (23.5)	25.0 (30.0)	3.0	1.2	0.05	33
M_7 (M_2 +GA3 0.5 ppm)	11	25.0 (30.0)	25.0 (30.0)	2.0	1.5	0.03	40
M_8 (M_2 +GA3 1 ppm)	12	16.6 (23.5)	25.0 (30.0)	3.1	1.4	0.03	113
M_9 (M_2 +BAP 0.5 ppm)	17	0 (0.28)	8.3 (16.4)	2.0	1.0	0.05	10
M_{10} (M_2 +BAP 1ppm)	16	0 (0.28)	8.3 (16.4)	1.0	1.7	0.06	15
Mean	11.2	10.81 (18.43)	16.64 (23.5)	1.82	1.55	0.037	41.54
SEd	0.207	0.246	0.302	0.040	0.035	0.0003	1.022
CD (P=0.05)	0.433	0.514	0.630	0.083	0.074	0.0005	2.133

DAS – Days after sowing (Values in parentheses are arc sine transformed values)

Table 5. Effect of different growth medium on *in vitro* germination and seedling vigour of two-year old true seeds

Treatments	Days taken for initial emergence	14 DAS germination (%)	28 DAS				
			Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter production (g/10 seedlings)	Vigour index
M_1 - (Agar)	0	0 (0.28)	0 (0.28)	0	0	0	0
M_2 - (½ MS)	15	8.3 (16.4)	8.3 (16.4)	1.0	1.5	0.05	21
M_3- (M_2 +IAA 0.5 ppm)	0	0 (0.28)	0 (0.28)	0	0	0	0
M_4 - (M_2+IAA 1 ppm)	12	8.3 (16.4)	8.3 (16.4)	2.0	1.0	0.03	25
M_5 - (M_2+IBA 0.5 ppm)	0	0 (0.28)	0 (0.28)	0	0	0	0
M_6 - (M_2+ IBA 1 ppm)	0	0 (0.28)	0 (0.28)	0	0	0	0
M_7 -(M_2 +GA3 0.5 ppm)	0	0 (0.28)	0 (0.28)	0	0	0	0
M_8 - (M_2 +GA3 1 ppm)	17	0 (0.28)	8.3 (16.4)	1.7	2.0	0.04	31
M_9- (M_2 +BAP 0.5 ppm)	0	0 (0.28)	0 (0.28)	0	0	0	0
M_{10}- (M_2 +BAP 1ppm)	14	8.3 (16.4)	8.3 (16.4)	1.3	1.1	0.02	20
Mean	5.8	2.49 (8.1)	3.32 (9.9)	0.6	0.56	0.014	9.62
SEd	0.211	0.085	0.116	0.011	0.030	0.0006	0.413
CD (P=0.05)	0.440	0.177	0.243	0.023	0.064	0.0013	0.862

DAS – Days after sowing(Values in parentheses are arc sine transformed values)

Table 6. Quantification of biochemical promoters present in teak true seeds

Treatments	Retention time (Min)	Area	Gibberellic acid (mg/kg)
Teak fresh drupes	2.5	0,4541	0.22
Teak one-year old drupes	2.5	0,5655	0.27
Teak two-year old drupes	2.5	1,3571	0.65

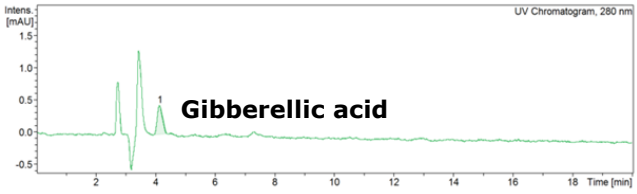

Fig. 2. *Detection of biochemical promotor gibberellic acid standard by HPLC*

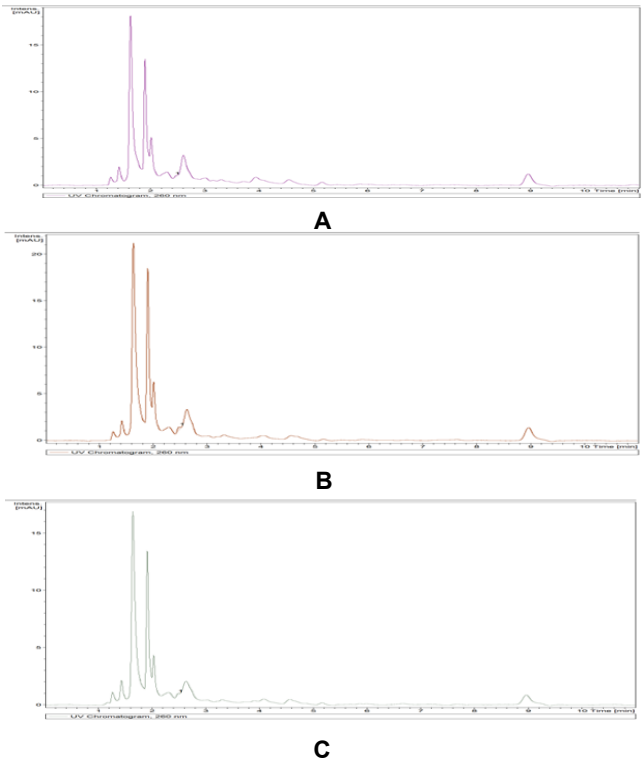

Fig. 3. *Detection of biochemical promoters and inhibitors in teak fresh (A), one-year (B) and two-year (C) old true seeds*

Conclusion

This study concluded that two-year-old treated teak drupes recorded the highest germination of 32 % compared to fresh drupes. In the case of *in vitro* germination, true seeds obtained from fresh teak drupes with an agar medium recorded maximum germination of 58.3 per cent. It was evident that physical and mechanical dormancy were mainly involved in lowering fresh teak drupes germination. On the other hand, fresh teak true seeds had 58.3 per cent, but the remaining 41.7 per cent of true seeds did not germinate. This might be because morphological dormancy was involved in true seeds to lower germination. Furthermore, SEM images' results also verified the presence of morphological dormancy. It is recommended that when forester/nursery men need seedling production from the drupes collected from the same year, *in vitro* germination of true seeds is an advisable method. However, it needs in-depth study for efficient and cost-effective hardening procedures for better establishment in the main field is necessary for the *in vitro* germinated seedlings.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Abdul Baki, A.A. & Anderson, J.D. (1973). Vigour determination in soybean seed by multiple criteria. *Crop Science.,*13,630-633.https://doi.org/10.2135/cropsci1973.0011 183X001300060013x
2. Akram, M. & Aftab, F. (2016). Fruit size and sampling sites effect on dormancy, viability and germination of teak (*Tectona grandis* L.) seeds. *Pakistan Journal of Botany.,* 48, 511-518.
3. Anandalakshmi, R., Singh, BG., Sivakumar, V., Warrier, R.R. and Chevanan, G. (2005). Effect of duration of accelerated ageing on physiological dormancy in Teak (*Tectona grandis*) Seeds: Preliminary Observations. *Seed Research.,* 33, 70-75.
4. Barrera, G.N., Calderon-Dominguez, G., Chanona-Perez, J., Gutierrez-Lopez, G.F., Leon, A.E. & Ribotta, P.D. (2013). Evaluation of the mechanical damage on wheat starch granules by SEM, ESEM, AFM and texture image analysis. *Carbohydrate Polymers*., 98,1449-1457.https://doi.org/10.1016/j.carbpol.2013.07.056
5. Butola, J.S. &Badola, H.K. (2004). Effect of pre-sowing treatment on seed germination and seedling vigour in *Angelica glauca*, a threatened medicinal herb. *Current Science*., 87, 796–799.https://www.jstor.org/stable/241 093 59
6. Chen, S.Y., Kuo, S.R. & Chien, C.T. (2007). Storage behaviour of seeds of *Cinnamomumos mophloeum* and *Neolitsea aciculata* var. variabillima (Lauraceae). Seed Science and Technology., 35, 237–243.https://doi.org/10.15258/sst.2007.35.1.22
7. Cornea-Cipcigan, M., Pamfil, D., Sisea, C.R., &Margaoan, R. (2020). Gibberellic acid can improve seed germination and ornamental quality of selected cyclamen species grown under short and long days. *Agronomy.*, 10,516.https://doi.org/10.3390/agronom y10040516
8. Dah, U.S. & Baw, U.S. (2001). Regional teak marketing and trade, Proceedings of the third regional seminar on teak, Indonesia. pp.1-14.
9. De Sousa Soares., Octavio, G., da Costa Leite, R., da Silva Junior, G.D., da Silva Reis, A., de Sousa Soares,

J.L. & Pereira Leite, M.R. (2017). Methods for overcoming dormancy in teak diaspores. *Pesquisa Agropecuaria Tropical.,* 47, 384-389.
10. Dhaka, R.K. & Jha, S.K. (2017). Evaluation of Provenances for Drupe, Seed and Germination Traits in Teak (*Tectona grandis* L.F). *International Journal of Current Microbiology and Applied Sciences.,* 6, 1721-1727. https://doi.org/10.20546/ijcmas.2017.611.208
11. Dharmalingam, C. &Masilamani, P. (1997). Radiography technique for testing the quality of teak (*TectonagrandisLinn.f)* seeds. *Bangladesh Journal of Forest Science.,* 26,51-55.
12. Emmanuel, C.J.S.K. & Dharmaswamy, S.S. (1991). Seed source variation in storage life of teak seed. *SilvaeGenetica.,*40,249-250.
13. Gunaga, R.P., Vasudeva, R. & Kanfade, A.M., (2008). Germination of Fresh and One Year Stored Fruits of Teak (*Tectona grandis* Linn. f) Collected from Several Seed Production Areas in Karnataka, South India. *Seed Technology*., 30, 70-75.https://www.jstor.org/stable/23433372
14. Gupta, B.N. & Pattanath, P.G. (1975). Factors affecting germination behaviour of teak seeds of eighteen Indian origins, *Indian Forester.,* 101, 584-588.
15. Hartmann, H. T., Kester, D. E., Davies, F. T. & Geneve, R. L. (1997). Plant propagation: principles and practices. Prentice Hall, Upper Saddle River.
16. International Seed Testing Association. (1985). International rules for seed testing.*Seed Science Technology.,*13,229-355.
17. Joshi, M. D. & Kelkar, S. P. (1971). Germination of seed in dry teak(*Tectona grandis L.)*Preliminary studies in fruit development and seed dormancy. *Indian Forester*.,97,210 - 216.
18. Keiding, H. (1985). Seed leaflet No 4 Teak (*Tectona grandis* Linn. f). Danida. Humlebeak, Denmark.
19. Keiding, H. (1993). *Tectona grandis.* Seed Leaflet No. 4. Danida Forest Seed Centre, Denmark. PP. 23.
20. Masilamani, P., Alex Albert, V., Baskar, M., Govindaraj, M., Vinothini, R. &Benaseer, S. (2018). Effect of different organic addition on seedling growth and stump quality of Teak (*Tectona grandis* Linn f). *Indian Journal of Forester.,* 41,363-367.
21. Masilamani, P., Dharmalingam, C. & Annadurai. K. (2002). Effect of natural ageing on germination and seedling Vigour of Teak (*Tectona grandis Linn. F*) drupes. *Indian Journal of Forestry*.,25,122-126.
22. Masilamani, P., Rajanbabu, V. and Venkatesan, S. (2020). Effect of Drupe size Grading on in Vivo and in Vitro Germination and its Dormancy Mechanism of Teak (*Tectona grandis* Linn. F). *Bioscience Biotechnological Research Asia.,* 17,673-683.http://dx.doi.org/10.13005/bbra/2871
23. Murashige, T. & Skoog, F. (1962). A Revised medium for rapid growth and bio assays with tobacco tissue cultures, *Physiological Plantarum.,*15, 473-497.
24. Onyekwelua, J.C. & Fayose, O.J. (2007). Effect of storage methods on the germination and proximate composition of *Treculia africana* seeds. in Proceedings of the International Conference on Agricultural Research for Development, University of Kassel Witzenhausen and University of Gottingen, Tropentag.
25. Panse, V.G. &Sukhatme, P.V. (1995). *Statistical methods for agricultural workers*. Indian Council of Agricultural Research Publications, New Delhi. 330.
26. Pradhan, B.K. &Badola, H.K. (2008). Seed germination response of populations of Swertiachirayita following periodical storage, *Seed Technology*., 30, 63–69.https://www.jstor.org/stable/23433371
27. Rajput, A. & Tiwari, K. P. (2001). Effect of alternate chilling/heating on germination of fresh teak (*Tectona grandis* L.f) drupes, without scarification of felty mesocarp. *Seed science and technology*., 29, 57-64.
28. Ravichand, A. & Gunaga, R.P. (2021). Seed Biology and Seed Orchard Dynamics in Teak. In *The Teak Genome*, 139-153. Springer. https://doi.org/10.1007/978-3-030-79311-1_9
29. Slator, N.J., Callister, A.N. & Nichols, J.D. (2013). Mechanical but not physical dormancy is a cause of poor germination in teak (*Tectona grandis* L. f). *New Forests.,* 44,39-49.https://doi.org/10.1007/s11056-011-9298-0
30. Solaiman, R. & Al-Zehouri, J. (2017). Determination of coumarin in methanol extract of cinnamon (*Cinnamomum cassia* Blume) using reversed-phase high performance liquid chromatography. *Journal of Pharmacognosy Phytochemistry.,* 6, 726-729.
31. Solberg, S. O., Yndgaard, F., Andreasen, C., Von Bothmer, R., Loskutov, I. G., & Asdal, Å. (2020). Long-term storage and longevity of orthodox seeds: A systematic review. *Frontiers in Plant Science*, 1007. https://doi.org/10.3389/fpls.2020.01007
32. Suangtho, V. (1980). *Factors Controlling Teak (Tectona Grandis Linn. F.) Seed Germination and Their Importance to Thailand*. The Australian National University, Australia.
33. Tiwari, S.K., Tiwari, K.P. Siril, E.A. (2002). An improved micropropagation protocol for teak. *Plant Cell, Tissue and Organ Culture.,*71, 1-6.https://doi.org/10.1023/A:1016570000846
34. Troup, R.S. (1921). *The Silviculture of Indian trees.* Clarendon Press, Oxford. PP. 1184.
35. Venkatesan, S., Masilamani, P., Eevera, T., Janaki, P., Sundareswaran, S., & Rajkumar, P. (2022). Effect of presowing seed treatments on teak (*Tectona grandis* L. F) drupes dormancy and germination. *Journal of Applied and Natural Science*, 14, 172-179.https://doi.org/10.31018/jans.v14i1.3316
36. Vernon-Parry, K.D. (2000). Scanning electron microscopy: an introduction, *III-Vs Review*.,13, 40-44.
37. Vichien, S., Hedegart, T. &Lauridsen, E.B.(1974). Compendium and manual for Teak improvement centre staff. Teak improvement centre, Internal paper.
38. Yahya, M.F., Hassan, N.H., Abdullah, N. & Wahid, M.S.A. (2020). Direct shoot regenaration of *Tectona grandis* (teak) for production of planting material. *International Journal of Agriculture, Forestry and Plantation.*, 10,398-403.
39. Yasodha, R, R Sumathi, R. & Gurumurthi, K. (2005). Improved micropropagation methods for teak. *Journal of tropical forest science*., 63-75.
40. Zhou, W., Apkarian, R., Wang, Z.L. and Joy, D. (2006). Fundamentals of scanning electron microscopy (SEM). In Scanning microscopy for nanotechnology. Springer, New York, NY. pp.1-40.
41. Zinsmeister, J., Leprince, O. & Buitink, J. (2020). Molecular and environmental factors regulating seed longevity. *Biochemical Journal*, 477, 305-323. https://doi.org/10.1042/BCJ20190165