Journal of Applied and Natural Science
14(1), 172 - 179 (2022)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

Effect of presowing seed treatments on teak (*Tectona grandis* L. F) drupes dormancy and germination

Subramanian Venkatesan*
Anbil Dharmalingam Agricultural College and Research Institute, Tamil Nadu Agricultural University (TNAU), Tiruchirappalli- 27 (Tamil Nadu), India
Poomaruthai Masilamani
Anbil Dharmalingam Agricultural College and Research Institute, TNAU (Tamil Nadu), India
Tamilmani Eevera
Agricultural College and Research Institute, TNAU, Coimbatore-03 (Tamil Nadu), India
Ponnusamy Janaki
Agricultural College and Research Institute, TNAU, Coimbatore-03 (Tamil Nadu), India
Sendrayaperumal Sundareswaran
Agricultural College and Research Institute, TNAU, Coimbatore-03 (Tamil Nadu), India
Perumal Rajkumar
Agricultural Engineering College and Research Institute, TNAU, Coimbatore-3 (Tamil Nadu), India

*Corresponding author. Email: svengat95@gmail.com

Article Info
https://doi.org/10.31018/jans.v14i1.3316
Received: January 27, 2022
Revised: March 4, 2022
Accepted: March 7, 2022

How to Cite
Venkatesan, S. *et al.* (2022). Effect of presowing seed treatments on teak (*Tectona grandis* L. F) drupes dormancy and germination. *Journal of Applied and Natural Science*, 14(1), 172 - 179. https://doi.org/10.31018/jans.v14i1.3316

Abstract
Poor seed germination is a major issue in teak (*Tectona grandis)* propagation. Teak seed dormancy is thought to be the reason for delayed germination. So far, specific dormancy mechanisms have not yet been identified. In order to study the influence of presowing treatments on germination, seedling vigour, and biochemical attributes of fresh teak drupes collected from the seed production area of Top Slip in Tamil Nadu. The collected drupes were subjected to different presowing treatments viz., T1 - control, T2 - soaking and drying for 6 days, T3 – T18 (soaking and drying for 5 days + soaking in different concentrations of thiourea, potassium nitrate, hydrogen peroxide and calcium oxychloride for 12 hours). Treated drupes were placed for germination in earthen pots and kept in open sunlight. In parallel, true seeds extracted from untreated drupes were also subjected to germination under *in vitro* conditions as a check. A higher percentage of germination (40%) was recorded in true seeds under *in vitro* conditions when compared to the treated and untreated drupes under *in vivo* conditions. Among the treated drupes sown under *in vivo* conditions, the drupes given soaking + drying for 5 days + soaking in 2% calcium oxychloride ($CaOCl_2$) recorded higher germination (17.16) with better seedling vigour. Analysis of teak true seeds and mesocarp extract in high-performance liquid chromatography showed that gibberellic acid was found only in true seeds, whereas the other compounds, viz., indole-3-acetic acid, indole butyric acid, abscisic acid and coumarin, were not present in the true seed or mesocarp.

Keywords: Biochemical constituent, Germination, Presowing treatment, Teak drupe, True seeds

INTRODUCTION

Teak (*Tectona grandis* L. F) has been cultivated for timber production for over 500 years (Evans and Turnbull, 2004). It is one of the most important timber species in the world, and in India, approximately 50% of the global teak plantations (1.7 m ha) are established in different environmental conditions and different altitudes (Krishnamoorthy *et al*., 2016). Teak is grown in moist deciduous forests and can also be found in evergreen forests to some extent. This large deciduous tree can reach a height of 30 meters and a circumference of over 2 meters when grown under ideal conditions. In tropical and subtropical regions of the world, the species is highly valued and farmed as a plantation tree (Santosa *et al*., 2022). Teak is regarded as one of the finest and most economically valuable tropical timber species, possessing the majority of important technical and ornamental characteristics (Raghu *et al*., 2020). Seed emptiness, fewer viable seeds, seed dormancy

This work is licensed under Attribution-Non Commercial 4.0 International (CC BY-NC 4.0). © : Author (s). Publishing rights @ ANSF.

and other factors contribute to low seed germination in teak (Ravichand and Gunaga, 2021). Nevertheless, there are difficulties in establishing large-scale plantations of teak because of poor and protracted germination. The nature of barriers that prevent germination can be physiological (presence of germination inhibitors in felty mesocarp and true seed), physical (thick and hard endocarp) and morphophysiological (hormone imbalance and immature embryo in true seeds), which results in low germination (Masilamani *et al*., 2008; da Silva Junior *et al*., 2017). Extremely low germination rates are a significant problem for the teak plantation industry (Kaosaard *et al*., 1998) as well as the deployment of planting material from breeding programs (Tewari, 1992). Several authors have worked on different presowing seed treatments to break dormancy and improve germination in teak (Amadi *et al*., 2019; Pamei *et al*., 2017; Billah *et al*., 2015; Omokhua & Alex, 2015). This delayed and irregular germination of seeds in the nursery is a serious constraint for teak for efficient nursery management and plantation establishment. Therefore, it is essential to determine presowing treatments to ensure early and successful germination in teak. Against the stalemate, a study was conducted to determine the influence of presowing treatments on germination, seedling vigour and biochemical attributes of teak drupes collected from top slip seed production areas of Tamil Nadu, India.

MATERIALS AND METHODS

Seed collection

The experiment was conducted at the Anbil Dharmalingam Agricultural College and Research Institute, Trichirappalli, Tamil Nadu, in September 2020. Teak (*Tectona grandis* L. F) drupes (fruit with seeds) were collected from 10 randomly selected plus trees in the top slip seed production area and bulked ($74^0$34'E $15^0$07'N 750 m MSL). The bulked drupes were properly dried and cleaned by removing shrivelled and insect-damaged drupes. Finally, drupes <9 mm in size were discarded, and 9 mm – 12 mm drupes alone were used as study materials in this experiment.

Physical parameters

100 drupe weight (g)

The 100 drupe weight was recorded using 100 drupes with eight replications using a highly precise electronic balance (International Seed Testing Association, 1985).

Drupe moisture content (%)

Drupe moisture content was estimated immediately after collection using a low constant temperature oven at 103 ± 1 $^{\circ}$C for 16 ± 1 h. For this purpose, ten grams of seed samples were used. After drying, the drupes were placed in desiccators containing calcium chloride for 30 minutes and weighed. The percent moisture content was calculated as per the International Seed Testing Association (1999) protocol.

Drupe diameter (mm)

Using a digital Vernier calliper, collected drupes were subjected to diameter (mm) measurement. One hundred drupes were size graded with a diameter greater than 9 mm in 8 replications.

Drupe seed ratio

One hundred drupes were randomly taken, and cutting tests were performed to assess how many true seeds were present in a single drupe. The recorded values are expressed as percentages.

Seed filling (%)

The drupes were cleaned and processed to ensure physical purity, and a cutting test was performed. Two hundred drupes were soaked in water for 24 hours, and then individual drupes were cut horizontally with the help of an areca nut cutter. The empty locules and one-seeded, two-seeded, three-seeded and four-seeded drupes were counted, and the mean numbers were recorded. Seed filling was expressed as a percentage (Masilamani *et al*., 2020).

True seed extraction

A true seed is a mature and fertilized ovule consisting of an embryo, with or without an external food reserve enclosed by a seed coat. The true seeds were extracted 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. After extracting the true seeds from the fruit, 100 true seeds were weighed (g). These seeds were only used for *in vitro* germination studies.

Seed viability (%)

One hundred numbers of true seeds were soaked in 2% 2,3,5 triphenyl tetrazolium chloride solution for 24 hours under dark conditions. After 24 hours of soaking, the number of fully stained, partially stained and unstained seeds were segregated and counted. Seed viability was expressed as a percentage (Keiding, 1993). This experiment was conducted with four replications.

Physiological parameters

Presowing drupe treatment

The drupes were subjected to the following presowing treatments: T1 - control, T2 - soaking and drying for 6 days, T3 – T6 (soaking and drying for 5 days + soaking in 0.5, 1, 1.5 and 2% thiourea for 12 hours), T7 - T10 (soaking and drying for 5 days + soaking in 0.5, 1, 1.5 and 2% potassium nitrate for 12 hours), T11 – T14

(soaking and drying for 5 days + soaking in 0.5, 1, 1.5 and 2% hydrogen peroxide for 12 hours) and T15 – T18 (soaking and drying for 5 days + soaking in 0.5, 1, 1.5 and 2% calcium oxychloride for 12 hours). The pre-conditioned and control drupes were placed for germination in sand taken in earthen pots (30 cm height and 30 cm upper width) and kept in open sunlight (Masilamani et al. 2020). The experiment was conducted in a completely randomized block design, and 10 replications of 30 drupes were used. Germination, number of seedlings/100 drupes, time taken for initial emergence, root length, shoot length, dry matter production and vigour index were recorded 28 days after sowing (ISTA, 1985). The Vigour index was also calculated (Abdul Baki & Anderson, 1973).

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

Media preparation for *in vitro* germination

To perform in vitro germination, half strength Murashige and Skoog medium (MS media) was prepared by adding 50% of the recommended dose of macro, micro and minor elements of the MS medium (Murashige & Skoog, 1962). The full strength of vitamins, 3% sucrose and 0.22 µM BAP was added to the medium, and the pH was adjusted to 5.8. After pH adjustment, 0.8% agar was added, and the media was melted to homogenize the agar. The melted media was evenly distributed in culture bottles up to 50 ml per vessel. Finally, the culture vessels with media were autoclaved at 121°C plus 15 psi pressure for 20 minutes.

Sterilization and inoculation of true seeds

The true seeds 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 washing, the true seeds were washed with sterile distilled water. Then, 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 test**

The sterilized seeds were carefully inoculated into a half MS media bottle under a laminar airflow chamber by following the ascetic techniques. Six seeds were inoculated per bottle and replicated eight times. Then, the culture vessels were placed in a primary growth room maintained at 25°C with 16 hours of light and 8 hours of dark conditions. Once in a day, the in vitro seed inoculates were observed for the time taken for initial emergence, and germination percentages were taken 14 and 28 days after sowing (ISTA, 1985). For the estimation of dry matter production, three seedlings were selected at random 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 calculated as per Abdul Baki & Anderson (1973).

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

Biochemical parameters

Methanol extract was prepared individually from teak true seeds and mesocarp to analyse biochemical promotors (indole-3-acetic acid, indole butyric acid and gibberellic acid) and inhibitors (abscisic acid and coumarin) using HPLC (Thermo Scientific ultimate 3000) with a C18 column. During analysis, the following machine settings were maintained: column temperature at 30°C using mobile phase A (methanol) and mobile phase B (water, 1% (v/v) acetic acid) in an isocratic program (50% A:50% B) with a flow rate of 1 mL/min. The injection volume was 10 µL for quantitative analysis. The biochemical promoter standard peak eluted at 4.4 min of retention time for gibberellic acid with an area of 5,2957 for 10 ppm, 7.4 min of retention time for IAA with an area of 105,36 for 10 ppm and 16.0 min of retention time for IBA with an area of 61,056 for 10 ppm. The biochemical inhibitor standard peak eluted with a retention time of 7.4 min for coumarin with an area of 169.964 for 10 ppm and a retention time of 10.4 min for ABA with an area of 344.74 for 10 ppm (Solaiman & Zehouri, 2017).

Statistical analysis

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

RESULTS AND DISCUSSION

Physical parameters

The results revealed that the freshly collected drupes of teak had 100 drupe weights of 69.99 g at a moisture content of 11.15%, a drupe diameter of 13.27 mm, a drupe seed ratio of 1:1.26, a seed filling of 75% and true seed viability of 27% (Table 1). The results of the tetrazolium test are not in line with the germination results. In our results, true seed viability was only 27%, but germination of true seeds under *in vitro* conditions up to 40% showed no correlation between seed viability and germination. Hence, the tetrazoliumim-based viability test is not the best option to estimate the viability in teak. A larger drupe size in teak resulted in better germination (Samapudhi, 1967; Kumar, 1979). The drupes were phenotypically varied in colour, shape, and size from ten separate provenances (seed sources) in Karnataka, Kerala, and Tamil Nadu (Jose &

Table 1. Physical parameters of *T. grandis*

S. No.	Parameters	
1	100 drupe weight (g)	69.99
2	Moisture content (%)	11.15
3	Drupe diameter (mm)	13.27
4	Drupe seed ratio	1:1.26
5	Seed filling (%)	75
6	Seed viability (%)	27

Indra, 2010). Similarly, Jayasankar *et al*. (1999) found more variability in physical traits and germination behaviour among teak drupes obtained from seven provenances in Kerala, India. Teak has a wide range of quantitative and qualitative characteristics depending on provenance and land race (Keiding *et al*., 1986). According to Dhaka & Jha (2017), the Mandvi provenance had the best seed length, seed breadth, 100-seed weight, filling percentage, drupe, and seed germination.

Physiological parameters

Drupe germination

The presowing treatments followed in this experiment tended to significantly (0.05%) influence germination, number of seedlings/100 drupes, and seedling emergence. The results revealed that T15 – T18 soaking-drying for 5 days + soaking in 0.5, 1.0, 1.5 and 2% $CaOCl_2$ for 12 hours recorded the highest germination of 14.20, 15.18, 15.84 and 17.16 percent, respectively, compared to all other treatments, including the control. The germination of teak drupes increased with increasing concentrations of $CaOCl_2$, followed by all other treatments. The experimental outcome of the tetrazolium test is not in line with the germination results. In this experiment, 27 percent of the true seeds alone were recorded as viable, but under in vitro germination conditions, true seeds recorded up to 40 percent germination. From this experiment, it is inferred that there is no correlation between seed viability and true seed germination under in vitro conditions. Hence, a tetrazoliumim-based viability test is not the best option to estimate the viability in teak. The larger drupe size in teak had better germination, as said by many authors (Samapudhi, 1967; Kumar, 1979).

Regarding seedlings/100 drupes, the highest number of 31.0 seedlings was produced in S-D for 5 days + soaking in 2% $CaOCl_2$ for 12 hours compared to all other treatments. Drupes treated with S-D for 5 days + soaking in 1.5% thiourea, 1% KNO_3, 0.5% H_2O_2 and 0.5, 1.5% $CaOCl_2$ for 12 hours required a minimum of 14 days for seedling emergence compared to all other treatments, including the control. In the case of root length, S-D for 5 days + soaking in 2% H_2O_2 and 1.5% $CaOCl_2$ for 12 hours recorded the highest root length of

Table 2. Effect of presowing treatments on germination and seedling vigour of fresh teak drupes

Treatments	Germination (%)	Seedling/100 drupes	Days taken for seedling emergence	Root length (cm)	Shoot length (cm)	Dry matter production (mg seedling^{-1})	Vigour index
T1 – Control	2.64 (8.130)	3.31	24	4.4	4.0	23	22.176
T2 - S-D for 6 days	3.30 (9.974)	4.20	19	5.2	4.3	39	31.350
T3 - S-D for 5 days + Soaking in 0.5% Thiourea 12 hours	3.32 (9.974)	5.10	16	5.2	4.0	40	30.544
T4 - S-D for 5 days + Soaking in 1% Thiourea 12 hours	3.98 (9.974)	6.21	15	5.3	4.1	39	37.412
T5 - S-D for 5 days + Soaking in 1.5% Thiourea 12 hours	3.86 (9.974)	6.00	14	5.1	4.2	41	35.898
T6 - S-D for 5 days + Soaking in 2% Thiourea 12 hours	4.13 (11.537)	7.20	15	5.4	4.1	42	39.235
T7 - S-D for 5 days + Soaking in 0.5% KNO3 12 hours	6.32 (14.179)	9.80	15	5.9	4.9	48	68.256
T8 - S-D for 5 days + Soaking in 1% KNO3 12 hours	7.51 (15.342)	12.20	14	5.2	4.3	45	71.345
T9 - S-D for 5 days + Soaking in 1.5% KNO3 12 hours	6.28 (14.179)	10.20	15	5.3	4.2	47	59.660
T10 - S-D for 5 days + Soaking in 2% KNO3 12 hours	6.41 (14.179)	10.70	16	5.8	4.1	40	63.459
Mean	4.775 (11.537)	7.496	16.29	5.28	4.21	40.40	45.93
Sed	0.100	0.145	0.341	0.094	0.076	1.037	0.827
CD (P=0.05%)	0.209	0.304	0.713	0.197	0.159	2.164	1.726

(Figures in parentheses indicate arc sine value)

Table 3. Effect of presoaking treatments on germination and seedling vigour of fresh teak drupes

Treatments	Germination (%)	Seedling/100 drupes	Days taken for seedling emergence	Root length (cm)	Shoot length (cm)	Dry matter production (mg seedling^{-1})	Vigour index
T1 – Control	2.64 (8.130)	3.31	24	4.4	4.0	23	22.176
T2 - S-D for 6 days	3.30 (9.974)	4.20	19	5.2	4.3	39	31.350
T3 - S-D for 5 days + Soaking in 0.5% H_2O_2 12 hours	6.31 (14.179)	8.20	14	5.8	4.9	41	67.517
T4 - S-D for 5 days + Soaking in 1% H_2O_2 12 hours	7.31 (15.342)	10.10	15	5.1	4.6	39	70.907
T5 - S-D for 5 days + Soaking in 1.5% H_2O_2 12 hours	6.80 (14.179)	9.30	16	5.7	5.0	40	72.760
T6 - S-D for 5 days + Soaking in 2% H_2O_2 12 hours	7.45 (15.342)	8.21	16	6.3	5.3	42	86.420
T7 - S-D for 5 days + Soaking in 0.5% $CaOCl_2$ 12 hours	14.20 (21.973)	23.50	14	5.6	4.4	43	142.000
T8 - S-D for 5 days + Soaking in 1% $CaOCl_2$ 12 hours	15.18 (22.787)	17.54	15	5.4	4.8	38	154.836
T9 - S-D for 5 days + Soaking in 1.5% $CaOCl_2$ 12 hours	15.84 (22.787)	19.30	14	6.3	5.0	40	178.992
T10 - S-D for 5 days + Soaking in 2% $CaOCl_2$ 12 hours	17.16 (24.350)	31.00	15	5.6	5.2	44	185.328
Mean	9.61 (17.458)	13.46	16.21	5.53	4.75	38.90	101.21
Sed	0.205	0.271	0.346	0.078	0.092	0.853	2.200
CD (P=0.05%)	0.428	0.565	0.723	0.164	0.193	1.780	4.590

(Figures in parentheses indicate arc sine value)

6.3 cm compared to all other treatments. The highest shoot length of 5.3 cm was recorded in S-D for 5 days + soaking in 2% H_2O_2 for 12 hours followed by all other treatments. S-D for 5 days + soaking in 0.5% KNO_3 for 12 hours resulted in significantly higher dry matter production in the 48 mg/seedling treatment than in the other treatments. In the case of the vigour index, S-D for 5 days + soaking in 1.5% $CaOCl_2$ for 12 hours had a higher vigour index value of 185.32 compared to all other treatments, including the control (Tables 2 and 3). Slaked lime Ca(OH)2 is blended with $CaOCl_2$ (bleaching powder), calcium hypochlorite Ca(OCl)2, and basic chloride $CaOCl_2$, H_2O. The chlorine in calcium chloride acts as a bleaching agent and is responsible for the evaluation of nascent oxygen in the living system. Bleaching may erode and soften the mesocarp of teak drupes and manage the osmotic pressure gradient, allowing for sufficient moisture to activate enzymes and cell development (Osborne, 1972; Morry *et al*., 1972). However, the mechanism by which $CaOCl_2$ improves germination is unknown, and more research is needed. Many authors have reported similar results in a variety of crops. Fresh and 10-month-old teak drupes treated with 4% $CaOCl_2$ increased germination and seedling vigour, according to Masilamani *et al*. (2008). Masilamani *et al*., (2015) for *Psedium gujava*; Masilamani *et al*., (2013) for *Terminalia catappa*; Seenu, (1987) for acid lime; and Maideen *et al*., (1990) for *Casuarina equisetifolia* observed similar results.

True seed germination

When compared to drupe germination, true seeds had the highest germination rate of 40%, the shortest time to initial emergence (6 days), the longest root length (8.54 cm), the highest dry matter production (64.7 mg), and the highest vigour index (454.8). (Table 4). True seed germination (40%) was much higher than control drupe germination at 28 days after sowing (2.64%).

Table 4. Drupe and true seed germination in teak

Parameter	Days taken for initial emergence	Germination (%) 14 DAS	28 DAS				
			Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter production (mg/10seedlings)	Vigour index
Drupe	24	0	2.64	4.4	4.0	23.0	22.1
True seed	6	33.3	40.0	8.54	2.83	64.7	454.8

DAS- Days after sowing

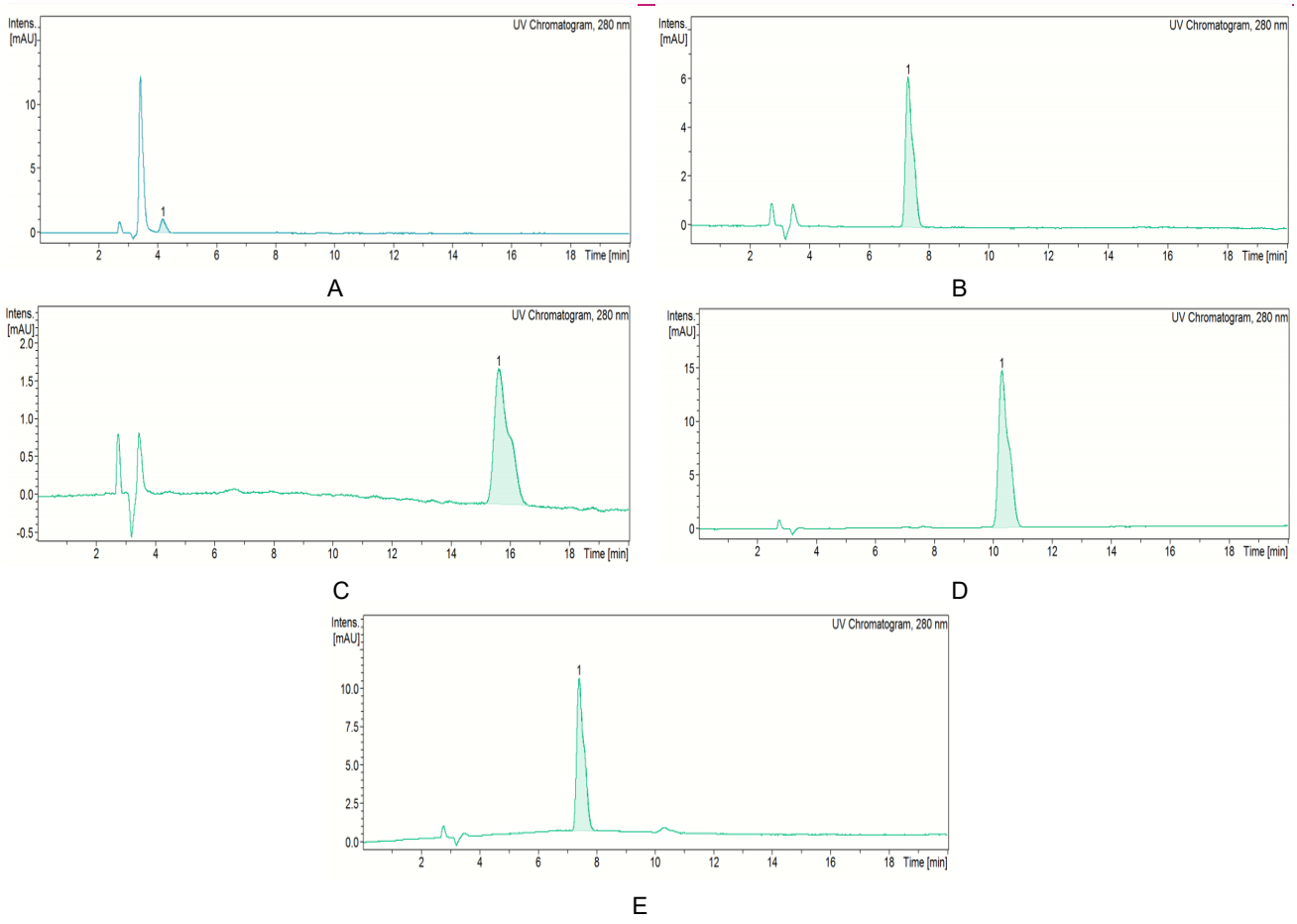

Fig. 1. *Detection of biochemical promotors and inhibitor standards by HPLC (A) - gibberellic acid, (B) - indole-3-acetic acid, (C) - indole-3-butyric acid, (D) - abscisic acid and (E) - coumarin*

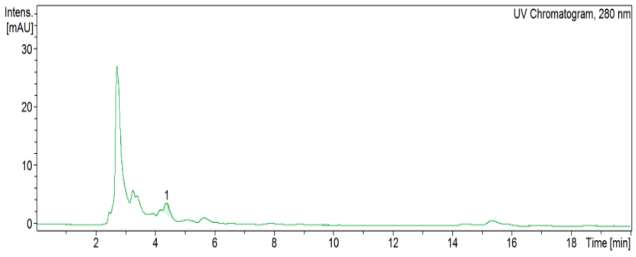

Fig. 2. *Detection of biochemical promotors in fresh true seeds by HPLC*

The germination rate of the drupe soaked in 2% $CaOCl_2$ was 17.6%, which was 1.25 times lower than the germination rate of true seeds. This method was used to demonstrate the occurrence of mechanical dormancy in teak seeds. As a result, teak germination has been found to be restricted by a barrier such as a hard rocky endocarp (Rajput & Tiwari, 2001; Slator *et al*., 2013; Masilamani *et al*., 2020). Dhaka and Jha, 2017 also found that teak drupes collected from five different provenances showed that only 13.55 percent of drupes will be germinated in all provenances, but in the case of true seeds, 54 percent germination was observed, which is four times higher than drupes. These results clearly indicate that teak governs physical and mechanical dormancy. Yashodha *et al*. (2005) also reported an in vitro promising method for producing high-quality teak seedlings. True seed germination was only 40% in this study, and the remaining ungerminated true seeds may possess morphological dormancy.

Biochemical parameters

HPLC analysis showed that gibberellic acid was present (1.91 mg/kg), whereas the other biochemicals, viz., IAA, IBA and inhibitors (ABA and coumarin), were not present in the extract of fresh teak true seeds and mesocarp (Table 5-6 and Fig 1-2). Gibberellic acid (GA_3) is a natural plant regulator with multiple applica-

Table 5. Quantification of biochemical inhibitors present in the mesocarp extract of teak drupes

Top slip seed production area	Retention time (Min)	Area	Coumarin (mg/kg)
	-	-	-

Table 6. Quantification of biochemical promotors and inhibitors present in teak true seeds

Biochemical promotors and inhibitors	Retention time (Min)	Tamil Nadu (Top slip) – Bulk seeds
Gibberellic acid	4.4	1.91
Indole-3-acetic acid	7.4	-
Indole-3-butyric acid	16.0	-
Abscisic acid	10.4	-
Coumarin	7.4	-

tions in the agriculture and horticulture industries due to its positive impact on plant growth and development. Gibberellins (GA) are essential plant regulators of multiple plant development processes, including seed germination, stem elongation, leaf extension, pollen maturation, and flowering induction (Cornea-Cipcigan *et al*., 2020). Gibberellins stimulate germination by inducing hydrolytic enzymes that weaken barrier tissues such as the endosperm or seed coat, inducing mobilization of seed storage reserves and stimulating the expansion of the embryo (Bewley and Black, 1994; Thomas, *et al*., 2005).

Conclusion

Fresh teak drupes taken from Tamil Nadu's top slip seed production area responded favourably to the calcium oxychloride seed treatment. True seeds isolated from teak drupes were implanted in vitro, and up to 40% germination rates were observed. This is a threefold increase in germination above drupe germination in vivo. The presence of physical dormancy in the drupe gathered from the indicated place is demonstrated by this finding. Furthermore, HPLC research results verified the presence of physical dormancy. The *in vitro* approach is an alternative way to produce more, uniform, and healthier teak seedlings for larger-scale production. The problem of physical and mechanical dormancy can be solved with this technology.

ACKNOWLEDGEMENTS

The authors are thankful to the Department of Science and Technology, SERB (EMR/2016/006171), Government of India for funding and Centre of Excellence in Sustaining Soil Health, Anbil Dharmalingam Agricultural College and Research Institute, Tamil Nadu Agricultural University, Trichy, Tamil Nadu for the facilities provided.

Conflict of Interest

The authors declare that they have no conflicts 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/cropsci1973.0011183X001300060013x.
2. Amadi, D. C. A., Glory, J., Kwada, D. K., & Thlama, D. M. (2019). The Effect of Three Pre-Germination Treatments on the Germination and Early Growth Studies of *Tectona Grandis* (Teak) For Rural Afforestation. *Journal of Agriculture & Agricultural Technology*., 19, 1-15.
3. Bewley, J. D. & Black, M. (1994). Seeds: Physiology of Development and Germination, Plenum, New York. pp.134.
4. Billah, M. A. S., Kawsar, M. H., Titu, A. P., Pavel, M. A. A., & Masum, K. M. (2015). Effect of presowing treatments on seed germination of Tectona grandis. *International Journal of Bioinformatics and Biomedical Engineering*, 1, 37-42.
5. 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. doi:10.3390/agronomy10040516
6. Da Silva Junior, G. D., da Silva Reis, A., Leite, M. R. P., da Costa Leite, R., de Sousa Soares, G. O., & de Sousa Soares, J. L. (2017). Methods for overcoming dormancy in teak diaspores. *Pesquisa Agropecuária Tropical*.
7. 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
8. Evans, J. & Turnbull, J. W. (2004) Plantation forestry in the Tropics. Oxford University Press, New York, NY, USA, pp. 482.
9. International Seed Testing Association. (1985). International rules for seed testing, *Seed Science and Technology.,* 13, 229-355.
10. International Seed Testing Association. (1999). International Rules of Seed Testing. *Seed Science and Technology.,* 27, 27-32.
11. Jayasankar, S., Babu, L. C., Sudhakara, K. & Unnithan, V. K. G. (1999). Provenance variation in seed and germination characteristics of teak (*Tectona grandis* L. f.). *Seed Science and Technology,* 27, 131–139.
12. Jose, S & Indira, E. P. (2010). Variability of seed related characters in teak (*Tectona grandis* L. f) from Western Ghat region. *Gregor Mendel Foundation Journal,* 1, 39-44.
13. Kaosaard, A., Suangtho, V. & Kjaer, E. D. (1998). Genetic improvement of teak (*Tectona grandis*) in Thailand. *Forest Genetic Resources,* 26, 21-29.
14. Keiding, H. (1993). *Tectona grandis*. *Seed Leaflet*, pp *4*.
15. Keiding, H., Lauridsen, E. B. & Wellendorf, H. (1986). Evaluation of an international series of teak provenance trials. Danida Forest Seed Centre, Denmark.

16. Krishnamoorthy, M., Palanisamy, K., Francis, A. P., & Gireesan, K. (2016). Impact of Environmental Factors and Altitude on Growth and Reproductive Characteristics of Teak (*Tectona grandis* Linn. f.) in Southern India. *Journal of Forest and Environmental Science*, 32, 353-366. https://doi.org/10.7747/JFES.2016.32.4.353
17. Kumar, A. (1979). Effect of fruit size and source on germination of teak seeds, Sri Lanka forester. 14:58-63.
18. Maideen, S. K., Selvaraj, J. A. & Rai, R. V. (1990). Presowing chemical treatment to hasten germination of *Casuarina equisetifolia*. *International Tree Crops Journal,* 6, 173-181. https://doi.org/10.1080/01435698.19 90.9 752882
19. Masilamani, P., Dharmalingam, C. and Annadurai, K. (2008). Effect of Calcium Oxychloride Presowing Treatment to Hasten Germination of Teak (*Tectona grandis* Linn. f.) Drupes. *Indian Forester,* 134, 1680-1685. DOI**:** 10.36808/if/2008/v134i12/878
20. Masilamani, P., Nagaraja, A., Yadav, M. P. & D. K. Srivastava. (2015). Influence of Different Presowing Treatments on Germination and Seedling Vigour of Guava (*Psedium guajava* L.) Seeds. *Journal of Non-Timber Forest Products,* 22, 7-9.
21. Masilamani, P., Rajanbabu, V. & 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. DOI: http://dx.doi.org/10.13005/bbra/2871
22. Masilamani, P., Yasodha, P. & Annadurai, K. (2013). Influence of Seed Pretreatments and Sowing Conditions on Germination and Initial Seedling Vigour of Indian Almond (*Terminalia Catappa* L.). *Indian Forester.,* 139, 248-252.
23. Morry, Y. Y., Chem, D. & Savid, S. (1972). Inset of deoxyribonucleic acid synthesis in germination of wheat embryos. *Plant Physiology,* 49, 20-23.
24. Murashige, T. & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiological Plantarum,* 15, 473-497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x
25. Omokhua, G. E. & Alex, A. (2015). Improvement on Teak (*Tectona Grandis* Linn F.) germination for large scale afforestation in Nigeria. *Nature and Science*, 13, 68-73.
26. Osborne, J. (1972). Can weed problems be reduced by improving crop establishment. come possibilities for enhancing seed germination. Proc. 11th British weed control conference. Pp. 1161-1172.
27. Pamei, K., Larkin, A., & Kumar, H. (2017). Effect of different treatments on the germination parameters and seedling quality index of Tectona grandis (Teak) under nursery condition. *International Journal of Communication Systems*, 5, 2418-2424.
28. Panse, V. G. & Sukhatme, P. V. (1995). *Statistical methods for agricultural workers*. Indian Council of Agricultural Research Publications, New Delhi. PP. 330.
29. Raghu, H. B., Ashwin, R., Ravi, J. E., & Bagyaraj, D. J. (2020). Microbial consortium improved growth and performance of teak (Tectona grandis Lf) in nursery and field trials. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*., 90, 903-909.
30. 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.
31. Ravichand, A., & Gunaga, R. P. (2021). Seed Biology and Seed Orchard Dynamics in Teak. In *The Teak Genome* Springer, Cham. Pp. 139-153. DOI: 10.1007/978-3-030-79311-1_9
32. Samapudhi, K. A. (1967). Country Report on Teak in Thailand. FAO Forestry Paper, Rome. 7. 18.
33. Santosa, S., Soekendarsi, E., Priosambodo, D., & Kasim, A. H. (2022). Water content, resorption of N and P, and the growth of teak Tectona grandis Lf seedlings on four types of growing media under drought stress. *Journal of Biological Research*. Accepted for publication. https://doi.org/10.4081/jbr.2022.9715
34. Seenu, G. (1987). Seed quality studies in Acid lime. *M. Sc.(Ag.) thesis. Tamil Nadu Agricultural University,* Coimbalore, 143.
35. 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 Forest.,* 44, 39-49. DOI 10.1007/s11056-011-9298-0
36. 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 and Phytochemistry,* 6, 726-729.
37. Tewari, D. N. (1992). A monograph on teak (*Tectona grandis* Linn. f.). International book distributors, Dehra Dun pp. 479.
38. Thomas, S. G., Rieu, I. & Steber, C. M. (2005). Gibberellin metabolism and signaling. *Vitam Horm*., 72, 289- 338. https://doi.org/10.1016/S0083-6729(05)72009-4
39. Yasodha, R., Sumathi, R. & Gurumurthi, K. (2005). Improved micropropagation methods for teak. *Journal of tropical Forest Science*, 63-75.