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RESEARCH ARTICLE

Evaluation of Dormancy Mechanism in Teak (*Tectona grandis* Linn.f) True Seeds Collected from Multiple Provenances and Forest Types of India

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ABSTRACT

Teak drupes were collected from seven geographical locations spread over four forest types (dry deciduous, moist deciduous, very moist deciduous, and semi-deciduous) from India. In order to study the dormancy mechanism associated with teak true seeds. The true seeds present inside the drupes from each provenance were extracted without causing any damage to the true seeds. The extracted true seeds were used to study the morphological and physiological characteristics. The true seeds were carefully inoculated in MS medium to observe germination. Twenty-eight days after sowing, the result revealed that Andhra Pradesh provenance recorded the highest true seed germination of 58 percent compared to all other provenances. These results clearly indicate that not only physical and mechanical dormancy is involved in teak for decreased germination but also morphological/physiological dormancy plays a major role in teak true seed germination.

Keywords: *Teak, provenance, forest types, true seeds, dormancy, in-vitro germination*

INTRODUCTION

Teak (*Tectona grandis* L.) is a timber-producing deciduous tree belonging to the family Verbenaceae. It is native to Myanmar, India, Laos, and Thailand and is considered among the top five tropical hardwood species worldwide (De Gyves *et al*., 2007). India has the largest teak forest and teak plantation area i.e., 9.0 million ha (Bhat and Ma, 2004). Difficulties in establishing large-scale plantations of teak are due to poor and erratic germination. Extremely low germination rates are a significant problem for the teak plantation industry (Tewari, 1992) as well as the deployment of planting material from breeding programs (Kaosard *et al*., 1998). The nature of barriers that prevent germination in teak can be physiological (presence of germination inhibitors in felty mesocarp and true seed), physical (thick and hard endocarp), and morphological (immature embryo in true seeds), which results in low germination (Masilamani *et al*., 2008; Venkatesan *et al*., 2023).

This delayed and irregular germination of seeds in the nursery is a serious constraint for teak for efficient nursery management and plantation establishment. In vitro propagation technique has become an efficient way for producing plants as uniform as possible on a large scale and in a short time for the plantation industry (Tiwari, 2002). Therefore, the present study has been taken to extract true seeds from drupes collected from seven provenances involving four forest types of India and used for in vitro germination to find out the dormancy mechanism involved in teak true seeds.

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MATERIAL AND METHODS

Drupe collection

Teak drupes were collected from seven provenances involving four forest types of India viz., Moist deciduous forest (Tamil Nadu), very moist deciduous forest (Kerala), dry deciduous forest (Andhra Pradesh, Telangana and Karnataka) and semi deciduous forest (Maharashtra and Madhya Pradesh) (Table1: Fig.1). From each provenance, teak drupes were collected from 10 randomly selected plus trees and bulked. The bulked drupes were properly dried, and cleaned by removing shrivelled and insect damaged drupes. Finally, drupes were size graded and the drupes with more than 9mm diameter were used in this experiment.

True seed extraction

The true seeds were extracted from drupes with a wooden mallet. True seeds located inside the locules of the drupes were removed carefully without any damage to the cotyledon and seed coat. Extracted true seeds were used for conducting in vitro germination study (Masilamani *et al*., 2020a).

True Seed weight

Following the International Seed Testing Association (ISTA 1985), 100-true seed weight was measured using 100 true seed and a highly precise electronic balance with eight replications.

True seed area, diameter, perimeter, roundness and fullness ratio

An image analyser was used to determine the physical properties of true seed retrieved from the seven provenances, including area, diameter, perimeter, roundness, and fullness ratio. With five replications, a total of 25 true seed were examined. The true seed were placed in an upright position on the platform, and images were recorded using QWin software and a CCD camera. The images were then calibrated to actual scale. QWin was used to analyse the acquired and calibrated images (Sivakumar *et al*., 2002).

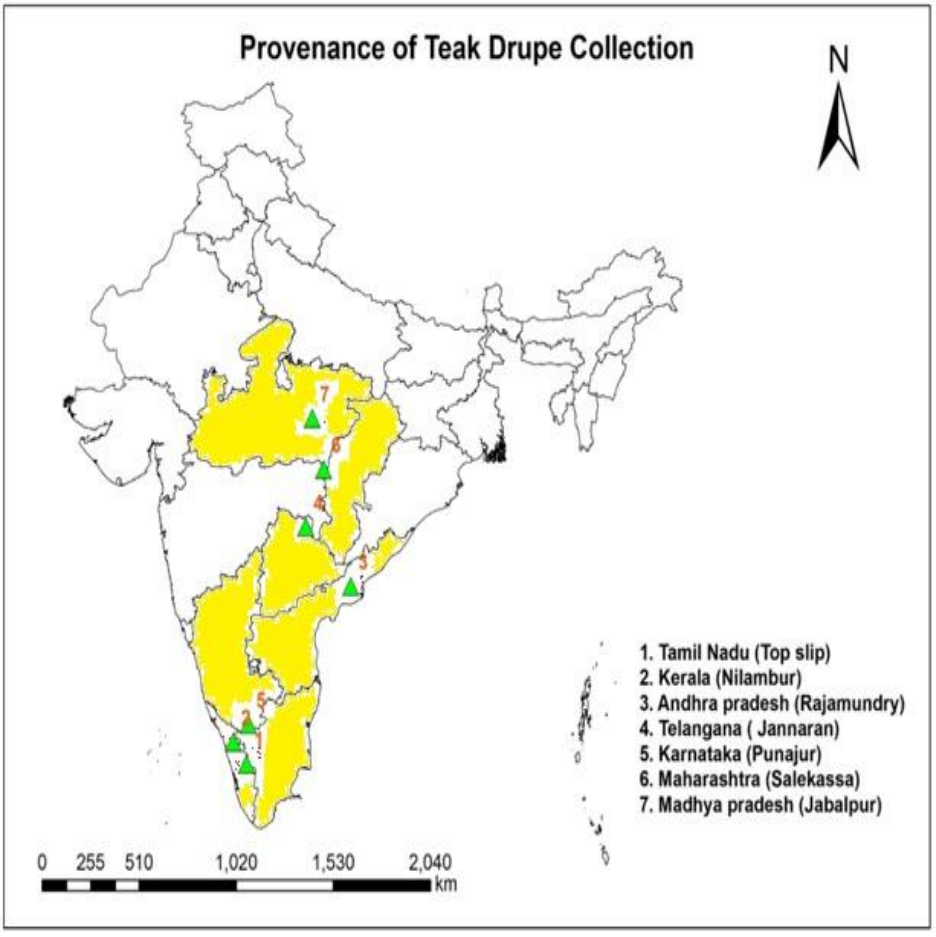

Fig 1. Provenances of teak drupe collection

Table 1. Description of the seed Provenances

Provenance	Teak forest type	Longitude	Latitude	Altitude (m)	Rainfall (mm)
Tamil Nadu (Top slip)	Moist Deciduous	74°34'E	15°07'N	750	1800.0
Kerala (Nilambur)	Very Moist Deciduous	76°13'E	11°16'N	400	2952.0
Andhra Pradesh (Rajamundry)	Dry Deciduous	81°46'E	17°0'N	14	959.0
Telangana (Jannaram)	Dry Deciduous	78°99'E	19°11' N	293	1080.0
Karnataka (Punajur)	Dry Deciduous	76°56'E	11°55'N	661	799.3
Maharashtra (Salekassa)	Semi Deciduous	80°29'E	21°18' N	300	1578.5
Madhya Pradesh (Jabalpur)	Semi Deciduous	79°57'E	23°10'N	416	1279.5

Media preparation

Half strength MS media was prepared for in vitro germination by adding 50% of the necessary amount of macro, micro, and minor components (Murashige and Skoog, 1962). The medium containing full strength vitamins, 3 percent sucrose, and 0.22 M BAP, and the pH was adjusted to 5.8. After adjusting the pH, 0.8 percent agar was added and the media, heated to homogenise the agar. 50 mL of melted medium was uniformly distributed into culture bottles. Finally, the culture bottles were autoclaved for 20 minutes at 121°C + 15 psi pressure.

Sterilization and inoculation of true seeds

Prior to sterilization, the true seeds were sun-dried for 1 hour. For five minutes, the seeds were immersed in distilled water, 0.1 percent Bavistin and 0.1 percent Tween 20 with constant shaking. Following the Bavistin treatment, the seeds were rinsed with tap water for one minute and then in 70% ethanol for 30 seconds. The true seeds were rinsed with sterile distilled water after being washed with ethanol. The seeds were then sterilised in a 0.1 percent mercuric chloride ($HgCl_2$) solution for five minutes while being constantly shaken. The true seeds were rinsed three times with sterile distilled water after being sterilised with HgCl2.

In vitro true seed germination test

The sterilised seeds were carefully inoculated in a half MS medium bottle under a laminar airflow chamber. Six seeds were inoculated in culture bottle with eight replications. Then the culture bottles were placed in primary growth room maintaining 25°C temperature, 16 hours light and 8 hours dark conditions. The in vitro seed inoculates were monitored once a day for the time taken for first emergence and germination % , 14 and 28 days after planting (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 derived from the formula (Abdul Baki and Anderson, 1973).

Statistical analysis

As per Panse and Sukhatme, (1995) the results were subjected to analysis of variance and assessed (t-test) for significant differences (p=0.05). Prior to statistical analysis, percentage values were converted to arc sine values.

RESULTS AND DISCUSSION

Physical Parameters

True seeds from the dry deciduous forest (Karnataka) had highest 100 true seed weight (2.57 g) than those from the rest of the provenances. Drupes from semi-deciduous forest (Maharashtra) had the lowest 100 true seed weight of 2.01g (Table 2). True seeds from the dry deciduous forest (Karnataka) had significantly higher area (0.182 cm2), higher length (0.182 cm), higher breadth (0.410 cm), highest roundness (1.843) and highest fullness ratio (1.594) than those from the rest of the provenances. Seeds from semi deciduous forest (Madhya Pradesh) recorded the highest perimeter (91.875 cm) when compared to rest of the provenances. Seeds from dry

Table 2. Effect of provenance on true seeds physical parameters of teak

Provenance	100 true seed weight (g)	Area (cm^2)	Length (cm)	Breadth (cm)	Perimeter (cm)	Roundness	Fullness ratio
Tamil Nadu (Top slip)	2.53	0.141	0.528	0.362	85.219	1.497	1.396
Kerala (Nilambur)	2.40	0.162	0.571	0.384	89.297	1.598	1.495
Andhra Pradesh (Rajamundry)	2.10	0.148	0.548	0.365	83.672	1.530	1.431
Telangana (Jannaram)	2.10	0.131	0.528	0.341	86.719	1.480	1.359
Karnataka (Punajanur)	2.57	0.182	0.602	0.410	86.484	1.843	1.594
Maharashtra (Salekassa)	2.01	0.132	0.531	0.343	82.500	1.449	1.361
Madhya Pradesh (Jabalpur)	2.06	0.144	0.558	0.356	91.875	1.559	1.429
Mean	2.25	0.14	0.55	0.55	86.53	1.56	1.43
SEd	0.045	0.003	0.009	0.009	1.344	0.037	0.026
CD (P=0.05)	0.097	0.006	0.019	0.019	2.884	0.079	0.056

(Figures in parentheses indicate arc sine value)

deciduous forest (Telangana) recorded significantly lowest area (0.131 cm2) and lowest fullness ratio (1.359) and semi deciduous forest (Maharashtra) and dry deciduous forest (Telangana) recorded lowest breadth (0.341 cm) and semi deciduous forest (Maharashtra) recorded lowest perimeter of 82.50 cm. and the lowest roundness (1.449) (Table 2). The size of teak drupes varied greatly according on the seed source (Jijeesh and Sudhakara, 2013), this may influence in the true seed also. The performance of a provenance is influenced by the place and seed source (Glover, 1987; Chadhar, 1994).

Several authors are investigating the provenance effect of physical characteristics in teak drupes not only in true seed. According to Krishnamoorthy *et al*. (2016), teak drupes from Kerala's Nilambur area is superior. High rainfall and site characteristics may be the primary source of the increase in drupe size in moist forest types. According to Surendra *et al*. (2018) the drupes harvested from Karnataka's Ponnampet seed source would have the maximum seed length and breadth. This huge variability can be attributed to various conditions associated with genetic and physiological components that prevailed both in dry and moist sources (Gupta and Pattanath, 1973). The results of the provenance effect of physical characteristics in true seed may reflect in the same trend as teak drupes.

Physiological Parameters

In vitro true seed germination

Significant differences were observed among all provenances. Andhra Pradesh provenance recorded the highest in vitro germination of 33.3 % on 14 days after sowing and 58.3 % on 28 days after sowing and was followed by all other provenance. Kerala and Maharashtra provenance recorded the lowest in vitro germination of 16 % on 28 days after sowing.

Andhra Pradesh provenance recorded the longest root length of 4.1 cm and the shortest root length was recorded by Madhya Pradesh (1.3 cm). Maharashtra provenance recorded the longest shoot length of 4 cm and the shortest shoot length was recorded in Andhra Pradesh (1.7 cm). Telangana provenance recorded the highest dry matter production of 0.072mg and the lowest dry matter production of 0.032mg was recorded in Kerala provenance. Higher vigour index (340.4) was recorded in Andhra Pradesh provenance. The lowest vigour index (101.2) was recorded in Maharashtra provenance (Table 3; Fig.2).

Many of the authors observed that the teak predominantly governs physical and mechanical dormancy for decreased germination. Surprisingly, our data showed that true seed germination had only been 58% in this study, and the remaining un-germinated true seed may possess morphological/

Table 3. Effect of provenance on germination and seedling vigour of true seed *in vitro* germination in teak

Provenance	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
Tamil Nadu (Top slip)	6	33 (35.06)	43 (40.97)	3.6	2.9	0.058	281.4
Kerala (Nilambur)	6	16 (23.57)	16 (23.57)	1.5	2.2	0.032	61.42
Andhra Pradesh (Rajamundry)	7	33 (35.06)	58 (49.60)	4.1	1.7	0.064	340.4
Telangana (Jannaram)	7	33 (35.06)	33 (35.06)	4.3	4.4	0.072	289.7
Karnataka (Punajanur)	7	16 (23.57)	33 (35.06)	3.7	2.0	0.054	191.4
Maharashtra (Salekassa)	7	16 (23.57)	16 (23.57)	2.1	4.0	0.035	101.2
Madhya Pradesh (Jabalpur)	7	33 (35.06)	33 (35.06)	1.3	2.5	0.035	126.5
Mean	6.71	26 (30.65)	33 (35.06)	2.94	2.81	0.050	198.8
SEd	0.155	0.345	0.375	0.071	0.055	0.001	5.707
CD (P=0.05)	0.333	0.741	0.805	0.153	0.119	0.002	12.242

(Figures in parentheses indicate arc sine value) DAS – Days after sowing

DAS – Days after sowing

Fig 2: Effect of provenance on *in vitro* germination of teak true seeds

physiological dormancy (Dabral, 1976, Slator *et al*., 2013). It implies an extensive study into the germination behaviour of true seed, regardless of provenance. Under in vitro conditions, there was a substantial connection between survival rate and seedling growth. In this study, the Andra Pradesh seed source had quite a better seedling growth and survival rate in vitro, reflecting the findings of Akram and Aftab (2016) and Dhaka and Jha (2017). At 28 days after sowing, true seed germination was recorded at 58% of the true seeds from dry deciduous forest (Andhra Pradesh). The occurrence of mechanical dormancy in teak seed was demonstrated in this way. As a result, a barrier such as a hard stony endocarp was reported to restrict teak germination. Mechanical dormancy has been established by Rajput and Tiwari (2001) as a stony endocarp of teak fruit acting as a mechanical barrier to germination that emerging radicles cannot traverse without opening valves (Hill, 1933). Mechanical dormancy, rather than physical hibernation, was demonstrated by Slator *et al*., 2013. Teak's slow germination is sometimes related to the endocarp's participation in softening (Keiding, 1985; Tewari, 1992; Yadav, 1992; Tewari *et al*., 2004; Dhaka and Jha, 2017; Masilamani *et al*., 2020).

The true seeds from dry deciduous forest (Maharashtra) exhibited low true seed germination than those from the rest of the provenances. Hartmann *et al*., (1997) stated that the morphological embryo dormancy involves a requirement for embryo maturation or after-ripening after dispersal in young seeds. The very low rate of germination in fresh drupes compared to 1 year old that embryo dormancy is present in teak seed as reported previous by Dabral, 1976; Joshi and Kelkar, 1971; Suangtho, 1980.

For the plantation sector, in vitro propagation has become an effective approach of creating plants that are as uniform as possible on a big scale and in a short period of time (Pandey and Brown, 2000). True seed germination under in vitro condition was between 16-58 per cent across the provenance studied. This result is in line with the Dhaka and Jha, 2017, they reported that true seed germination was 54.0 per cent, collection of teak drupes from five different provenance. But the exact reason for low viability and high germination percent under in vitro condition may be due to morphological dormancy (Dabral, 1976; Slator *et al*., 2013; Masilamani, 1996). Yashodha *et al*., 2005 and Senthil Kumar, 2015 reported that in vitro seed germination holds promise for enhancing germination potential of teak on MS medium for early growth and good quality seedling production. It saves time and labour cost against germination under nursery conditions which is useful for tree species having high seed dormancy and low germination potential.

CONCLUSION

From this study it could be concluded that in vitro germination of seeds from dry deciduous forest (Andhra Pradesh provenance) recorded highest germination of 58 percent when compared to rest of the provenances. It is unequivocally stated that the germination of teak true seeds, which is regulated by morphological and physiological dormancy, necessitates an after-ripening period. This study useful for the precious genetically improved small size seed useful for establishing large scale teak plantations.

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Ethics statement

No specific permits were required for the described field studies because no human or animal subjects were involved in this research.

Originality and plagiarism

This is original research work and any work and/or words of others, has been appropriately cited.

Consent for publication

All the authors agreed to publish the content.

Competing interests

There were no conflict of interest in the publication of this content

Data availability

All the data of this manuscript are included in the MS. No separate external data source is required.

Author contributions

Research grant - PM, Idea conceptualization - PM, Experiments- PM, SV, Guidance - PM, VR, TE, SS, PR, Writing original draft - PM, SV, Writing- reviewing & editing - PM, SV, TE, VR, SS, PR

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