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Research Article

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Seed germination behaviour of *Terminalia paniculata* Roth (Combretaceae), an economically important endemic tree to peninsular India

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ABSTRACT

The present study was conducted to investigate the seed germination behaviour of *Terminalia paniculata*, a commercially important tree component of deciduous forests of Peninsular India. The study included the identification of maturity index to determine the optimum collection period of the most viable seeds with good seed longevity. Species are characterized by very low germinability and poor regeneration status. Observations were made throughout the flowering and fruiting period on selected matured trees. Seed viability was tested at various stages of maturity and seed longevity was evaluated. The results, indicated that the 16th week after anthesis with red-colored fruits recorded the maximum viability (2.60% germination) that shows it is the best period for the collection of seeds. Seed emptiness is very high in *T. paniculata* (4 to 2%) compared to other *Terminalia* species (low emptiness). Pre-sowing treatments was not effective in enhancing seed germination and viability. Seeds can be stored up to 6 months in an air-tight metallic container at 4°C and 45 ± 5 % relative humidity. The results of the study can be taken as a reference for various other tree improvement programs and further studies.

KEYWORDS: Germination, Maturity Index, Flowering Murdah, Seed Emptiness, Seed Longevity

INTRODUCTION

Terminalia L. (Combretaceae), commercially important taxa with winged fruits, is distributed in tropical and sub-tropical regions mainly in semi-evergreen, dry and moist deciduous forests. It is well known for its timber and traditional medicinal uses. Sixteen species of *Terminalia* were reported from India, and among them 12 species from mainland including 2 exotics, 4 from Andaman and Nicobar Islands, India (Gangopadhyay & Chakrabarty, 1997). *Terminalia paniculata* Roth is one of the multipurpose tree species endemic to Peninsular India and is distributed in Karnataka and Kerala. Massive fruiting in the deep red color of the species during the summer gives red coloration to the canopy and which leads the naming Flowering Murdah (Figure 1). Normally the tree grows up to 30 m height and more than 2.50 m diameters at breast height and distribution ranged from 800- 1200 MSL (Pillai, 2017). Wood is commonly used for construction, agricultural implements, boat building, plywood, blackboards, packing cases, and non-wood products are used for drug preparation, tannins, gums, oils, fodder and certain organic compounds (Narayanan *et al*., 2011; Nazma *et al*., 1981; Trotter, 1959). FAO, Botanical Garden Conservation International and several other agencies listed *T. paniculata* as one of the common commercially important tree species in India (FAO, 1984; Mark *et al*., 2014; Nair, 1971; Nazma *et al*., 1981; Trotter, 1959).

The population of the species is declined due to low regeneration status followed by anthropogenic activities especially massive felling of the trees for various wood and non-wood products (Pillai, 2017; Rani & Swaminath, 1999). Seed emptiness and poor seed germination are the general characteristic feature of the *Terminalia* genus and are very high in *T. paniculata* (Chacko *et al*., 2002). Low regeneration status of *T. paniculata* is due to high seed emptiness, poor seed germination, and climatic factors such as rain, temperature and moisture, which affect declining of the tree population (Chacko *et al*., 2002; Murali, 1997; Pillai, 2017). Population management and conservation of species are essential due to its multipurpose usage and endemicity. For the conservation of *T. paniculata* in the native range, detailed

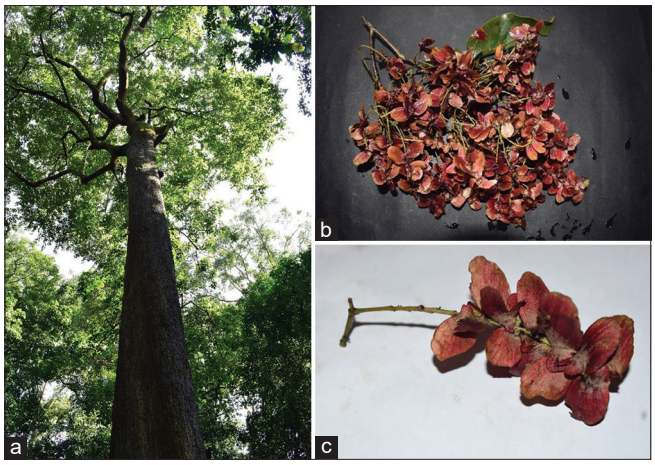

Figure 1: Tree habit located in South Nilambur forest division, Kerala, India (a), fruit twigs (b) and single fruit twig (c) of *T. paniculata* in Karulai forest range, South Nilambur forest division, Kerala, Peninsular India

knowledge on seed characteristics and analysis regarding seed quality parameters is needed. The information on seed behavior includes seed maturation, viability and seed longevity. Keeping all these factors in view, the present study was carried out to full-fill the above-mentioned objectives.

MATERIALS AND METHODS

Study Area

Maturity index analysis conducted in Peechi Forest Range of Peechi Wildlife Division of Kerala have an area of 126.73 sq. km. Peechi is bordered with Thrissur and Nenmara Divisions in the northwest and northeast side respectively and Chalakkudy Division and Chimmony Wildlife Sanctuary in the southern side (N 100 26'-100 40'; E 760 15'- 760 28'). Seed germinability, viability and longevity studies conducted by using the seeds collected from Karulai Forest Range of South Nilambur Division, Kerala have an area of 265.61 sq. km. Karulai is bordered with North Nilambur Division in the north, Kalikavu Range in the west and Tamil Nadu in the southeast side (N 110 14'- 110 23; E 760 19'- 760 34'). Laboratory experiments were conducted at Kerala Forest Seed Centre, Thrissur, Kerala, India.

Maturity Index

Thirty mature trees of *T. paniculata* were randomly selected from the study area for detailed investigation. Total height and circumference of trees ranging from 2.35–3.68 m ($\bar{x}$= 29.67 m) and 22–34 m ($\bar{x}$=3.15 m) respectively and crown dimensions such as crown diameter and crown length ranging from 12–26 m ($\bar{x}$= 19.6 m) and 10–17 m ($\bar{x}$= 12.4 m) respectively. Ten inflorescences from each tree were selected, marked, and labelled during flowering initiation as per standard protocol developed by Walton et al. (2016). Periodical (weekly) observations were done from the period of fruit initiation up to mature fruit fall initiation covering the entire fruiting phenophases. Fruits were collected weekly during different stages of fruiting phenophases and documented the details like color of drupe and seed using the Munsell soil color chart (Reeder *et al.*, 2014) and period of collection. Fruit characteristics such as fruit fresh mass, fruit dry mass, fruit large wing length, fruit large wing width and fruit small wings length were also documented. Shimadzu digital analytical weighing balance is used for estimating the fruit mass and Mitutoyo absolute digital Vernier Caliper is used for measuring the fruit large wing length, width and small wings length.

Seed Germinability

Seeds of *T. paniculata* were directly collected from thirty matured trees from the study area during 10th (when maturation starts) to 25th weeks (when abscission starts) after fruit set with an interval of one week. Fruit/seeds were processed and tested for different seed parameters such as seed weight (number of seeds per kg), moisture content (MC %), seed viability and seed longevity. The MC of seeds was estimated by hot air oven-dry method (dry seeds in hot-air oven for 1 hr at 130°C) as per ISTA rules (ISTA, 2005).

Seed Viability and Longevity

Data were documented for each stage of fruit development and determined maturity index and optimum time for seed collection. Seeds from the 16th week after fruit setting were collected periodically (weekly), determined MC% and tested viability in terms of rapid viability test (cutting test using seed cutter) and germination test. Different pre-sowing treatments were applied to study the treatment effect on seed germination. Germination trials were conducted in germination trays filled with vermiculite as media.

The treatment details are as follows:

1. Control (without any treatment)
2. Seeds soaked in water for 12 hrs
3. Seeds soaked in water for 24 hrs
4. Seeds soaked in water for 48 hrs
5. De-winged seeds soaked in water for 12 hrs
6. De-winged seeds soaked in water for 24 hrs
7. De-winged seeds soaked in water for 48 hrs

The seeds were soaked in tap water and a germination test was conducted as per ISTA (2005) rules. Treated seeds (n = 1000 x 8) were taken from each treatment and placed on wet vermiculite media in plastic trays (30 x 40.5 x 7 cm) and kept under laboratory conditions. Observations on seed germination like the initial day of germination, speed of germination were recorded throughout the germination period and the end of the period, the number of normal seedlings in each replication was counted and the germination was expressed in percentage (ISTA, 2005). Cleaned seeds were stored under cold (4°C) and ambient conditions in an air-tight plastic container for the study of seed longevity. Germination trials were conducted with monthly intervals with the stored seeds till the seeds express zero viability to estimate seed life span (Rajjou & Debeaujon, 2008). Data were recorded from the initial to the final day of seed

germination and determined different germination parameters as per the standard methods (Xu *et al.*, 2016).

Analysis

Seed moisture content and viability from 11th to 25th week were analysed according to the ordinary least square regression (r) analysis (Wang *et al.*, 2011). The effects of pre-sowing treatments were evaluated by Analysis of variance (ANOVA). For easier calculation and to explore the possible variation within the samples, statistical software PAST was used.

RESULT AND DISCUSSION

Maturity Index

Table 1 describes the colour of fruits/seeds, MC and germination per cent recorded during 11 – 25th week after seed setting. The developmental stage started with green coloured fruit with white-coloured non-viable seeds. From the 11th week after anthesis/seed setting, the greenish-red fruits have viable light brown seeds. Seed moisture content (4.69 ± 0.8%) and germinability (1.52 ± 0.76%) exhibited a slight increase up to 16th week and decreases afterwards. Seed moisture content and germinability exhibited a slight increase up to 16th week and decreases afterwards. Maximum moisture content and germinability noticed during the 16th week after fruit setting (59% MC and 2.60 ± 0.1% germination). The observation revealed that the seeds exhibited viability only up to 24th week and appears in dark red fruits with dark brown seeds. Sixteenth week was characterized by HUE 10R 4/6 red-coloured fruits with the help of the Munsell Soil Colour Chart. Morphological characteristics of fruits during the 16th week of fruit development is as follows - fruit colour (HUE 10R 4/6 red), fruit fresh mass (0.1849 ± 0.019 g), fruit dry mass (0.0370 ± 0.005 g), fruit large wing length (21.44 ± 1.16 mm), fruit large wing width (12.93 ± 0.79 mm), and fruit small wings length (14.66 ± 2.34 mm).

Seed Viability

Seed germination graph plotted against the moisture content and ordinary least square regression analysis revealed that there is a significant strong positive correlation ($r^2 = 0.80756$; $p = 0.001$) between the variables (Figure 2). The seed germination rate of different treatments was varied from 2.40 to 2.63% (Figure 3) and the maximum germination rate (2.63 ± 0.58%) was recorded in water soaking pre-sowing treatment for 24 hours (Table 2) but the effect of pre-sowing treatments found to be non-significant among the treatments. The study revealed that the germination initiated on the 14th day and required 21-22 days for completion.

Seed Longevity

Seeds of T. paniculata were able to maintain viability up to 6 months under cold condition (4oC with 45 ± 5 % RH) in an air-tight plastic container. However, a gradual decline from 2.60 ± 0.06 % to 0.20 ± 0.1 % of seed viability was observed during 6 months after storage (Figure 4). Table 3 depicts the longevity of seeds under storage. Seeds show 99.80% futile rate after six months under cold storage condition.

The size and weight of the seeds of *T. paniculata* are very small compared to other species of the genus- *T. tomentosa*, *T. arjuna*, *T. bellerica*, *T. catappa*, *T. chebula*, etc (Chacko *et al.*, 2002). The present study showed that the size of the fruit is 6.1 ± 2.07 g and one kilogram contains 18,216 ± 6,872 fruits with mean moisture content is 46.4%. Seed dispersal of *T. paniculata* is anemochorous

Table 1. Fruit/seed characteristics of T. paniculata under different maturity stages

Sl. No.	Weeks after anthesis	Color of fruit	Color of seed	MC (%)	Ger. (%)
1.	11	Greenish Red	Light Brown	44	0.6 ± 0.1
2.	12	Greenish Red	Light Brown	47	1.0 ± 0.1
3.	13	Light Red	Light Brown	50	1.6 ± 0.1
4.	14	Light Red	Light Brown	53	2.0 ± 0.1
5.	15	Red	Light Brown	56	2.1 ± 0.2
6.	16	Red	Light Brown	59	2.6 ± 0.1
7.	17	Red	Deep Brown	56	2.4 ± 0.1
8.	18	Red	Deep Brown	53	2.2 ± 0.2
9.	19	Red	Deep Brown	50	1.9 ± 0.1
10.	20	Red	Deep Brown	47	1.8 ± 0.1
11.	21	Deep Red	Deep Brown	44	1.8 ± 0.1
12.	22	Deep Red	Deep Brown	41	1.4 ± 0.1
13.	23	Deep Red	Deep Brown	38	1.2 ± 0.1
14.	24	Deep Red	Deep Brown	35	0.2 ± 0.1
15.	25	Deep Red	Deep Brown	30	0.0 ± 0.1

MC (%): Moisture content; Ger. (%): germination per cent

Table 2: Germinability of *T. paniculata* seeds under different pre-treatments

Sl. No.	Treatment	Imbibition period (days)	Germination duration (days)	Germination (%)
1.	Control	15	22	2.40 ± 0.00
2.	12 hr Water soaking	15	22	2.40 ± 0.00
3.	24 hr Water soaking	14	21	2.63 ± 0.58
4.	48 hr Water soaking	14	21	2.53 ± 0.58
5.	12 hr Dewinged water soaking	14	21	2.57 ± 0.58
6.	24 hr Dewinged water soaking	14	21	2.47 ± 0.58
7.	48 hr Dewinged water soaking	14	21	2.47 ± 0.58

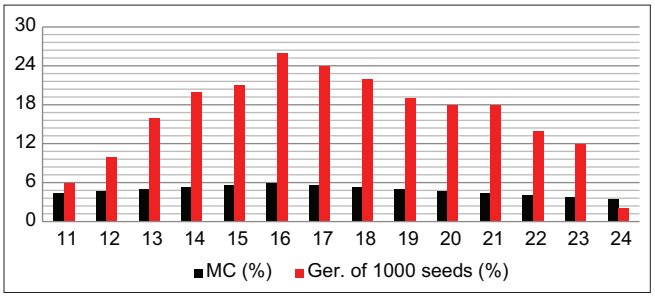

Figure 2: Seed moisture content on seed germination during 11th to 24th week after fruit setting in *T. paniculata* (MC: moisture content; Ger.: germination percent)

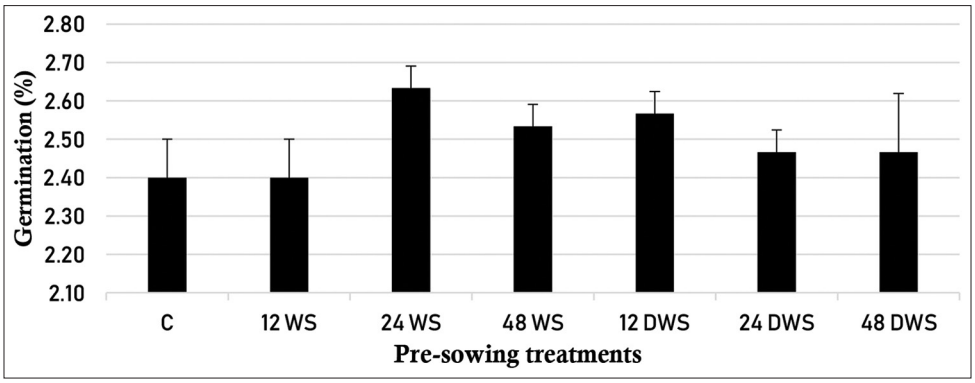

Figure 3: Influence of pre-sowing treatments on *T. paniculata* seed germination. Lines on the top of the bars indicate the standard error of the sample. C: control; 12WS: 12 hr water soaking; 24WS: 24 hr water soaking; 48WS: 48 hr water soaking; 12DWS: 12 hr dewinged water soaking; 24DWS: 24 hr dewinged water soaking; 48DWS: 48 hr dewinged water soaking

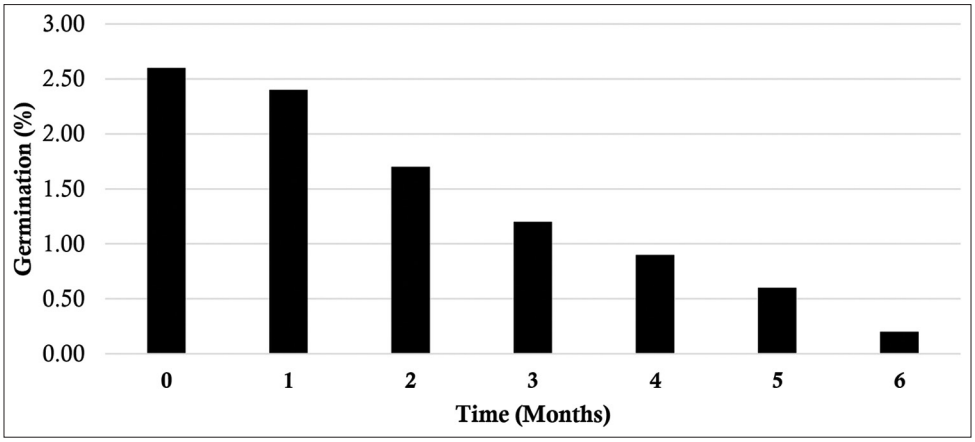

Figure 4: Influence of storage time on *T. paniculata* seed germination. Number 0 is the initial germination after collection.

like *T. arjuna* and *T. tomentosa* due to its winged fruit character. *T. paniculata* is characterized by high seed emptiness compared to associate tree species such as Grewia tilifolia, Lagerstroemia microcarpa, Tectona grandis, Pterocarpus marsupium, Xylia xylocarpa, etc (Chacko *et al*., 2002). Pre-sowing treatments are generally used to enhance seed germinability and speed of germination. There were different treatments applied to enhance germinability in *Terminalia* such as water soaking, scarification, hot water, acid scarification, hormone application, de-pulping, etc (Hossain *et al*., 2005; Hossain *et al*., 2014; Likoswe *et al*., 2008; Mewded *et al*., 2018). The application of 6 pre-sowing treatments in *T. paniculata* resulted that the pre-treatments are futile. Germinability of *T. paniculata* in natural conditions is very low (<1%) due to seed emptiness and high pest infestation and in laboratory conditions is 2.60% (Chacko *et al*., 2002). Storage study of the species confirmed that under cold conditions in an air-tight container, seed longevity shall be extended up to 6 months. Compared to other *Terminalia* species (360 days) or other associate winged species such as Pterocarpus marsupium (360 days), *Swietenia macrophylla* (360 days) and Dalbergia latifolia (360 days), seed longevity period is very low (Murali, 1997; Tompsett, 1986).

The rate of seed emptiness is very high in *T. paniculata* compared to other species belongs to *Terminalia* (Chacko *et al*., 2002). Seed emptiness is very less in T. *arjuna*, *T. bellerica*, *T. catappa*, *T. chebula* and their germinability is 61%, 85%, 80%, 60 % respectively (Chacko *et al*., 2002). There are sixteen species of *Terminalia* are distributed in India, four of them are producing winged fruits including *T. paniculata*. Earlier studies confirmed that de-winging is the best pre-treatment of seed germination of winged seeds including in the case of *Terminalia*s (Pillai & Chandrasekhara, 2011; Zoysa & Ashton, 1992). The results of the present study showed that the rate of seed germination varied between treatments (2.40 to 2.60%) and the differences were not statistically significant. Germination pattern showed that maximum germination rate was (2.60%) in water soaking treatments for 24 hours and about 21-22 days were required for completion of germination. Table 3 depicts the longevity

Table 3. Pattern of seed longevity of *T. paniculata* under storage

Sl. No.	Storage period (in months)	Germination (%)	Futile (%)	Difference in Germination (%)
1.	0 (fresh seeds)	2.6 ± 0.06	97.4	-
2.	1	2.4 ± 0.06	97.6	- 0.2
3.	2	1.7 ± 0.06	98.3	- 0.7
4.	3	1.2 ± 0.15	98.8	- 0.5
5.	4	0.9 ± 0.06	99.1	- 0.3
6.	5	0.6 ± 0.06	99.4	- 0.3
7.	6	0.2 ± 0.1	99.8	- 0.4

of seeds under storage and the seeds were able to maintain viability up to 6 months within the air-tight metallic container at 4°C. However, a gradual decline from 2.60 ± 0.06 to 0.20 ± 0.10 % of seed viability was noticed during the six months after storage at 4°C.

CONCLUSIONS

Study documented seed characteristics of *T. paniculata* such as maturity index, germination pattern and seed longevity. The study concluded that during the 16th week after fruit setting, fruits are red with maximum germinability. It is very easy to identify the best fruit collection period with the help of the Munsell Colour Chart than identifying the 16th week of fruit development. Germination studies concluded that 2.60% is the maximum germinability and pre-sowing treatments do not enhance the germination rate. However, water-soaking for 24 hours helps to improve seed germination and reduce the germination duration. Seed longevity study revealed that seeds shall be stored up to six months under cold condition in an airtight plastic container. Results of this study will be helpful for tree improvement programs and other research programs of the species and similar species.

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