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Aeration Quality of Root Trainer and Rooting Media in the Artificial Regeneration Studies of *Myristica malabarica* Lam.

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ABSTRACT

Tropics are known for the species richness and the evergreen rain forests are blessed with many tree species, which are of economic importance. *Myristica malabarica* is an important evergreen tree species, which urgently require artificial regeneration technology for plantation trials. Hence this work has great importance. The present study was carried out using seeds from two forest localities (L1 and L2) and sown in four sowing media (SM1-SM4) and control (SM5) in root trainers and reusable poly-pots. Germination trials using fresh and stored seeds with and without seed treatments were also performed. Higher germination percentage of 44% was exhibited by seeds collected from L1 and sown in perlite and soil in root trainer (SM3). Least germination percentage of 12.75 % was found in seeds collected from L2 and sown in garden soil in nursery bed (SM5). Higher germination value (GV) of 0.515 was recorded in seeds collected from L1 and sown in perlite and soil (SM3) and least value of 0.024 in seeds collected from L2 and sown in garden soil (SM3). Seeds soaked in water (T3) and sown in perlite and soil (SM3) and nutmeg pericarp and soil (SM4) showed higher germination percentage of 84.5 % and 83.5 % respectively while seeds without pretreatment (T1) and sown in vermiculite and soil (SM2) exhibited 21.75 % of germination in root trainer. Germination percentage was higher in seeds sown in perlite and soil in root trainer. Seeds sown in vermiculite and soil in pots recorded greater MGT of 40.57 days and least in seeds sown in perlite and soil in root trainer. Germination rate and CV_t was higher in all sowing medium in root trainer compared to that in pots. Greater germination value of 3.085 were recorded in seeds sown in perlite and soil in root trainer and least values in seeds sown in vermiculite and soil in pots. Thus the results directly proves the ability of root trainers in environmentally safe artificial generation of seedlings for plantation purpose.

Key words: *Root trainer,* Myristica malabarica *Lam., perlite, vermiculite, rooting media, Germination value (GV).*

Introduction

Myristica malabarica is an indigenous forest tree species with multifarious use. It is well known as a non-wood forest product (NWFPs) yielding plant among the forest produce collectors and foresters. Artificial regeneration of the tree species is important in the present era as the natural regeneration in the natural stands is low and the exploitation is more. Data generation through field study is dependent on several features and it is highly unpredictable as the climate together with the set of management practices changes from crop to crop and region to region. For seed germination of industrial crops in arid and semi-arid regions, drought and salinity stresses are the main limitation factors (Rasheed *et al.,* 2019). The

variability increases when the experimentation is with plantation trees as it takes longer time. Several studies have shown that drought and salinity can delay or completely inhibit germination (Toscano *et al.,* 2017; Hu *et al.,* 2018; Tang *et al.,* 2019). Tropical land may be safe as many of the adverse conditions is not applicable. Hence this study gains its importance in developing the technology of the plantation crop by raising the seedlings in root-trainers and its trials in many rooting media.

Materials and Methods

The dehiscence of the pericarp indicates the seed maturity in *Myristica malabarica.* Collecting fallen fruits was avoided as they may be old and deteriorated. Fruits were collected in gunny bags.Pest-infested and diseased fruits were examined and recorded from the field itself. They were labeled and brought to the laboratory and infected regions of the fruits were critically scrutinized.The rind of *Myristica malabarica* split open and the aril was removed. Extraction and conditioning were done carefully to avoid seed damage as the damages caused during seed handling reduce viability in storage.

Water absorption percentage was worked out using the equation (McWatters *et al.,* 2002; Shafaei *et al.,* 2016).

$$W_a = \frac{W_f - W_i}{W_i} \times 100$$

Where W_a isthe water absorption percentage, W_f is the weight of seeds after soaking in water and W_i is the initial weight of the seed.

Similarly, moisture content wascalculated using the formula (Hart *et al.,* 1959; Bonner, 1981).

$$\text{Moisture content (\%)} = \frac{\text{Wetbiomass} - \text{Dry biomass x 100}}{\text{Wetbiomass}}$$

Total phenol content was analyzed using Folin Ciocalteu's method (Singleton *et al.,* 1999). The seed viability test using 2,3,5- triphenyl tetrazolium chloride was performed. Dissection, staining, and evaluation methods in TZ Test may vary from species to species. A tetrazolium staining test was performed to determine the viability of seeds (ISTA, 1985).

Germination and growth studies were conducted in the poly house located at Dr. T.C Joseph Memorial Botanical Garden, Department of Botany, Union Christian College, Aluva with coordinates 10°7′30"N and 76°20′3"E (Figure 1). The temperature inside the poly house was maintained around 25-35 °C and humidity 70-85% during the period of study.

Fig. 1 a & b. Inside and outside view of Poly house

Root trainers (RT) of 36 cm length, 24 cm width, and 10 cm height with 24 cells and 100 cc volumes in each cell and plastic pots (PT) of 500 cc volumes were used as sowing containers for the study.

Water holding capacity of the sowing medium was determined using the equation

$$\text{Moisture content\%} = \frac{\text{A-B} \times 100}{\text{B}}$$

Where A: Weight of the saturated surface dried sample and B: Dry weight of the sample. Coir pith showed a higher water holding capacity of 626.6±63.9% followed by meg pith (397.2 ± 29.8%). The least water-holding capacity was recorded in perlite (Table 1).

Table 1. Water holding capacity of sowing medium

Sl No.	Sowing medium	Dry weight (B) (g)	Wet weight (A) (g)	Moisture content %
1	SM 1Coir pith	500	3633 ± 319	626.6 ± 63.9
2	SM 2 Vermiculite	500	820 ± 19	66.7 ± 3.8
3	SM 3Perlite	500	658 ± 13	29.8 ± 2.6
4	SM 4Meg pith	500	2490 ±149	397.2 ± 29.8

Data were represented as mean ± SD, n=5

In the present study, 100 seeds each were used for germination testsin each trial during each year from 2018-2021. Seeds were sown in coir pith and soil (SM1), vermiculite and soil (SM2), perlite and soil (SM3), fermented organic material and soil (SM4)in root trainers and pots, and garden soil in nursery bed (SM5).

Seeds without treatment (control) (T1), seed coat removed (T2), seeds soaked in water (T3), seeds treated with hot water (T4), and seeds treated with gibberellic acid (T5) were used for the study. Different pretreated seeds such as control (T1), seed coat removed or cut (T2), seeds soaked in water (T3), seeds treated with hot water (T4), and seeds treated with gibberellic acid (T5) were used ingermination studies. Germination parameters of seeds stored for one month (SS1), two months (SS2), and three months (SS3) were analyzed. The duration taken for seed germination was noted for each trial. The germination count was recorded on daily basis**.** The germination value was calculated as per Czabator (1962).

Germination value GV= MDG x PV

Where MDG is the mean daily germination calculated asthe cumulative percentage of total seed germinated at the end of the test divided by the number of days taken to complete the germination test. Peak value (PV) is the maximum MDG recorded at any time of the test.

Mean Germination time ($\bar{t}$) was proposed by Haberlandt (1875) and mentioned by Labouriau (1983). The mean germination time is calculated as

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

Where t_i is the time from the start of the test to the i^{th} observation, n_i is the number of seeds germinated at the time i, and k is the last time of germination.

According to Labouriau (1970), the mean germination rate is the reciprocal of the mean germination time. Mean germination rate $\bar{v} = 1/\bar{t}$

The coefficient of variation of the germination time (CVG) as mentioned by Ranal *et al.* (2009) is calculated by the expression $CV_t = \frac{S_t}{\bar{t}}\ 100$

Where Standard deviation of mean germination time $(S_t) = \sqrt{S_t^2}$ and $\bar{t}$ is the mean germination time. The variance of germination time is calculated by the expression (S_t^2)

$$S_t^2 = \sum_{i=1}^{k} n_i (t_i - \bar{t})^2 / \sum_{i=1}^{k} n_i - 1$$

Where $\bar{t}$ is the mean germination time, t_i is the time between the start of the experiment and the i^{th} observation, n_i number of seeds germinated in the time *i*, and *k* is the last time of germination.

All the data were subjected to statistical analysis by one-way ANOVA and two ways ANOVA using SPSS version 16.0 to study the influence of different medium, pretreatments, different containers, locations, and stored seeds upto 3 months on germination characteristics. The significant difference between means was detected by Duncan's multiple range test at a significance level of $p<0.05$ (Figure 3.14).

Results and Discussion

Myristica malabarica Lam., Mem. Acad. Sci. Paris 162. 1791; Hook.f. & Thomson, Fl. Ind. 1:163.1855; Bedd., Fl. Sylv. T. 269. 1872; Hook.f., Fl. Brit. India 5: 103.1886; Gamble, Fl. Madras: 1213.1925; Mani. & Sivar., Fl. Calicut 249. 1982; Saldanha, Fl. Karnataka 1: 54. 1984; Nayar *et al.*, Fl. Pl. Western Ghats 1: 664. 2014. According to *the IUCN Red List of Threatened Species Myristica malabarica* is listed as vulnerable (CAMP, 1998).

Myristica malabarica belongs to Myristicaceae family. It is locally known as Kattujathi, Kattujathikka, Kottappannu, Pathiripoovu, Panampalka, Ponnampannu, Ponnampayin, Ponnampanu, and Ponnampu in Malayalam, Colaivenkai, Pahiri, Katjathika and Kattujatikkai in Tamil, Kanage, and Doddajajikai in Kannada.

It is a tree with smooth greenish-black bark, about 15-20 m. height and 40–50-inch diameter Wood is

Fig. 2. a & b. Germination of seeds sown in root trainers and pots

yellowish brown with grey shade, moderately hard, nondurable. Aril of *Myristica malabarica* is used to cure burning sensation, fever, cough and bronchitis. Fat extracted from seeds are used to treat rheumatism, indolent ulcers, analgesics, muscle pain, sprains and sores (Chelladurai and Ramalingam, 2017). Tribals of Western Ghats used *Myristica malabarica* for treatment of hepatitis. Hepatoprotective activity of seed aril extract provided scientific evidence for this (Manjunatha *et al.*, 2011).

Fruits and seeds were found to be infected with fungus. The fungus was identified as *Penicillium sp.* (Figure 3.a & b). Few fruits were decaying and were infested with worms and bugs.

Fig. 3. a & b. Fruits Infected with *Pencillium sp.*

Hand section of *Myristica malabarica* pericarp was taken. Numerous trichomes arise from epidermis which were segmented upto 11 celled. Epicarp consists of thick walled narrowly oblong cells. Parenchymatous thin walled mesocarp contained oil cavity. Small vascular strands were distributed in mesocarp (Figure 4. A, b & c).

Seed coat is hard and measures upto 1.5 mm in thickness. Perisperm of seed penetrates into endosperm forming dark brown coloured radial, lines upto 8 mm long. Perisperm cells contain dark amorphous inclusions (Figure 5. a-d). Aril is flat and isobilateral in the transectional view. Numerous large celled oil bearing idio blast were clustered in ground tissue towards the epidermis. Seeds remain attached to seedlings even after three months of growth (Figure 6 a-c) (Figure 7. A-c).

Water uptake by intact seeds with seed coat was measured at 12 h interval. Seeds of *Myristica malabarica* soaked in water showed uptake of 252.45 ± 4.68 ml of water per 100 seeds (15.710 ± 0.186%) at 12 h of soaking and it continued upto 192 h with maximum uptake of 737.73 ± 1.23 ml of water per 100 seeds (45.913 ± 0.241%) (Figure 8).

About 50% of water uptake took place within 36 h of hydration and after 132 h the imbibition curve was nearly asymptotic. Minimal amount of water uptake was continued up to 5-8 days. Seed coat turned slimy after 5-6 days of soaking.

Data on wet biomass and dry biomass of 100

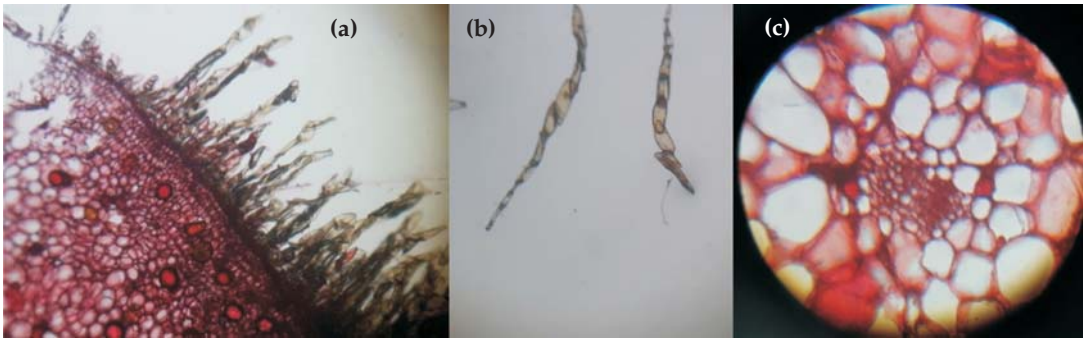

Fig. 4.1.5. T.S of fruit with trichome (b) trichomes (c) mesocarp with vascular bundle

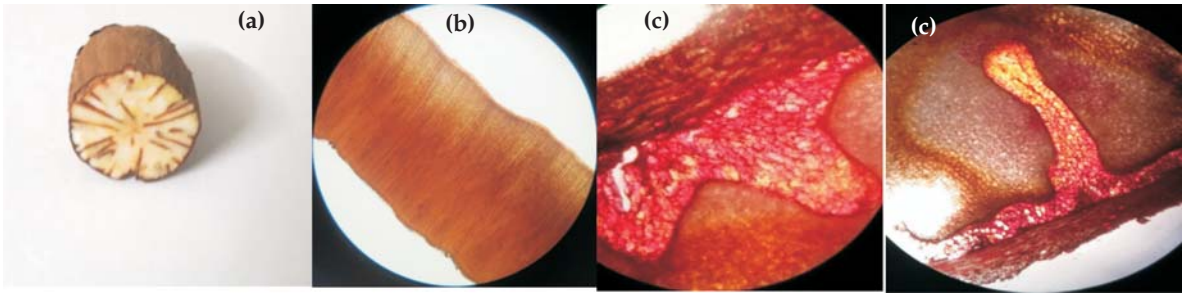

Fig. 5. (a) A mature cross cut seed (b) (c) and (d) C.S of seed coat and seed

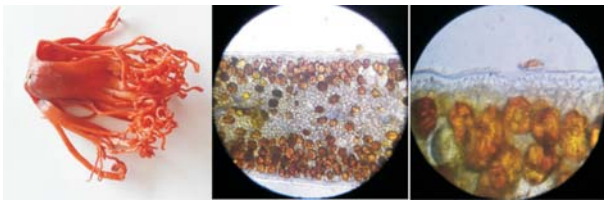

Fig. 6. (a) Aril of *Myristica malabarica* (b) and (c) C.S of aril under 10x and 65x

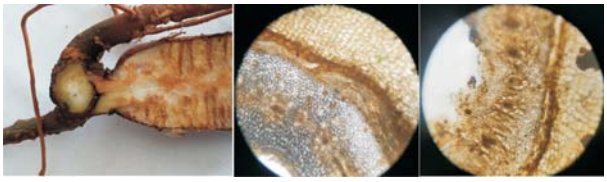

Fig. 7. a-c. *Myristica malabarica* (a) Seedling attached to seed (b)(c) C.S of seed attached to seedling

seeds each were recorded from seeds collected from Mullaringad (L1) and Kodanad (L2) and percentage of moisture content calculated. Higher moisture content was recorded in seeds collected from L2 (30.71 ± 0.703%) and lower moisture content of 29.15 ± 0.838% in seeds collected from L1 (Table 2).

Total phenol content was higher in methanol extract of seeds and aril compared to chloroform extract and water extract. Seed aril exhibited greater total phenol content than seeds of *Myristica malabarica* in all extracts (Table 3).

Table 3. Quantitative analysis of total phenol content of seeds and aril of Myristica malabarica

Total phenol	Methanol	Chloroform	Water
Seed	1.132±0.072	0.325±0.014	0.141±0.027
Aril	1.760±0.044	0.439±0.024	0.219±0.024

Tetrazolium test were conducted to test the viability percentage of fresh seeds of *Myristica malabarica*. Three lots of 50 seeds each were subjected to tetrazolium test and a mean of 87.33 ± 3.06 % viability was recorded (Figure 9).

Fig. 9. *Myristica malabarica* seeds longitudinally bisected and stained with tetrazolium

Table 2. Mean Seed moisture content percentage

Location	Wet biomass/ 100 seeds (g)	Dry biomass/ 100 seeds (g)	Moisture content (%)
L1	1636.75 ± 51.97	1159.50 ± 27.00	29.15 ± 0.838
L2	1715.25 ± 56.93	1188.25 ± 33.93	30.71 ± 0.703

Data were represented as mean ± SD, n=4

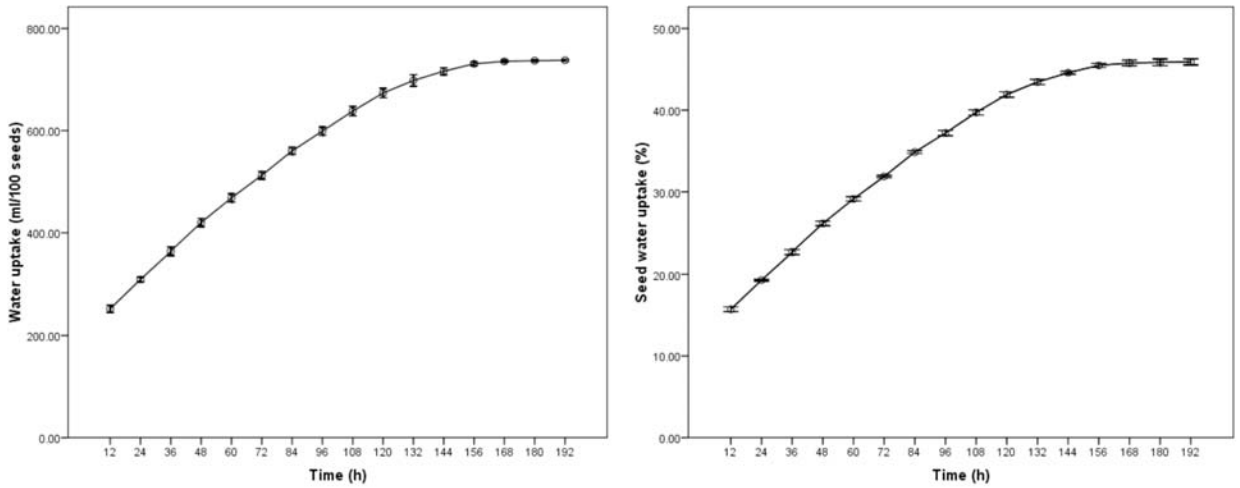

Fig. 8. a & b. (a) Mean water uptake per 100 seeds and (b) percentage of water absorbed by *Myristica malabarica* seeds at 12 h intervals during imbibition.

Few seeds of *Myristica malabarica* stored in containers for one month were found to be infected with fungi. Incidence percentage of 28.75% was detected from stored seeds collected from L1 during 2018. Cottony, fluffy grayish patches appeared on infectious seeds. Fungus was identified as *Lasiodiploidiatheobromae* (Pat.) Griffon and Maubl. Seeds collected from L1 during 2020, stored in containers were infected with fungus after two months of storage. The fungus was identified as *Pleurotus* sp (Figure 10 a-d).

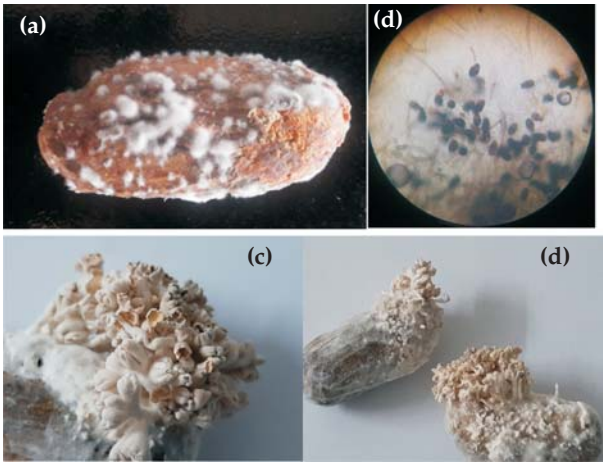

Fig. 10. a-d. Stored seeds of *Myristica malabarica* (a) Infected with fungus *Lasiodiploidatheobromae* (b) *Lasiodiploidatheobromae* spores under light microscope (45x) (c) and (d) infected with fungus *Pleurotus*sp.

Germination characteristics of fresh seeds in different sowing medium and location

Seeds collected from (L1) and (L2) without pretreatment sown in different sowing medium was subjected to germination study. Higher germination percentage of 44% was exhibited by seeds collected from L1 and sown in perlite and soil in root trainer (SM3). Least germination percentage of 12.75 % was found in seeds collected from L2 and sown in garden soil in nursery bed (SM5). Seeds collected from L2 and sown in garden soil in nursery bed (SM5) recorded higher Mean germination time (MGT) of 72 days and lowest in seeds collected from L1 and sown in perlite and soil (SM3) of 41.8 days. Maximum germination rate per day was 0.024 for seeds collected from L1 and sown in perlite and soil (SM3) and least germination rate of 0.014 per day in seeds collected from L1 and L2 and sown in garden soil (SM5). Lower CV_t values of 8.327 % and 8.358 % were found in seeds collected from L1 and L2 respectively and sown in garden soil in nursery bed (SM5) and greater CV_t value of 22.23 % in seeds collected from L1 and sown in perlite and soil (SM3). Higher germination value (GV) of 0.515 was recorded in seeds collected from L1 and sown in perlite and soil (SM3) and least value of 0.024 in seeds collected from L2 and sown in garden soil (SM3) (Table 4).

Germination characteristics of pretreated fresh seeds in different sowing medium

Seeds subjected to different pretreatments T1 (control), T2 (seed coat removed), T3 (soaked in water), T4 (treated with hot water) and T5 (treated with GA) were sown in different sowing medium. Seeds soaked in water (T3) and sown in perlite and soil (SM3) and nutmeg pericarp and soil (SM4) showed higher germination percentage of 84.5 % and 83.5 % respectively while seeds without pretreatment (T1) and sown in vermiculite and soil (SM2) exhibited 21.75 % of germination in root trainer. MGT was

Table 4. Germination characteristics of fresh seeds of *Myristica malabarica* without pretreatment

Sowing Medium	Location	Germination %	MGT (day)	Germination rate(day-1)	CVt (%)	GV
SM1	L1	31.00	60.04	0.017	16.183	0.178
	L2	27.50	60.90	0.016	10.105	0.140
SM2	L1	21.75	62.65	0.016	11.924	0.091
	L2	19.75	62.57	0.016	7.555	0.072
SM3	L1	44.00	41.80	0.024	22.230	0.515
	L2	35.00	45.08	0.022	12.510	0.305
SM4	L1	34.75	52.87	0.019	17.081	0.298
	L2	28.25	50.47	0.020	10.060	0.200
SM5	L1	14.25	70.87	0.014	8.327	0.038
	L2	12.75	72.00	0.014	8.358	0.024

lower in seeds soaked in water (T3) and sown in all four sowing medium compared to other pretreatments. Higher germination rate of 0.036 per day was found in seeds soaked in water (T3) sown in perlite and soil (SM3) and nutmeg pericarp and soil (SM4). Seeds soaked in water (T3) and sown in all three medium except vermiculite and soil (SM2) recorded higher CV_t value than in other pretreated seeds. Higher germination value of 3.085 were obtained in seeds soaked in water (T3) and sown in perlite and soil (SM3) while lower in seeds without pretreatment (T1) and sown in vermiculite and soil (SM2) (Table 5). Pretreatment with 100 ppm IBA hormone induced rooting in endangered Proteaceae plant *Leucadendron laxum* in bark, sand and polystyrene mixture as medium (Laubscher and Ndakidemi, 2008). Similarly, in olive seeds, scarification of seeds using 97% sulfuric acid soaking for 6 hrs broke the dormancy and resulted in effective seed germination (Rostami and Shasavar, 2009). Recently Javaid *et al.* (2022) reported that environmental factors such as temperature, salinity and seed burial depth influence the rate of seed germination in rye grass.

Germination characteristics of fresh seeds in different sowing medium and container

Seeds soaked in water (T3) were sown in different sowing medium in root trainers of 100 cc volume and pots of 500 cc volume. Germination percentage was higher in seeds sown in perlite and soil in root

Table 6. Germination characteristics of seeds of *Myristica malabarica* in different containers

Sowing Medium	Container	Germination %	MGT($\bar{t}$) (day)	Germination rate (day^{-1})	CVt (%)	GV
SM1	Root trainer (100cc)	74.25	28.97	0.035	36.18	2.279
SM2		63.00	32.78	0.031	22.55	2.047
SM3		84.50	27.49	0.036	36.34	3.085
SM4		83.50	28.09	0.036	37.46	2.656
SM1	Pots (500cc)					
	66.50	38.37	0.026	12.88	2.281	
SM2		49.50	40.57	0.025	14.67	0.939
SM3		75.25	38.24	0.026	12.79	2.894
SM4		68.75	39.13	0.026	13.39	2.112

Table 5. Germination characteristics of pretreated seeds of *Myristica malabarica*

Sowing Medium	Pretreatment	Germination %	MGT($\bar{t}$) (day)	Germination rate(day^{-1})	CVt (%)	GV
SM1	T1	31.00	60.04	0.017	16.183	0.178
SM2		21.75	62.65	0.016	11.924	0.091
SM3		44.00	41.80	0.024	22.230	0.515
SM4		34.75	52.87	0.019	17.081	0.298
SM1	T2	41.50	39.87	0.025	13.125	0.894
SM2		30.75	39.94	0.025	12.828	0.487
SM3		59.25	37.14	0.027	21.663	1.321
SM4		53.75	39.58	0.025	21.275	1.017
SM1	T3	74.25	28.97	0.035	36.18	2.279
SM2		63.00	32.78	0.031	22.55	2.047
SM3		84.50	27.49	0.036	36.34	3.085
SM4		83.50	28.09	0.036	37.46	2.656
SM1	T4	30.75	49.37	0.020	17.01	0.235
SM2		24.25	55.49	0.018	16.40	0.105
SM3		45.75	45.76	0.022	16.19	0.713
SM4		42.00	47.13	0.021	16.59	0.507
SM1	T5	66.75	45.68	0.022	16.84	1.414
SM2		58.50	47.29	0.021	16.37	0.845
SM3		71.75	36.35	0.027	18.53	2.627
SM4		67.75	41.29	0.024	14.02	1.694

trainer. Seeds sown in vermiculite and soil in pots recorded greater MGT of 40.57 days and least in seeds sown in perlite and soil in root trainer. Germination rate and CV_t was higher in all sowing medium in root trainer compared to that in pots. Greater germination value of 3.085 were recorded in seeds sown in perlite and soil in root trainer and least values in seeds sown in vermiculite and soil in pots (Table 6).

Germination characteristics of stored seeds in different sowing medium

Seeds stored for 1 month (SS1), 2 month (SS2) and 3 month (SS3) were sown in different sowing medium in root trainers. Seeds failed to germinate after a storage period of 1 month. Germination percentage of SS1 was highly reduced compared to fresh seeds. Higher germination percentage of 12.25% was recorded in SS1 sown in perlite and soil (SM3). MGT was 87.38 days in SS1 sown in vermiculite and soil (SM2) that was higher than seeds sown in other three medium. Germination rate was higher in SS1 sown in perlite and soil (SM3) and $CV_t$was higher in vermiculite and soil (SM2). Higher germination value of 0.018 was obtained in SS1 sown in SM3 (Table 7), (Figure 11).

Analysis of Germination characteristics of fresh seeds in different sowing medium and location

A two-way ANOVA was conducted to compare effect of location of collection, medium and their interaction in germination characteristics of fresh seeds without pretreatment. In all germination characteristics F value was significant at 1% level among sowing medium and interaction between sowing medium and location. Germination percentage, CVt and GV was significant at 1% level and germination rate at 5% level among different location. There was no significance for MGT among location of collection (Table 8). Maximum mean germination percentage of 39.5 % was recorded in seeds sown in perlite and soil (SM3) in root trainer and lower of 13.5% in garden soil (SM5). Among different locations higher mean germination percentage of 29.15 % were recorded in seeds collected from L1. Seeds sown in SM3 in root trainer showed minimum mean germination time of 43.44 days. Rashid *et al* (2018) reported the positive effect of root trainer size on the sturdiness and quality of seedlings in comparison of growth parameter studies in *Jatrophacarcus plant.* Trainer size (600 cc) displayed maximum expression in growth parameters such as number of leaves, root length, sturdiness ratio and quality index in *Jatropha* seedlings. MGT of seeds collected from different location were homogenous. Maximum mean germination rate was recorded in seeds sown in SM3 and seeds collected from L1. Significantly higher mean CV_t of 17.37 % was recorded from seeds sown in SM3. Mean CV_t of seeds collected from L1 was significantly higher compared to seeds collected from L2. Seeds collected from L1 recorded higher mean GV of 0.224 and seeds sown in SM3 showed a sig-

Fig. 11a-c. Germination of seeds in root trainers and pots

Table 7. Germination characteristics of stored seeds of *Myristica malabarica*

Sowing Medium	Seed storage	Germination %	MGT($\bar{t}$) (day)	Germination rate (day^{-1})	CVt (%)	GV
SM1	SS1	9.75	81.89	0.012	12.27	0.011
SM2		6.75	87.38	0.011	15.02	0.004
SM3		12.25	76.01	0.013	14.70	0.018
SM4		10.25	80.27	0.012	11.42	0.013

Table 8. A two-way ANOVA examining the effect of sowing medium and location of collection of seeds and their interactions on seed germination characteristics of *Myristica malabarica*

Germination characteristics	Source of Variation	Sum of Squares	Df	Mean Square	F-value	P-value
Germination (%)	Medium	3222.600	4	805.650	178.373	<0.001**
	Location	202.500	1	202.500	44.834	<0.001**
	Medium * Location	81.000	4	20.250	4.483	0.006**
	Error	135.500	30	4.517		
	Total	32586.000	40			
MGT($\bar{t}$) (day)	Medium	3679.476	4	919.869	652.834	<0.001**
	Location	3.136	1	3.136	2.226	0.146ns
	Medium * Location	34.020	4	8.505	6.036	0.001**
	Error	42.271	30	1.409		
	Total	137959.544	40			
Germination rate (day-1)	Medium	<0.001	4	9.901E-5	718.366	<0.001**
	Location	7.290E-7	1	7.290E-7	5.289	0.029*
	Medium * Location	7.181E-6	4	1.795E-6	13.025	<0.001**
	Error	4.135E-6	30	1.378E-7		
	Total	0.013	40			
CVt(%)	Medium	401.315	4	100.329	49.904	<0.001**
	Location	295.023	1	295.023	146.745	<0.001**
	Medium * Location	104.605	4	26.151	13.008	<0.001**
	Error	60.313	30	2.010		
	Total	7044.734	40			
Germination value	Medium	0.718	4	0.180	161.752	<0.001**
	Location	0.057	1	0.057	51.502	<0.001**
	Medium * Location	0.053	4	0.013	12.042	<0.001**
	Error	0.033	30	0.001		
	Total	2.245	40			

**Significant at 1% level; * Significant at 5% level; ns-non significant at 5% level

Table 9. Comparison of germination characteristics of seeds in different medium

Sowing Medium	Germination %	MGT($\bar{t}$) (day)	Germination rate (day^{-1})	CVt (%)	GV
SM1	29.25c	60.47c	0.0165c	13.14b	0.159c
SM2	20.75d	62.61b	0.0160d	9.74c	0.081d
SM3	39.50a	43.44e	0.0230a	17.37a	0.410a
SM4	31.50b	51.67d	0.0194b	13.57b	0.249b
SM5	13.50e	71.43a	0.0140e	8.34c	0.031e

Mean with same superscript letter indicates no significant difference in Duncan multiple range test at $p \leq 0.05$

Table 10. Comparison of germination characteristics of seeds from different location

Location	Germination %	MGT($\bar{t}$) (day)	Germination rate (day^{-1})	CVt (%)	GV
L1	29.15a	57.64a	0.0179a	15.15a	0.224a
L2	24.65b	58.20a	0.0176b	9.72b	0.148b

Mean with same superscript letter indicates no significant difference in Duncan multiple range test at $p \leq 0.05$

nificant higher mean GV of 0.410 and lower in seeds collected from L2 and sown in SM5 (Table 9), (Table 10).

Analysis Germination characteristics of pretreated fresh seeds in different sowing medium

Impact of pretreatment, sowing medium and their interactions in germination characteristics were analysed by two- way ANOVA. All germination characteristics F value was significant at 1% level between sowing medium, pretreatment and their interaction (Table 11). Maximum mean germination percentage of 61.05% was recorded in perlite and soil (SM3) among sowing medium and 76.31% in seeds soaked in water (T3) among different pretreatments. Lower germination percentage was exhibited by seeds sown in vermiculite and soil (SM2) and without pretreatment (T1). Minimum MGT of 37.71 days was recorded in SM3 and of 29.33 days in T3. Germination rate and CV_t was higher in SM3 and T3. A maximum mean GV of 1.652 was recorded in SM3 and 2.517 in T3. Least GV was obtained in SM2 and T1 (Table 12), (Table 13). Ibrahim *et al.* (2001) reported increased seed germinating effects of pre-

Table 11. A two-way ANOVA examining the effect of sowing medium and pretreatments of seeds and their interactions on seed germination characteristics of *Myristica malabarica*

Germination characteristics	Source of Variation	Sum of Squares	Df	Mean Square	F-value	P-value
Germination (%)	Medium	5243.35	3	1747.783	218.019	<0.001**
	Pretreatment	23283.45	4	5820.863	726.095	<0.001**
	Medium * Treatment	484.15	12	40.346	5.033	<0.001**
	Error	481.00	60	8.017		
	Total	241466.00	80			
MGT($\bar{t}$) (day)	Medium	1081.737	3	360.579	519.104	<0.001**
	Treatment	5949.083	4	1487.271	2141.135	<0.001**
	Medium * Treatment	562.501	12	46.875	67.483	<0.001**
	Error	41.677	60	0.695		
	Total	155399.379	80			
Germination rate (day^{-1})	Medium	0.000	3	9.707E-5	270.837	<0.001**
	Treatment	0.002	4	0.001	1632.960	<0.001**
	Medium* Treatment	8.800E-5	12	7.333E-6	20.460	<0.001**
	Error	2.150E-5	60	3.584E-7		
	Total	0.051	80			
CVt (%)	Medium	529.731	3	176.577	148.9	<0.001**
	Treatment	3436.042	4	859.010	724.367	<0.001**
	Medium * Treatment	618.514	12	51.543	43.464	<0.001**
	Error	71.153	60	1.186		
	Total	36780.561	80			
Germination value	Medium	9.420	3	3.140	80.218	<0.001**
	Treatment	56.199	4	14.050	358.919	<0.001**
	Medium * Treatment	2.428	12	0.202	5.170	<0.001**
	Error	2.349	60	0.039		
	Total	176.247	80			

**Significant at 1% level; * Significant at 5% level; ns-non significant at 5% level

Table 12. Comparison of germination characteristics of seeds in different medium

Sowing Medium	Germination %	MGT($\bar{t}$) (day)	Germination rate(day^{-1})	CVt (%)	GV
SM1	48.85c	44.79b	0.0237c	19.87c	1.000c
SM2	39.65d	47.63a	0.0221d	16.01d	0.715d
SM3	61.05a	37.71d	0.0273a	22.99a	1.652a
SM4	56.35b	41.79c	0.0250b	21.29b	1.234b

Mean with same superscript letter indicates no significant difference in Duncan multiple range test at $p \leq 0.05$

treatments such as soaking, scarification and acid dip promoting seed dormancy breakage.

Analysis of germination characteristics of fresh seeds in different sowing medium and container

Effect of container volume, sowing medium and their interaction in germination characteristics of seeds were examined by two -way ANOVA. In all germination characteristics F value appeared significant at 1% level between sowing medium and between containers. Interaction between container volume and sowing medium found significant at 1% level in MGT, germination rate and CV_t and 5% significance in germination value and no significant effect on germination percentage (Table 14). Among sowing medium higher mean germination percentage of 79.88% was recorded in seeds sown in perlite and soil (SM3) and among containers root trainer showed higher mean germination percentage of 76.31%. Prolonged water retensive capacity of coir pith was reported by Paramanandham *et al.* (2014) at its maximum, specifically at 500-micron particle

Table 13. Comparison of germination characteristics of different pretreated seeds

Treatment	Germination %	MGT ($\bar{t}$) (day)	Germination rate (day^{-1})	CV_t (%)	GV
1	32.88^{e}	54.34^{a}	0.0189^{e}	16.85^{b}	0.270^{d}
T2	46.31^{c}	39.13^{d}	0.0256^{b}	17.22^{b}	0.930^{c}
T3	76.31^{a}	29.33^{e}	0.0343^{a}	33.14^{a}	2.517^{a}
T4	35.69^{d}	49.44^{b}	0.0203^{d}	16.55^{b}	0.390^{d}
T5	66.19^{b}	42.65^{c}	0.0237^{c}	16.44^{b}	1.645^{b}

Mean with same superscript letter indicates no significant difference in Duncan multiple range test at $p \leq 0.05$

Table 14. A two-way ANOVA examining the effect of sowing medium and volume of container and their interactions on seed germination characteristics of *Myristica malabarica*

Germination characteristics	Source of Variation	Sum of Squares	Df	Mean Square	F-value	P-value
Germination (%)	Medium	2580.094	3	860.031	70.627	<0.001**
	Container	1023.781	1	1023.781	84.074	<0.001**
	Medium * Container	67.094	3	22.365	1.837	0.167ns
	Error	292.250	24	12.177		
	Total	16371.00	32			
MGT($\bar{t}$) (day)	Medium	68.098	3	22.699	30.854	<0.001**
	Container	759.30	1	759.330	1032.108	<0.001**
	Medium * Container	13.279	3	4.426	6.016	0.003**
	Error	17.657	24	0.736		
	Total	38297.789	32			
Germination rate (day^{-1})	Medium	6.367E-5	3	2.122E-5	28.465	<0.001**
	Container	0.001	1	0.001	802.790	<0.001**
	Medium* Container	2.449E-5	3	8.163E-6	10.948	<0.001**
	Error	1.789E-5	24	7.456E-7		
	Total	0.029	32			
CVt(%)	Medium	236.887	3	78.962	68.725	<0.001**
	Container	3106.296	1	3106.296	2703.581	<0.001**
	Medium * Container	373.654	3	124.551	108.404	<0.001**
	Error	27.575	24	1.149		
	Total	21090.806	32			
Germination value	Medium	9.060	3	3.020	25.331	<0.001**
	Container	1.694	1	1.694	14.210	0.001**
	Medium * Container	1.427	3	0.476	3.990	0.019*
	Error	2.861	24	0.119		
	Total	182.355	32			

**Significant at 1% level; * Significant at 5% level; ns-non significant at 5% level

size which promoted seed germination. Effect of specific planting media which is loamy soil: sandy soil: vermiculite in (2:1:1) ratio on seed germination and robustness of seedlings were reported by Mahmoud *et al.* (2019). In Kiwi fruit seedlings maximum seed germination was observed in (1:1) Peat: perlite ratio by Maghdouri *et al.* (2021). Seeds sown in vermiculite and soil (SM2) and in pots showed least germination percentage. Seeds sown in pots exhibited a maximum mean MGT of 39.08 days. Mean germination rate and CV_t was greater in SM3 and root trainer and lesser in SM2 and pots. Higher mean GV of 2.989 was noted in SM3 among different sowing medium and of 2.517 in root trainer among containers (Table 15), (Table 16).

Analysis of germination characteristics of stored seeds in different sowing medium

Germination characteristics of one month stored seeds in different sowing medium were analysed. Germination percentage, MGT, germination rate and GV of stored seeds exhibited a significance level of 1% among different sowing medium, but no significant difference found in CV_t (Table 17).

Mean germination percentage was higher (12.25%) in stored seeds sown in perlite and soil

Table 15. Comparison of germination characteristics of seeds in different medium

Sowing Medium	Germination %	MGT($\bar{t}$) (day)	Germination rate (day^{-1})	CVt (%)	GV
SM1	70.38c	33.67b	0.0303b	24.53a	2.280b
SM2	56.25d	36.67a	0.0276c	18.61b	1.493c
SM3	79.88a	32.87b	0.0313a	24.56a	2.989a
SM4	76.13b	33.61b	0.0306ab	25.43a	2.384b

Mean with same superscript letter indicates no significant difference in Duncan multiple range test at $p \leq 0.05$

Table 16. Comparison of germination characteristics of seeds from different container

Container	Germination %	MGT($\bar{t}$) (day)	Germination rate (day^{-1})	CVt (%)	GV
Root trainer	76.31a	29.33a	0.034a	33.14a	2.517a
Pot	65.00b	39.08b	0.026b	13.43b	2.056b

Mean with same superscript letter indicates no significant difference in Duncan multiple range test at $p \leq 0.05$

Table 17. A one-way ANOVA examining the effect of sowing medium in germination characteristics of stored seeds of *Myristica malabarica*

Germination characteristics	Source of Variation	Sum of Squares	df	Mean Square	F-value	P-value
Germination (%)	Between groups	62.00	3	20.667	10.783	0.001**
	Within groups	23.00	12	1.917		
	Total	85.00	15			
MGT ($\bar{t}$) (day)	Between groups	265.123	3	88.374	11.423	0.001**
	Within groups	92.842	12	7.737		
	Total	357.965	15			
Germination rate (day^{-1})	Between groups	<0.001	3	<0.001	10.720	0.001**
	Within groups	<0.001	12	<0.001		
	Total	<0.001	15			
CVt(%)	Between groups	37.880	3	12.627	1.784	0.204ns
	Within groups	84.945	12	7.079		
	Total	122.826	15			
Germination value	Between groups	<0.001	3	<0.001	14.446	<0.001**
	Within groups	<0.001	12	<0.001		
	Total	0.001	15			

**Significant at 1% level; * Significant at 5% level; ns-non significant at 5% level

Table 18. Comparison of germination characteristics of stored seeds in different medium

Sowing Medium	Germination %	MGT($\bar{t}$) (day)	Germination rate (day^{-1})	CVt (%)	GV
SM1	9.75b	81.89b	0.0122b	12.28a	0.0108b
SM2	6.75c	87.38a	0.0114c	15.02a	0.0040c
SM3	12.25a	76.01c	0.0132a	14.70a	0.0180a
SM4	10.25ab	80.27bc	0.0124b	11.42a	0.0130b

Mean with same superscript letter indicates no significant difference in Duncan multiple range test at $p \leq 0.05$

(SM3) and least (6.75%) in vermiculite and soil (SM2). Longest mean MGT of 87.38 days were recorded in seeds sown in SM2. Germination rate and GV was higher in seeds sown in SM3 and least in SM2 (Table 18). Wawrzyniak *et al.* (2020) reported that seed storage has an impact on seedling emergence and seed germination but it does not affect seedling growth after first growing season.

Conclusion

Artificial propagation using seed in *Myristica malabarica* in several sowing media will be a breakthrough in the field of forestry. Data generation through field study is dependent on several features and it is highly unpredictable as the climate together with the set of management practices changes from crop to crop and region to region. Root trainer experiment results have a stable value in many cases and hence it is reliable as a structure for space effective seedling generation in the field of agronomy and plantation crop regeneration. Aeration within the root trainer is another effect, which is proved through the use of different media used. Coir pith and perlite are known for their aeration and water holding capacity.

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