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Effect of Drupe Size Grading on *in vivo* and *in vitro* Germination and its Dormancy Mechanism of Teak (*Tectona grandis* Linn. F)

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The effect of drupe size on *in vivo* and *in vitro* germination and its dormancy mechanism in teak have been studied in this paper. Teak drupes were size graded to five categories namely very large with more than 15 mm, large with 13 to <15 mm, medium with 11 to <13 mm, small with 9 to <11 mm and very small with less than 9 mm of diameter. Under *in vivo* pot culture condition very low germination percent of drupes was observed. It was observed that very large size drupe had highest 100 drupe weight, 100 true seed weight and seed filling capacity, but it was negatively correlated with *in vivo* germination of teak drupes. When true seeds isolated from the drupes were grown under *in vitro* condition in half strength MS medium the germination percent was significantly increased up to 54.1. True seeds obtained from small size graded drupes recorded highest germination per cent in MS media. This showed that the drupe size did not play major role in the germination and early growth in teak. Very small size graded drupes have low seed filling capacity, and it leads to decrease the germination percent. Poor germination of drupes showed the presence of mechanical and physiological dormancy in drupes. Similarly, the presence of morphological and embryo dormancy in teak may limit the true seed germination potential to 54.1 per cent. In addition, *in vitro* germination of true seeds will be a promising approach to obtain large number of saplings in teak.

Keywords: Teak, drupe size grading, dormancy, *in vivo* - *in vitro* germination.

Teak (*Tactona grandis* Linn.f) is one of the most durable and highly preferred plantation timber species which is indigenously distributed in four countries (Myanmar, Lao PDR, Thailand and India) across the globe[1]. Internationally traded teak timber plays a significant role in the forest economy with an annual trade of 1 million m^3 which is worth US$ 487 million[2]. The reputation of teak is due to its matchless combination of qualities - termite, fungal and weather resistance, lightness with strength, attractiveness, workability and seasoning capacity without splitting, cracking warping or materially altering shape[3]. In recent years a number of private enterprises have come up for teak growing in many tropical countries including India. Guiding forces behind this have perhaps been initial fast growth and successful agroforestry practices resulting in early returns. Teak is a seed propagated tree although vegetative means such as bud and / or root grafting are possible. However, the later has little practical feasibility to measure up to the vast demand for planting material every year[4]. Seed size has been found to influence germination and seedling growth and ultimately

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the population in the field[5]. Seed grading has been suggested as an integral part in post-harvest operation to enhance the planting value of seed lots. In teak, drupe size exerts a significant influence and fillingness, germination as well as seedling vigour[6]. This will help nursery men to minimize the production cost of seedlings on a broad scale. Till date, there was no detailed experiment to evaluate the effects of drupe size on true seed dormancy mechanism and germination of teak. Poor seed germination is a significant problem for propagation of teak. Seed dormancy is the putative cause for delayed and sporadic germination of teak seed but specific dormancy mechanisms have not been proven[7]. The whole drupe physical dormancy mechanisms require exclusion of water from the embryo via an impermeable fruit wall or endocarp[8] and physiological dormancy prevails in the mesocarp[9,6] water soluble germination inhibitors. Minimal germination of viable true seeds may have been influenced by morphological embryo dormancy[10,11,12]. Against the stalemate an experiment was conducted to evaluate the effect of drupe size on fillingness *in vivo* germination of dupe and *in vitro* germination of true seed to find out the dormancy mechanism in teak drupe as well as true seed.

MATERIALS AND METHODS

Seed Collection

Teak drupes (fruit with seed) were collected from existing 20-year-old plantation at Agricultural Engineering College and Research Institute, Kumulur, Tiruchirappalli, Tamil Nadu (10º4' N; 78º5 E; 70 msl). Newly fallen matured drupes were collected during April, 2020. After collection, the drupes were dried and cleaned and removing the insect infected and shriveled drupes. The study samples were drawn from those lots.

Size grading

The drupes were size graded using sieves of different mesh sizes. The different size grades were 15 mm retained (>15 mm diameter), 13 mm retained (13 - <15 mm), 11 retained (11- <13 mm), 9 mm retained (9 - < 11 mm), and 9 mm pass (< 9 mm). The smallest drupes were discarded. After grading the drupes, weighed 100 drupes weigh (g) grade wise **Fig 1 (a)**.

Cutting test

Cutting test was made grade wise and two hundred drupes were soaked in water for 24 hour and then individually cut form horizontally with the help of areca nut cutter. The empty locules, one seeded, two seeded, three seeded and four seeded drupes were counted and mean numbers recorded. The fillingness was expressed as percentage **Fig 1 (b)**.

Drupe germination test

The drupes were subjected to soaking in water followed by drying at 12 hours interval for 6 days and 7th day the pre-conditioned drupes were placed for germination in sand taken in earthen pots (30 cm height and 30 cm upper width) and kept in open sun light. Experiment was conducted in Completely Randomized Design and 10 replications of 30 drupes were used in all grads. A germination period of 28 days as recommended by[13] was adopted. Time taken for initial emergence was observed. The normal seedlings (one (or) more) produced by single drupe was counted as one seedling and germination (expressed as percentage) was computed, total number of seedlings produced by 30 drupes were also counted and the mean value was expressed as 100^{-1}. For the estimation of dry matter production, ten seedlings were selected at random and kept in a hot air oven maintained at 85^{o}C for 24 hours after measuring their root and shoot length. The vigour index was derived from the formula[14].

VI=Percent germination X Total seedling length (cm)

True seed extraction

The true seeds were extracted grade wise 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, weighed 100 true seed weight (g) grade wise. Those seed only used for *in vitro* germination studies **Fig 1 (c)**.

Media preparation for *in vitro* germination

To perform *in vitro* germination, half strength MS media was prepared by adding 50 % of the recommended dose of macro, micro and minor elements of the MS medium[15]. 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 to culture bottles up to 50 ml per vessel. Finally, the culture vessels with media was autoclaved at 121°C plus 15 psi pressure for 20 minutes.

Sterilization and inoculation of true seeds

Each grade of the true seeds was dried for 1 hour in sun light prior to sterilization. The seeds were placed in distilled water containing 0.1 % Bavistin and 0.1 % Tween 20 for five minutes with constant shaking. After Bavistin treatment the seeds were washed in tap water for one minute and washed in 70 % ethanol for 30 seconds. After ethanol wash the true seeds were washed with sterile distilled water. Now the seeds were sterilized in 0.1 % mercuric chloride ($HgCl_2$) solution for five minutes with constant shaking. After $HgCl_2$ sterilization the true seeds have been washed three times with sterile distilled water.

***In vitro* true seed germination test**

The sterilized seeds were carefully inoculated in to a half MS media bottle under a laminar airflow chamber by following the ascetic techniques. Six seeds were inoculated per bottle and eight replicates were made for each treatment. Then the culture vessels were placed in a primary growth room maintaining 25°C temperature, 16 hours light and 8 hours dark conditions. Once in a day the *in vitro* seed inoculates were observed for time taken for initial emergence and germination percent were taken 14 and 28 days after sowing[13]. 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[14].

VI=Percent germination X Total seedling length (cm)

Statistical analysis

The results were subjected to analysis of variance and tested (t-test) for significant difference (p=0.05) as suggested[16]. Percentage values were transformed into arc sine values prior to statistical analysis.

RESULTS AND DISCUSSION

Size grading on seed filling

Effect of drupe size and germination of teak revealed that significant difference was found among the treatments. The large sized drupes with 15 mm diameter recorded highest 100 drupe weight of 105.19 g and drupe diameter of 14.16 mm followed by 13 - < 15 mm retained had 76.68 g 100 drupe weight and 12.67 mm drupe diameter, 11 - < 13 mm retained had 58.18 g 100 drupe weight and 11.28 mm drupe diameter, 9 - < 11 mm retained had 55.95 g 100 drupe weight and 10.28 mm drupe diameter and 9 mm passed drupes recorded lowest 100 drupe weight of 42.79 g and drupe diameter of 9.47 mm. When seed filling percent of 15 mm retained recorded highest seed filling of 68 % with

Table 1. Effect of drupe size on frequency of seed development (%) in teak drupes

Treatments (Size grade)	No. of seeds/drupe					
	Empty	One	Two	Three	Four	Seed filling
$T_1$15 mm retained (> 15 mm dia.)	32(34.45)	22(27.97)	40(39.23)	6(14.17)	0(0.286)	68(55.55)
$T_2$13 mm retained (13 - < 15 mm dia.)	38(38.05)	40(39.23)	22(27.97)	0(0.286)	0(0.286)	62(51.94)
$T_3$11 mm retained (11- < 13 mm dia.)	46(42.70)	42(40.39)	8(16.43)	4(11.53)	0(0.286)	54(47.29)
$T_4$9 mm retained (9 - < 11 mm dia.)	40(39.23)	50(45.00)	10(18.43)	0(0.286)	0(0.286)	60(50.76)
$T_5$9 mm pass (< 9 mm dia.)	42(40.39)	54(47.29)	4(11.53)	0(0.286)	0(0.286)	58(49.60)
Mean	39.6(38.64)	41.6(39.81)	16.8(23.57)	5(12.92)	0(0.286)	60.4(50.76)
SEd	0.617	0.523	0.083	0.033	-	0.583
CD (P= 0.01)	1.819	1.542	0.246	0.100	-	1.720

(Figures in parentheses indicate arc sine value)

Table 2. Effect of drupe size on 100 drupe weight, drupe diameter, germination and seedling vigour of teak

Treatments	100 drupe weight (g)	Drupe diameter (mm)	Days taken for initial emergence	14 DAS Germination (%)	Germination (%)	28 DAS Root length (cm)	28 DAS Shoot length (cm)	No. of leaves	Dry matter production (mg/10 seedlings)	Vigour index
$T_1$15 mm retained (> 15 mm dia.)	105.19	14.16	-	0(0.286)	0(0.286)	0	0	0	0	0
$T_2$13 mm retained (13 - < 15 mm dia.)	76.68	12.67	15	0(0.286)	10(18.43)	2.4	3.5	4	0.068	59.0
$T_3$11 mm retained (11- < 13 mm dia.)	58.18	11.28	21	0(0.286)	3.3(9.97)	2.2	3.4	6	0.017	18.48
$T_4$9 mm retained (9 - < 11 mm dia.)	55.95	10.28	22	0(0.286)	3.3(9.97)	2.5	1.8	4	0.025	14.19
$T_5$9 mm pass (< 9 mm dia.)	42.79	9.47	17	0(0.286)	3.3(9.97)	2.0	4..0	2	0.019	19.8
Mean	67.7	11.8	15	0(0.286)	3.98(9.97)	1.82	2.54	3.2	0.025	22.29
SEd	0.890	0.121	0.306	-	0.089	0.026	0.016	0.069	0.0006	0.548
CD (P= 0.01)	2.623	0.358	0.901	-	0.264	0.078	0.047	0.204	0.0018	1.615

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

Table 3. Effect of drupe size on 100 true seed weight and *in vitro* germination and seedling vigour of true seeds of teak

Treatments	100 true seed weight (g)	Days taken for initial emergence	14 DAS Germination (%)	Germination (%)	Root length (cm)	28 DAS Shoot length (cm)	No. of leaves	Dry matter production (mg/10 seedlings)	Vigour index
$T_1$15 mm retained (> 15 mm dia.)	1.78	4	33.3(35.06)	33.3(35.06)	2.1	2.25	4.20	86	144.85
$T_2$13 mm retained (13 - < 15 mm dia.)	1.58	8	33.3(35.06)	41.6(39.81)	2.81	1.56	3.0	83	181.79
$T_3$11 mm retained (11- < 13 mm dia.)	1.48	5	41.6(39.81)	45.8(42.13)	2.95	1.77	2.75	69	216.17
$T_4$9 mm retained (9 - < 11 mm dia.)	1.34	5	50.0(45.00)	54.1(47.29)	2.0	2.03	3.62	78	218.02
$T_5$9 mm pass (< 9 mm dia.)	1.21	4	25.0(30.00)	29.1(32.58)	2.68	2.02	3.91	52	136.77
Mean	1.47	5.2	36.64(36.87)	40.78(39.23)	2.50	1.92	3.49	73.6	179.52
SEd	0.023	0.06	0.44	0.59	0.03	0.02	0.06	0.663	1.94
CD (P= 0.01)	0.068	0.13	0.93	1.26	0.06	0.05	0.13	1.955	4.14

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

a greater number of two seeded drupes, followed by 13 - < 15 mm retained drupes recorded 62 % seed filling, 9 - < 11 mm retained drupes recorded 60 % seed filling, 9 mm pass 58 % and 11- <13 mm retained 54 % seed filling with a greater number of single seeded drupes compared to 15 mm retained **(Table 1 and Fig 4)**. These results indicated that the number of seeds per drupe was not found to decrease with decreasing drupe size.

Size grading on drupe *in vivo* germination

The results of drupe weight showed that 100 drupe weight decreases with decrease in drupe diameter. Variation in drupe seed germination per cent, root length, shoot length, number of leaves per seedling, seedling dry matter production and vigour index was observed among the different groups of size graded seeds. 13 - < 15 mm retained showed highest drupe germination of 10 % followed by 11- < 13 mm retained, 9 - < 11 mm retained, 9 mm pass had 3.3 % of germination and none of the drupes was germinated in 15 mm retained. In case of root length 9 - < 11 mm retained drupes showed highest root length of 2.5 cm followed by 2.4 cm of 13 - < 15 mm retained, 2.2 cm 11- < 13 mm retained, 2.0 cm of 9 mm pass drupes. 9 mm pass drupes had highest shoot length of 4 cm followed by 3.5 cm of 13 - < 15 mm retained, 3.4 cm of 11 - < 13 mm retained, 1.8 cm of 9 - < 11 mm retained drupes. 11 - < 13 mm retained showed highest total number leaves (6) followed by (4) 13 - < 15 mm retained; 9 - < 11 mm retained (2) of 9 mm pass drupes. 13 - < 15 mm retained drupes

Fig. 1. (a) Size grading of teak drupes

Fig. 1. (b) Frequency of seed development (%) in teak drupes

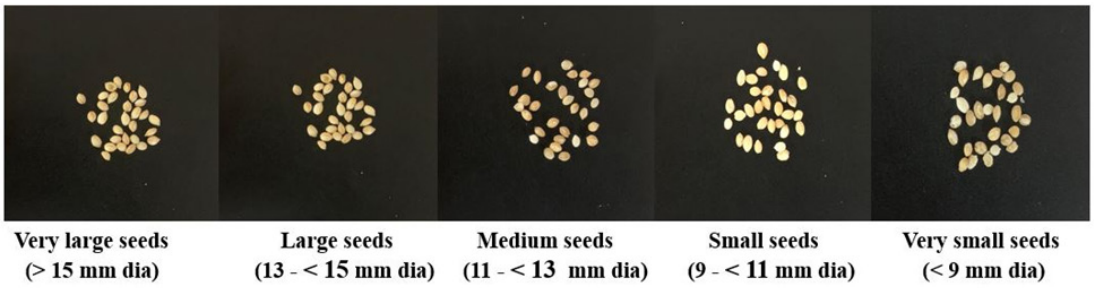

Fig. 1. (c) Extracted true seeds from teak drupes

showed highest dry matter production on 0.068 g followed by 0.025 g of 9 - < 11 mm retained drupes, 0.019 g of 9 mm pass drupes and 0.017 g of 11- < 13 mm retained drupes had lowest dry matter. 13 - < 15 mm retained drupes recorded highest vigour index of 59 followed by 19.8 of 9 mm pass, 18.48

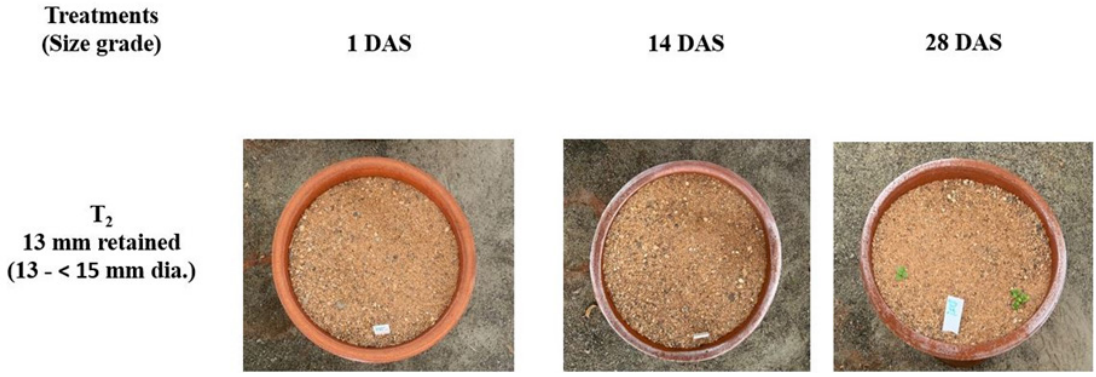

Fig. 2. In vivo germination o teak drupes (13 - <15 mm dia.)

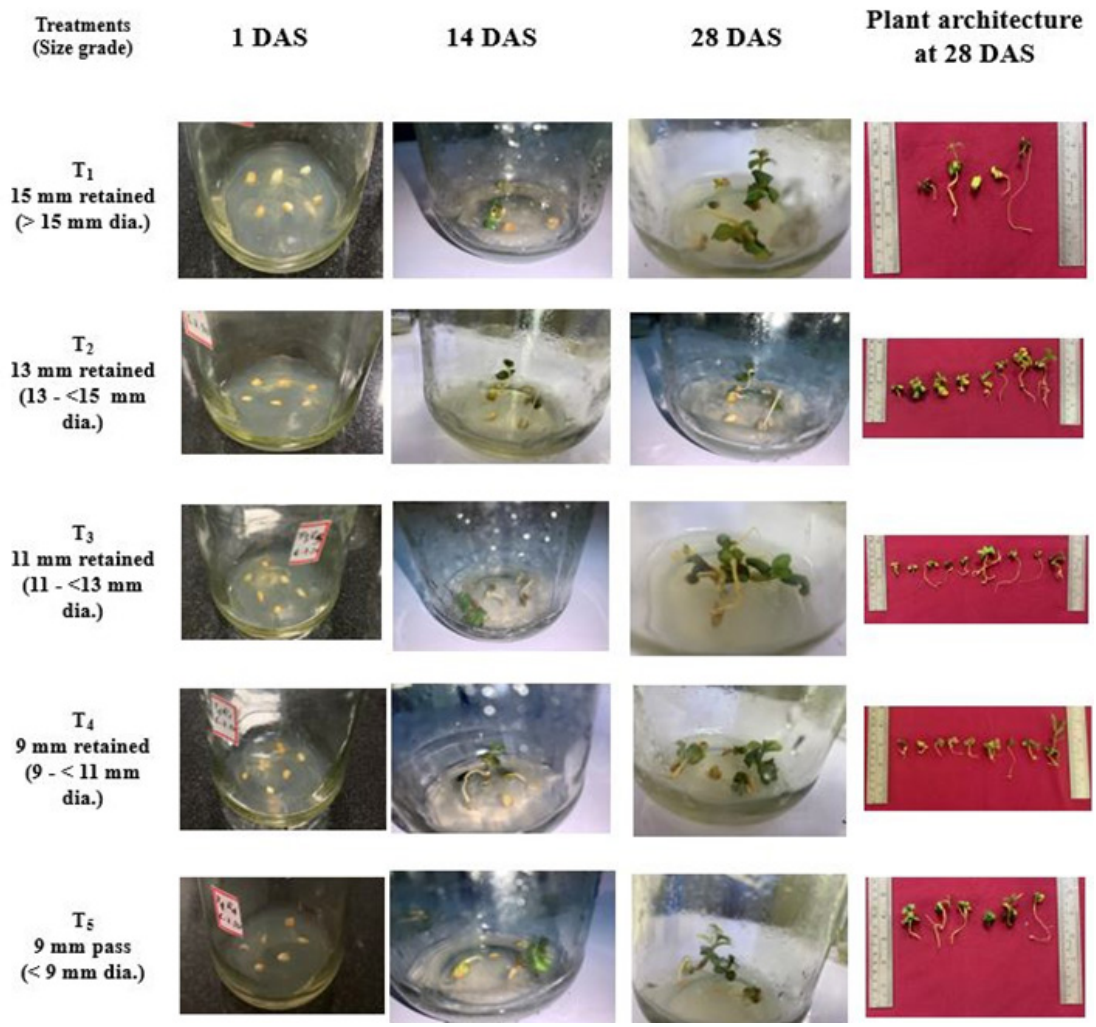

Fig. 3. In vitro germination of teak true seeds

of 11- < 13 mm retained drupes and 9 - < 11 mm retained drupes recorded lowest vigour index of 14.19 **(Table 2 and Fig 4)**. These results showed that the drupe size and filling per cent were found to be negatively correlated with drupe germination in teak.

Size grading on *in vitro* true seed germination

The true seeds were extracted from five diameter groups of teak drupes based on their size grades. 15 mm retained had the highest 100 true seed weight of 1.78 g followed by 13 - < 15 mm retained (1.58 g), 11- < 13 mm retained (1.48 g), 9 - < 11 mm retained (1.34 g) and 9 mm pass (1.21 g). These results clearly indicated that decreases in drupe weight with decrease in true seed weight.

True seeds of different grades were germinated in half MS media under *in vitro* condition and observed for 14 and 28 days after sowing. Variation in true seed germination per cent, root length, shoot length, number of leaves per seedling, seedling dry matter production and vigour index was observed among the different groups of size graded seeds. 9 - < 11 mm retained showed highest *in vitro* germination per cent of 50.0 % at 14 days after sowing (DAS), and 54.1 % germination in 28 DAS. The 11- < 13 mm retained group recorded 41.6 % germination at 14 DAS and 45.8 % germination at 28 DAS, 13 - < 15 mm retained group showed 33.3 % germination in 14 DAS and 41.6 % germination in 28 DAS, 15 mm retained group had 33.3 % germination in 14 DAS and 33.3 % germination in 28 DAS. Very small seed group of 9 mm pass had lowest germination value of 25 % in *in vitro* conditions at 14 DAS and 29.1 % germination at 28 DAS.

11 - < 13 mm retained showed highest root length of 2.95 cm followed by 2.81 cm for 13 - < 15 mm retained, 2.68 cm for 9 mm pass, 2.1 cm for 15 mm retained and 9 - < 11 mm retained had lowest root length value of 2.0 cm. 15 mm retained recorded highest shoot length of 2.25 cm followed by 2.03 cm for 9 - < 11 mm retained, 2.02 cm for 9 mm pass, 1.77 cm for 11- < 13 mm retained and 13 - < 15 mm retained had lowest shoot length value of 1.56 cm. 15 mm retained showed highest number of leaves per plant (4.20) followed by (3.91) for 9 mm pass, (3.62) for 9 - < 11 mm retained, (3.0) for 13 - < 15 mm retained and 11- < 13 mm retained had lowest number of leaves (2.75) compare to all treatments. 9 - < 11 mm retained had highest seedling vigour with a vigour index value of 218.02, followed by 216.17 for 11- < 13 mm retained, 181.79 for 13 - < 15 mm retained, 144.85 for 15 mm retained and 136.77 for 9 mm pass **(Table 3 and Fig 4).** These results showed that the drupe size and filling per cent were found to be negatively correlated with *in vitro* germination in teak.

The present results were not agreed with earlier studies indicated that larger drupe size in

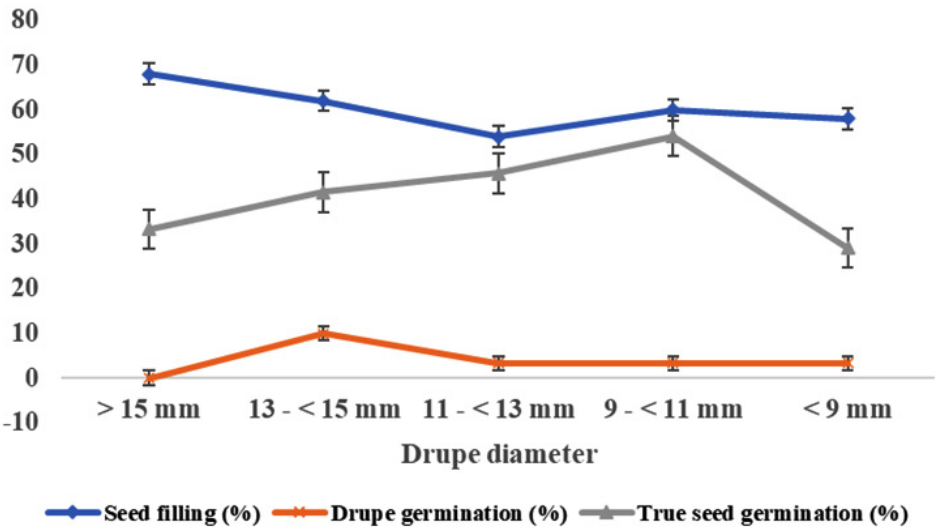

Fig.4. Effect of drupe size on seed filling (%), drupe germination and true seed *in vitro* germination (%) of teak

teak have better germination[17,18,19]. Size of the drupe is primary factors for determining germination per cent[20]. The results confirm that the variability between different population within the species in seed characters and germination percentage is due to strong genetic influence[21,22,23]. In earlier studies showed that number of seeds per drupe was found to decrease with drupe size[24,25,26]. The drupe weight and endocarp weight that are positively correlated with filling per cent but not significantly correlated with germination per cent[25]. the higher endocarp ash may due to the presence of higher inorganic compounds like calcium which can help to increase cell wall rigidity and thickness and it may affect germination[27]. While, direct correlation of mesocarp weight on germination was found to be negatively correlated. High mesocarp proportion affects germination because of more phenolic content is present [28,29,30]. Present studies also confirm that the larger drupe size has no relation with germination, confirm the results of[31,32,33]. There was strong interaction between survival rate and seedling growth under *in vitro* and *in vivo* condition. Better seedling growth and survival rate of 9 - < 11 mm retained drupes (true seeds) under *in vitro* compare to *in vivo* condition, it confirming the result of [26].

The present study reveals that the average seed filling percentage was 60.4, irrespective of the size of the drupe were studied, where as in an average germination of drupe only 3.98 percent, when the drupe was placed for germination in sand method. Whereas the true seeds were extracted from the drupe and placed for germination in *in vitro* condition in half MS medium recorded 40.78 percent, around 36.8 percent higher than the drupe germination. Improved germination in MS medium is not well understood. It is assumed that the nutrient composition of the media might be enhanced the germination of true seed. Poor germination of whole drupe might be due to physical[25], Physiological[6] and morpho-physiological dormancy[34]. There is contradictory view regarding physical dormancy the report[7], demonstrates that teak drupes generally have mechanical not physical dormancy. The above findings supported with the present study, where the true seed extracted from the drupe enhanced the germination when compared to sowing the drupe as it is. The endocarp of the drupe might be given the mechanical resistance to the germinating seed. In the present study, true seed recorded only 40.78 present germination the remaining un-germinated true seed may have been affected by morphological embryo dormancy[11,35,7]. It need in-depth study regarding the germination behavior of true seed.

CONCLUSION

From this study it could be concluded that 15 mm retained drupes had highest 100 drupe weight, 100 true seed weight and seed filling but it has negatively correlated with germination. In *in vitro* condition 9 - < 11 mm retained drupes (true seeds) recorded highest germination compared to all other grades. It is clearly indicated that small size grades seeds also give highest germination compared to large grade drupes. This study useful for the precious genetically improved small size seed useful for establishing large scale teak plantations.

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Conflict of Interest

We confirm that we do not have any conflict of interest.

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