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BIOCHEMICAL MECHANISMS ASSOCIATED WITH DORMANCY IN TEAK (*TECTONA GRANDIS*) DRUPES AND TRUE SEEDS

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Poor seed germination is a significant obstacle in the propagation of teak and may be due to dormancy but the specific mechanism has not been explored. In order to investigate the physiological mechanisms (promoters–inhibitors) involved in teak seed dormancy, teak drupes were collected from seven provenances of India ranging across four forest types. The collected drupes were used to study morphological, physiological and biochemical characters. Teak drupes collected from moist deciduous forest (Tamil Nadu) recorded significantly higher physiological parameters while drupes collected from semi-deciduous forest (Maharashtra) recorded the highest seed filling of 94%. Gibberellic acid, was found in all seven provenances of teak seeds. The highest levels of gibberellic acid (1.96 mg kg^{-1}) in seeds were found in very moist deciduous forests in Kerala and dry deciduous forests in Andhra Pradesh. The presence of biochemical inhibitor coumarin in teak seeds of highly moist deciduous forest (Kerala) (1.88 mg kg^{-1}) is reported for the first time in this paper. The findings suggest that coumarin is leached out during alternate soaking and drying before seeding teak drupes and this did not influence the germination of teak. Drupe and seed extracts of teak did not contain growth regulators such as indole-3-acetic acid, indole butyric acid and abscisic acid.

Keywords: Propagation, physical parameters, seed filling, germination, gibberellic acid, coumarin

INTRODUCTION

Teak (*Tectona grandis*), a member of the Verbenaceae family, is one of the most popular tropical hardwoods valued for its aesthetic beauty, strength and durability. Teak is indigenous to India and South-East Asia (Myanmar, Thailand and Laos) where it is a significant source of wood. The annual trading volume of teak wood is about 1 million m^3, worth at USD487 million (Kollert & Walotek 2015).

Teak is mostly reproduced through seeds (Tiwari et al. 2002). Teak seeds have combined dormancy, which consists of two or more different dormancy mechanisms (Keiding 1985). The different dormancy mechanisms are physical dormancy, mechanical dormancy, chemical dormancy and embryo dormancy. Some species have 'hard' seeds, where germination is prevented by water-impermeable seed coats. Hard seededness or physical dormancy is present in few plant families, of which Leguminosae is the largest (Paulsen et al. 2013). Physical dormancy relates to seeds with water-impermeable coats (Schmidt 2000, Baskin & Baskin 2014). The endocarp and mesocarp of teak seed are covered on the outside by waxy cuticles, have a layer of palisade cells, and have strong walls, which cause seed dormancy and consequently poor germination rate (Soares et al. 2017).

Mechanical dormancy is described as the inability of a seed to expand and for the radicle to initiate growth due to a rigid mechanical barrier (Hartmann et al. 1996, Schmidt 2000). The stony endocarp of teak fruit has been identified as a mechanical barrier to germination (Rajput & Tiwari 2001) and if unopened, it cannot be penetrated by emerging plumule and radicle. Mechanical dormancy in teak results in delayed and sporadic germination (Dias et al. 2009).

Chemical dormancy occurs when chemical germination inhibitors prevent embryo growth (Hartmann et al. 1996). Morphological dormancy refers to an underdeveloped embryo condition, where embryo grows prior to germination (Hartmann et al. 1996, Baskin & Baskin 2004, Soares et al. 2017).

A multitude of problems are associated with teak drupes (fruit with seed) such as emptiness (i.e. absence of seed), poor and protracted germination and prolonged nursery period (Masilamani et al. 2015). The complications of dormancy and how to overcome them are critical challenges. As a result, much research has been conducted on various pre-treatment procedures to increase the germination of freshly harvested teak drupes. Nevertheless, teak seed germination is still difficult despite the fact that several investigations on the physical, physiological and morphological dormancy of teak seed have been carried out. Teak seed germination is not improved by the available pre-sowing seed treatment technologies for removing dormancy. Since the biochemical features of teak seed dormancy have not been investigated in depth, a study was therefore conducted to identify the role of germination promoters and inhibitors in teak seed dormancy.

MATERIALS AND METHODS

Experimental material

After obtaining permission from the local authorities and Tamil Nadu Agricultural University, Coimbatore, India, teak drupes were collected from seven different geographical locations at four different forest types in India (Table 1). Drupes were collected and bulked at each location from 10 randomly chosen plus trees. After eliminating any drupes that were shrivelled or damaged by insects, the drupes were properly cleaned and dried. The drupes were then size graded, and those with 9-mm or larger diameter were kept and utilised in the current study (Masilamani et al. 2020).

Physical parameters

Drupe weight

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

Drupe moisture content

Drupe moisture content was estimated by low constant temperature oven method at 103 ± 1 °C for 16 ± 1 hours with known weight of seed samples. After drying, the seed samples were placed in desiccators containing calcium chloride for 30 min and weighed. The percentage of moisture content was calculated using the following formula (ISTA 1999).

$$\text{Moisture content (\%)} = \frac{M_2 - M_3}{M_2 - M_1} \times 100$$

where, M_1 = weight of empty moisture bottle with lid (g), M_2 = weight of moisture bottle with sample before drying (g) and M_3 = weight of moisture bottle with sample after drying (g).

Table 1 Description of the seed provenances

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

Drupe area, diameter, perimeter, roundness and fullness ratio

An image analyser was used to determine the physical properties of drupes retrieved from the seven provenances, including area, diameter, perimeter, roundness, and fullness ratio. With five replications, a total of 20 drupes were examined. The drupes 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).

Seed filling

A total of 200 teak drupes were soaked in water for 24 hours before being chopped horizontally using a cutter. The empty locules, one-seeded, two-seeded, three-seeded, and four-seeded drupes were counted and averaged. The percentage of seed filling was calculated based on Masilamani et al. (2020).

$$\text{Seed filling (\%)} = \frac{\text{Total number of filled drupes}}{\text{Total number of drupes}} \times 100$$

Physiological parameter

Drupe germination

Fresh drupes collected from the seven provenances were preconditioned by alternating soaking and drying at 12-hour intervals for six days. On the seventh day, the treated and control drupes were sown in earthen pots (30 cm height and 30 cm top width) containing sand media and kept under direct sunlight for assessing the germination (Masilamani et al. 2020). The experiment was carried out using a completely randomised block design with 10 replications (30 drupes per replication) from each provenance. After 28 days, the number of seedlings per 100 drupes, germination, time taken for first emergence, root length and shoot length were recorded (ISTA 1985). To calculate dry matter production, the seedlings were shade dried for 24 hours and dried again in a hot-air oven maintained at 85 ± 2 °C for 24 hours and cooled in desiccator filled with silica gel for 30 min. The dry weight of seedlings was recorded using an electronic balance and expressed as mg per 10 seedlings. The seedling vigour index was determined by multiplying the percent germination and total seedling length (Abdul-Baki & Anderson 1973)

$$\text{Seedling vigour index} = \text{Germination (\%)} \times \text{total seedling length (cm)}$$

Vigour testing does not only measure the percentage of viable seeds in a sample, it also reflects the ability of those seeds to produce normal seedlings under less than optimum or adverse growing conditions similar to those which may occur in the field. Seeds may be classified as viable in a germination test which provides optimum temperature, moisture and light conditions to the growing seedlings; however, they may not be capable of continuing growth and completing their life cycle under a wide range of field conditions.

Biochemical studies

Sample preparation

Seeds were carefully extracted from the drupes using a wooden mallet without damaging the cotyledon and seed coat. The extracted seeds were subjected to biochemical analysis.

Seed extract

Teak seeds samples (0.5 g) were mixed with 2.5 mL methanol and pulverised before centrifugation for 10 min at 3000 rpm. The supernatant was collected and dried. The final volume was adjusted to 1 mL by adding methanol and mixed thoroughly. Impurities present in the prepared sample were removed by passing through a 0.2-μm nylon membrane filter. An amount of 1 mL sample was used for the analysis (Solaiman & Al-Zehouri 2017).

Mesocarp extract

A total of 20 mL 80% methanol was added to a centrifuge tube containing 5 g mesocarp from teak drupes. The mixture was sonicated for 30 min and centrifuged for 10 min at 3000 rpm. The supernatant was collected and dried. The final volume was adjusted to 1 mL using methanol and mixed thoroughly. The extract was passed through a 0.2-μm nylon membrane filter and 1 mL of the filtered sample was used for analysis (Solaiman & Al-Zehouri 2017).

UHPLC instrumentation and chromatographic conditions

Ultra-high-performance liquid chromatography (UHPLC) with C18 column was used for the biochemical analysis. During analysis, methanol and 1% (v/v) acetic acid was used as mobile phase with isocratic programme of 50% each. The flow rate of mobile phase was set at 1 mL min^{-1} while the column temperature, 30 °C. For quantitative analysis, 10 µL of sample was used and UV detector at a wavelength of 270 nm was applied for all growth regulators and inhibitors studied in this experiment (Solaiman & Al-Zehouri 2017). Peaks corresponding to standards of gibberellic acid, indole acetic acid (IAA), indole butyric acid (IBA), abscisic acid (ABA) and coumarin appeared at the retention times 4.4, 6.8, 16.1, 10.5 and 7.8 min respectively. Limits of detection of coumarin, gibberellic acid, IAA, IBA and ABA are given in Table 2.

Statistical analysis

Results were subjected to analysis of variance and assessed (*t*-test) for significant differences (p = 0.05) (Panse & Sukhatme 1995). Prior to statistical analysis, percentage values were converted to arc sine values, and correlation was explored using SPSS software.

RESULTS

Physical parameters

Drupes from the dry deciduous forest (Karnataka) had higher 100-drupe weight (90.08 g) than those from the rest of the provenances (Table 3). Drupes from semi-deciduous forest (Maharashtra) had the lowest 100-drupe weight at 50.33 g. Drupes from very moist deciduous forest in Kerala had higher moisture content of 15.63% than the other provenances. Drupes from semi-deciduous forest (Maharashtra) had the lowest moisture content of 10.88%. Drupes from moist deciduous forest (Tamil Nadu) had the highest area, length, breadth, perimeter, roundness and fullness ratios (Table 3).

Seed filling

Drupes from semi-deciduous forest (Maharashtra) had the highest seed filling of 94%, followed by Kerala very moist deciduous forest (89%) (Table 4). Dry deciduous forest (Karnataka) and semi-deciduous forest (Madhya Pradesh) had 88% seed filling. Drupes from dry deciduous forest (Andhra Pradesh) had the lowest seed filling rate of 54%. Dry deciduous forest (Telangana) had the highest number of one-seeded drupe (61%), while, semi-deciduous forest (Maharashtra) had the maximum number of two-seeded drupes (41%). Drupes from the dry deciduous forest in Andhra Pradesh had the highest percentage of empty (46%) and four-seeded drupes (4%) compared with the rest of the provenances (Table 4).

Physiological parameters

Drupe germination

The provenance with the very moist deciduous forest, Kerala, had the highest germination percentage (17.0%), number of seedlings per 100 drupes (23.3), root length (6.0 cm), dry matter production per 10 seedlings (42.0 mg), and vigour index (196.0) (Table 5). Semi-deciduous forest in Maharashtra exhibited the lowest drupe germination percentage (4.0%),

Table 2 Limits of detection and quantification for the growth regulators and inhibitors

Growth regulator/inhibitor	Limit of detection (µg mL^{-1})	Limit of quantification (µg mL^{-1})
Coumarin	0.80	2.42
Gibberellic acid	7.38	22.4
Indole acetic acid	0.33	1.01
Indole butyric acid	0.56	1.69
Abscisic acid	0.03	0.10

Table 3 Effects of provenance on drupe physical parameters of teak

Provenance	100-drupe weight (g)	Moisture content (%)	Drupe diameter (mm)	Area (cm^2)	Length (cm)	Breadth (cm)	Perimeter (cm)	Roundness	Fullness ratio
Tamil Nadu (Topslip)	69.99	11.24 (19.37)	13.27	3.47	2.27	2.00	7.41	7.41	6.76
Kerala (Nilambur)	70.65	15.63 (22.78)	13.72	2.06	1.76	1.53	5.55	1.12	0.98
Andhra Pradesh (Rajamundry)	64.80	14.15 (21.97)	11.37	2.12	1.76	1.56	5.66	5.26	1.13
Telangana (Jannaram)	75.94	13.23 (21.13)	14.75	2.17	1.80	1.56	5.70	5.70	5.31
Karnataka (Punajanur)	94.08	12.67 (20.26)	14.53	2.73	2.01	1.76	6.51	6.51	5.97
Maharashtra (Salekassa)	50.33	10.88 (18.43)	10.80	1.61	1.55	1.36	4.95	1.14	0.98
Madhya Pradesh (Jabalpur)	60.65	12.98 (20.26)	11.57	1.70	1.58	1.39	5.08	5.08	4.72
Mean	74.77	13.12 (21.13)	12.85	2.26	1.82	1.60	5.85	4.59	3.70
SE	1.200	0.312	1.962	0.061	0.047	0.039	0.099	0.103	0.092
CD ($p = 0.05$)	2.574	0.670	4.209	0.132	0.101	0.085	0.212	0.222	0.198

Figures in parentheses indicate arc sine values; SE = standard error, CD = critical difference

Table 4 Effect of provenance on percentage of seed filling in teak

Provenance	Empty (%)	One-seeded (%)	Two-seeded (%)	Three-seeded (%)	Four-seeded (%)	Seed filling (%)
Tamil Nadu (Topslip)	25.0 (30.00)	33.0 (35.06)	34.0 (35.66)	7.0 (15.34)	1.0 (5.73)	75.0 (60.00)
Kerala (Nilambur)	11.0 (19.37)	47.0 (43.28)	28.0 (31.94)	12.0 (20.26)	2.0 (8.13)	89.0 (70.63)
Andhra Pradesh (Rajamundry)	46.0 (42.70)	32.0 (34.45)	11.0 (19.37)	7.0 (15.34)	4.0 (11.53)	54.0 (47.29)
Telangana (Jannaram)	24.0 (29.33)	61.0 (51.35)	9.0 (17.45)	5.0 (12.92)	1.0 (5.73)	76.0 (60.66)
Karnataka (Punajanur)	12.0 (20.26)	44.0 (41.55)	32.0 (34.45)	11.0 (19.37)	1.0 (5.73)	88.0 (69.73)
Maharashtra (Salekassa)	6.0 (14.17)	39.0 (38.64)	41.0 (39.81)	11.0 (19.37)	3.0 (9.97)	94.0 (75.82)
Madhya Pradesh (Jabalpur)	12.0 (20.26)	46.0 (42.70)	32.0 (34.45)	7.0 (15.34)	3.0 (9.97)	88.0 (69.73)
Mean	19.4	43.1	26.7	8.5	2.1	80.5
SE	0.252	0.427	0.384	0.213	0.074	1.008
CD (p = 0.05)	0.542	0.917	0.824	0.457	0.160	2.163

Figures in parentheses indicate arc sine values; SE = standard error, CD = critical difference

Table 5 Effect of provenance on germination and seedling vigour of teak drupe

Provenance	Drupe germination (%)	Number of seedlings/ 100 drupes	Days taken for initial emergence	Root length (cm)	Shoot length (cm)	Dry matter production (mg/10 seedlings)	Vigour index
Tamil Nadu (Topslip)	11.0 (19.37)	14.3	17.0	5.7	4.8	40.0	117.6
Kerala (Nilambur)	17.0 (24.35)	23.3	16.0	6.0	5.2	42.0	196.0
Andhra Pradesh (Rajamundry)	8.0 (16.43)	10.0	19.0	5.4	5.0	36.0	89.4
Telangana (Jannaram)	7.0 (15.34)	12.3	20.0	5.5	4.9	38.0	81.1
Karnataka (Punajanur)	9.0 (17.45)	13.3	17.0	5.8	5.4	36.0	104.6
Maharashtra (Salekassa)	4.0 (11.53)	6.6	24.0	5.1	4.2	37.0	39.9
Madhya Pradesh (Jabalpur)	8.0 (16.43)	8.6	18.0	5.2	4.9	38.0	86.8
Mean	9.0 (17.45)	12.6	18.7	5.5	4.9	38.1	102.1
SE	0.098	0.137	0.203	0.069	0.073	0.482	1.448
CD (p = 0.05)	0.204	0.285	0.422	0.145	0.152	1.002	3.012

Figures in parentheses indicate arc sine values; SE = standard error, CD = critical difference number of seedlings per 100 drupes (6.6), and root and shoot lengths (5.1 and 4.2 cm respectively). The highest shoot length (5.4 cm) was in the dry deciduous forest in Karnataka. Initial emergence ranged from 16 (Kerala) to 24 days (Maharashtra). The dry deciduous forests (Karnataka and Andhra Pradesh) had the lowest dry matter production of 36.0 mg per 10 seedlings. The lowest (39.9) vigour index was in the semi-deciduous forest (Maharashtra) provenance (Table 5).

Biochemical parameters

Gibberellic acid was present in the methanolic extracts of teak seeds collected from all the seven provenances with values ranging from 1.38 (Madhya Pradesh) to 1.96 mg kg^{-1} (Kerala and Andhra Pradesh) (Table 6). Coumarin, a biochemical inhibitor, was detected only in fresh teak seeds collected from very moist deciduous forest (Kerala) at a concentration of 1.88 mg kg^{-1}. Coumarin was also present in mesocarp extracts of semi-deciduous forest (Madhya Pradesh) at 1.7 mg kg^{-1} and dry deciduous forest (Andhra Pradesh) at 1.6 mg kg^{-1}. Table 7 shows the correlation between different physical characters of teak drupes, seed filling and germination percentage and Table 8 shows that of number of seeds per drupe and drupe germination percentage.

DISCUSSION

Physical parameters

Significant differences in physical parameters of drupes were found among the seven provenances evaluated in this study. Drupes from the dry deciduous forest (Karnataka) were the largest, while those from semi-deciduous forest (Maharashtra) were the smallest (Table 3). Moist deciduous forest (Tamil Nadu) drupes had higher roundness value and thus were more spherical than drupes from the rest of the provenances. The seed collected from dry deciduous forest (Karnataka) was heavier than the rest of the provenances (Table 3). Until now, research shows that the longest and widest drupes are from the Ponnampet region of Karnataka (Surendra et al. 2018). Teak wood from Nilambur region of Kerala is reported to be superior in growth and wood quality (Prabhu et al. 2013). High rainfall and site characteristics, among other contributing factors, could be the main causes of the increase in drupe size in moist forest types (Krishnamoorthy et al. 2016). Dhaka and Jha (2018) reported that maximum average drupe length (12.67 mm), width (14.84 mm) and weight (0.708 g) were recorded in very moist teak forest. This huge variability in seed length and breadth can be attributed to various conditions associated with genetic and physiological components that prevail both in dry and moist sources (Gupta & Pattanath 1975).

Table 6 Quantification of biochemical inhibitors and promoters present in teak seeds and mesocarp

Provenance	Seed		Mesocarp
	Coumarin (mg kg^{-1})	Gibberellic acid (mg kg^{-1})	Coumarin (mg kg^{-1})
Tamil Nadu (Topslip)	BDL	1.91	BDL
Kerala (Nilambur)	1.88	1.96	BDL
Andhra Pradesh (Rajamundry)	BDL	1.96	1.60
Telangana (Jannaram)	BDL	1.80	BDL
Karnataka (Punajur)	BDL	1.84	BDL
Maharashtra (Salekassa)	BDL	1.92	BDL
Madhya Pradesh (Jabalpur)	BDL	1.38	1.70

BDL = below detection limit

Table 7 Correlations between different physical characters of teak drupes, seed filling and germination percentage

	MC	DD	DA	DL	DB	DP	DR	DFR	DG	S/100D	DTIE	DVI	SF	100D	DW	MW	SW	D/S
MC	1	0.274	-0.263	-0.217	-0.235	-0.260	-0.267	-0.423	0.657	0.631	-0.464	0.663	-0.287	0.242	0.363	0.150	0.517	0.039
DD		1	0.518	0.568	0.525	0.525	0.372	0.562	0.443	0.612	-0.536	0.472	0.084	0.883**	0.850*	0.741	0.766*	0.584
DA			1	0.998**	0.999**	0.999**	0.702	0.677	0.292	0.326	-0.535	0.292	-0.260	0.311	0.446	0.439	0.418	0.305
DL				1	0.998**	0.998**	0.690	0.669	0.321	0.369	-0.552	0.324	-0.259	0.359	0.496	0.477	0.468	0.332
DB					1	0.999**	0.706	0.666	0.303	0.337	-0.549	0.304	-0.285	0.321	0.469	0.459	0.439	0.321
DP						1	0.710	0.680	0.285	0.321	-0.539	0.287	-0.263	0.321	0.464	0.464	0.422	0.333
DR							1	0.841*	-0.138	-0.176	-0.388	-0.144	-0.469	0.469	0.520	0.555	0.386	0.487
DFR								1	-0.068	-0.045	-0.374	-0.069	0.042	0.544	0.439	0.501	0.254	0.494
DG									1	0.955**	-0.820*	0.998**	0.035	0.075	0.229	0.091	0.347	0.023
S/100D										1	-0.721	0.966**	0.103	0.256	0.372	0.210	0.486	0.091
DTIE											1	-0.819*	0.119	-0.250	-0.453	-0.425	-0.375	-0.410
DVI												1	0.052	0.103	0.267	0.138	0.362	0.070
SF													1	-0.118	-0.295	-0.127	-0.512	0.082
100D														1	0.898**	0.770*	0.822*	0.590
DW															1	0.925**	0.852*	0.751
MW																1	0.594	0.939**
SW																	1	0.297
D/S																		1

MC = moisture content (%), D = drupe diameter (mm), DA = area (cm^2), DL = length (cm), DB = breadth (cm), DP = perimeter (cm), DR = roundness, DFR = fullness ratio, DG = drupe germination (%), S/100D = seedlings/100 drupes, DTIE = days taken for initial emergence, DVI = drupe vigour index, SF = seed filling (%), 100D = 100 drupe weight (g), DW = drupe weight (g), MW = mesocarp weight (g), SW = shell weight (g), D/S = drupe/shell weight (g); *correlation is significant at the 0.05 level, **correlation is significant at the 0.01 level

Table 8 Correlations between number of seeds drupe^{-1} and percentage of germination in teak

Parameter	One-seeded (%)	Two-seeded (%)	Three-seeded (%)	Four-seeded (%)	Drupe area (cm^2)	Drupe diameter (mm)	Germination (%)
One-seeded	1	-0.348	-0.185	-0.446	-0.305	0.587	0.050
Two-seeded		1	0.614	-0.076	0.056	-0.288	-0.045
Three-seeded			1	0.022	-0.153	-0.039	0.313
Four-seeded				1	-0.656	-0.882**	-0.265
Drupe area (cm^2)					1	0.518	0.292
Drupe diameter (mm)						1	0.443
Germination (%)							1

**Correlation is significant at the 0.01 level

Semi-deciduous forest (Maharashtra) seed source had the highest seed filling (Table 4). Drupe emptiness ranged from 6.0% in semi-deciduous forest (Maharashtra) to 46.0% in dry deciduous forest (Andhra Pradesh). Average percentages of empty, one-, two-, three- and four-seeded drupes from Parambikulam seed source were 37, 43.9, 15.3, 3.4, and 0.5% (Sivakumar et al. 2002). However, in the study by Kamra (1973), five seed provenances had 30.8, 48.4, 16.6, 3.2, and 1.0% of empty, one-, two-, three-, and four-seeded drupes respectively. Masilamani et al. (2020) observed that drupes > 15-mm diameter had higher seed filling (68%). In another study, Mandvi, a dry deciduous forest with black soil type, has the highest seed filling of 77% (Dhaka & Jha 2018). Seed filling depends on the forest source. Drupes from dry and semi-deciduous forest loaded up better than those from very moist and moist deciduous forest types. Physiological or environmental influences, or a combination of both, may influence blooming and maturing of drupes leading to the seed filling variation. Emptiness in teak drupes is common and seedling propagation is directly proportional to the amount of well-developed seeds in the drupes. The quality of drupes to be used for raising the expected number of seedlings is based on the filling percentage, anatomy and dormancy of the seed.

Physiological parameters

Very moist deciduous forest (Kerala) exhibited better germination percentages than the other sources in this study. Germination of seeds from Nilambur and Konni is quite high among the diverse Kerala sources (Jayasankar et al. 1999). In this study, drupe germination was poor in the drupes collected from the Maharashtra seed source (Table 5). One of the most critical criteria in determining germination is the size of drupe (Banik 1997). There is considerable genetic influence for variation in seed traits and germination over populations within a species (Hellum 1976, Vakshasya et al. 1992). Research has indicated that the number of seeds per drupe decreases with decrease in drupe size (Banik 1997, Sivakumar et al. 2002, Akram & Aftab 2016). Teak is naturally distributed in different climatic and edaphic zones. It has developed different ecotypes during the process of evolution. Teak exhibits great variability between provenances and land races in various quantitative and qualitative traits (Keiding 1985). Drupes from Kerala exhibited superior germination. This information unequivocally demonstrates that true seeds from the very moist deciduous forest in Kerala contain coumarin, which may have been washed out over repeated cycles of soaking and drying.

Deep dormancy in drupes caused by the combined action of physical, physiological and morphological traits restricting seed germination may be the logical reason for poor germination of drupes collected from the arid zones of Andhra Pradesh, Telangana and Karnataka. Drupes from dry provenances have thick endocarp and nutrient imbalances (Gupta & Pattanath 1973, Dhaka & Jha 2017). Prasad and Jalil (1986) also reported that germination was higher in drupes from moist localities, ranging from 60 to 80%, than in dry regions, with only less than 30% germination. Very moist deciduous forest (Kerala) provenance had the highest drupe germination (17%). Teak drupes collected from moist deciduous forest (Kerala) provenance contained coumarin.

Biochemical parameters

Biochemical analysis revealed the presence of gibberellic acid in all seeds of seven provenances (Table 6). Presence of coumarin was found only in very moist deciduous forest (Kerala). Simple coumarins are found in almost all plant families. Coumarins act as growth inhibitors (anti-auxins) for plants and play an important role in the defence function (Chattha et al. 2018). Xu et al. (2001) reported that hydration and dehydration of radish seeds in the presence of coumarin delay germination and reduce seedling growth. When coumarin is removed naturally by rain, the drupe will germinate. Immediately after sowing, coumarin is leached by alternate wetting and drying and, thus, it cannot influence the germination of teak. Coumarin delays seed germination by inhibiting ABA catabolism in rice (Chen et al. 2019). Drupes collected from all seven provenances studied did not have ABA, IAA and IBA.

Correlation studies

Physical parameters of drupe characters were positively correlated with drupe germination and seed filling percentage. Sivakumar et al. (2002) observed positive correlation between drupe weight and seed filling percentage in teak collected from 30 different sources, but negative correlation between mesocarp weight and drupe/shell weight ratio and germination percentage. In a study of *in vivo* and *in vitro* germination and dormancy mechanism of teak, Masilamani et al. (2020) observed that the 100-drupe weight, 100-true seed weight and seed filling capacity were the highest in very large size drupes, but they were inversely correlated with *in vivo* germination. Germination percentage is positively correlated with mesocarp weight, drupe/shell weight and filling percentage (Sivakumar et al. 2002). Teak fruit size has positive significant association with seedling survival percentage (Suresh et al. 1998).

Seed filling percentage was not substantially connected with drupe, mesocarp or shell weight, which are all positively correlated with drupe germination. Drupe/shell weight, on the other hand, was positively linked with seed filling and drupe germination. Similarly, drupe germination was positively correlated with 100-drupe weight (Table 7).

Drupe area had positive correlation with two-seeded drupes. In the correlation analysis, area and diameter of the drupes demonstrated positive relationship with drupe germination. The percentage of one- and three-seeded drupes was also found to be positively correlated with drupe germination (Table 8). Sivakumar et al. (2002) observed that the size of drupe was positively correlated with the percentage of two- and three-seeded drupes, while germination was positively correlated with the proportion of two-seeded drupes

CONCLUSION

Physical traits and germination characteristics of teak drupes were highly varied among forest types and provenances. Very moist deciduous forest (Kerala) provenance had the highest drupe germination (17%) compared with the rest of the provenances. The biochemical mechanisms involved in teak seeds revealed the presence of gibberellic acid in all seven geographical locations. The condition in very moist deciduous forest such as in Kerala favoured the formation and accumulation of coumarin (1.88 mg kg^{-1}) in drupes. From the results it can be concluded that during alternate soaking and drying before sowing of teak drupes, coumarin is leached out and, thus, cannot influence the germination of teak drupes.

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