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Phenological and Seed Developmental Studies of *Hopea parviflora* Bedd.: an Economically Important Endemic Trees of Western Ghats

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Abstract

The Western Ghats forest of Kerala is gifted with a vast spectrum of ecological habitats hosting a wide variety of tree species including many endemic and endangered ones. But unfortunately, the forest lands, habitats, ecosystems and species have been declining at an alarming rate particularly during the last 150 years. This has caused fragmentation of habitats and population that leads to many endemic species live in isolated refugia and become unviable in the long run. In addition to this, many physical and physiological parameters are responsible for the loss of these genetic resources, the diversity in general and on endemic species in particular. Therefore, the dwindling forest wealth accompanied by poor productivity is putting severe stress on the existing forest cover and the gap between demand and supply are widening day by day. In this backdrop, the present study makes an effort to understand the different parameters responsible for the seed development. The leaf flushing occurred between October to November, flowering extended from January to March and peak in the mid-wet season of February and fruits during June – July. Anthesis, stigma receptivity, pollen ovule ratio, pollen fertility etc. were ascertained. The results on phonological studies have also helped to understand the causal factors leading to rarity of these species. The dry matter accumulation gradually increased with seed development. The biochemical changes including sugars, starch, phenols, amino acids, lipids etc. were estimated and they served as biochemical markers for detecting the optimum period of harvest and associated features. The lipid and starch account for major proportion of the reserve in this species. Thus, the study focused the implementation of new conservation strategies for the sustainable utilization of this valuable species.

Key words: *Hopea parviflora, Seed Development, Dry matter accumulation, Phenology, Biomolecules*

Introduction

Western Ghats forest of Kerala is gifted with a vast spectrum of ecological habitats among the tropics and hosts a wide variety of tree species including many endemic and endangered ones. Most of the economically important tree species of Western Ghats belongs to the primitive families like Dipterocarpaceae. *Hopea parviflora* Bedd. is an important element in the productive family Dipterocarpaceae. It is a large, elegant, evergreen tree reaching a height of 30-40 m with a clean cylindrical bole of 20 m and girth of 4-5 m and indigenous to the evergreen forests of the Western Ghats from North Kanara to Kerala. The tree is a well known for its timber value as well as their medicinal importance. The silviculture and regeneration of this species is difficult and the complexity in maintaining regeneration in the absence of regular good seed year, further aggravates their sustainability. An in-depth study in to the vegetative as well as reproductive phenology along with the dry matter accumulation and biochemistry of seeds during development would shed more light on their population dynamics as enabling the formulations of appropriate conservation strategies.

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Material and methods

Species for the present study were located from Kallar, Kulathupuzha and Palode forest patches of Kerala (Southern Western Ghats). The forest patches are situated between 8° 45[1]& 8° 47[1]N latitude and 77°1[1]&77° 4[1]longitude at an altitude of 150-700 m above sea level. Phenological studies cover both vegetative as well as reproductive stages. The data such as leaf flushing, insect pest incidences on leaves, follicolous fungal infections, period of flowering, time of anthesis, mode of pollination, type of pollinators, stigma receptivity, pollen ovule ratio, pollen fertility etc. were also recorded. Samples for seed development studies were collected at various stages of their development from ear marked trees of the candidate species. Branches of several trees having the same blossom period were labelled and seeds in their initial developmental stages were collected for moisture content analysis by Low Constant Air Oven Method (ISTA1985) and primary metabolites analysis. This process was continued at every two weeks interval until seed maturity. A total of 12 stages are used to designate periods of seed development, with stage - 1 representing the earliest stage, approximately 2 weeks after anthesis and the stage - 12 being the mature seed. The size range of 50 seeds from each period of collection was also ascertained by recording length, breadth, fresh and dry weights. Different stages of seeds during development were collected and immediately subjected to various tests

such as moisture content analysis and biochemical estimations.

Moisture content determination during seed development

Moisture content was determined by the difference between fresh and dry weight. For dry weight determination, the seed material was taken in a pre-weighed bottle and weighed in an electronic balance, dried in an hot air oven at 103°C for 17 hours (until the constant weights are obtained) (ISTA 1985). The dry weight was recorded in every harvest, after cooling to room temperature in a desiccator. Moisture content of each sample was ascertained based on the average of 10-15 replicates.

Biochemical estimations

Biochemical studies were conducted to elucidate the biomolecular transformations of metabolites taking place simultaneously with the change during different stages of the seed development.

Extraction of Biomolecules

Weighed (1g) dry samples (48 hours of drying at 80 °C) from different periods of development were homogenized in known volume (10 ml) of 80 % (v/v) ethanol in distilled water centrifuged at 3000 rpm for 10 min. The residue was washed thrice with 80 % ethanol (v/v) centrifuged and these combined supernatant served as the source for phenol and amino acid estimation. A known volume (5 ml) of the supernatant was taken in a crucible and dried in a hot air oven at 70 °C and is dissolved in known volume (5-10 ml) of distilled water by using a fine polished glass rod and centrifuged at 3000 rpm for 10 min and this supernatant served as the source for total soluble sugar estimation. The left over residue was ground with 30 and 15 % PCA (Perchloric acid) respectively at two times, centrifuged at 3000 rpm for 10 min each and the combined supernatant used for the extraction of starch.

Soluble sugar content was estimated by using Phenol-Sulphuric acid method (Montgomery *et al*.1957). The starch is assayed by anthrone method (Mc Cready *et al*. 1950). Total phenols were estimated following the method of Swain and Hillis (1959). Total Amino acid was estimated by the method of Sadasivam and Manickam (1996).

Lipids were extracted following the method of Bligh and Dyer (1959). Weighed samples (1g) were homogenized in a mixture of chloroform and methanol (2:1 v/v) and kept overnight at room temperature in dark. Further addition of 20 ml chloroform and 20 ml distilled water was made and centrifuged at 5000 rpm for 15 minutes. Of the three layers, the clear lower layer of chloroform containing all lipids was carefully collected evaporated and the amount of lipid was determined gravimetrically.

Statistical analysis

The data from different experiments were analyzed following one way Analysis of Variance (ANOVA) and the ratio obtained were checked for significance at 1 and 5 % probability (P) level. From this calculated ANOVA the means of each treatment were separated following the Least Significance Difference (LSD) by Duncan's multiple range tests at 1 % and 5 % P level.

Results

Vegetative phenology

In *Hopea parviflora*, yellowish green foliage appeared from October onwards, imparting a very impressive appearance and peak flushing was observed during November. The newly developed leaves appeared along with mature leaves and the colour changed to light green and then to dark green on maturity. During the process of leaf maturity severe attack of treehoppers, caterpillars of moths, butterflies etc. were noticed. Fungal infections were also noted on the leaves of *H. parviflora* and the pathogen was identified as new species of the genus *Asterina hopeae* (Hosagoudar and Kamarudeen 2002). The flower initiation starts during the light green stage of leaves. Observations revealed that majority of flowers appeared at the tip region of the branches.

Flowering and fruiting behaviour of *Hopea parviflora* was studied and the results showed that flowering was infrequent, with gregarious flowering occurring once in 2-5 years followed by sporadic, irregular flowering activities. The disparity in the timing (period) of flower initiation was also noted in the species. The flower initiation started from December onwards. Insect attack and premature abscission of flower buds were noticed. Flowers are bisexual, creamy white and fragrant. Leaf flushing occurred every year, but flowering was noted only at 2-5 years interval. Good seed years often occur after a gap of 3-5 years. Certain plants located in isolated habitats showed some unusual flowering behaviour during off-season. Development of flower bud to full bloom required 30-40 days. Anthesis was noted between 2-5 pm. In *H. parviflora* the pollination is mainly through wind (anemophily). Around 15 anthers can be seen in a flower. Stigma receptivity continued for 8-12 hours. The ovary consists of 6 ovules i.e. 2 ovules in each locule. All the ovules except one are aborted during development. Each flower consists of 2, 18,400 pollen grains and pollen ovule ratio is (6240: 6) 1040: 1. Acetocarmine staining technique revealed that 93 % of pollengrains are fertile. The petals of the flower remain only for 3 - 4 days after fertilization. The fruit primordia are initially yellowish white in colour, and then they turned to green and finally brownish as they attain maturity. Most of the insect attacks (including Mealy-bugs attack for sucking juice from pedicel) and abscissions were noted during the initial fruit primordial stage. In general, during flowering process in *H. parviflora*, heavy shedding of abortive immature fruits occurred during the first two weeks following anthesis and about 4 months are required for fruit maturity and they ripened during May-July. Throughout their development, premature abscissions of fruits were noted. The fruits were damaged due to the attack of weevils belonging to the order Coleoptera (for laying eggs). Mature fruits were predated by Giant Squirrel, Bats etc.

and the fallen fruits by Spiny mouse. Severe infestations by soil born weevils were also noted on fallen fruits, which considerably reduced the soil seed bank and subsequent germination. Around 2 - 4 lakhs flowers were present in a plus tree during a peak flowering season. But the number of fruit setting compared to flowering is very less about 1.5 %. Natural germination of seeds exclusively depends up on climatological conditions i.e. if conditions are not favourable during shedding, the percentage of regeneration was very poor and also the seeds lose their viability within 7 days due to recalcitrant nature. Mature fruits are with brownish fruit wall and persistent calyx. The seeds shed with high moisture content. The fruit have compact cotyledon with large embryonal axis and the endosperm is observed like a thin dried film during maturity.

Dry matter accumulation in seeds

Length and breadth of the *Hopea parviflora* seeds gradually increased during their development (i.e. from 0.3-0.4´0.2-0.3cm to 1.6-2.0´.8-0.9cm). Dry matter accumulation in *H. parviflora* seeds during their development showed a definite lag in the early period of development up to 55 DAA followed by significant increase in the fresh weight and dry weight of seeds throughout their development. After separation of embryo at 70 DAA, gradual reduction of moisture content was noted in the tissues of cotyledons and embryo till the maturity (Table 1). Around 35% of the initial moisture content (after 55 DAA) was reduced when the seed attained maturity. However the dry matter accumulation drastically increased with the reduction in moisture content. The total variations in fresh and dry weight of seed represent mainly the variations in the cotyledons. The moisture content was lower in the cotyledons (41 %) than that of the embryonic axes (52 %) in the mature seeds (Table 1).

Biochemical changes during seed development

i) ***Total Soluble Sugar accumulation (TSS)***

Level of TSS in the developing *Hopea parviflora* seeds increased considerably up to 130 DAA (10.2 mg/g dwt. to 84.6 mg/g dwt.) and followed by a minimal increase up to 160 DAA. After that the level of TSS was almost stable up to the maturity stage (Table 2).

ii) ***Starch accumulation***

Starch content of *Hopea parviflora* seeds increased rapidly through out the seed development. Around 90% of starch accumulation happened after 40 DAA (3rd stage onwards). Starch accumulation attained a maximum (411mg/g dwt.) at the 10th stage of their development (145 DAA), followed by a gradual decline at maturity stage. High level of starch accumulation was noted in cotyledons than in embryonal axes. At the final stage of seed development, the starch level of embryonic axes showed rapid decline (Table 2).

iii) ***Phenols accumulation***

Total phenol content in *Hopea parviflora* increased gradually from 111 mg/g dwt. at 0 DAA to 163 mg/g dwt. at maturity. But a slight reduction was noticed at 130 and 145 DAA followed by gradual increase. Phenol content was maximum in embryonal axes (168 mg/g dwt.) than cotyledon (132 mg/g dwt.) at the maturity stage (Table 2).

iv) ***Lipid accumulation***

Total lipid accumulation was maximum (92 mg/g dwt.) during the mature stages of seed development in *Hopea parviflora* (Table 2). Around 80% of lipid accumulation occurred between 5th and 12th stages (70 DAA and 175 DAA).

v) ***Amino acid accumulation***

It is evident from the data in Table-2, that amino acid accumulation of *Hopea parviflora started* only at 25 DAA. Maximum level of amino acid accumulation was found in the embryonal axes of the mature seed (4.7 mg/g dwt.) than in cotyledons (2.1 mg/g dwt.).

Table.1. Dry matter accumulation of *Hopea parviflora* seeds during different seed developmental stages

Stages (DAA)	Length (cm)	Breadth (cm) (g) *	F.wt/10 replicates (g) *	D.wt/10 replicates	Moisture Content (%) ±SE *
0DAA	0.3 - 0.4	0.2 - 0.3	0.0065 g	0.0036 f	33.0 ± 1.965 a*
10DAA	0.4 - 0.6	0.3 - 0.4	0.014 g	0.0076 f	45.0 ± 0.714 b*
25DAA	0.4 - 0.7	0.3 - 0.4	0.023 g	0.0106 f	53.0 ± 0.913 f**
40DAA	0.5 - 0.8	0.3 - 0.5	0.0305 g	0.0131 f	57.0 ± 0.149 c*
55DAA	0.6 - 0.9	0.4 - 0.5	0.037 g	0.0133 f	64.0 ± 0.854 g**
70DAA WS	0.7-1.0	0.5-0.6	0.0475 g	0.019 f	60.0± 00 h
COT	– – – –	– – – –	0.0445 5**	0.020 5	55.0±1.20 1*
EMB	– – – –	– – – –	0.003 G	0.0009 F	70.0±00 A*
85DAA WS	0.8-1.3	0.5-0.8	0.06 75 e**	0.0283 f	58± 1.074 e**
COT	– – – – –	– – – – –	0.0595 3**	0. 0286 3*	51.0±0.932 2*
EMB	– – – – –	– – – – –	0.008 A*	0.0026 F	67.0±0.500 C**

100DAA WS	0.8-1.6 — —	0.6-0.8 — —	0.0975 c**	0.0458 c**	53± 1.025 d*
COT	— — — — —	— — — — —	0.0775 4**	0.0405 4**	49.0±0.056 4**
EMB	— — — — —	— — — — —	0.018 B*	0.0061 D**	66.0±0.111 F
115DAA WS	1.2-1.8	0.6-0.9	0.120 d**	0.0625 d*	47.0±0.916 h
COT	— — — — —	— — — — —	0.093 1*	0.0502 1*	46.0±0.020 3**
EMB	— — — — —	— — — — —	0.027 D**	0.0094 E**	65.0±0.185 F
130DAA WS	1.5-1.8	0.6-0.9	0.165 a*	0.0890 a*	46.0±0.460 h
COT	— — — — —	— — — — —	0.132 2*	0.0726 2*	45.0±000 6
EMB	— — — — —	— — — — —	0.033 C**	0.0122 F	63.0±1.30 F
145DAA WS	1.5-1.9	0.6-0.9	0.197 b**	0.1084 b*	44.0± 1.974 h
COT	— — — — —	— — — — —	0.156 6	0.0874 5	43.0±0.974 4**
EMB	— — — — —	— — — — —	0.041 E**	0.016 C**	60.0±0.975 D**
160DAA WS	1.5-2.0	0.7-0.9	0.215 f**	0.1226 e**	42.0± 1.276 h
COT	— — — — —	— — — — —	0.167 6	0.0969 5	41.0±1.476 5**
EMB	— — — — —	— — — — —	0.048 F**	0.0206 B*	57.0±0.083 E**
175DAA WS	1.6-2.0	0.8-0.9	0.228 g	0.1344 f	41.0± 1.536 h
COT	— — — — —	— — — — —	0.174 6	0.1027 5	41.0±0.976 6
EMB	— — — — —	— — — — —	0.054 G	0.0254 A*	52.0±0.963 B*

[Each columns · represent 3 factors WS (Whole Seed), COT (Cotyledon), EMB (Embryonal axes) and significant difference represented by small alphabets, arabic numerals & capital alphabets respectively]+

ANOVA (done separately), *significant at P = 0.01, **significant at P= 0.05 level, ±SE: Standard Error of the mean and the figures superscribed by same letters in columns without asterisks are not significant (at 1% and 5% P level) based on LSD (Duncan's Multiple Range Test-DMRT). DAA: Days After Anthesis, Fwt.: Fresh weight, Dwt.: Dry weight.

Table.2. Quantitative estimation of biomolecules during seed development of *Hopea parviflora*

Stages (DAA)	Moisture Content (%) ±SE · ±SE ·	Total Soluble Sugars(TSS) mg g -1 d.wt	Starch mg g-1d. wt ±SE ·	Total Phenol mg g-1 d.wt ±SE ·	Lipid mg g-1d.wt ±SE ·	Amino acid mg g-1d.wt ±SE ·
0DAA	33.0 ± 1.965 a*	10.2±0.029 d*	16.7±0.008 j	111.0±1.308 e*	8.0±0.131 g**	Nil
10DAA	45.0 ± 0.714 b*	19.0±0.364 c*	18.4±0.056 j	126.0±0.976 b*	12.0±0.208 d*	Nil
25DAA	53.0 ± 0.913 f**	28.7±1.210 h**	21.6±0.170 i**	171.5±1.390 c*	18.37±0.122 j	Nil
40DAA	57.0 ± 0.149 c*	34.0±0.290 e*	36.7±0.310 c*	195.0±1.098 d*	19.0±0.390 i**	0.20±0.003 d**
55DAA	64.0 ± 0.854 g**	42.4±0.745 i**	96.0±1.050 e*	212.0±2.447 a*	16.0±0.080 e*	0.40±0.005 c*
70DAAWS	60.0± 00 h	45.3±0.216 f*	144.0±1.89 a*	145.0±0.675 i	22.0±0.134 h**	0.90±0.002 e**
COT	55.0±1.20 1*	18.9±1.08 3**	124.0±0.786 1*	164.0±0.320 2*	— — — — — —	0.60±0.003 4**
EMB	70.0±00 A*	23.7±0.240 C**	118.0±0.187 C*	231.6±0.224 A*	— — — — — —	1.30±0.008 D
85DAAWS	58± 1.074 e**	51.5±0.830 a*	218.0±1.36 b*	148.0±1.06 i	25.4±0.079 j	0.80±0.002 d**
COT	51.0±0.932 2*	21.8±0.117 4**	209.1±0.490 2*	112.7±0.845 1*	— — — — — —	0.50±0.003 3*
EMB	67.0±0.500 C**	28.0±0.298 A*	190.0±1.107 G**	151.0±0.338 F	— — — — — —	1.20±0.004 D
100DAAWS	53± 1.025 d*	68.2±1.254 g*	287.0±0.200 f*	152.2±0.313 i	28.0±0.260 f**	1.00±0.003 f
COT	49.0±0.056 4**	24.3±0.600 2*	290.0±1.589 4*	123.0±0.710 3 **	— — — — — —	.80±0.002 4**
EMB	66.0±0.111 F	39.8±1.300 B*	210.0±0.396 D*	149.5±0.087 B*	— — — — — —	01.30±0.005 D
115DAAWS	47.0±0.916 h	73.9±0.665 b*	333.0±2.089 d*	152.3±0.050 h**	33.0±0.108 a*	1.00±0.003 a*
COT	46.0±0.020 3**	29.5±0.147 1*	343.1±0.630 5*	132.0±0.260 4**	— — — — — —	0.90±0.003 1*
EMB	65.0±0.185 F	46.3±0.300 D**	267.0±1.120 F**	169.0±0.910 C**	— — — — — —	1.20±0.005 A*
130DAAWS	46.0±0.460 h	84.6±0.281 k	387.0±1.830 h**	144.5±0.140 i	78.0±0.239 j	2.00±0.004 b*
COT	45.0±000 6	36.0±0.400 5	396.0±0.221 7**	129.0±0.089 6	— — — — — —	1.70±0.002 5
EMB	63.0±1.30 F	49.5±0.557 E	310.8±0.982 B*	161.0±0.100 E**	— — — — — —	2.70±0.006 C*
145DAAWS	44.0± 1.974 h	85.3±0.057 k	411.0±2.385 g**	140.3±0.230 g**	76.0±0.190 c*	2.80±0.013 f
COT	43.0±0.974 4**	35.5±0.110 5	421.2±0.940 3*	120.0±0.540 4**	— — — — — —	1.63±0.005 2*
EMB	60.0±0.975 D**	48.2±0.243 E	398.0±2.190 E*	154.7±0.105 F	— — — — — —	3.30±0.010 B*

160DAAWS	42.0± 1.276 ^{h}	86.0±0.016 ^{k}	382.0±1.600 ^{j}	151.0±0.300 ^{f**}	83.5±0.166 ^{b*}	2.80±0.020 ^{d**}
COT	41.0±1.476 ^{5**}	37.5±0.109 ^{5}	367.5±3.160 ^{6**}	127.8±0.410 ^{5**}	– – – – – –	2.00±0.008 ^{4**}
EMB	57.0±0.083 ^{E**}	46.5±0.380 ^{E}	342.0±0.740 ^{A*}	160±0.203 ^{D**}	– – – – – –	4.30±0.021 ^{E*}
175DAAWS	41.0± 1.536 ^{h}	52.0±0.963	48.4±0.710 ^{E}	250.0±2.900 ^{H}	168.0±2.100 ^{F}	2.60±0.009 ^{f}
COT	41.0±0.976 ^{6}	83.5±0.200 ^{k}	378.0±0.490 ^{j}	163.3±0.600 ^{i}	92.0±0.450 ^{j}	2.10±0.012 ^{5}
EMB	52.0±0.963 ^{B*}	36.5±0.189 ^{5}	362.3±0.860 ^{8}	132.0±1.700 ^{6}	– – – – – –	4.70±0.026 ^{D}

Discussion

The results of the phenological studies covering both vegetative and reproductive dynamics of the selected species (leaf flushing, flowering and fruiting episodes, floral biology such as anthesis, pollen-ovule ratio, pollen fertility, stigma receptivity, fruit development, premature abscission of fruits, weevil infestations, fruit predations, incidence of insect-pests, fungal infections etc.) shed more light on the reproductive efficiency, maturity index, regeneration performance and related complexities of the species and to identify the causal factors for the reduction of the species in their respective habitats/ communities. Leaf flushing of *Hopea parviflora* started from September onwards and extended up to December. The leaves change its colour from yellowish green to dark green on maturity. During the entire leaf development severe insect pest incidences, follicolous fungal infections etc. were noticed in both the species. The irregular flowering and fruiting cycles observed in *H. parviflora* is in agreement with the findings of Troup (1921) and Anonymous (1959, 1976). The disparity in the period/time of flower initiation was also noted in the species. Similar observations were made in *H. parviflora* by Kader (2001). The pollen count of *H. parviflora* flower is 6240 which indicates the pollination behaviour of *H. parviflora* ie by entemophily. The severe premature fruit abscission, insect-pest attacks especially weevil incidences, irregular fruiting cycles, habitat specificity, recalcitrant seeds etc. of the species may be some of the factors, which responsible for their rarity in the long run. According to Murali and Sukumar (1994), Lokesha and Vasudeva (1997), Jose (2001) the premature abscission of fruits, weevil incidence and other phenological irregularities leads to the rarity of the species in their respective habitats. The present study also in general agreement with the above findings. It is evident that the size of the developing *H. parviflora* seeds increased gradually and attained maximum at the maturity stage. Dry matter accumulation starts immediately after anthesis in the species and showed a definite lag phase in the early period of development, but increased greatly after 55 DAA. The dry matter accumulation during different developmental stages revealed that maximum dry matter accumulation occurred during 175 DAA i.e. at the harvesting maturity stage. Merouani *et al*. (2003) observed the morphological, physiological and biochemical characteristics of *Quercus suber* seeds during their development and suggested that the reduction in moisture content differed greatly between tissues i.e. the pericarp and embryo losing less water than cotyledon during the maturation processes. In the present study during seed development in *H. parviflora*, the reduction in moisture content varied among the seed tissues (cotyledon, embryonal axes etc.). In *H. parviflora,* water losses from the cotyledon were greater during maturation process than embryonal axes. Ockenden *et al.* (2001) suggested that the developing embryos of *Cucurbita maxima* seeds increased markedly in weight and size and this is true in case of *H. parviflora*. Gradual increase in the fresh and dry weight characteristics of seed development was observed in this species. Similar observations were made in *Medicago sativa* seeds by Xu *et al*. (1991), in *Nicotiana tobaccum* by Li *et al* (2015). Seed moisture was found to be a good indicator of seed development. During maturity the seed moisture of the species decreased gradually but these seeds lacked pronounced drying at the maturity stage. Sliwinska (2003) found out in sugar beet that seed maturation and seed quality may help to estimate the optimal harvest time. The presence of high moisture content in fully mature stages of *H. parviflora* characterize their recalcitrant nature. Berjak *et al*. (1993) suggested that plant species with recalcitrant seeds are usually of woody taxa and their seeds are shed with high moisture content and they lack pronounced drying unlike orthodox seeds. The seed size, the rate of dry matter accumulation, moisture content, colour changes etc. of the seeds of the selected species often served as the markers for detecting the optimum maturity of the seeds, which is in line with the observations of Lima *et al*. (2000) in *Ceiba pentandra*, Gorecki *et al*. (1997) in *Lupinus luteus*, Pimpini *et al*. (2002) in radicchio, Nayal *et al*. (2002) in *Grewia optiva*.

Biochemical studies of *Hopea parviflora* seeds during development revealed that total soluble sugar (TSS) accumulation gradually increased up to the maturity stage, however the maximum accumulations was observed up to 70 DAA and thereafter no significant changes were noticed. Bertossi *et al*. (2003) pointed out the role of moisture and sugars in the acquisition of desiccation tolerance in developing oil palm (*Elaeis guineensis*) embryos. Starch accumulation in *H. parviflora* seeds during development recorded a maximum at 145 DAA followed by a reduction. However the rate of starch accumulation gradually increased during the entire stages of seed development. Around 90% of the starch accumulation in *H. parviflora* seeds occurred after 40 DAA. The result of the present study is in agreement with the observations of Brown *et al*. (2001), who suggested that reserve accumulation in developing *Durio zibethinus* seeds occurred in the later stages of their development. According to Bhattacharya *et al*. (2002), the maximum starch accumulation in *Camellia sinensis* seeds is coincided with the maximum dry matter accumulation i.e. the embryo maturation phase. Many workers pointed out that accumulation of metabolites

occurred during the seed development and are metabolically active with high level of carbohydrate for immediate translocation to axis during germination (Kermode and Bewley 1989, Kermode *et al*. 1989, Hong and Ellis 1992, Lahuta *et al*. 2000, Modi *et al*. 2000, Gosslova *et al*. 2001 etc.). In *H. parviflora*, during seed development, TSS accumulation recorded very high in embryos after differentiation, which promoted quick germination, Farrant *et al*. (1992a) and *Freitas et al (2016)* observed, recalcitrant seeds predominantly accumulate soluble reserves, which may cause high degree of desiccation sensitivity in these seeds. According to Farrant *et al*. (1993) high levels of complex reserves stored in the vacuoles of seeds might contribute towards the degree of tolerance (desiccation tolerance) to a certain extent.

Phenol accumulation in the species gradually increased throughout their development except for a slight decline in the mid maturity stage at 130 DAA. But lipid and amino acid accumulation gradually increased throughout the developmental period. The results of the present study partially support the view of Singh *et al*. (1981), who reported that the total phenolic content showed an increase during the initial period of ovule development in cotton and decreased when the seeds are matured. According to Kefeli and Kutacek (1977), the physiological role of phenolic substance is controversial because these are localized in vacuoles and hence remote in the control of physiological processes.

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