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Phenological and Seed Developmental Studies of *Vateria indica* L.

An Economically Important Endemic Tree Species of the Western Ghats

Kamarudeen M, G Jee, Babu K P**, Anilkumar. C** Pandurangan. A G** and Krishnan. P N ****

Department of Botany, University of Kerala, Kariavattom, Trivandrum, Kerala, India
*Sree Sankara College, Mattoor, Kalady, Kerala, India
**Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Palode, Kerala
***Malabar Botanic Garden and Institute for Plant Sciences, Olavanna, Kozhicode, Kerala
Corresponding author: drmkamar313@gmail.com

Abstract

The tropical rain forests found to occur along the slopes of the Western Ghats in Kerala is gifted with ~~a~~ vast spectrum of various ecological niches hosting wide range of tree species. Nevertheless, the deforestation in this region owing to various reasons such as anthropogenic activities and catastrophic events causing sever damages on various growing stocks and fragmentation of habitats. Besides, several other physical and physiological parameters are also accountable for the loss of genetic resources including endemics from this bio- geographical zone. 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 December to March and peaking in the mid-wet season of February and fruits during June – July. Anthesis, stigma receptivity, pollen ovule ratio, pollen fertility etc. were also being ascertained. The results on phonological studies leading to rarity of the species. The dry matter accumulation gradually increased with the seed development and the estimation of bio molecules such as sugars, starch, phenols, amino acids and lipids were served as biochemical markers for detecting the optimum period of harvest and associated features.

Key words: *Vateria indica, Seed Development, Dry matter accumulation, Phenology, Biomolecules*

Introduction

The Western Ghats, located between the latitudes 8º20′ and 20º4′ N and longitudes 73º and 77º E covers the biogeographical zone of about 1,60,000 km^2 from the Tapti River (Gujarat) in North extending towards Kanyakumari (Tamilnadu) in South. Tropical warm and humid climatological features with strong influence of both Southwest and Northeast monsoons facilitate luxuriant growth of tropical rain forests with several economically important tree species. *Vateria indica* L. is an elegant evergreen tree species with 30 to 40 m in height with cylindrical bole of around 15 m and girth of about 4 to 5 m belonging to the family Dipterocarpaceae found to occur from North Kanara to Kerala. The tree is a well known species for making commercial plywood and yields an oleo-resin called white dammar. The resin is with expectorant properties against throat troubles, chronic bronchitis, piles, diarrhea, rheumatism, tubercular glands etc used as tonic and carminative. It is also used against gonorrhea and ulcer (FAO 1985). 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. In *Vateria indica*, prolific seeding occurs in 3-5 years with one to two poor seed years and one to two average seed years between them (Anonymous 1976). 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.

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 (ISTA 1985) 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 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 *Vateria indica*, crimson red coloured 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 yellowish 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 *V. indica* and the pathogen was identified as new species of the genus *Echidnodella* (Hosagoudar and Kamarudeen 2002 b). 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 *V.indica* 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 20-25 days. Anthesis was noted between 4-9 am. The pollination is mainly through wind (anemophily). Around 50-56 anthers can be seen in a flower. Stigma receptivity continued for 15-16 hours. The ovary consists of 6 ovules i.e. 2 ovules in each 3 locules. All the ovules except one are aborted during development and in certain cases fruits show polyembryony. Each flower consists of 2,18,400 pollen grains and pollen ovule ratio is (2,18,400: 6) 36400: 1. Acetocarmine staining technique revealed that 98 % of pollengrains are fertile. The petals of the flower withstand 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 *V. indica*, 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 %. The seeds of *V. indica* attain physiological maturity around 20 days before the harvesting stage and the early harvested seeds showed only 40 % germination. Its seedlings were often eaten by short horn treehoppers (Acredidae). 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 18 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 *V. indica* seeds gradually increased during their development (i.e. from 1.3-1.35´0.4-0.45cm to 7.2-7.25´4.9-4.93cm). Dry matter accumulation in *V. indica* 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 90 DAA, gradual reduction of moisture content was noted in the tissues of cotyledons and embryo till the maturity. But the endosperm tissues recorded only a slight reduction in the moisture content during the initial period followed by significant reduction till 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

(44 %) than that of the embryonic axes (51 %) in the mature seeds (Table 1).

Biochemical changes during seed development

i) Total Soluble Sugar accumulation (TSS)

Level of TSS in the developing *V. indica* seeds increased considerably up to 70 DAA (14.66 mg/g dwt. to 33.9 mg/g dwt.) and followed by a slight decline and then gradual increase noted up to 130 DAA. After that the level of TSS was almost stable up to the maturity stage (Table 2).

ii) Starch accumulation

Starch content of *V. indica* seeds increased slowly throughout the seed development. Around 80% of starch accumulation happened after 70 DAA. Starch accumulation attained a maximum (108mg/g dwt.) at the 12th stage of their development (170 DAA). High level of starch accumulation was noted in cotyledons than in embryonal axes (Table 2).

iii) Phenols accumulation

Total phenol content in *V. indica* seeds increased gradually up to 5th stages (70 DAA) of their development from 170.2 mg/g dwt at 0 DAA to 380.5 mg/g dwt.) at 70 DAA. But a slight reduction was noticed at 130 and 145 DAA followed by gradual increase. Phenol content was maximum in cotyledon (350 mg/g dwt.) than embryonal axes (233 mg/g dwt.) at the maturity stage (Table 2).

iv) Lipid accumulation

Total lipid accumulation was maximum (313 mg/g dwt.) during the mature stages of seed development in *V. indica* (Table 2). Around 80% of lipid accumulation occurred between 5th and 12th stages (70 DAA and 180 DAA).

v) Amino acid accumulation

It is evident from the data in Table-2, that amino acid accumulation of *V. indica* seeds increased gradually up to the maturity stage from 1.5 mg/g dwt. at 0 DAA to 8.8 mg/g dwt. at 180 DAA. Maximum level of amino acid accumulation was found in the embryonal axes of the mature seed (8.9 mg/g dwt.) than in cotyledons (7.15 mg/g dwt.). However the amino acid level of endosperm and seed coat tissues gradually declined throughout the development.

Discussion

The results of the phenological studies covering both vegetative and reproductive dynamics of the selectedspecies(leafflushing,floweringandfruiting episodes, floral biology such as anthesis, pollen-ovule ratio, pollen fertility, stigma receptivity, fruit development, pre- mature 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 *Vateria indica* started from October to November. The leaves change its colour from crimson red to dark green on maturity. During the entire leaf development severe insect pest incidences, follicolous fungal infections etc. were noticed. The irregular flowering and fruiting cycles observed in *V.indica* 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 *V.indica* flower is 2,18,400 which indicates the pollination behaviour of *V.indica* ie by anemophily. 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 *V.indica* 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 180 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 *V.indica*, the reduction in moisture content varied among the seed tissues (cotyledon, embryonal axes etc.). 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 *V.indica*. 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 *V.indica* 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 *V.indica* seeds during development revealed that maximum total soluble sugar (TSS) accumulation was observed up to 70 DAA and thereafter significant decreases were observed. 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 content of *V. indica* seeds increased slowly throughout the seed development and its accumulation recorded a maximum at 180 DAA. Around 80% of starch accumulation happened after 70 DAA.. High level of starch accumulation was noted in cotyledons than in embryonal axes (Table 2). 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 *V indica*, 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, lipid and amino acid accumulations were gradually increased throughout their seed development. 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.

Table.1.
Dry matter accumulation of Vateria indica seeds during different developmental stages.

Stages (DAA)	Length (cm)	Breadth (cm)	F.wt/10 replicates (g) •	D.wt/10 replicates (g) •	Moisture Content (%) ±SE•
0DAA	1.30-1.35	0.40-0.45	0.087 ^{j}	0.0265 ^{i}	69.50±0.42 ^{a*}
10DAA	1.65-1.72	0.60-0.63	0.107 ^{j}	0.0360 ^{i}	66.30±0.51 ^{j}
25DAA	1.95-2.01	0.75-0.78	0.127 ^{j}	0.0350 ^{i}	72.40±0.40 ^{e*}
35DAA	2.50-2.58	1.20-1.24	0.353 ^{j}	0.0800 ^{i}	77.30±0.31 ^{g**}
55DAA	3.60-3.64	2.10-2.14	4.900 ^{g**}	1.0000 ^{i}	79.50±0.98 ^{j}

70DAA	3.90-3.96	2.40-2.45	12.30 ^{c**}	3.4000 ^{h**}	78.80±0.57 ^{c*}
90DAA WS	4.85-4.91	2.85-2.91	30.597 ^{a*}	6.5000 ^{f**}	78.70±0.68 ^{d*}
COT	---------	---------	20.673 ^{3**}	6.800 ^{2*}	67.10±1.43 ^{4*}
EMB	---------	---------	0.041 ^{D}	0.007 ^{F}	82.90±0.268 ^{B*}
END	---------	---------	0.108 $^{(a)*}$	0.008 $^{(c)**}$	92.5±0.925 $^{(b)*}$
110DAA WS	5.10-5.14	3.20-3.25	37.230 ^{e**}	10.000 ^{e**}	73.10±0.39 ^{f**}
COT	---------	---------	28.30 ^{4**}	8.300 ^{6}	70.6±.380 ^{5**}
EMB	---------	---------	0.195 ^{A**}	0.056 ^{B*}	71.28±0.205 ^{A*}
END	---------	---------	0.200 $^{(b)*}$	0.010 $^{(d)}$	95.0±1.30 $^{(e)}$
130DAA WS	5.40-5.47	3.55-3.58	48.300 ^{b*}	14.600 ^{d*}	69.00±0.72 ^{i**}
COT	---------	---------	37.60 ^{2*}	13.600 ^{3*}	63.8±0.612 ^{2*}
EMB	---------	---------	0.244 ^{D}	0.080 ^{E**}	67.2±1.30 ^{D**}
END	---------	---------	0.180 $^{(e)}$	0.030 $^{(a)*}$	83.3±0.711 $^{(d)*}$
145DAA WS	5.60-5.63	3.90-3.97	54.663 ^{f**}	20.000 ^{b*}	63.40±0.42 ^{e**}
COT	---------	---------	43.40 ^{5**}	18.00 ^{4*}	58.5±0.303 ^{3*}
EMB	---------	---------	0.310 ^{B**}	0.110 ^{C**}	64.5±0.916 ^{F}
END	---------	---------	0.130 $^{(c)**}$	0.040 $^{(b)**}$	70.3±1.82 $^{(c)*}$
160DAA WS	6.10-6.12	4.30-4.33	62.000 ^{d**}	30.500 ^{a*}	50.00±0.86 ^{b*}
COT	---------	---------	54.80 ^{1*}	28.00 ^{1*}	48.9±0.608 ^{1*}
EMB	---------	---------	0.372 ^{C**}	0.160 ^{A*}	56.9±0.943 ^{C*}
END	---------	---------	0.093 $^{(d)**}$	0.048 $^{(c)**}$	48.3±0.719 $^{(a)*}$
170DAA WS	6.70-6.74	4.80-4.85	65.500 ^{h**}	35.500 ^{c*}	45.80±1.20 ^{h**}
COT	---------	---------	58.30 ^{6}	31.70 ^{5**}	45.6±0.878 ^{6**}
EMB	---------	---------	0.385 ^{D}	0.189 ^{D**}	53.5±1.13 ^{E**}
END	---------	---------	0.095 $^{(e)}$	0.0495 $^{(d)}$	47.8±0.416 $^{(e)}$
180DAA WS	7.20-7.25	4.90-4.93	68.000 ^{i**}	38.100 ^{g**}	43.90±0.800 ^{j}
COT	---------	---------	60.40 ^{6}	33.70 ^{6}	44.2±0.970 ^{7}
EMB	---------	---------	0.389 ^{D}	0.190 ^{F}	51.10±1.36 ^{F}
END	---------	---------	0.094 $^{(e)}$	0.0496 $^{(d)}$	47.2±0.578 $^{(e)}$

[Each columns • represent 4 factors WS (Whole Seed), COT(Cotyledon), EMB (Embryonal xes), END (Endosperm) and significant difference represented by small alphabets, arabic numerals, capital alphabets and small alphabets in parenthesis 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 (DMRT). DAA: Days After Anthesis, Fwt.: Fresh weight, Dwt.: Dry weight.

Table.2.
Quantitative estimation of biomolecules during seed development of Vateria indica

Stages (DAA)	Moisture Content (%) ±SE•	Total soluble sugars (TSS) mg g^{-1} d.wt ±SE •	Starch mg g^{-1} d.wt ±SE •	Total Phenol mg g^{-1} d.wt ±SE •	Lipid mg g^{-1} d.wt ±SE •	Amino acid mg g^{-1} $d.wt^{-1}$ ±SE •
0DAA	69.50±0.42 ^{a*}	14.66±0.300 ^{a*}	35.00±0.880 ^{a*}	170.2±2.67 ^{a*}	56±1.05 ^{a*}	1.5±0.008 ^{a**}
10DAA	66.30±0.51 ^{j}	17.00±1.09 ^{b**}	43.20±0.106 ^{b**}	225.5±0.45 ^{b*}	69.4±3.65 ^{b**}	2.10±0.005 ^{b**}
25DAA	72.40±0.40 ^{e*}	24.66±0.190 ^{c**}	44.80±0.140 ^{c}	250.0±0.32 ^{c*}	73.7±0.460 ^{c}	2.45±0.010 ^{c**}
35DAA	77.30±0.31 ^{g**}	33.32±1.340 ^{d**}	42.70±0.240 ^{c}	265.8±0.73 ^{d**}	88.0±0.760 ^{d**}	2.75±0.035 ^{d**}
50DAA	79.50±0.98 ^{j}	30.5±0.205 ^{e**}	40.80±0.100 ^{c}	390.0±0.882 ^{e*}	114.0±0.85 ^{e*}	3.88±0.029 ^{e**}
70DAA	78.80±0.57 ^{c*}	33.90±0.670 ^{f**}	50.00±1.30 ^{d**}	380.5±0.43 ^{f**}	130.0±0.430 ^{f**}	3.98±0.100 ^{f}

90DAA WS COT EMB END SC	78.70±0.68 d* 67.10±1.43 4* 82.90±0.268 B* 92.5±0.925 (b)* 52.70±0.400 (1)*	17.46±0.140 g** 15.90±0.060 1** 15.20±0.100 A** 11.65±0.230 (a) 10.93±0.100 (1)*	60.40±0.19 e* 27.0±1.750 1** 5.98±0.120 A** 38.3±0.226 (a)** 10.45±0.530 (1) *	288.0±0.935 g* 120.3±1.23 1* 32.4±0.340 A* 17.2±0.129 (a)* 31.6±0.078 (1) *	159.2 ±0.360 g*	4.25±0.042 g** 5.88±0.078 1** 5.67±0.050 A** 1.67±0.008 (a)* 4.10±0.170 (1)
110DAA WS COT EMB END SC	73.10±0.39 f** 70.6±.380 5** 71.28±0.205 A* 95.0±1.30 (e) 47.40±0.560 (1)*	25.9±0.085 h** 24.73±0.820 2* 17.00±0.210 B** 9.6±0.108 (b)** 10.54±0.07 (2) **	63.6±0.105 f ** 40.40±0.410 2** 6.98±0.040 B 47.50±0.36 (b)** 66.50±1.200 (2) *	274.0±0.145 h** 159.0±0.325 2** 83.0±0.430 B* 28.4±0.220 (b)** 35.6±0.068 (2) *	187.0±2.37 h*	6.50±0.060 h* 6.35±0.028 2** 7.0±0.054 B** 2.0±0.005 (b)** 3.10±0.044 (2) *
130DAA WS COT EMB END SC	69.00±0.72 i** 63.8±0.612 2* 67.2±1.30 D** 83.3±0.711 (d)* 42.10±0.160 (3)	28.80±0.11 i** 26.97±0.335 3** 18.65±.042 C** 0.050±0.002 (c)* 5.11±0.060 (3) *	67.20±0.45 g** 128.0±1.650 3* 10.88±.156 C* 20.16±1.208 (c)* 67.00±1.420 (3)	316.0±2.89 i* 208.0±1.05 3* 264.6±0.870 C* 40.5±0.290 (c)** 50.66±0.210 (3) *	246.6±1.26 i*	7.46±0.012 i** 6.90±0.027 3** 8.10±0.007 C** 1.8±0.013 (c)** 0.95±0.003 (3) *
145DAA WS COT EMB END SC	63.40±0.42 e** 58.5±0.303 3* 64.5±0.916 F 70.3±1.82 (c)* 40.4±0.580 (3)	17.30±0.102 j 14.35±0.36 4** 13.80±0.030 D Tr 4.98±0.120 (4)	72.40±0.30 h** 132.0±1.60 4 12.90±0.320 B 17.20±0.087 (d) 63.50±0.21 (4)**	327.0±1.430 j** 220.0±0.650 4* 258.0±1.30 D** 11.2±0.210 (d)* 61.5±0.420 (4) *	258.0±0.40 j**	7.90±0.020 j** 6.95±0.009 4 8.21±0.015 D** 1.75±0.006 (d) 0.8±0.008 (4) **
160DAA WS COT EMB END SC	50.00±0.86 b* 48.9±0.608 1* 56.9±0.943 C* 48.3±0.719 (a)* 39.40±0.025 (2)*	10.66±.095 k** 11.9±0.310 5** 15.8±0.056 E** Tr 4.53±.028 (5) **	93.0±1.020 i* 166.0±0.650 5** 20.5±0.320 D** 10.4±0.41 (e)** 59.0±0.100 (5) **	375.2±0.651 k* 290.0±1.40 5* 246.5±0.200 E** 2.89±0.180 (e)** 72.0±0.0.85 (5) **	292.5±1.06 k*	8.50±0.016 k** 7.12±0.009 5* 8.52±0.014 E** 1.0±0.0.004 (e)** 0.5±0.002 (5) **
170DAA WS COT EMB END SC	45.80±1.20 h** 45.6±0.878 6** 53.5±1.13 E** 47.8±0.416 (e) 34.28±0.900 (3)	10.23±0.008 j 9.64±0.060 6** 15.20±0.350 D Tr 4.32±0.04 (6) **	108.0±1.44 j* 174.5±2.89 4 38.40±0.930 E* 8.60±0.180 d 58.10±0.085 (3)	381.0±0.215 l 316.9±0.336 6** 239.0±0.130 F** 3.89±0.360 (f) 74.3±0.118 (6)	297.0±2.77 c	8.70±0.011 f 7.15±0.003 4 8.70±0.021 F 0.91±0.002 (d) 0.41±0.006 (1)
180DAA WS COT EMB END SC	43.90±0.800 j 44.2±0.970 7 51.10±1.36 F 47.2±0.578 (e) 33.30±0.056 (3)	10.10±0.020 j 9.20±0.089 7** 15.90±0.565 D Tr 4.20±0.022 (4)	254.0±1.86 k* 224.8±2.35 6* 76.0±0.760 F* 4.30±0.209 (f)** 56±0.243 (6) **	390.0±0.25 m** 350.0±2.67 7** 233.0±1.22 G 3.75±0.088 (f) 79.0±0.231 (7) **	313.0±1.93 i**	8.80±0.015 f 7.10±0.005 4 8.90±0.014 G** 0.50±0.002 (f)* 0.35±0.003 (1)

[Each columns • represent 5 factors WS (Whole Seed), COT (Cotyledon), EMB (Embryonal axes), END (Endosperm), SC (Seed coat tissues) and significant difference represented by small alphabets, arabic numerals, capital alphabets, small alphabets in parenthesis and arabic numerals in parenthesis 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 (DMRT). DAA: Days After Anthesis, dwt.: dry weight, Tr: Trace.

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