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Comparative Analysis of Developmental Stages of Haustorium from Three Varieties of *Cocos nucifera* L.

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Abstract

The primary objective of the study was to monitor the development of the haustorium during the germination of coconuts, particularly in terms of growth and morphological changes._The distal part of the embryo produced a soft, spongy mass of tissue called the haustorium during the germination of coconut seeds. Three varieties of coconut cultivars such as Chowghat Orange Dwarf (COD), Malayan Green Dwarf (MGD) and West Coast Tall (WCT) were used for the study. The results revealed that the three coconut varieties under study were quite equivalent in terms of the morphological changes that took place during seed germination and seedling growth.

Key words: *Cocos nucifera* L., Haustorium, Seedling growth, Developmental stage, Comparative analysis

The coconut palm, or *Cocos nucifera* L., is one of the most beneficial plants cultivated throughout tropical regions. It belongs to the Arecaceae family, also referred to as the Palmae. Due to its many uses, it is referred to in Indian literature as "kalpavriksha" (the all-giving tree). This includes different forms of edible oils extracted from dried coconut kernel, and fresh kernel milk. Coconut palms are mainly classified into two basic groups: tall and dwarf, based on various traits like stature, tree growth characteristics and the early flowering and nut features. The most popular tall coconut cultivar grown commercially is West Coast Tall (WCT). The significant dwarf types grown are Malayan Green Dwarf (MGD), Chowghat Orange Dwarf (COD) etc. The coconut seed has a high amount of endosperm and a small embryo. The distal part of the embryo grows larger to create a haustorium, which stays inside the seed and swells significantly when the endosperm vanishes. Coconut haustorium is a soft, spongy mass of tissue that forms inside the cavity of the coconut fruit during the sprouting process. Haustorium is divided into two unique sections; the outer yellow section is rich in oil while the inner white section is rich in carbohydrates. The haustorium continues to expand until the seed is entirely filled. Based on these structural alterations within the seed, it is thought that the haustorium may be crucial to germination and initial seedling growth [1].

The literature that is currently available indicates that very little research has been done on potential relationship between coconut haustorium growth and development. The main goal of the study was to monitor how the haustorium changed morphologically and developed throughout the germination of coconuts.

MATERIALS AND METHODS

Three distinct coconut varieties, including the Chowghat Orange Dwarf (COD), Malayan Green Dwarf (MGD) and West Coast Tall (WCT), were chosen for the present study. The varieties were procured from a local plantation in Thiruvananthapuram, Kerala, India. Identically sized and weighed nuts were chosen and arranged in horizontal rows, covered with soil to a depth of two third, and watered periodically to keep the soil moist. Germination of the nuts was checked on a regular basis. The initial day of germination was identified as the time the shoot tip first appeared on the surface of the nut. Morphological changes during different developmental stages beginning from initial Day After Germination (DAG) to 100 DAG were studied. The parameters recorded were shoot length (SL), haustorium fresh weight (HFW), kernel weight (KW) and nut water volume (NWV). In coconut, the embryo is situated just beneath the soft eye of the nut. It is difficult to determine the precise timing of germination since the distance between the surface of the coconut and the soft eye varies. Thus, the first day of germination in the current study was observed as the emergence of the shoot at the surface of the nut. Sampling was done at every 20 days intervals up to 100 DAG (Days after germination). After the appearance of shoot, the shoot length was recorded by using a measuring scale at different developmental stages. To avoid ambiguity, the shoot length (SL) was calculated from the soft eye, where the shoot emerges after dehusking. Germinated nuts were dehusked, broken carefully and used for haustorium collection. Using a weighing balance, the haustorium's fresh weight was

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measured. After removing the haustorium, the remaining meat (kernel) were scraped out and weighed. After dehusking, the nuts were carefully broken, the coconut water was collected, and its volume was calculated using a measuring cylinder.

Statistical analysis

The data were subjected to one way analysis of variance (ANOVA), Duncan's multiple range test (DMRT) and Tukey post-hoc tests to statistically evaluate the data obtained from the morphological changes in three varieties of coconut during different developmental stages using the software SPSS version 16 for Windows, and significant differences between the morphological characteristics examined in the coconut haustorium were found at $p<0.05$. All the morphological parameters were further subjected to correlation analysis based on Pearson's coefficient of correlation to evaluate the relatedness of various traits.

RESULTS AND DISCUSSION

The collected data on the morphological changes in the three varieties of coconut during different developmental stages are presented in the (Table 1, Fig 1). The data collection procedure started on the initial day after germination (DAG) and continued for 100 DAG (Fig 2-4). The shoot length (SL) and haustorium fresh weight of all three varieties increased from initial to 100 days after germination. According to Konan *et al.* [2], between the second and fourth months of germination in Malaysian Yellow Dwarf, the fresh weight of the coconut haustorium increased rapidly from 20.98 g to 88.47 g. All three coconut varieties showed a considerable reduction in kernel weight after 60 DAG. During germination, kernel weight and thickness decrease due to the continual growth of haustorium inside the nut [3]. The studies of Manivannan *et al.* [4] showed that shoot length ranges from 27.6-60.5cm and haustorium weight ranges from 80.2-131 g at 60 days after germination in WCT variety.

Nut water volume was gradually reduced during different developmental stages and completely disappeared at 100 DAG. From the first day of germination to the 80 DAG, all three varieties of coconut showed a considerable decrease in nut water volume (Table 1). Li *et al.* [5] found that during germination, the length of the seedling and development of the haustorium increased steadily, but after 4.5 and 8.5 months of collection, the weight of the haustorium and the amount of solid and liquid endosperm in the Hainan Tall and Red Dwarf of coconut decreased slightly. Our study shows that the coconut water (liquid endosperm) and kernel (solid endosperm) both declines sequentially while the haustorium fills the nut gradually.

Table 1 Morphological changes in three varieties of coconuts during different developmental stages

Parameters	Varieties	Days after germination (DAG)					
		Initial DAG	20 DAG	40 DAG	60 DAG	80 DAG	100 DAG
Shoot length (SL) (cm)	COD	2.89±0.27^{a}	11.24±1.23^{b}	15.76±0.84^{c}	24.08±2.61^{b}	35.88±4.06^{b}	56.00±5.09^{b}
	MGD	3.12±0.25^{a}	10.02±0.93^{b}	13.82±0.87^{b}	21.92±2.63^{a,b}	31.96±4.00^{a,b}	50.82±5.51^{a,b}
	WCT	4.61±0.38^{b}	6.5±0.60^{a}	11.04±0.62^{a}	18.82±3.07^{a}	27.44±4.22^{a}	44.68±4.17^{a}
Haustorium fresh weight (HFW) (g)	COD	4.59±0.66^{b}	20.88±2.28^{b}	64.12±5.04^{b}	98.94±5.02 ^{a}	113.10±4.61 ^{a}	126.24±7.18 ^{b}
	MGD	4.12±0.67^{b}	20.10±1.17^{a,b}	60.84±3.33^{b}	96.70±4.40 ^{a}	107.52±6.20 ^{a}	117.48±2.71 ^{a}
	WCT	3.26±0.47^{a}	17.40±2.46^{a}	54.22±3.56^{a}	94.8±5.27 ^{a}	111.74±6.41 ^{a}	123.66±4.79 ^{a,b}
Kernel weight (KW) (g)	COD	260.44±5.53 ^{a}	244.62±4.51 ^{a}	231.32±4.42 ^{a}	200.48±7.83 ^{a}	153.9±4.99 ^{b}	128.20±7.31 ^{b}
	MGD	261.78±3.23 ^{a}	245.24±4.91 ^{a}	239.44±3.79 ^{b}	195.9±7.20 ^{a}	136.1±5.28 ^{a}	118.68±3.61 ^{a}
	WCT	280.90±5.24 ^{b}	265.12±4.70 ^{b}	256.48±5.87^{c}	227.54±9.57 ^{b}	164.62±6.66 ^{c}	137.46±6.32 ^{c}
Nut water volume (NWV) (ml)	COD	107.8±4.00 ^{a}	91.00±5.08 ^{b}	48.94±5.37 ^{a}	17.60±4.05 ^{a,b}	3.04±3.14 ^{a}	0
	MGD	106.92±6.45 ^{a}	83.90±3.48 ^{a}	43.82±3.18 ^{a}	15.00±3.69 ^{a}	2.00±2.03 ^{a}	0
	WCT	116.05±5.60 ^{b}	97.1±5.23 ^{b}	55.00±3.76 ^{b}	22.400±4.29 ^{b}	0	0

Results are represented as mean ± standard deviation.
Mean ± standard deviation followed by different letter, within a line indicates a significant difference (P<0.05) as determined by Duncan's multiple range test

Table 2 Tukey's HSD post-hoc tests on shoot length of different coconut varieties

Dependent variable	(I) Coconut	(J) Coconut	Mean difference (I-J)	Standard Error	Significance
Initial DAG	MGD	COD	0.23	0.197	0.496
	WCT	COD	1.72*	0.197	0.000
		MGD	1.49*	0.197	0.000
20 DAG	COD	MGD	1.22	0.605	0.151
		WCT	4.74*	0.605	0.000
	MGD	WCT	3.52*	0.605	0.000
40 DAG	COD	MGD	1.94*	0.499	0.006
		WCT	4.72*	0.499	0.000
	MGD	WCT	2.78*	0.499	0.000
60DAG	COD	MGD	2.16	1.75	0.460
		WCT	5.26*	1.75	0.028
	MGD	WCT	3.10	1.75	0.223
80 DAG	COD	MGD	3.92	2.59	0.320
		WCT	8.44*	2.59	0.017
	MGD	WCT	4.52	2.59	0.230
100 DAG	COD	MGD	5.18	3.13	0.263
		WCT	11.32*	3.13	0.009
	MGD	WCT	6.14	3.13	0.165

*The mean difference is significant at the 0.05 level

Table 3 Tukey's HSD post-hoc tests on haustorium fresh weight of different coconut varieties

Dependent variable	(I) Coconut	(J) Coconut	Mean difference (I-J)	Standard Error	Significance
Initial DAG	MGD	WCT	0.86	0.388	0.109
	COD	MGD	0.47	0.388	0.470
		WCT	1.33*	0.388	0.013
20 DAG	COD	MGD	0.78	1.30	0.823
		WCT	3.48*	1.30	0.049
	MGD	WCT	2.70	1.30	0.137
40 DAG	COD	MGD	3.28	2.56	0.433
		WCT	9.90*	2.56	0.006
	MGD	WCT	6.62	2.56	0.058
60DAG	COD	MGD	2.16	3.10	0.771
		WCT	4.14	3.10	0.405
	MGD	WCT	1.98	3.10	0.803
80 DAG	COD	MGD	5.58	3.66	0.316
		WCT	1.36	3.66	0.927
	WCT	MGD	4.22	3.66	0.503
100 DAG	COD	MGD	8.76	3.30	0.052
		WCT	2.58	3.30	0.722
	WCT	MGD	6.18	3.30	0.190

*The mean difference is significant at the 0.05 level

Table 4 Tukey's HSD post-hoc tests on kernel weight of different coconut varieties

Dependent variable	(I) Coconut	(J) Coconut	Mean difference (I-J)	Standard Error	Significance
Initial DAG	MGD	COD	1.34	3.02	0.898
	WCT	COD	20.46*	3.02	0.000
		MGD	19.12*	3.02	0.000
20 DAG	WCT	COD	20.50*	2.97	0.000
		MGD	19.88*	2.97	0.000
	MGD	COD	0.62	2.97	0.976
40 DAG	WCT	COD	25.16*	3.01	0.000
		MGD	17.04*	3.01	0.000
	MGD	COD	8.12*	3.01	0.048
60DAG	WCT	COD	27.06*	5.22	0.001
		MGD	31.64*	5.22	0.000
	COD	MGD	4.58	5.22	0.665
80 DAG	WCT	COD	10.72*	3.60	0.029
		MGD	28.52*	3.60	0.000
	COD	MGD	17.80*	3.60	0.001
100 DAG	WCT	COD	9.26	3.77	0.072
		MGD	18.78*	3.77	0.001
	COD	MGD	9.52	3.77	0.064

*The mean difference is significant at the 0.05 level

Table 5 Tukey's HSD post-hoc tests on nut water volume of different coconut varieties

Dependent variable	(I) Coconut	(J) Coconut	Mean difference (I-J)	Standard Error	Significance
Initial DAG	COD	MGD	0.88	3.44	0.965
	WCT	COD	8.26	3.44	0.080
		MGD	9.14	3.44	0.052
20 DAG	WCT	COD	6.10	2.95	0.139
		MGD	13.20*	2.95	0.002
	COD	MGD	7.10	2.95	0.079
40 DAG	WCT	COD	6.06	2.66	0.098
		MGD	11.18*	2.66	0.003
	COD	MGD	5.12	2.66	0.175
60DAG	WCT	COD	4.80	2.54	0.184
		MGD	7.40*	2.54	0.033
	COD	MGD	2.60	2.54	0.577
80 DAG	COD	MGD	1.04	1.70	0.817
		WCT	0.34	1.70	0.978
	WCT	MGD	0.70	1.70	0.912

*The mean difference is significant at the 0.05 level

To ascertain whether there is a statistically significant difference between the three varieties, Tukey HSD test was performed following the one-way ANOVA. The post hoc tests showed that the mean scores for the initial day after germination and the 20th day after germination for shoot length were significantly different between Chowghat Orange Dwarf

(COD) and West Coast Tall (WCT), and Malayan Green Dwarf (MGD) and West Coast Tall (WCT) ($p<0.05$), but not between Chowghat Orange Dwarf (COD) and Malayan Green Dwarf (MGD). The mean scores for 40 DAG for shoot length were significantly different between all three varieties ($p<0.05$). The mean scores for 80-100 DAG for shoot length were statistically significant between COD and WCT ($p<0.05$), but not between MGD and WCT and COD and MGD. So, from this analysis it can be inferred that the shoot length showed statistically significant difference between COD and WCT during different days after germination.

The haustorium fresh weight showed significant difference between COD and WCT variety up to 40 days after germination ($p<0.05$), but no significant difference in the haustorium fresh weight was noticed during later stages of development (Table 3). It is clear from the (Table 4) that the mean score of kernel weight during different germination stages were significantly different between WCT and the other two dwarf varieties ($p<0.05$). The nut water volume did not show significant difference between WCT and COD whereas statistically significant difference was noticed between COD and MGD from 20 DAG to 60 DAG (Table 5).

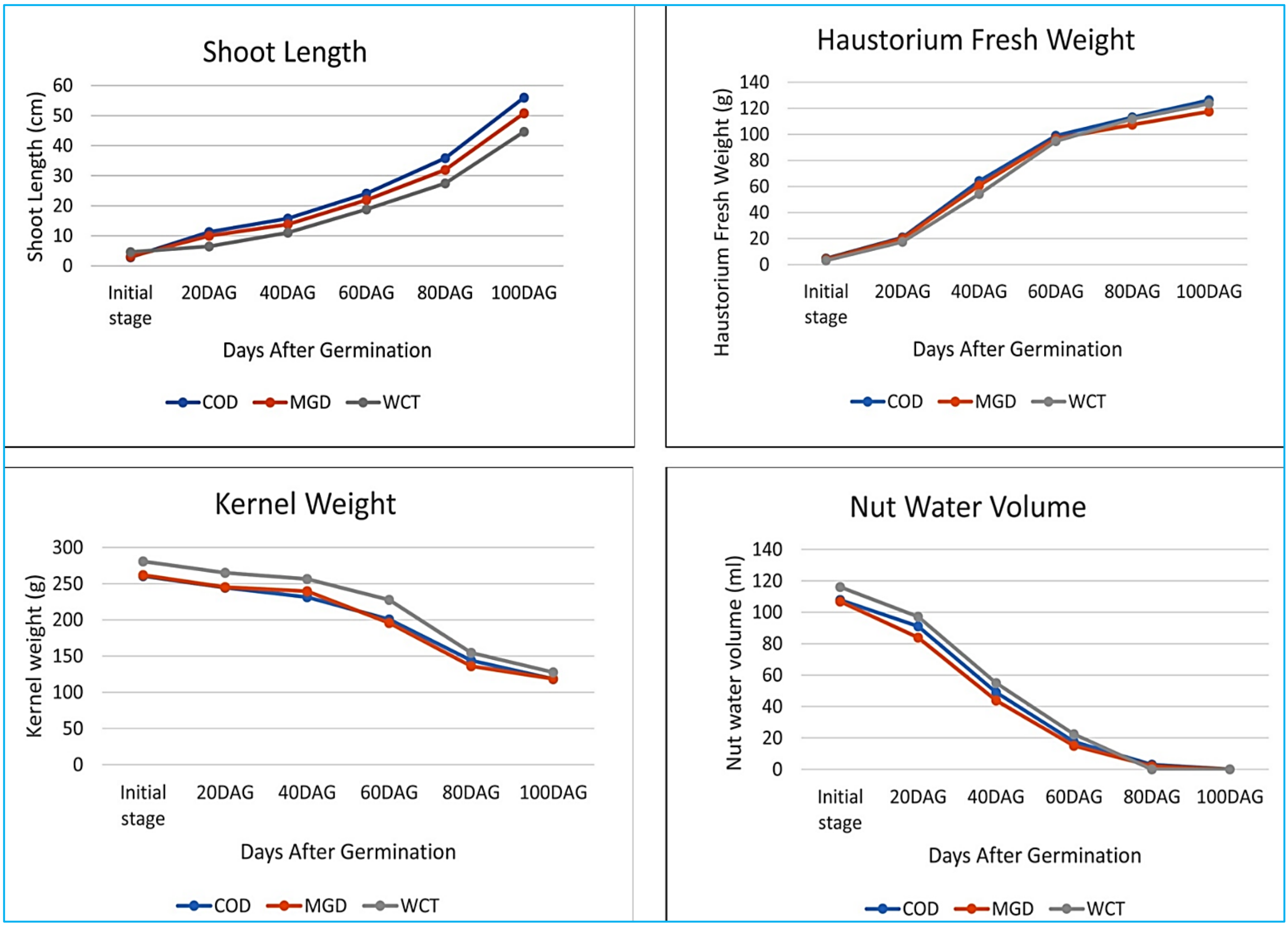

Fig 1 Variation of selected parameters in three coconut varieties during different developmental stages

Pearson correlation analysis

Pearson's coefficient of correlation among selected parameters during different days after germination are presented in the (Table 6).

With an increase in days, there was a significant positive association between shoot length and haustorium fresh weight, however there was a significant negative correlation between nut water volume and kernel fresh weight for all three varieties, with a p value < 0.01 (Table 6). This is in accordance with previous research by Li *et al*. [5], which showed that the haustorium gradually fills the nut as the liquid and solid endosperm inside the coconut exhaust sequentially. They also found that the haustorium had a significant positive correlation with the seedling, but a significant negative correlation with the liquid and solid endosperms in Hainan Tall and Red Dwarf coconuts.

Table 6 Pearson correlation among the shoot length, haustorium fresh weight, Kernel weight and nut water volume during different days after germination of coconut varieties

	Days after germination	Shoot length	Haustorium fresh weight	Kernel weight	Nut water volume
Days after germination	1	0.958**	0.976**	-0.974**	-0.964**
Shoot length		1	0.894**	-0.963**	-0.868**
Haustorium fresh weight			1	-0.927**	-0.991**
Kernel weight				1	0.909**
Nut water volume					1

**Correlation is significant at the 0.01 level

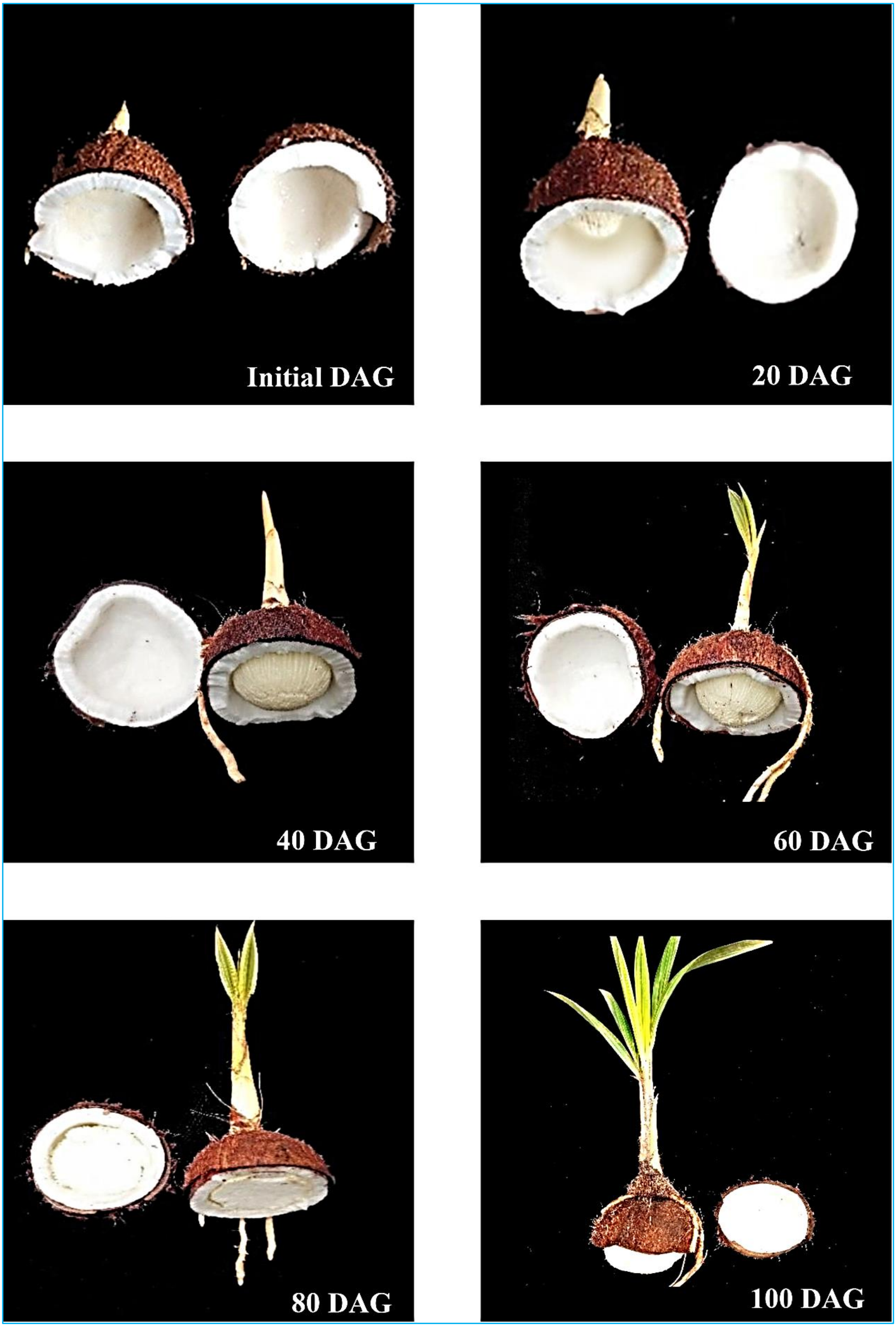

Fig 2 Development of COD haustorium at different growth stages

Fig 3 Development of MGD haustorium at different growth stages

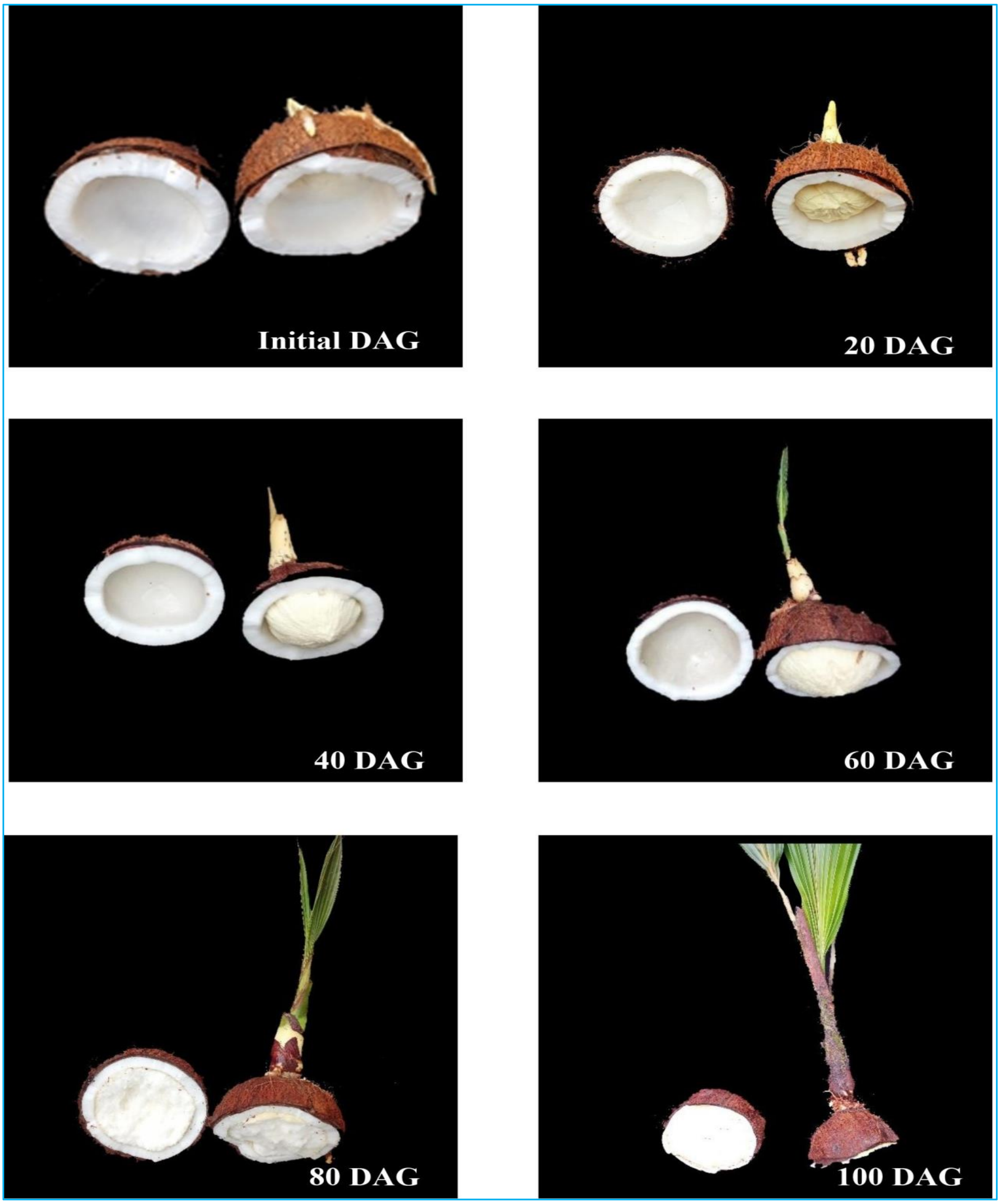

Fig 4 Development of WCT haustorium at different growth stages

CONCLUSION

Haustorium plays a crucial function in nutrient mobilization during coconut germination and seedling growth by absorbing energy from the liquid and solid endosperms (coconut water and kernel). The weight and volume of the kernel and coconut water decreased as the haustorium formed and the seedling grew. The coconut haustorium absorbs nutrients from the kernel, particularly during the later stages of coconut germination.

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