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Response of Physiological Parameters and Leaf Dry Matter Accumulation in Different Improved Coconut (*Cocos nucifera* L.) Cultivars to Environmental Conditions in DL_3 and IL_{1a}

A.S.M.P.M. WICKRAMASINGHE[1], C.S. RANASINGHE[2] and D.C. ABEYSINGHE[1]

[1] *Department of Plantation Management, Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila (NWP)*

[2] *Plant Physiology Division, Coconut Research Institute, Sri Lanka*

ABSTRACT

This study was conducted to find out the response of photosynthetic parameters and leaf dry matter accumulation of six improved coconut cultivars (DGT, DBT, DGSR, TDB, TSR and TT) under wet (Early January) and moderately dry (March) periods in IL_{1a} (Raddegoda) and DL_3 (Wanathawilluwa) . The chlorophyll a (Ch_a), chlorophyll b (Ch_b), carotenoid (*Caro*), leaf dry matter (*LDM*), total canopy dry matter (*TCDM*) contents, rate of photosynthesis (*A*), leaf nitrogen (*LN*) percentage in youngest fully opened leaf were measured. DBT has the highest total chlorophyll content ($Ch_a + Ch_b$) and the rate of photosynthesis. DGSR has shown the highest carotenoid content. The highest and lowest leaf nitrogen was observed in TSR and TT respectively. The total canopy dry matter content was not significantly different among the varieties but the highest leaf dry matter content was observed in TDB. All considered parameters except photosynthesis were higher in IL_{1a} than DL_3. The highest Ch_a, Ch_b, leaf dry matter, total canopy dry matter contents, rate of photosynthesis and leaf nitrogen percentage were observed in moderately dry period. The highest carotenoid content was observed in wet period. Different interaction effects of time, location and variety were present among physiological parameters.

KEYWORDS: *Cocos nucifera*, Dry matter, Physiological parameters

INTRODUCTION

Coconut (*Cocos nucifera* L.) is the most widely grown plantation crop in Sri Lanka contributing 19 % of the total arable land. Coconut is important in daily diet of Sri Lankans and it is an important raw material for many coconut based industries. Nearly 80% of the total national production is used for domestic consumption with per capita consumption of 109 nuts. However, there is a considerable gap between the national coconut production and demand.

The endemic coconut germplasm in Sri Lanka are grouped into three distinct varieties such as Typica (tall), Nana (dwarf) and Aurantiaca based on the morphological characters and breeding habits. San Raman (SR) is an exotic tall variety introduced from Philippines. New coconut hybrids were recently developed by crossing tall × tall and tall × dwarf forms. These different forms show different physiological characteristics and performance in the field. The rate of photosynthesis is one of the indicators to analyze physiological performance of coconut. Chlorophylls and carotenoids, the photosynthetic pigments are found in the chloroplast of higher plants which have a strong relationship with the photosynthetic rate of coconut. Chlorophyll is primarily involved in absorbing light energy for photosynthesis and carotenoid is known as a photo protective pigment as it reduces the photo-oxidative damage to the photosynthetic apparatus (Andrew *et al.*, 2007). Rubiuolose bisphosphate carboxylase (Rubisco) is the most abundant protein in leaves and it is the most important enzyme in C_3 plants in the photosynthetic assimilates production. It constitutes about 25% of the total leaf nitrogen, making it as the largest nitrogen containing component in leaf (Caldera, 2012). At the early stages of coconut seedling growth (first 10 years), the vegetative dry matter production, especially the leaf, is useful for studying crop responses and varietal differences to environmental conditions.

Kurunegala and Puttlam are major coconut cultivating districts in Sri Lanka belong to intermediate (IL_{1a}) and dry (DL_3) zones respectively. More frequent and long drought is expected in intermediate and dry zones of Sri Lanka with projected climate change. Therefore, with increasing importance of coconut in national economy, it is vital to identify the cultivars which can perform better under various environment conditions. However, detail scientific information on these physiological parameters of the newly improved coconut hybrids is scarce. Therefore, the objective of this research was to identify the most suitable coconut cultivars for intermediate and dry zones with respect to photosynthetic related parameter.

MATERIALS AND METHODS

Site and Plant Description

The research was conducted in Plant Physiology Division of Coconut Research Institute, Lunuwila, Sri Lanka from January to April 2013. Seven to eight year old six improved coconut forms known as the Tall × Tall (TT), Dwarf Green × San Raman (DGSR), Tall × San Raman (TSR), Dwarf Brown × Tall (DBT), Tall × Dwarf Brown (TDB), Dwarf Green × Tall (DGT) were selected from Raddegoda (IL_{1a}) and Wanathawilluwa (DL_3) (field evaluation trials of Genetic and Plant Breeding Division in CRI). Six palms from each variety were used at two time periods; wet (early January following a continuous rainy period) and moderately dry (end of March following a low rainfall period) in two Agro Ecological Regions (DL_3 and IL_{1a}).

Data Collection and Analysis

Leaflets of youngest fully opened leaf of palms were used for measurements. Chlorophyll *a* and *b*, carotenoid contents, leaf nitrogen percentage, rate of photosynthesis, leaf dry matter (dry weight) and total canopy dry matter contents were measured under wet and moderately dry conditions.

Results were analyzed using Statistical Analysis Software (SAS 9.1, 1999). Comparisons between means were made using the completely randomized design (CRD) and GLM procedure.

Chlorophyll and Carotenoid Contents

Coconut leaflets were sampled to labeled polythene bags and put on ice immediately and those samples were brought to the laboratory for analysis of chlorophyll *a* (Ch_a) (mg/g fw, mg/cm^2), chlorophyll b (Ch_b) (mg/g fw ,mg/cm^2), and carotenoid (*Caro*), (mg/g fw,mg/cm^2). Leaf samples were cleaned with a paper tissue, leaf discs were cut avoiding major veins in both sides of a leaflet, leaf discs (1.13 cm^2) were weighed, cut into small pieces and crushed in 10 ml of acetone for 1 min. using an electric crusher. Crushed samples were centrifuged for ten min. at 3500 rpm and absorbance of the supernatant containing chlorophyll and carotenoid were measured at 663,646 and 470 nm wavelengths using a Uv-Visible spectrophotometer (Shimadzu,Japan).

Chlorophyll and Carotenoid contents were calculated using modified equation of Lichtenthaler and Wellburn(1983).

$$Ch_a=12.21\,A_{663}-2.81\,A_{646}$$

$$Ch_b=20.13A_{646}-5.03A_{663}$$

$$Caro=\frac{1000A_{470}-3.27Ch_a-104Ch_b}{229}$$

Where, A_{663}, A_{646}, A_{470} are absorbance at 663,646, 470 nm respectively.

Estimation of Leaf Dry Matter (frond)

Cross section area (width × depth) of the petiole at the point of attachment of the lowest leaflet in fully open youngest leaf of each palm was measured using a vernier caliper .Total dry matter content of the leaf (frond) was calculated using the modified equation of Friend and Corley, (1994).

$$W = 0.13C - 0.25$$

Where, W=Total leaf dry matter (kg) and C= depth × width of the petiole (cm^2).

Photosynthesis

Rate of Photosynthesis of youngest fully opened leaf was measured using the LI-COR portable photosynthesis meter (LI-6200, USA).

Total Leaf Nitrogen

Twelve leaflets of youngest fully opened leaf were selected for total leaf nitrogen analysis. Middle portion of leaflets were separated and cleaned. Leaf samples were put into labeled paper bags and oven dried. Dried leaf samples were powdered using an electric grinder,. 0.1 g of powdered sample was weighed into a digestion tube, 2.0 g of anhydrous sodium sulphate, few drops of distilled water (to moisten the sample), 4 ml of 0.4% selenium sulphuric solution were added. The tubes were placed in the digestion block, kept for 6 hr under four different temperatures (150 ^{0}C for 20 min, 250 ^{0}C for 30 min, 300 ^{0}C for 30 min, 380 ^{0}C for 2.5 hr). The tubes were removed from the block, allowed to cool to room temperature; 25 ml distilled water added and mixed well using a whirly mixture. Digested samples were diluted up to 75 ml with distilled water and allowed to stand overnight. The content was transferred to another container and the total nitrogen content of each sample was analyzed using an Autoanalyzer (BRAN+LUBEE, Germany).

Total Canopy Dry Matter Content

Number of leaves (fronds) of the canopy was counted. Total canopy dry matter content was calculated by multiplying number of leaves by single leaf dry matter content

Climate Data

Monthly rainfall and temperature data were obtained from nearest meteorological stations at Puttlam and Kurunegala.

RESULTS AND DISCUSSION

Variation of Physiological Parameters between Wet and Moderately Dry Seasons

The dry mater accumulation in newly developed leaf, total canopy dry matter content and rate of photosynthesis were significantly higher under the moderate drought compared to wet period (Table 1).Whilst chlorophyll *a* (Ch_a), Chlorophyll *b* (Ch_b) and carotenoid contents were significantly affected by period of sampling percentage of leaf nitrogen was unaffected by the time (Table 3). Contents of Ch_a and Ch_b were significantly lower in wet (January) than in moderately dry (March) period. The higher rate of photosynthesis in moderately dry seems to be associated with higher Ch_a and Ch_b contents and this has led to higher leaf dry matter content in moderately dry compared to wet period. The results revealed that photosynthetic parameters and leaf dry mater accumulation prefers to climatic conditions during moderately dry period, probably with more solar radiation intensity and more favourable temperature than the continuous wet period with less than optimum temperature. The low carotenoid content in moderately dry was not affected the rate of photosynthesis, because chlorophyll molecules were able to utilize the absorbed energy into photosynthesis without the need for dissipation of energy by caroteniods (Caldera, 2012).

Table 1. Mean values of Rate of Photosynthesis (*A*) (μmol CO_2 $m^{-2}s^{-1}$), leaf dry matter (*LDM*) (kg) and total canopy dry matter content (*TCDM*) (kg) at two different time periods

Time	A	LDM	TCDM
Wet (Early January)	7.30^b	2.67^b	57.55^b
Moderately Dry (March)	8.11^a	3.15^a	68.37^a

For each variable, means followed by the same superscript letter are not significantly different

Variation of Physiological Parameters between Two Agro Ecological Regions

Whilst there was no location effect on contents of Ch_a (mg/g fw, mg/cm^2), Ch_b (mg/g fw), carotenoids (mg/g fw, mg/cm^2), leaf dry matter and leaf nitrogen percentage, there was a significant location effect on rate of photosynthesis, total canopy dry matter content and Ch_b (g/cm^2) (Tables 2 and 4). Earlier studies have shown that supra-optimal temperature in DL_3 can positively affect for the vegetative growth characteristics of these seedlings (Dissanayaka *et al.*, 2012) and this may be associated with observed higher rates of photosynthesis in present study. Further the results indicated that the plants have not been exposed to supra optimal temperature during this study which plant experience during severe dry period. But, the highest total canopy dry matter content was observed in Raddegoda (Table 2). This was due to the observed higher number of leaves in IL_{1a} than DL_3.

Table 2. Mean values of Photosynthesis (*A*) (μmol CO_2 $m^{-2}s^{-1}$), leaf dry matter (*LDM*) (kg) and total canopy dry matter content (*TCDM*) (kg) at two locations

Location	A	LDM	TCDM
IL_{1a}	7.09^b	2.85^a	74.92^a
DL_3	8.29^a	2.99^a	54.00^b

For each variables, mean followed by the same superscript letters are not significantly different

Variation of Physiological Parameters among Varieties

When considering the variety effect, DBT has the highest ch_a (mg/g fw) content and it was significantly higher than that of DGT and DGSR (DG female crosses) and not differently from TT, TSR, and TDB (Tall female crosses) (Table 5). Ch_a content per unit area was not significantly different. Ch_b (mg/g fw) was also highest in DBT and it was not significantly different from its reciprocal cross (TDB), but significantly higher than that of DGT, DGSR, TT and TSR. When Ch_b values were expressed in mg/ cm^2, the highest and the lowest values were observed in DBT and DGT respectively (Table 5). The highest carotenoid content per unit weight and area was observed in DGSR. Rate of photosynthesis was not statistically different between varieties, but DGSR showed the highest value. The highest leaf dry matter content was observed in TDB and it was not significantly different from two tall forms (TT, TSR) and it's reciprocal cross DBT (Table 6). It shows that partitioning of dry matter into vegetative part was different among varieties, but, there was no significant difference in total canopy dry matter content among cultivars. This was due to lower number of leaves in TDB, TT, and TSR (Table 6). TSR has the highest leaf nitrogen content.

Table 3. Mean values of Chlorophyll a (Ch_a) (mg/g fw, mg/cm^2), Chlorophyll b (Ch_b) (mg/g fw, mg/cm^2), Carotenoid (*Caro*) (mg/g fw, mg/cm^2) contents and Leaf Nitrogen (*LN*) (%) at different time periods

Time	Ch_a		Ch_b		*Caro*		*LN*
	(mg/g fw)	(mg/cm²)	(mg/gfw)	(mg/cm²)	(mg/ g fw)	(mg/cm²)	
Wet (Early January)	1.85^b	0.059^b	0.97^b	0.03^b	0.32^a	0.010^a	1.99^a
Moderately Dry (March)	2.15^a	0.066^a	1.67^a	0.06^a	0.15^b	0.005^b	1.89^a

For each variable, means followed by the same superscript letter are not significantly different

Table 4. Mean values of Chlorophyll a (Ch_a) (mg/g fw, mg/cm^2), Chlorophyll b (Ch_b) (mg/g fw, mg/cm^2), Carotenoid (*Caro*) (mg/g fw, mg/cm^2) contents and Leaf Nitrogen (*LN*) (%) at two locations

Location	Ch_a		Ch_b		*Caro*		LN
	(mg/g fw)	(mg/cm²)	(mg/g fw)	(mg/cm²)	(mg/g fw)	(mg/cm²)	
IL_{1a}	2.06^a	0.06^a	1.38^a	0.052^a	0.23^a	0.01^a	1.89^a
DL_3	1.96^a	0.07^a	1.30^a	0.044^b	0.23^a	0.01^a	1.99^a

For each variable, means followed by the same superscript letter are not significantly different

Table 5. Mean values of Chlorophyll a (Ch_a) (mg/g fw, mg/cm^2), Chlorophyll b (Ch_b) (mg/g fw, mg/cm^2), Carotenoid (*Caro*) (mg/g fw, mg/cm^2) contents and Leaf Nitrogen (*LN*) (%) among different varieties

Variety	Ch_a		Ch_b		*Caro*		*LN*
	(mg/g fw)	(mg/cm²)	(mg/g fw)	(mg/cm²)	(mg/g fw)	(mg/cm²)	
DGT	1.88^b	0.06^a	1.19^b	0.043^c	0.24^{ab}	0.008^{ab}	1.93^{ab}
DBT	2.19^a	0.07^a	1.60^a	0.055^a	0.21^b	0.007^{bc}	1.95^{ab}
DGSR	1.92^b	0.06^a	1.19^b	0.045^{bc}	0.27^a	0.009^a	1.92^{ab}
TT	1.97^{ab}	0.06^a	1.30^b	0.046^{bc}	0.21^b	0.006^c	1.86^b
TSR	2.05^{ab}	0.07^a	1.34^b	0.047^{bc}	0.24^{abc}	0.008^{abc}	2.09^a
TDB	2.04^{ab}	0.07^a	1.39^{ab}	0.050^{ab}	0.21^{bc}	0.007^{bc}	1.89^{ab}

For each variable, means followed by the same superscript letter are not significantly different

It was significantly higher than TT, but it was not significantly different from DGT, DBT, DGSR and TDB (Table 5).The rate of photosynthesis of plant depends on mesophyll capacity for photosynthesis. Mesophyll capacity depends, amongst other factors, on the activities of Ribulose1-5 bisphosphate carboxylase oxygenase (Rubisco) and the capacity for Rubiolose bisphospate (Rubp) regeneration (Jayasekara *et al.*, 1996).The lowest leaf nitrogen content in TT may have contributed to reduce a mesophyll capacity for photosynthesis in TT.

Although, effect of time × location was significant for Ch_a and Ch_b per unit weight and area and thus chlorophyll *a* and chlorophyll *b* were higher in IL_{1a} during wet period. The effect of time × variety for all considered parameters was statistically insignificant except carotenoid values were expressed in mg/cm^2.Thus the carotenoids content (mg/cm^2) of DGT was higher during the wet period compared to other varieties. There was a significant location × variety effect on Ch_a (mg/g fw), Ch_b (mg/cm^2), carotenoid contents (mg/g fw, mg/cm^2), leaf dry weight and total canopy dry matter content. But that was not significant for Ch_a (mg/cm^2), Ch_b (mg/g fw), rate of photosynthesis and leaf nitrogen percentage. The highest values for both Ch_a (mg/g fw), *Chl b* (mg/cm^2) were observed in DBT in DL_3. The highest carotenoid content was observed in DGT in DL_3 and DGSR in IL_{1a}. The highest total dry matter content was observed in TSR in IL_{1a} and TDB in DL_3. Time × variety × location was not statistically significant for all considered physiological parameters and leaf dry matter accumulation.

Table 6. Mean values of rate of Photosynthesis (*A*) (µmol CO_2 $m^{-2}s^{-1}$), leaf dry mater (*LDM*) (kg), total canopy dry matter content (*TCDM*) (kg) and no. of leaves per palm (*NL*) among different varieties

Variety	*A*	*LDM*	*TCDM*	*NL*
DGT	7.27^{a}	2.59^{c}	56.39^{a}	21.66^{a}
DBT	7.63^{a}	2.81^{abc}	63.21^{a}	22.04^{a}
DGSR	8.47^{a}	2.74^{bc}	60.01^{a}	22.13^{a}
TT	7.60^{a}	3.13^{ab}	67.95^{a}	21.88^{a}
TSR	7.49^{a}	3.11^{ab}	67.24^{a}	21.08^{a}
TDB	7.85^{a}	3.16^{a}	62.95^{a}	20.25^{a}

For each variables, mean followed by the same letter are not significantly different

Table 7. Monthly Mean Temperature (*T Mean*) (°*C*), Rainfall (*RF*) (*mm*), Sun Shine Hour (SSH) in DL_3 during the experimental period

Year	Month	T Mean	RF	SSH
2012	Oct	27.30	365.20	5.40
	Nov	27.50	250.80	5.00
	Dec	26.30	307.50	4.80
2013	Jan	25.60	87.40	6.20
	Feb	26.50	167.70	6.00
	Mar	27.00	147.60	6.20

Table 8. Monthly Mean Temperature (*T Mean*) (°*C*), Rainfall (*RF, mm*), Sun Shine Hour (SSH) in IL_{1a} during the experimental period

Year	Month	T Mean	RF	SSH
2012	Oct	28.30	430.30	5.10
	Nov	27.50	247.70	4.80
	Dec	26.20	431.90	3.10
2013	Jan	25.50	194.80	5.80
	Feb	27.20	52.90	6.20
	Mar	27.30	119.50	6.00

The palms were not exposed to severe drought periods during the experimental period. (Tables 7 and 8).Therefore, it is suggested to continue the data collection following a longer dry period to identify physiological changes among varieties.

CONCLUSIONS

The study indicated that the pigments involve in photosynthesis and photo-protection of leaves can be used to screen cultivars for drought and heat resistance in the early growth stages of coconut. For drought prone areas, DG× T and DB × T hybrids seem to be more suitable and for areas with optimum conditions for coconut, DG × SR seems to be more suitable compared to other crosses.

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REFERENCES

Andrew, J.S., Helene, M., Marcel, K., Gaelle, P., Chnwei, L., Steave, T. and Jame, M. (2007). An investigation of carotenoid biosynthesis in *Coffea canephora* and *Coffea arabica. Journal of plant physiology*. **165**, 1087-1106.

Caldera, H.I.V. (2012). Plant response to environmental change with special emphasis on anatomical and physiological adaptations of stomata across an altitudinal gradient. Ph.D. Thesis, SL, university of Colombo.

Dissanayaka, H.M.D.C., Attanayaka, R.B., Fernando, A.A., Jayathlaka, R., Padmasiri, H.M.L., Herath, H.M.N.B., Chandasiri, S. A.S., Perera, S.A.C.N., Megahakumbura, M.K., and Perera L. (2012). Development of promising coconut hybrids using novel brown dwarf coconut variety. Proceeding of 4th symposium on plantation crop research.20-21 September 2012.11-25.

Friend, D. and Corley, R.H. (1994). Measuring coconut palm dry mater production. *Expl Agric.* **30** (10), 223-235.

Jayasekara, C., Nainayaka, A. P. A. D. and Jayasekara, K. S. (1996). Photosynthetic characteristics and Productivity of coconut palm. *Journal of Plantation Crop.* **24**, 538-547.

Lichtenthaler, H.K. and Wellburn, A.R. (1983). Determination of total carotenoids and chlorophyll a and b of leaf extracts in different solvents. *Biochemical society transaction*, 591-693.