Response of Stomatal Characteristics of Different Cultivars of *Cocos nucifera* L. to Environmental Factors in IL_{1a} and DL_3

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ABSTRACT

This study was conducted to identify the response of stomatal density, stomatal conductance, guard cell length, potential conductance index, leaf area and total canopy development of six coconut cultivars (DGT, DBT, DGSR, TDB, TSR and TT) in two agro-ecological zones (IL1a and DL3) under two different time periods (wet and moderately dry). The cultivars DGT, DBT, and DGSR showed higher leaf area development in youngest fully opened leaf with compared to other three cultivars. The stomatal density in all six cultivars was reduced during moderately dry periods in order to minimize water loss via stomatal openings. DBT showed the highest guard cell length during moderately dry period. TDB showed better performances than other cultivars with respect to vegetative growth (leaf area and total canopy development). However, further studies are needed to confirm these results and to determine the differential responses for severe stress conditions.

KEYWORDS: Guard cell length, Potential conductance index, Stomatal conductance, Stomatal density

INTRODUCTION

Coconut (*Cocos nucifera* L.) is one of the major plantation crops in Sri Lanka, being the fourth largest coconut growing country in the world. This monocot tree crop grows well in warm, humid weather with an estimated 120 sunshine hours monthly. The ideal mean temperature is around 27 ^{0}C with a diurnal variation of 5 ^{0}C to 10 ^{0}C. Coconut can be successfully grown in areas where the annual precipitation is between 1300 mm and 2500 mm or above (Abid *et al.*, 2007). Prolonged dry spells lasting for more than two months adversely affect the palms; this statement was the early belief, but it is not correct. The majority of coconut growing areas are fed by two monsoons and rain free periods of four months or more between two monsoons with supra optimal temperatures have often adversely affected coconut yield (Jayasekara *et al.*, 1996).

Stomata are small pores present on the aerial parts of land flora, predominantly on the abaxial surface of leaves. They play a crucial role in linking the atmosphere and terrestrial biosphere through the inward diffusion of CO_2 during photosynthesis and outward diffusion of water vapor during transpiration (Caldera *et al.*, 2011). Under drought conditions, canopy water use and CO_2 uptake can be controlled by reducing stomatal density in the newly developing leaves or reducing stomatal size as adaptations to such environmental conditions.

The previous studies have shown the stomatal density and size of stomata in typica×pumila hybrids (Manthriratna and Sambasivam, 1974). In this particular experiment, the stomatal characteristics of different coconut cultivars were studied with respect to the different environmental factors in dry zone and intermediate zone.

The physiological parameters of coconut cultivars recently introduced by the CRI, Sri Lanka have yet to be broadly studied. Therefore, this particular study was conducted to determine the environmental adaptations of those coconut cultivars with respect to stomatal characteristics.

MATERIALS AND METHODS

Location

The research was conducted at Coconut Research Institute of Sri Lanka, Lunuwila from January to April 2013. The data were collected from two research fields situated at Raddegoda, Kurunegala district (IL_{1a}) and Wanathawilluwa, Puttlam district (DL_3) in two different time periods; wet (during early January after a continuous rainy period) and moderately dry (end of March).

Materials

Six improved coconut cultivars known as Dwarf Brown × Tall (DBT), Tall × Dwarf Brown (TDB), Dwarf Green × Tall (DGT), Dwarf Green × San Ramon (DGSR), Tall × Tall (TT), and Tall × San Ramon (TSR) were selected for the study. Six palms from each cultivar, altogether 36 palms were randomly selected and the fully opened youngest leaf of each palm was used for data collection.

Stomatal Density and Guard Cell Length

Epidermal imprints, made of clear nail varnish were taken from the abaxial surface of proximal, middle and distal portion of each leaflet. Number of stomata was counted under light microscope at × 40 magnifications (Leitz-Dialux 20 EB). Stomatal density was calculated as the number of stomata mm^{-2} of leaf. Length of guard cells (μm) was measured using a calibrated eye piece graticule.

Potential Conductance Index

It was assumed that the stomatal aperture area is proportional to the guard cell length squared and a unitless "potential conductance index" was calculated as below (Holland and Richardson, 2009).

$$PCI = (\text{guard cell length})^2 \times \text{stomatal density} \times 10^{-4}$$

Stomatal Conductance

The rate of passage of carbon dioxide (CO_2) entering, or water vapor exiting through the stomata of the leaves, which is called stomatal conductance was measured by the LI-COR photosynthesis meter during 8.00 am to 1.00 pm.

Leaf Area Measurement

Twelve leaflets from the fully opened youngest leaf were detached from both sides of the frond. The area of these leaflets was measured by LI-3100C area meter and the total area of the frond was calculated as below (Jayasekara and Mathes, 1992).

$$Y = -5832 + 12.08X$$

Where Y = area of the total frond and X = area of the 12 leaflets (cm^2).

Canopy Size

Number of fronds of the canopy was counted. Total canopy area was determined by multiplying single leaf area by the number of leaves.

Climatic Data

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

Statistical Analysis

Statistical analysis software (SAS 9.1,) was used to perform three factor factorial experimental designs considering cultivar, time and location as three factors.

RESULTS AND DISCUSSION

Variation of the Leaf Physiological Parameters among Cultivars

There was a significant effect of cultivar on leaf area development of the youngest fully opened leaf (Table1). The varieties TDB, TSR, and TT showed a significantly higher leaf area than DGT, DBT and DGSR. These results clearly showed that the cultivars with tall mother palms produced bigger leaves than the hybrids with dwarf mother palms. However the total leaf area development of the canopy was not affected by the cultivar. This was due to higher number of leaves in DGT, DBT and DGSR (22.95) compared to other three cultivars (21.07).

Table 1. Mean values of leaf area (LA) (m^2), total canopy area (TCA) (m^2) and stomatal conductance (g_s) (mol H_2O $m^{-2}s^{-1}$), of different cultivars

Cultivar	LA	TCA	g_s
DGT	4.74 ^{b}	102.01^a	0.30 ^{a}
DBT	4.80 ^{b}	106.46^a	0.30 ^{a}
DGSR	4.65 ^{b}	103.07^a	0.27 ^{a}
TDB	5.32 ^{a}	104.64^a	0.29 ^{a}
TSR	5.26 ^{a}	112.32^a	0.25 ^{a}
TT	5.30 ^{a}	117.28^a	0.26 ^{a}

Within each column values with same superscript letters are not significantly different

Even though, the stomatal conductance (g_s) and guard cell length (GCL) were not significantly different between cultivars, stomatal density (SD) significantly varied between cultivars (Tables 1 and 2). The highest SD was observed in DGSR in all proximal, middle and distal positions of the leaflets. Considering the SD of proximal end, DGT and DGSR cultivars showed a significantly higher SD than other cultivars except TSR, whereas in the middle portion of the leaflets, DGSR showed a significantly higher SD than DBT, TDB and TT. The SD of distal end was not significantly different between cultivars. There was a significant cultivar effect on potential conductance index (PCI) of the proximal end of leaflets. The PCI values of DGT and DGSR were significantly higher than that of TT. However the PCI of proximal end of the leaflets in all five cultivars, except TT, was not significant from each other. PCI of the middle and distal position of leaflets was not affected by the cultivar. The results revealed that the response of stomata distribution pattern (SD), size

Table 2. Mean values of guard cell length (GCL) (μm), stomatal density (SD) (no/mm²) and potential conductance index (PCI) of different cultivars

	GCL			SD			PCI		
Cultivar	p	m	d	p	m	d	p	m	d
DGT	32.51ª	35.21ª	34.34ª	191.93ª	189.04ªᵇ	174.86ª	25.20ª	22.40ª	20.60ª
DBT	36.76ª	34.34ª	42.82ª	168.75ᵇ	165.15ᶜ	163.48ª	23.17ªᵇ	22.59ª	26.65ª
DGSR	35.23ª	34.45ª	33.90ª	192.77ª	194.38ª	180.70ª	23.81ª	22.01ª	19.81ª
TDB	34.38ª	34.88ª	34.48ª	172.99ᵇ	169.87ᵇᶜ	167.21ª	22.36ªᵇ	27.74ª	26.67ª
TSR	36.33ª	35.53ª	40.99ª	184.01ªᵇ	175.71ªᵇᶜ	171.32ª	23.48ªᵇ	22.17ª	21.02ª
TT	35.82ª	34.56ª	42.76ª	173.37ᵇ	162.78ᶜ	158.67ª	20.78ᵇ	19.34ª	19.43ª

Within each column values with same superscript letters are not significantly different; p, m and d denote proximal, middle and distal positions of leaflets respectively

(GCL) and consequently, the PCI can vary depending on cultivar as well as the position of a leaf.

Variation of the Leaf Physiological Parameters between Wet and Moderately Dry Periods

The leaf area development and the PCI were not affected by the period of sampling (Tables 3 and 4).

Table 3. Mean values of leaf area (LA) (m²), total canopy area (TCA) (m²) and stomatal conductance (g_s) (mol H_2O $m^{-2}s^{-1}$), of two different time periods

Time period	LA	TCA	g_s
Wet	5.10ª	108.01ª	0.36ª
Moderately dry	4.92ª	107.26ª	0.17ᵇ

Within each column values with same superscript letters are not significantly different

Stomatal conductance (g_s) during wet period was significantly higher than that of moderately dry period. The GCL at proximal and distal ends were not affected by the period, however the GCL of middle portion of leaflets were significantly higher during moderately dry period compared to the wet period. That indicated the size of stomata in different positions has responded differently to environmental conditions. The SD of all three positions of leaflets (*p, m,* and *d*) was significantly higher during wet period than during moderately dry period. This higher SD has increased stomatal conductance (g_s) during wet period compared to moderately dry period.

Variation of the Leaf Physiological Parameters between two Agro-Ecological Regions, IL_{1a} (Radddegoda) and DL_3 (Wanathawilluwa)

The leaf area development (youngest fully opened leaf) and the PCI were not affected by the agro-ecological region (Tables 5 and 6). But the total canopy area (TCA) development was significantly higher in IL_{1a} than that of DL_3 (Table 5). The g_s in IL_{1a} area was significantly higher than that of DL_3. The GCL of middle and distal portion of the leaflets were not affected by the agro-ecological region, whereas the GCL of the proximal end was significantly higher in IL_{1a} than that of DL_3. The SD of the proximal end of leaflets was significantly higher in IL_{1a} while it was the other way round in the middle position of the leaflets. The SD of the distal portion was not affected by the agro-ecological region. This revealed that the higher gs in IL_{1a} palms was mainly attributed to the higher number of stomata (SD) and greater size of stomata (GCL) at the proximal position of leaflets compared to DL_3.

Table 5. Mean values of leaf area (LA) (m²), stomatal conductance (g_s) (mol H_2O $m^{-2}s^{-1}$), and total canopy area (TCA) (m²) of two agro-ecological regions

Location	LA	TCA	g_s
IL_{1a}	4.95ª	128.57ª	0.39ª
DL_3	5.08ª	86.69ᵇ	0.33ᵇ

Within each column values with same superscript letters are not significantly different

Several interactions were figured out between the three factors; cultivar, time period and location. Location × cultivar effect on leaf area was significant. The highest leaf area development was observed in TDB cultivar at Raddegoda (IL_{1a}) and it was significantly different from other location × cultivar combinations. This reveals that TDB has a mechanism to perform better than other cultivars under near optimum conditions (soil and climate) for coconut prevailed in

Table 4. Mean values of guard cell length (µm), stomatal density (no./mm^2) and potential conductance index (PCI) of two different time periods

	GCL			SD			PCI		
Time period	p	m	d	p	m	d	p	m	d
Wet	35.73^a	33.71^b	33.27^a	185.83^a	186.21^a	179.68^a	23.93^a	24.30^a	24.64^a
Moderately dry	36.10^a	35.93^a	34.54^a	175.35^b	165.98^b	159.05^b	22.33^a	21.15^a	20.15^a

Within each column values with same superscript letters are not significantly different

Table 6. Mean values of guard cell length (µm), stomatal density (no./mm^2) and potential conductance index (PCI)of two different agro-ecological regions

	GCL			SD			PCI		
Location	p	m	d	p	m	d	p	m	d
IL_{1a}	36.50^a	34.82^a	33.99^a	186.42^a	172.48^b	168.05^a	25.54^a	23.39^a	24.60^a
DL_3	35.34^b	34.82^a	33.82^a	176.92^b	185.38^a	173.67^a	23.29^a	21.82^a	20.88^a

Within each column values with same superscript letters are not significantly different

Raddegoda in IL_{1a}, with respect to its vegetative growth. There was a significant location × period effect on the GCL of proximal ends. The cultivars showed the highest GCL of proximal ends in DL_3 during wet period. The reason for the increased GCL in this region may be associated with that plants have developed bigger stomata during favorable periods enabling higher gas exchange, but with lower SD in DL_3. Cultivar × period interaction showed a significant effect on GCL. DBT expressed the highest GCL during moderately dry period while it was lowest during wet period. This shows DBT cultivar has a mechanism to change the size of stomata as a response to the changes in environment which is an adaptation to stress conditions. The plants were not exposed to severe drought condition during the experimental period (Tables 7 and 8). Therefore, further studies are suggested to determine the leaf anatomical parameters of new crosses/ hybrids under severe stress conditions.

Table 7. Monthly mean temperature (TEMP *Mean, °C*), rainfall (*RF, mm*), and sun shine hours (SSH) in DL_3 during the experimental period

Year	Month	TEMP 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 (TEMP *Mean, °C*), rainfall (*RF, mm*), and sun shine hours (SSH) in IL_{1a} during the experimental period

Year	Month	TEMP 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

CONCLUSIONS

Cultivars responded differently to different growth conditions during their early growth stage. The response to moderately dry conditions was mainly by reducing stomatal density, stomatal conductance and by increasing the stomatal size (GCL). For drought prone areas, DBT seem to be more suitable with its plasticity of stomatal characteristics and for areas with optimal conditions for coconut, TDB seem to be more suitable compared to other crosses.

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REFERENCES

Abid, R., Sharmeen, S. and Praveen, A. (2007). Stomata type of monocots within the flora of Karachi, Pakistan. *Pakistan Journal of Botany*, **39** **(1)**: 15-21.

Caldera, H.I.U., De Costa, W.A.J.M., Wijeratne, M.A. and Ranwala, S.M.W. (2011). Response of stomatal characters of Sri Lankan tea cultivars to environmental change. In proceedings of International Conference on the Impact of Climate Change on Agriculture, 114.

Holland, N. and Richardson, A.D. (2009). Stomatal length correlates with elevation of growth in four temperate species. *Journal of Sustainable Forestry*, **28**, 66-67.

Manthriratna, M.A.P.P. and Sambasivam, S. (1974). Stomatal density in varieties and forms of the coconut. *Ceylon Cocon. Q*., **25**, 105-106.

Jayasekara, C. and Mathes, D.T. (1992). A method to determine leaf area of a frond and the whole canopy of an adult coconut palm. *Indian Coconut Journal.* July, 7-8.

Jayasekara, K.S., Peries, R.R.A., and Jayasekara,C. (1996). Drought response and efficient water management in coconut. *Journal of Plantation Crops*, **24**, 633.