Proceedings of 15th Agricultural Research Symposium (2016) 455-459

Response of Leaf Physiological Parameters of Improved Coconut (*Cocos nucifera* L.) Hybrids to High Temperature and Drought Stress

U.V.P.N. UDADENIYA[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), 60170, Sri Lanka*

[2]*Plant Physiology Division, Coconut Research Institute, Lunuwila, 61150, Sri Lanka*

ABSTRACT

This study was conducted to determine the high temperature and drought tolerance of six improved coconut hybrids (*i.e.* TSR, TT, DGT, TDB, DGSR and DBT), using the response of leaf physiological parameters. The Cell membrane thermostability (CMT), rate of photosynthesis (photo), stomatal resistance (rs), canopy temperature depression (CTD), chlorophyll content (chl), chlorophyll stability index (CSI) and, leaf area (LA) of youngest fully opened leaf and, total canopy area (TCA) and leaf area index (LAI) of palms were measured under heat and drought stress. Hybrid TSR had the highest CMT and second highest LA, TCA and LAI. The highest and lowest LA, TCA and LAI were shown by TDB and DGT, respectively. Photo, rs, CTD, chl and CSI were not significantly different among the varieties, and therefore, less predictive in describing varietal tolerance to high temperature and drought. The study has identified TDB and TSR as more tolerant to heat and drought stress based on leaf physiological parameters. Except CMT, all other parameters showed a significant variation with sampling time and there was no variety × time interaction for any parameter.

KEYWORDS: *Cocos nucifera*, Heat and drought stress, Hybrid, Leaf physiological parameters

INTRODUCTION

Coconut (*Cocos nucifera* L.) is a plantation crop widely grown in Sri Lanka, belongs to the family *Arecaceae.* Total land area under cultivation is 440,000 ha and annual national nut production is about 2,600-3000 Mn nuts. Coconut palm has versatile uses which accounts for approximately 12% of all agricultural produce in Sri Lanka (Anon, 2014).

According to the morphological characters and breeding habits, coconut is grouped into three distinct varieties as Typica (tall), Nana (dwarf) and Aurantiaca. San Ramon is an exotic tall variety introduced to Sri Lanka from Philippines. Several coconut hybrids has been recently developed by the crosses between tall×tall and dwarf×tall forms.

The optimum climatic conditions for coconut are a year round warm and humid climate, mean annual temperature of 27-29 °C and a well distributed rainfall of 1,500-2,500 mm (Chan and Elevitch, 2006).

The rate of photosynthesis and chlorophyll contents are indicators to analyze the potential dry matter production of coconut palm. High temperature and water stress cause the stomatal closure decreasing the rate of photosynthesis. Chlorophyll stability index (CSI) is an indicator of stress tolerance in plants and the varieties with high CSI able to withstand heat stress (Mohan *et al.*, 2000). Cell membrane thermostability (CMT) measured as electrolyte leakage from leaf discs, is a predictor of the stability of the cell membrane under the influence of heat stress. Leaf area (LA) is important for the canopy photosynthesis and leaf area index (LAI) determine the efficiency of the photosynthesis and transpiration rate of the canopy. Canopy temperature depression (CTD) determines the ability to cool the canopy and the ability to avoid dehydration under heat and water stress.

The response of coconut varieties to the impacts of climate change may vary and this strategy could be used to screen heat and drought tolerant varieties. Therefore, the objective of this research was to identify high temperature and drought tolerant coconut hybrids with respect to leaf physiological parameters.

MATERIALS AND METHODS

Site and Plant Description

The research was conducted in the Plant Physiology Division of Coconut Research Institute, Lunuwila, Sri Lanka from January to April 2016. Six improved coconut hybrids; Tall×Tall (TT), Dwarf Green×San Ramon (DGSR), Tall×San Ramon (TSR), Dwarf Brown×Tall (DBT), Tall×Dwarf Brown (TDB) and Dwarf Green×Tall (DGT) were selected from Wanathawilluwa (DL_3) field evaluation trial of CRI. Eight palms from each variety were used from three blocks.

Data Collection and Analysis

Leaflets of the youngest fully expanded frond were used for physiological measurements. Rate of photosynthesis, stomatal resistance and canopy temperature depression were measured six times during 2nd week of January (2WJan), 2nd and 3rd weeks of February (2WFeb and 3WFeb), 2nd and 4th weeks of March (2WMar and 4WMar) and 4th week of April (4WApril). Chlorophyll stability index, chlorophyll content, cell membrane thermostability, leaf area, canopy area and leaf area index were measured three times during 2nd week of January (2WJan), 3rd week of March (3WMar) and 4th week of April (4WApril).

Results were analyzed using SAS statistical package by Analysis of Variance (ANOVA) with GLM procedure. Mean separation was done using Duncan's multiple range test.

Chlorophyll Stability Index (CSI)

CSI was assessed according to the procedure described by Gajanayake, *et al.* (2011). Two sets of leaf samples were prepared using two leaf discs (2.0 cm^2) from each palm and the discs were placed in tubes containing 4 mL of dimethyl sulphoxide (DMSO) for extraction of chlorophyll. One set of samples were incubated at room temperature in the dark for 24 h to allow complete extraction of chlorophyll pigments (control). Absorbance of the extraction was measured using a UV-Visible Spectrophotometer (UV 160A Shimadzu) at 470, 649 and 665 nm wavelengths. The other set of samples were incubated at 56 °C in a temperature controlled water bath for 1 h (treatment). The set of tubes were brought to 25 °C and absorbance was measured as described previously. Total chlorophyll content of samples was calculated using equations described by Wickramasinghe *et al.* (2013). The CSI was estimated using the following equation.

$$CSI(\%) = \frac{Total\ chl\ in\ treatment}{Total\ chl\ in\ control} \times 100$$

Chlorophyll Content

Chlorophyll content of the youngest fully expanded leaf was measured using chlorophyll meter (SPAD-502 plus).

Cell Membrane Thermostability (CMT)

Two sets of samples (control and treatment), each tube containing two leaf discs (each 1.3 cm^2) in 20 mL of deionized water were prepared. The leaf discs were thoroughly rinsed with deionized water to remove electrolytes adhering to the leaf surface and leachate from the cut surface. One set of tubes (control) were kept at room temperature (28-29 °C) and the other set (treatment) was incubated at 55 °C for 20 min in a temperature controlled water bath and initial conductance of control (CEC_1) and treatment (TEC_1) were measured using an electrical conductivity meter (Orion 145A+) at room temperature. Tubes were then autoclaved at 0.1 MPa for 12 min to kill the tissues completely and cooled to room temperature and final conductance (CEC_2 and TEC_2) was measured. Cell membrane thermostability (%) was estimated using the equation (Gajanayake *et al.*, 2011).

$$CMT(\%) = \frac{[1 - (TEC_1/TEC_2)]}{[1 - (CEC_1/CEC_2)]} \times 100$$

Rate of photosynthesis (photo) and stomatal resistance (rs)

Rate of photosynthesis and stomatal resistance were measured using the LI-COR portable photosynthesis meter (LI-6200, USA) during 9.00 a.m. to 12.00 noon under fully sunlight.

Canopy Temperature Depression (CTD)

Air temperature (Ta) and canopy temperature (Tc) of each cultivar was measured using LI-COR portable photosynthesis meter (LI-6200, USA) and CTD was estimated using the following equation.

$$CTD = Ta - Tc$$

Leaf Area (LA), Total Canopy Area (TCA) and Leaf area Index (LAI)

Area of twelve leaflets from youngest fully opened leaf (frond) of each cultivar was measured using the Area meter (LI 310°C, USA). LA was calculated using the equation [1] (Jayasekara and Mathes, 1992) and TCA and LAI were calculated using equations [2] and [3] (no of palms per ha is 160).

$$LA\ (m^2) = \frac{[Area\ of\ 12\ leaflets \times 12.08) - 5882]}{10^4} \quad [1]$$

$$TCA\ (m^2) = LA \times Number\ of\ fronds\ per\ palm \quad [2]$$

$$LAI = \frac{[Canopy\ area\ of\ a\ palm \times 160]}{10^4} \quad [3]$$

Climate Data

Monthly rainfall and temperature data were obtained from nearest meteorological station at Ambakale, Genetic Resource Center.

RESULTS AND DISCUSSION

Climatic Conditions during the Experimental Period

The palms were exposed to severe drought during the experimental period (Figure 1).

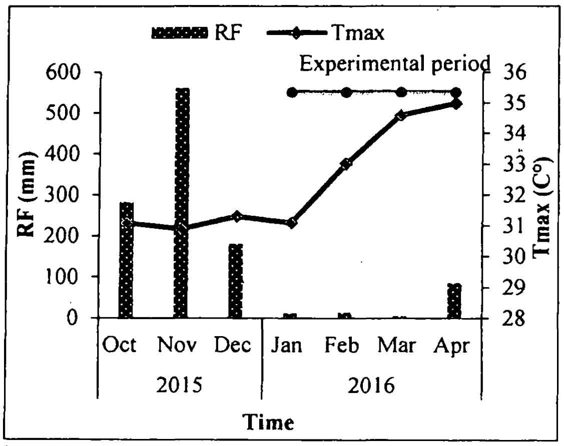

Figure 1. Monthly variation of maximum temperature (T_{max}) and rainfall (RF) from Oct 2015 to April 2016. *Experimental period is shown by a straight line*

Variation of Leaf Physiological Parameters among Varieties

Rate of photosynthesis (photo), rs and CTD were not significantly different among varieties and therefore, these parameters are not good indicators to differentiate the heat tolerance of varieties. Generally, TDB and TSR varieties showed the highest photo (Table 1). There was no variety × time effect on these parameters.

Table 1. Mean rate of photosynthesis (photo) ($\mu mol m^{-2}s^{-1}$), stomatal resistance (rs) (scm^{-1}) and canopy temperature depression (CTD) of different varieties

Variety	Photo ($\mu mol m^{-2}s^{-1}$)	rs (scm^{-1})	CTD (°C)
TSR	8.24	1.748	1.127
TT	7.81	1.684	1.251
DGT	7.92	1.633	1.172
TDB	8.32	1.645	1.227
DGSR	7.99	1.572	1.204
DBT	7.62	1.578	1.265

Hybrid TSR variety had significantly higher CMT (%) compared to all other varieties, except DGSR variety (Figure 2). Cell membrane thermostability measures the high temperature tolerance of palms through the electrolytes leakage from cell membrane under heat stress. Thus, TSR variety followed by DGSR variety were more tolerant to heat stress compared to TT, DGT, TDB and DBT varieties.

There was no significant difference in chl and CSI (%) among the varieties and there was no variety × time interaction effect (Table 2). Therefore, CSI and chl also could not be used to differentiate the heat tolerance of the varieties.

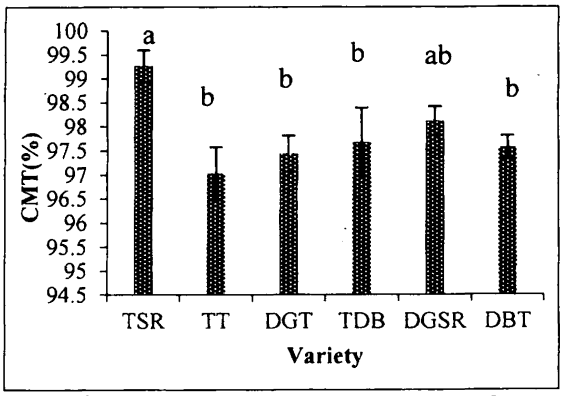

Figure 2. Mean cell membrane thermostability (CMT) (%) of different varieties. *Means followed by the same letters are not significantly different at $p<0.05$*

Table 2. Mean chlorophyll content (chl) and chlorophyll stability index (CSI) (%) of varieties

Variety	Chl (SPAD units)	CSI (%)
TSR	67.26	49.06
TT	64.42	57.31
DGT	66.13	50.97
TDB	67.66	52.67
DGSR	67.76	56.64
DBT	64.69	54.24

There was no significant difference in LA between TSR, TT and TDB varieties however the values were significantly higher than that of DGT, DGSR and DBT varieties (Figure 3). The lowest LA was observed in DGSR and DGT varieties. TDB and TSR varieties had the highest LAI which was not significantly different from the LAI of TT variety but significantly higher than that of DBT, DGSR and DGT varieties.

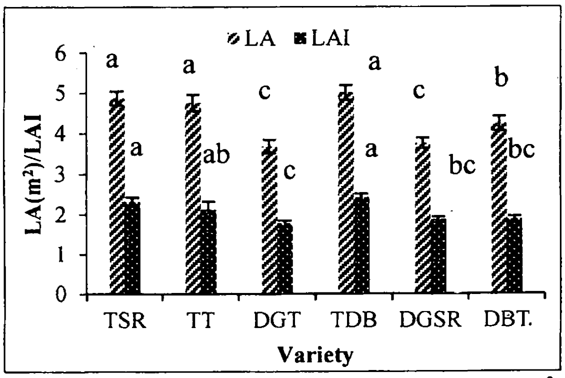

Figure 3. Mean values of leaf area (LA) (m^2) and leaf area index (LAI) among different varieties. *Means followed by the same letters are not significantly different at $p<0.001$*

TCA was significantly different between varieties (Figure 4). Highest TCA was observed in TDB and TSR varieties which was not significantly different from TT variety but significantly higher than that of DBT, DGSR and DGT varieties (Figure 4).

Therefore TDB and TSR varieties have adapted to heat and drought stress than other varieties with respect to leaf canopy development.

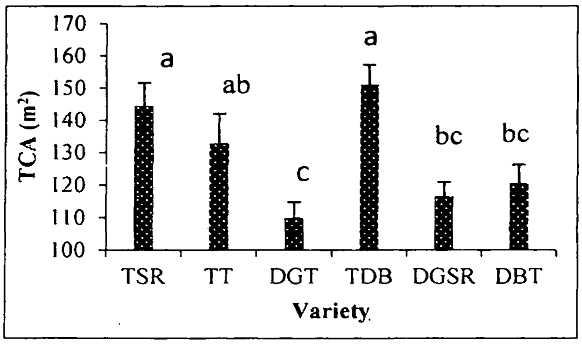

Figure 4. Mean values of total canopy area (TCA- m^2) between varieties. *Means followed by the same letters are not significantly different at $p<0.05$*

Variation of Leaf Physiological Parameters with Time of Sampling

The photo and rs were significantly varied with time (Figure 5). A significantly higher photo in 2WMar compared to other four events with moderate rs may be attributed to intermediate rainfall during second week of March and a significantly lower photo observed in 3WFeb with the highest rs was attributed to stomatal closure due to combined heat and water stress during this period.

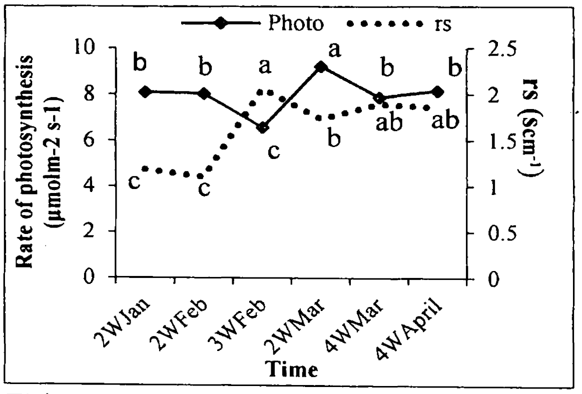

Figure 5. Mean rate of photosynthesis (photo- $\mu molCO_2m^{-2}s^{-1}$) and stomatal resistance (rs- scm^{-1}) over four months. *For each parameter, means followed by the same letters are not significantly different at $p<0.001$*

There was a significant difference in CTD with time (Figure 6). Canopy temperature depression was positive during 2WJan and 2WFeb and negative during other four times. Canopy temperature depression measures the plants' ability to lower the canopy temperature through transpirational cooling. Therefore, higher CTD indicates the higher cooling ability up to moderate stress condition and thereafter when the stress levels were very high the canopy cooling ability was not apparent (negative).

Cell membrane thermostability was not varied with the time and chl and CSI were significantly different with time (Table 3). The leaves emerged in 4WApril had significantly higher chl than other two months, and this may be possibly due to recovery from water stress after appreciable amount of rainfall. The highest CSI was observed in leaves emerged in 3WMar. Higher CSI indicates capability of the palms to tolerate heat stress.

The temperature (Tmax) was highest in 3WMar and this can be a possible adaptation in the leaves developed under high Tmax combined with three month long water stress (Figure 1).

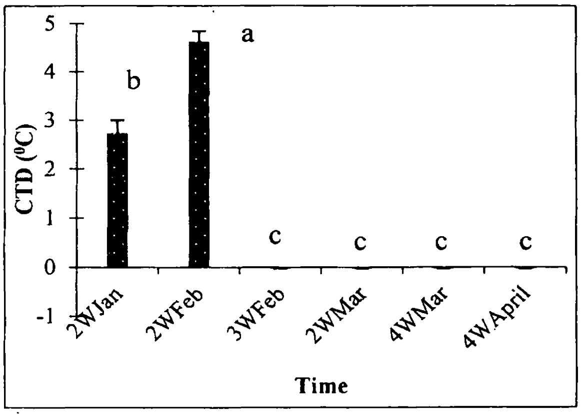

Figure 6. Canopy Temperature Depression (CTD- °C) over time. *Means followed by the same letters are not significantly different at $p<0.001$*

Table 3. Mean cell membrane thermostability (CMT) (%), chlorophyll content (chl) and chlorophyll stability index (CSI) (%) over time

Time	CMT (%)	chl (SPAD units)	CSI (%)
2WJan	97.29	65.33^{b}	50.93^{b}
3WMar	97.98	63.77^{b}	57.84^{a}
4WApril	98.28	69.86^{a}	51.67^{b}

For chlorophyll content (chl) and chlorophyll stability index (CSI), means followed by the same letters are not significantly different at $p<0.01$ and $p<0.05$ respectively; CMT = cell membrane thermostability

There was a significant difference in TCA with time (Figure 7). The TCA was higher in January compared to other two months and this may be possibly attributed to more favorable climatic conditions prevailed during leaf development stage for 2WJan compared to the combined stress affected on leaves of 3WMar and 4WApril.

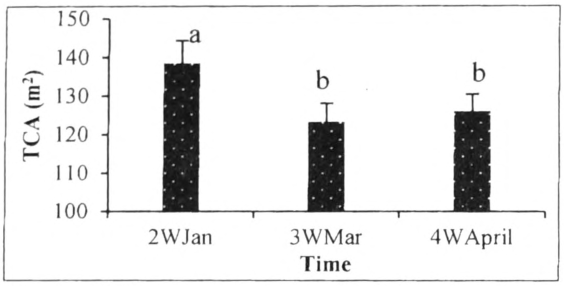

Figure 7. Mean values of Total Canopy Area (TCA- m²) over the experimental time. *Means followed by the same letters are not significantly different at $p<0.05$*

Area of the youngest leaf (LA) and LAI were significantly different with time (Figure 8). Highest LA and LAI were observed in 2WJan possibly due to favorable rainfall and temperature during previous three months before the leaf opening as described above. LA and LAI were bio-meteorological variables which intercept light, exchange heat, moisture and CO_2 with the atmosphere. The fronds developed under severe heat stress may have reduced the LA as an adaptation to the heat stress, thereby, to reduce the water losses through transpiration.

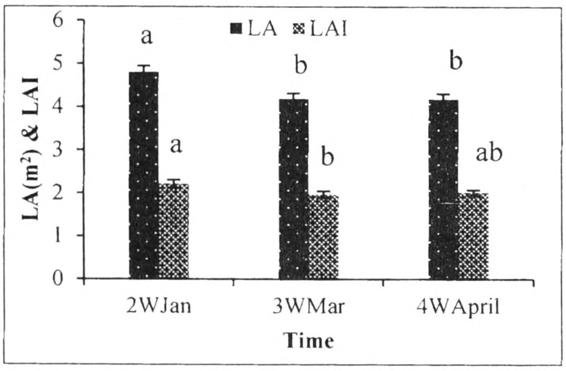

Figure 8. Mean values of Leaf Area (LA) (m²) and Leaf Area Index (LAI) over time. *For LA and LAI, means followed by the same letters are not significantly different at $p<0.001$ and 0.05 respectively*

Finally, it was imperative to note that although there are significant adverse effects of heat and drought stress on reproductive physiological parameters of different varieties of coconut (Ranasinghe, 2014), according to the present study, the effects on vegetative physiological parameters are comparatively low.

CONCLUSIONS

Cell membrane thermostability, LA, TCA and LAI varied among varieties under stress and those parameters can be used to screen coconut varieties for heat and drought tolerance. Photo, rs, CTD, chl and CSI were less predictive for selecting varieties. TSR and TDB varieties are more tolerant to drought and heat stress with respect to vegetative physiological parameters.

ACKNOWLEDGEMENTS

Authors wish to express their gratitude to Director and Deputy Director (Research), Coconut Research Institute of Sri Lanka for granting permission to conduct this study at CRI. We also wish to acknowledge all the staff of Plant Physiology Division, Crop Protection Division and Soil and Plant Nutrient Division of CRI for their grateful support and Head, Genetics and Plant Breeding Division for permitting us to use their evaluation trials for data collection.

REFERENCES

Anon. (2014). Sri Lanka coconut statistic, Coconut Department Authority.

Chan, E. and Elevitch, C.R. (2006). *Cocos nucifera* (Coconut). Species profile for pacific island agroforestry.

Gajanayake, B., Tarder, B.W., Reddy, K.R. and Harkess, R.L. (2011). Screening ornamental pepper cultivar for temperature tolerance using pollen and physiology parameters. *Hortscience*, **4** (6), 878-884.

Jayasekara, C. and Mathes, D. (1992). A method to determine leaf area of a frond and the whole canopy of an adult coconut palm. *Indian Coconut Journal*, **23**, 7-13.

Mohan, M.M., Narayanan, S.L. and Ibrahim, S.M. (2000). Chlorophyll stability index (CSI): its impact on salt tolerance in rice. *Journal of International Rice Research*, **2**, 38-39.

Ranasinghe, C.S. (2014). Climate change effects on organic coconut. in: organic coconut cultivation. Eds. H. P. M Gunasena, Occasional Publications. Coconut Research Institute, Lunuwila, Sri Lanka. 119-130.

Wickramasinghe, A.S.M.P.M., Ranasinghe, C.S. and Abeysinghe, D.C. (2013). Response of physiological parameters and leaf dry matter accumulation in different improved coconut (*Cocos nucifera* L.) to environmental conditions in DL3 and IL_{1a}. In proceedings of 12th Agricultural Research Symposium, August, 2013. Wayamba University of Sri Lanka. 274-278.