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Physiological responses for moisture stress and development of an index for screening coconut (*Cocos nucifera L.*) genotypes for drought tolerance

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ABSTRACT

The effect of moisture stress on leaf water potential [Ψ], relative water content [*RWC*], stomatal conductance [g_s], transpiration [*E*], rate of net photosynthesis [*A*], leaf temperature [T_{leaf}] and ratio of intercellular and atmospheric CO$_2$ concentration [C_i/C_a] of four coconut genotypes (Clovis [*CL*], Dwarf Green [*DG*], Dwarf Brown [*DB*] and Cameron Red Dwarf [*CRD*]) were studied, to identify factors that contribute to drought tolerance of some coconut genotypes and to develop an index for screening drought tolerant genotypes. All palms were about 15 years of age, grown in IL$_1$ Agro-Ecological Region and were exposed to 80-day natural drought. *RWC* of leaves showed that the three dwarf genotypes dehydrated faster than *CL*. The reduction in g_s in response to moisture stress was observed in all genotypes, even though the Ψ was not significantly reduced. This suggests that there is a signal from roots that induces a reduction in g_s. *CL* and *DB* appeared more drought tolerant by maintaining high *A* even under low soil moisture conditions and by having low g_s, while, *DB* responded quickly to subsequent rains by regaining its initial rates of photosynthesis and g_s. Moreover, *CL* maintained lowest T_{leaf} and highest C_i/C_a ratio during dry weather. *CRD* showed highest rate of reduction of g_s with the inception of dry spell, indicating its highest sensitivity to moisture stress conditions. Based on the index of stomatal performance, calculated by using g_s and Ψ CRD was selected as the most drought sensitive genotype, and *DB* as the most drought tolerant genotype.

Key words: Drought, coconut, stomatal conductance, drought tolerance, index for stomatal performances.

INTRODUCTION

Coconut (*Cocos nucifera* L.) is the most widely grown plantation crop in Sri Lanka (Fernando *et al.*, 1997). It is predominantly grown in the coconut triangle bordering Puttalam, Kurunegala and Gampaha Districts and also in the Southern coast. Sri Lanka is the fourth largest coconut producing country in the world (Liyanage, 1999). The average annual per capita consumption by way of oil and fresh nuts is

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about 110 nuts, with 90 being consumed as fresh nuts and the rest as by products (Fernando *et al.*, 1997).

The coconut industry in Sri Lanka annually experiences a marked reduction of yield and a loss of a large number of coconut palms due to drought. The amount of coconut lands distributed in dry, intermediate and wet zones are about 15, 69, 16 % respectively (Agriculture survey, 2002). The annual rainfall in the intermediate dry and dry zones is 1000-1500 mm and 1000 mm respectively, with four to seven month-long dry periods and one major monsoon rainy period. Lack of soil water and high temperature are the main limiting factors to palms growing in these regions. Meanwhile, due to long generation period of coconut, the adverse effects of drought can persist for a period of about two and a half years (Murray, 1977). Therefore, selection and breeding of drought tolerant genotypes of coconut is very important.

Plants possess several anatomical and physiological mechanisms to withstand drought. These mechanisms have been classified into two groups as avoidance and tolerance mechanisms (Taiz and Zeiger, 1991). In the case of avoidance mechanisms, the reduction of plant tissue hydration is low during drought, because of reduced plant development cycle, reduced size of transpirational surface, expanding root system and controlling water loss through stomatal regulation. In the case of tolerance mechanisms, plants maintain normal physiological activities despite a reduction in tissue hydration through osmotic adjustment by accumulating solutes and induce stomatal closure by increasing abscisic acid content in leaf tissues (Repellin *et al.*, 1994). Rajagopal and Ramadasan (1999) have shown that stomatal regulation and osmotic adjustment are the main mechanisms responsible for drought tolerance in coconut.

Various drought screening techniques have been proposed based on physiological, biochemical and vegetative changes occurring in plants in response to drought. Chandrasekara (1997) has used an index for stomatal performances (ISP) to select drought tolerant Hevea genotypes. Therefore, this study was aimed to identify physiological factors that contribute to drought tolerance of some coconut genotypes and to examine the applicability of index of stomatal performance to screen drought tolerant coconut genotypes.

MATERIALS AND METHODS

The experiment was carried out at the Plant Physiology Division of Coconut Research Institute of Sri Lanka (*CRISL*). Four different coconut genotypes *viz.* Clovis (*CL*), Sri Lankan Brown Dwarf (*DB*), Sri Lankan Green Dwarf (*DG*) and Cameroon Red Dwarf (*CRD*) were selected for the experiment. They were a part of the *ex- situ* coconut gene bank, with approximately 15 years of age and planted at Poththukulam Research Station (*PRS*), situated in the IL_1 Agro-Ecological Region. All palms were under the general management practices recommended by *CRISL*, from the time of planting. Eight adjacent palms from each of four genotypes were selected from the experimental plots arranged in a Completely Randomized Design (*CRD*).

Measurements were taken once a month during the rainy season and the frequency was increased to once a week during the dry spell. Leaflets from the middle portion of the ninth frond from top (Braconnier and Bonneau, 1998) were used for all physiological measurements. Measurements were conducted on excised leaves immediately after the excision and completed within two minutes during the period from 10.00 am to 12.00 noon.

Stomatal conductance (g_s) (mol m^{-2} s^{-1}), rate of net photosynthesis (A) (µmol m^{-2} s^{-1}), rate of transpiration (E) (mmol m^{-2} s^{-1}), leaf temperature (T_{leaf}) (°C), intercellular CO_2 concentration (Ci) (µmol mol^{-1}) and atmospheric CO_2 concentration (C_a) (µmol mol^{-1}) were measured employing a close system portable photosynthesis System (LI-COR Inc., Lincoln, Nebraska, USA). The leaf water potential (Ψ) (MPa) was measured using Scholander type portable pressure chamber (Soil moisture Equip. Corp, Santa Babara, California, USA) Leaflets were detached from the palm, and they were sealed in the chamber as soon as possible to prevent evaporation of water from the leaf. Compressed air was used to determine the pressure necessary to produce incipient sap exudation, which corresponds to the balancing xylem sap negative pressure. The Relative water content (*RWC*) (%) was calculated using the following formula (Turner, 1981).

$RWC = (FW - DW) / (TW - DW)$

FW = Fresh weight of the sample

DW = Dry weight of the sample

TW = Fully turgid weight of the sample

Daily rainfall (*RF*) (mm) and soil moisture content (%) at the depth of 50 cm and 1 m were measured (gravimetric method) at each measurement simultaneously with other physiological measurements. The *ANOVA* was used for primary data analysis and Duncan's New Multiple Range Test was used as the mean separation technique to identify the significance of differences between genotypes.

Index of stomatal performances

Each genotype was given a grade point based on the significant letters (a, b, and c) obtained from *DNMRT* for stomatal conductance and leaf water potential like 3, 2, and 1 respectively. The genotype obtained two significant letters from *DNMRT* was given average of two respective grade points (e.g. ab=3+2/2=2.5). The *ISP* was calculated using the following equation,

$$ISP = \sum_{x=1}^{t} X.\ FX$$

where,

ISP - Index of stomatal performance
t - Number of genotypes
X -Grade point obtained by the geno type for recordings of different Months
F - Frequency of the corresponding grade point

RESULTS AND DISCUSSION

Palms were under dry environmental conditions from 18th December 2004 to 7th March 2005 although three brief sporadic rains occurred during the experimental period. The marked reduction in monthly rainfall from 416.22 mm in November to 16.2 mm in January indicates the intensity of the dry spell (Fig. 1). The soil was sufficiently wet due to the heavy rains received prior to the dry spell and the soil moisture content (θ, %) reduced from around 10 % to levels as low as 2.5 % during the dry period.

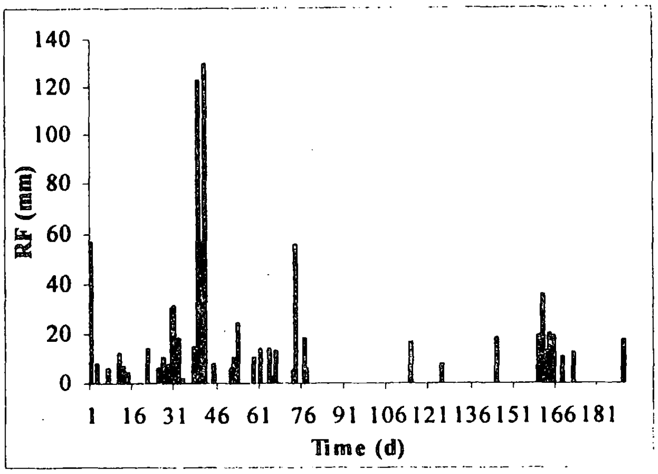

Figure 1. Variation of rainfall during the experimental period

Leaf water status:

During non-stressed conditions (i.e. day 56), *CRD* showed the highest relative water content but it did not differ significantly ($P>0.05$) from other genotypes (Fig. 2). All genotypes showed approximately similar *RWC* at the initial stage of the dry spell (i.e. day 103). However, as the dry spell progressed, *CL* and *DG* maintained significantly higher ($P<0.05$) *RWC* values than those of *CRD* and *DB*.

Both *CRD* and *DB* showed comparatively rapid reductions in leaf *RWC* with the progress of dry spell. This quick response in those two genotypes indicates their greater sensitivity to depleting soil moisture and their orientation towards the conservation of water and survival during water stressed conditions. However, no significant differences were observed between four genotypes. Thereafter on day 140, they could recover *RWC* to their pre-stress levels. The *RWC* of *CL* and *DG* was higher than other two genotypes during the period from day 118 to 140. This suggests the differences of root systems in absorbing water from the drying soil and deeper soil layers. *RWC* decreased from day 56 to day 118 in all genotypes. Thereafter, it increased again until day 151, then there was a reduction. These variations indicate that the level of moisture depletion was not so critical for all coconut genotypes tested in the present experiment. Variation of transpirational water losses due to varying stomatal resistances and three sporadic rainfalls that occurred during dry spell might have caused these uneven variations of *RWC*.

Maintenance of higher leaf water potential under water stressed conditions is a desirable trait, as it would enable tissues to maintain favourable metabolic activities to withstand desiccation. In the present experiment, short dry period and three small sporadic rains experienced towards the latter part of the dry spell appear to have substantial impact on minimizing water stress. Therefore, water potential did not vary significantly between genotypes during dry spell (Fig. 3). *DB* showed lower Ψ than those of other three genotypes during the experimental period. The Ψ of *CRD* was considerably higher than that of its non-stressed values at the end of the dry spell. On the other hand, coconut as a perennial crop with a large root system is able to maintain its internal water status without substantial reductions during short dry periods.

Gas exchange measurements:

Gas exchange parameters are important

because they are directly associated with carbon and water economy of plants. Stomatal conductance (g_s) of these four coconut genotypes decreased with moisture stress. This is in accordance with previous results, which showed that all the palms irrespective of the genotype were equally sensitive to soil water depletion and responded to water deficit by decreasing the g_s (Rajagopal *et al.*, 1990). Stomatal conductance declined by 55, 37, 63 and 53% (from day 56 to 151) in *CL*, *DG*, *CRD* and *DB* respectively while *CRD* showed the lowest (0.77 mol $m^{-2} s^{-1}$) absolute g_s (Fig. 4a). As shown by Jayasekara *et al.*, (1993) and Ranasinghe *et al.*, (2003) stomatal regulation was the key factor controlling the water balance of coconut. The quick and significant reductions observed in g_s in comparison to the *A*, in the present experiment, suggests the general tendency of coconut to conserve water by reducing the water loss by the reduced g_s. In the present experiment, *DB* and *CRD* showed higher g_s under well-watered conditions, while *CL* and *DG* showed similar values. But there was no statistically significant difference ($P<0.05$) between the g_s of different genotypes.

The rate of reduction of g_s was greater in *DB* and *CRD* than in *CL* and *DG* (Fig. 4a). Therefore, out of the tested genotypes, *DB* and *CRD* can be considered as more sensitive to water stress. *DB* and *CL* maintained higher g_s as compared to the other two genotypes at the end of the dry spell. *DB* and *CL* showed a quick recovery of g_s with re-wetting after rains, with *DB* being the quickest. This is an important characteristic of a plant better adapted to intermittent droughts. After rains, the g_s returned to its normal values within three weeks.

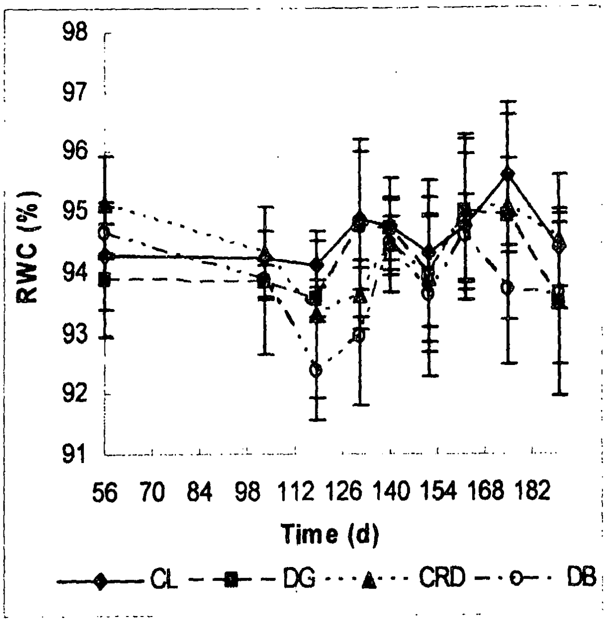

Fig. 2 The effect of drought on leaf Relative Water content (RWC, %)

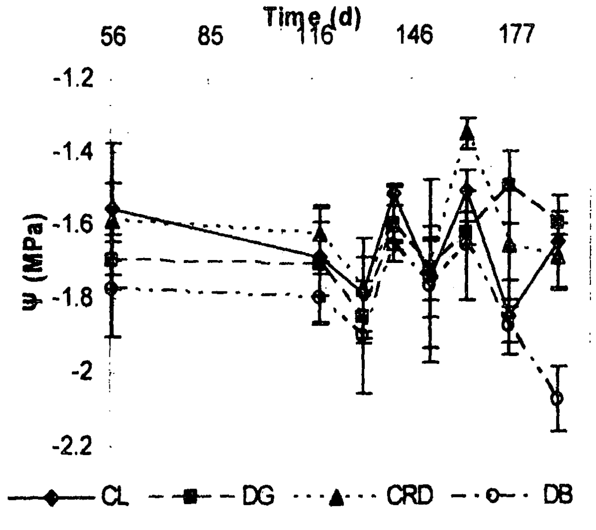

Fig. 3 The effect of drought on leaf water potential

Transpiration through leaves is necessary for healthy growth of a palm as it lowers leaf temperature during warm weather conditions. The rate of transpiration also declined with decreasing g_s showing the close relationship between those two parameters (Fig. 4c). *DB* showed a significantly higher *E*, whereas *CL* showed a lower rate under non-stressed conditions with no significant difference between those of *DG* and *CRD*. *DB* and *CRD* showed greater decreases in *E* than *DG* (Fig. 4c). *DB* maintained the highest *E* throughout the dry spell and also at the end of the dry spell. The lowest *E* was observed in *CL* and *CRD* and there was no significant difference between the *E* of these two varieties.

It is well known that one of the important physiological processes affected by water stress is the photosynthesis (*A*) (Flexas *et al.*, 2004). Both *CL* and *DB* showed significantly higher ($P<0.05$) *A* values at the inception of dry spell (i.e. days 103) while *CRD* showed the lowest (Fig. 4b). All genotypes showed a reduction in *A* with the progress of dry spell. However, both *CL* and *DB* maintained significantly higher *A* ($P<0.05$) than the other two genotypes throughout the dry spell. In contrast, *CRD* maintained the lowest *A*. Photosynthesis of all four genotypes showed a rapid response to rewetting with subsequent rains and *DB* was the quickest in regaining the initial rates. Stomatal responses to subsequent rains were also quick in *CL* and *DB*.

When water stress sets in, stomatal closure seems to be the main carbon assimilation-limiting factor in coconut palms. In addition, the stomatal closure increases leaf temperature by transpiring less water, thereby reducing transpirational cooling. Considerably high leaf temperature may affect photosynthetic regulation during prolonged water stress, by impairing metabolic activities.

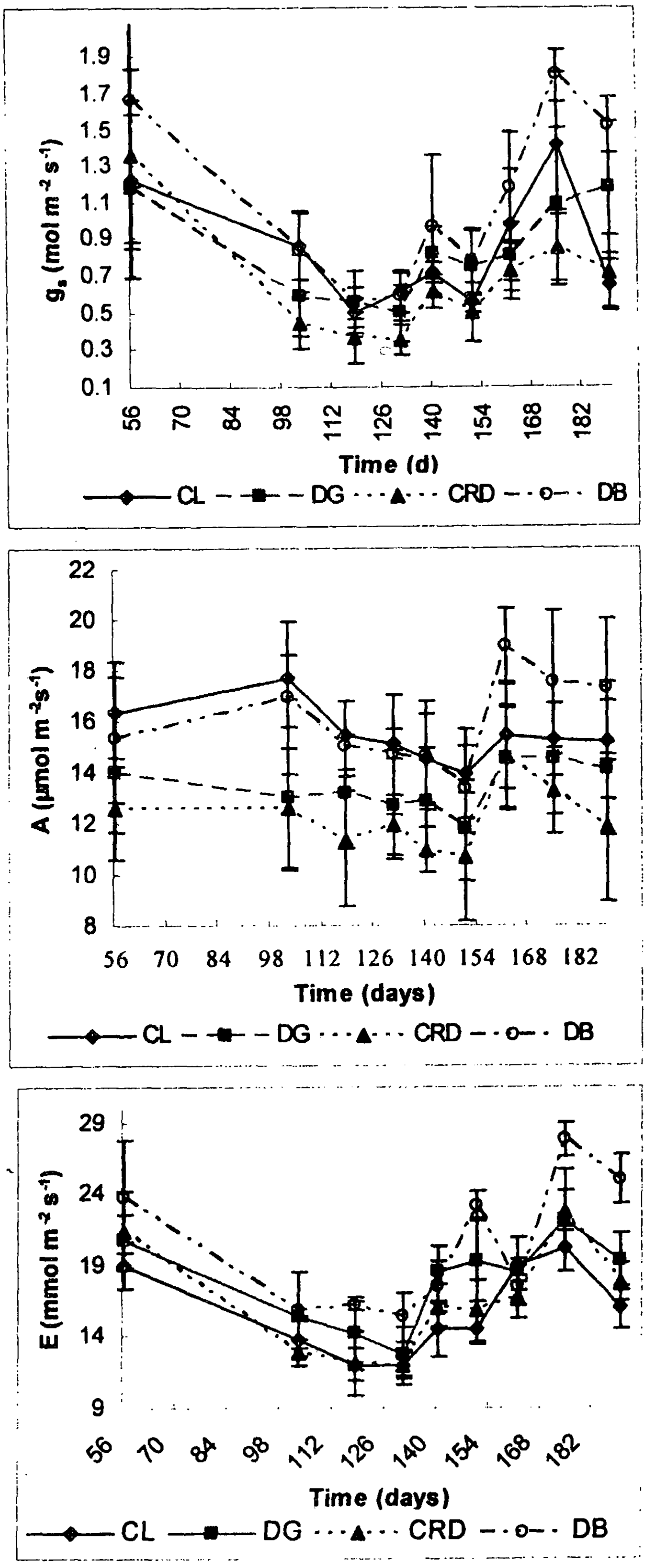

Fig. 4 The effect of drought on rate of stomatal conductance (a), photosynthesis (b) and transpiration (c).

The leaf temperature of *DB* and *CRD* was increased up to 40° C (Fig. 5a) and it could be one reason for *CRD* having the lowest A during the stress period.

Leaf temperature:

The general increase in leaf temperature (T_{leaf}, °C) with progressive drought was common for all genotypes.

CL showed the lowest ($P<0.05$) T_{leaf} at the inception and throughout the dry spell period while *CRD* showed the highest (Fig. 5a). T_{leaf} of *CRD* and *DB* reached almost 40° C while *DG* reached 39° C at the end of the dry period. However, *CL* was able to maintain T_{leaf} at a lower level close to 36° C.

Stomatal closure during drought led to a decrease in transpiration, which was a major adaptation to dry weather condition. This phenomenon could be observed in all four genotypes in the present experiment during dry spell. However, Grassi and Magnani (2005) observed that the direct effect of increased temperature on A is marginal in species, which have a broad temperature optimum. Coconut is also a sun-loving tropical plant, which can tolerate a substantial increase in leaf temperature with a minimal impact on A during early stages of a drought.

C_i/C_a ratio: C_i/C_a is a derived parameter that can be used to identify whether there is a stomatal or a biochemical limitation for the photosynthetic process under a given set of conditions. Although, there was no significant variation in C_i/C_a ratio among the four genotypes tested, the reduction in C_i/C_a with progressive of dry spell was a common feature in all four genotypes (Fig. 5b). This indicates that there was a stomatal limitation for diffusion of CO$_2$ in to the intercellular spaces. C_i/C_a ratio decreased up to day 131 and *CRD* showed the highest rate of reduction and lowest value at day 131 (0.01). In general, under severe water stress conditions C_i is increased due to impaired photosynthetic metabolism (Flexas *et al.*, 2004). However, in the present experiment, the increase of C_i after day 131 was not because of the impairment of bio-chemical pathways of photosynthesis but because of the increased g_s due to the brief rains experienced towards the latter part of the dry spell.

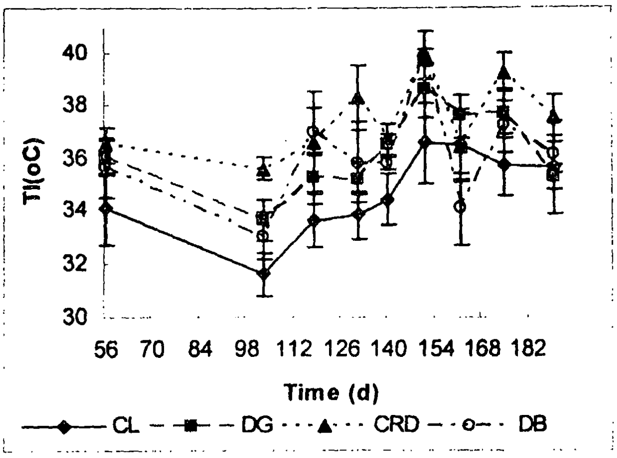

Fig. 5 The effect of drought on leaf temperature (a) and intercellular and atmospheric CO2 concentration (b).

Index of stomatal performance

This method was used by Chandrashekara (1997) to select drought tolerant *Hevea* genotypes. A genotype having a higher Index of stomatal performance (*ISP*) was considered more drought tolerant.

In this study, Ψ and g_s under moisture-limited conditions were used to calculate *ISP*. These values are presented in Tables 1 and 2. In general, drought tolerant genotypes should have higher stomatal conductance and water potential values. Therefore, significant letters (a, b and c) obtained from Duncan's New Multiple Range Test (*DNMRT*) were given grade point values 3, 2, and 1 respectively. A genotype obtaining more than one significant letter from *DNMRT* was given the average of the respective grade points (e.g. ab=3+2/2=2.5). Index for stomatal performance was calculated using these grade point and grade frequencies (Table. 4).

Table 1. Stomatal conductance of indivi ual leaves of four genotypes during the moisture stress period (i.e. from day 79 to 159).

Geno-	**Days after starting drought**			
type	**40**	**53**	**62**	**73**
CL	0.536^{a}	0.531^{a}	0.604^{b}	0.552^{bc}
DG	0.552^{a}	0.492^{ab}	0.815^{ab}	0.743^{ab}
CRD	0.364^{b}	0.352^{b}	0.605^{b}	0.496^{c}
DB	0.555^{a}	0.592^{a}	0.967^{a}	0.771^{a}

Note: Means followed by the same letter within a column are not significantly different at the probability level of 0.05 according to Duncan's New Multiple Range Test.

DB showed the highest *ISP* followed by *CL* and *DG* with minor differences. However, *ISP* of *CRD*, being the lowest of all, showed a substantial difference to those of others. Thus, *CRD* can be identified as a drought sensitive genotype compared to the rest.

A key factor in controlling the internal water status in coconut as well as in most other plants is g_s.

Table 2 . Leaf water potential of individual leaves of four genotypes during the moisture stress period (i.e. from day 79 to 159).

Geno-	Days after starting drought			
type	40	53	62	73
CL	-1.696^{a}	-1.787^{a}	-1.516^{a}	-1.771^{a}
DG	-1.142^{a}	-1.85^{a}	-1.60^{a}	-1.725^{a}
CRD	-1.625^{a}	-1.775^{a}	-1.60^{a}	-1.72^{a}
DB	-1.800^{a}	-1.525^{a}	-1.658^{a}	-1.75^{a}

Note: Means followed by the same letter within a column are not significantly different at the probability level of 0.05 according to Duncan's New Multiple Range Test.

Therefore, calculating the index for stomatal performance might be important in classifying coconut genotypes for drought tolerance. However, when considering the index for stomatal performance of these four genotypes, results were substantially different from the known conditions at the field level. *DB* was shown as the genotype with the highest drought resistance. However, it was known as a drought sensitive genotype under field conditions. Though the genotype CL was identified as the most drought tolerant

Table 3. Calculation of index for stomatal performance.

Grade	a	ab	b	bc	c		
Grade point	3	2.5	2	1.5	1	Index	Rank
	Grade frequencies						
CL	6		1	1		21.5	3
DG	5	3				22.5	2
CRD	4		3		1	19	4
DB	8					24	1

genotype under field conditions, in the present study it was identified as a genotype which is more drought sensitive than DG. However, CRD was identified as the most drought sensitive genotype.

CONCLUSION

The variation patterns of physiological parameters with the progress of dry spell were approximately similar among genotypes. However, some differences that were observed can be used to identify genotypic variation in drought tolerance in coconut varieties used in the present study. The eighty-day dry period during which physiological performance of coconut palms were evaluated, appeared not so critical for coconut palms. This was probably because their general hardy behaviour and the drought-relieving effect of the brief rains experienced towards the end of the dry spell.

Reduction of the rate of transpiration and stomatal conductance with the progress of the dry spell in all four genotypes, even when there were no substantial reductions in internal water status showed that there would be some other factors such as root-shoot communication that regulates stomatal functioning. Although clear distinctions between genotypes were not observed in gas exchange parameters due to the shorter duration of the dry spell than what is generally experienced in the region, *CL* appeared more drought tolerant than the other three dwarf genotypes. However, out of the three dwarf genotypes tested, *DB* showed some degree of tolerance by maintaining high rates of photosynthesis even under low soil moisture availability and by having low stomatal conductance durindry period.

These four genotypes can be ranked in order to drought tolerance in terms of ISP as DB>DG>CL>CRD. But the results are substantially different from known conditions of drought tolerance (CL>DB>DG>CRD) at the field level. Therefore it is important to repeat this study during much harsher and prolonged drought period to test and verify the applicability of this method.

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