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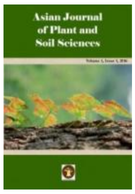

VARIATION OF LEAF GAS EXCHANGE PARAMETERS WITHIN THE CANOPY OF RUBBER (*Hevea brasiliensis)*

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The main objective of this study was to investigate the variation of leaf gas exchange parameters of Rubber (*Hevea brasiliensis* Muell. Arg.) within the canopy of tapped trees of two *Hevea* genotypes, I.e. RRIC121 and RRISL 211. The leaf gas exchange parameters, I.e.Stomatal conductance (gs),Intercellular CO2 concentration (Ci), Leaf temperature (Tleaf),Transpiration rate (E),Water use efficiency (WUE) and Leaf-air vapour pressure deficit (lvpd) were measured under optimal environmental conditions. Study findings clearly revealed that the intercellular CO2 concentration (Ci) gradually decrease with increasing light intensity in both clones. The same trend was observed in the different canopy layers under both tapping treatments.Clone RRISL 211 showed a higher leaf temperature than RRIC 121 under tapping. The highest leaf-air vapour pressure deficit (lvpd) was shown in the leaves of the upper canopy and lowest in the bottom canopy layer. Furthermore, the water use efficiency (WUE) of clone RRISL 211 was higher than that of clone RRIC 121 under tapping. In the tapped treatment, because of tapping, the photosynthetic rate is stimulated relative to the transpiration rate. The positive latex yield response to WUE and lvpd of top leaves indicates that, in top leaves when lvpd increases transpiration also increases accordingly. Clone RRIC 121 showed a greater contribution from the middle layer to overall canopy photosynthesis while RRISL 211 showed a lower contribution. This could have been due to the more open canopy architecture in clone RRIC 121 or maintenance of a higher A in low light levels.

Keywords: *Hevea*; leaf gas exchange parameters; canopy layers; tapping; clone; Photosynthesis.

1. INTRODUCTION

Rubber (*Hevea brasiliensis* Muell. Arg.) "is a latex producing species belonging to the family Euphorbiaceae". "Natural rubber is biosynthesized initiating from the end product of the photosynthetic process of the rubber tree" [1]. "The rate of latex production in the latex vessels of *Hevea* has been related to the supply of sucrose from the adjacent sieve tubes" [2]. "This suggests that a close link exists between the CO_2 assimilation capacity and the economically important component of the rubber tree" [3]. "The photosynthetic characters of leaves at different canopy positions are acclimatized to their own micro-environment. The major differences in photosynthetic activity in sun and shade adapted

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leaves within a canopy are correlated with differences in the concentration of the electron transport chain, photosystem activity and photosynthetic enzyme activity" [4]. "Therefore, it appears that canopy photosynthesis is naturally optimized by partitioning of photosynthetic capacity among the leaves with respect to natural light exposure. Thus gas exchange characters of a leaf from a particular canopy position cannot be considered necessarily to represent the photosynthetic characters of the entire canopy. Hence, estimating canopy photosynthesis has become an important aspect of plant productivity research" [5,4]. However for tree crops, a model that describes light attenuation within the canopy and the use of light response curves for leaves at different canopy positions to determine the CO2 assimilation potential may be the only practicable alternative to estimating canopy photosynthesis. Studies on canopy photosynthesis and yield have shown a clear positive correlation between them [6]. The variation of leaf photosynthetic characteristics with depth in the canopy is an important factor to consider in the prediction of canopy photosynthesis.

2. MATERIALS AND METHODS

2.1 Experimental Site and Plant Material

The present study was supplement to a longer experiment to determine the feasibility of early commencement of tapping of two *Hevea* genotypes i.e. RRIC121 and RRISL 211.The experiment was conducted in the Dartonfield Estate at the Rubber Research Institute, Agalawatta, Sri Lanka, located in the agro ecological zone WL1.Two *Hevea* genotypes (mature trees of same girth) with contrasting yield potential, i.e. RRIC 121 and RRISL 211 were selected for the study. All cultural practices were done according to the recommendations made by the Rubber Research Institute of Sri Lanka.

2.1.1 Experimental design and treatments

Four tapped and four untapped trees of *Hevea* from each of the two genotypes were selected for the study. Trees tapped at ½ S d/3 + E, i.e. the highest yielding treatment, was selected for this study.

Each day, leaflets were sampled from two trees (tapped and untapped) from each genotype and the following sequence was adopted to measure their gas exchange characteristics. Gas exchange measurements were made on twelve leaves on any given day. Sequence was reversed on the second day and measurements were taken on four days.

Sequence for measurements of Amax of tapped (T) and untapped (UT) trees of clones RRISL 211 and RRIC 121.

1	RRISL 211	T/t	2	RRIC	121	T/t
3	RRISL 211	UT/t	4	RRIC	121	UT/t
5	RRISL 211	T/m	6	RRIC	121	T/m
7	RRISL 211	UT/m	8	RRIC	121	UT/m
9	RRISL 211	T/l	10	RRIC	121	T/l
11	RRISL 211	UT/l	12	RRIC	121	UT/l

T - tapped tree, UT - un-tapped tree, t - top strata of the canopy, m - middle strata of the canopy, l -lower strata of the canopy

2.1.2 Protocol to use detached leaves for gas exchange measurements

With the Portable Infra Red Gas Analyzer, IRGA (Model LI – 6400, LICOR Inc., Lincoln, NE, USA), reaching the leaves of mature rubber canopy for the measurements of CO2 assimilation was extremely difficult. According to Korpilathi [7], use of detached leaves in the measurements of CO2 assimilation affects the accuracy. However, similar photosynthetic rates in attached leaves of field grown trees and detached leaves have been reported [8, 9,3].

2.1.3 Detached leaves for the gas exchange measurements

The canopy of each tree was visually divided into three strata as top, middle and bottom. Then twigs were cut from each stratum with a sharp knife and immediately put into a bucket of water. Thereafter, the middle leaflet of a healthy, bright green, mature leaf was separated (about 2 cm above the leaf base) from the petiole using a sharp blade and it was cut across the lamina under water. A further cut was made very closely after 2 minutes to remove any latex coagulated at the cut end of the leaf lamina. Then they were immediately put into a small beaker with their cut ends dipped in water and were taken to the laboratory under an air tight container. The leaves sampled were kept for about two hours in controlled environment, i.e. at 30^{o}C with low light ca.100 □ mol m-^{2} s-^{1}. These detached leaves were used for gas exchange measurements as per the sequence described previously.

2.2 Gas Exchange Measurements

Using an open system, portable Infra Red Gas Analyzer, IRGA (LI – 6400, LICOR Inc., Lincoln, NE, USA), CO2 uptake by leaves was measured at different light levels. An in-built artificial light source, 6400 – 02B Red/Blue (LICOR Inc., Lincoln, NE, USA), was used to provide the varying levels of incident PPFD. While taking the measurements of leaf photosynthesis, other related gas exchange parameters, i.e. transpiration rates (E), stomatal conductance (gs), intercellular CO2 concentration (Ci) and leaf temperature (T) were also recorded simultaneously.

2.2.1 CO_2 assimilation rate (A)

Photosynthetic and transpiration rates were measured by the LI – 6400 system based on differences in the mole fraction of CO2 and H2O of the outlet (analysis cell) and inlet (reference cell) air streams flowing through the leaf cuvette. The net CO2 assimilation rate, A (□mol m^{-2} s^{-1}) is given by the formula given below (Von Caemmerer and Farquhar, 1981).

$$A = \{ F (Cr - Cs) / 100 S \} - Cs E$$

Where, F, Cr, Cs, S and E are respectively molar flow rate of air entering the leaf chamber (μmol s^{-1}), mole fraction of CO_2 in the inlet of analysis cell (μmol CO_2 mol^{-1} air), mole fraction of CO2 in the outlet of analysis cell leaf area (cm^2) and transpiration (mol H_2O m^{-2} s^{-1}).

2.2.2 Transpiration rate (E)

The equation that the LI – 6400 uses for computing transpiration rate (E) is,

$$E = \frac{F\,(ws - wr)}{100\,S\,(1000 - ws)}$$

where,

F = flow rate (μmol s^{-1})
ws & wr= sample and reference water mole fractions (mmol H_2O mol $^{-1}$ air)
S= leaf area (cm^{-2})

2.2.3 Stomatal conductance (gs)

The equation that the LI – 6400 uses to compute stomatal conductance to water vapour (gsw) is,

where,

$$gsw = \frac{1}{1/gtw - kf/gbw}$$

gbw = boundary layer conductance to water vapour (mol H_2O m^{-2} s^{-1}) from one side of the leaf
gtw = total conductance to water vapour (mol H_2O m^{-2} s^{-1})
kf = stomatal ratio

The stomatal conductance to water vapour (mol H_2O m^{-2} s^{-1}) is obtained from the total conductance by removing the contribution from the boundary layer.

2.2.4 Intercellular CO_2 concentration (Ci)

The equation that the LI – 6400 uses for computing intercellular CO2 concentration (Ci), (μmol CO_2 mol^{-1} air) is,

$$Ci = \frac{(gtc - E/2)\,Cs - A}{gtc + E/2}$$

where,

gtc = total conductance to CO2

2.2.5 Water use efficiency (WUE)

The CO_2 and transpiration rates at saturating light levels were used in determining water use efficiency (WUE) as:

$$WUE = \frac{Photosynthetic\ rate\ (A)}{Transpiration\ rate\ (E)}$$

2.3 Data Analysis

Analysis of Variance (ANOVA) was carried out using the SAS (SAS Institute, Inc., Carry, NC, USA) statistical package. To evaluate the significance of differences between all possible pairs of treatment means, mean separation of treatments was performed with Duncan's Multiple Range Test (DMRT).

3. RESULTS

3.1 Response of Gas Exchange Parameters to Light

3.1.1 Stomatal conductance (gs)

Stomatal conductance at saturated light intensities (gsat) of both clones showed a similar pattern of response to tapping (Figs. 1a and b). Tapping decreased gsat in the top canopy layer in both clones. On the other hand, gsat increased due to tapping in the middle and bottom canopy layers of both clones. In general, gsat values of the middle and bottom canopy layers of RRISL 211 were higher than the corresponding values of RRIC 121. In contrast, the opposite was observed for the gsat of top canopy layers. In RRIC 121, the gsat values of the top canopy layer were significantly greater than corresponding values of the middle and bottom canopy layers (Fig. 1b). However, no such clear cut variation between the gsat values of different canopy layers could be observed in RRISL 211 (Fig. 1).

3.1.2 Transpiration rate (E)

Transpiration rates showed gradual increases with increasing light intensity in both clones. When the data for all canopy layers were pooled, the leaf transpiration rate of both tapped and untapped treatments of both clones showed a slight increase with increasing light intensity. In the tapped treatment, the overall transpiration rates of RRISL 211 was greater than that of RRIC 121, especially at higher light intensities. However, no such variation could be observed between the two clones in the untapped treatment. In the tapped treatment, while both top and middle canopy layers did not differ significantly between the two clones, the transpiration rate of the bottom canopy layer was significantly greater in RRISL 211 (Fig. 2).

3.1.3 Intercellular CO_2 concentration (Ci)

Intercellular CO2 concentration showed a decreasing trend with increasing light intensity up to a level of around 200 μmol m^{-2} s^{-1}. Thereafter, Ci was stable across all light levels above 200 μmol m^{-2} s^{-1}. In the tapped treatment, Ci of RRIC 121 was consistently greater than that of RRISL 211, for both overall mean and in different canopy layers. In the tapped treatments the Ci values of different canopy layers at all light levels were greater in RRIC 121 than in RRISL 211. This may be due to the lower overall photosynthetic capacity of RRIC 121. In the untapped treatments also, a similar trend was observed. However, the difference between the two clones was prominent only in the middle and bottom canopy layers (Fig. 3).

3.1.4 Leaf temperature (Tleaf)

The variation of leaf temperature with light intensity (LI) showed a common variation pattern across the two clones, the two tapping treatments and the three canopy layers. As the LI increased from zero, leaf T decreased until the LI reached around 200 μmol m^{-2} s^{-1}. Thereafter, the leaf T showed a gradual increase with increasing LI. In the tapped treatment, RRISL 211 showed a higher leaf temperature than RRIC 121. However, in the untapped treatment, there was no consistent variation between the two clones with respect to leaf temperature (Fig. 4).

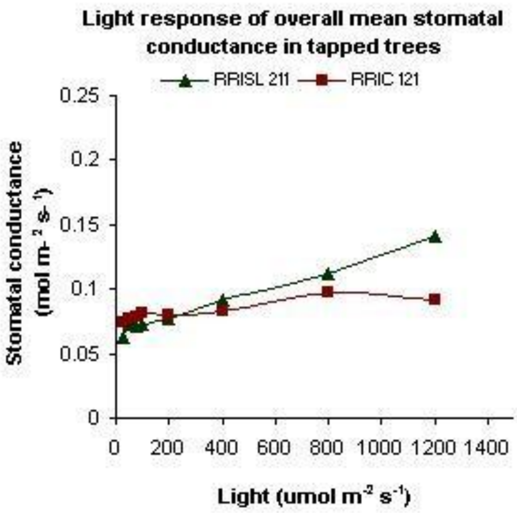
3.1.5 Water use efficiency (WUE)

Variation of WUE with light intensity was similar for both tapped and untapped treatments and for different canopy layers in both clones. The WUE increases with light intensity until it reached 200 μmol m^{-2} s^{-1}. Thereafter, the WUE did not vary significantly with light intensity. When the data of all canopy layers were pooled, in the tapped treatment WUE of RRISL 211 was higher than that of RRIC 121 at all light intensities except at 1200 μmol m^{-2} s^{-1} where, both clones had similar WUE (Fig. 5).

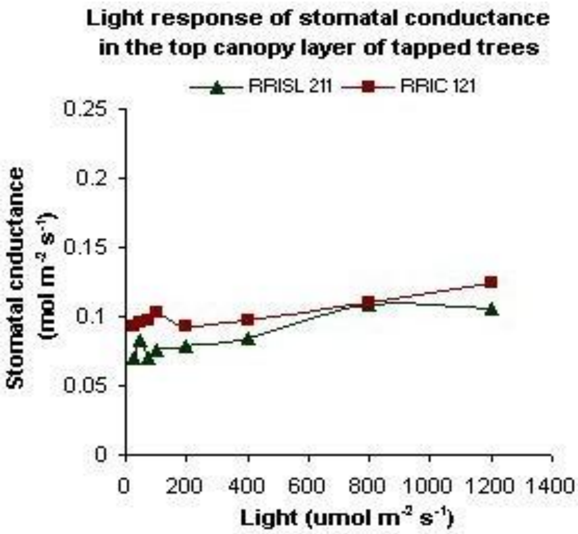
4. DISCUSSION

In this study, in the tapped treatment, the stomatal conductance of clone RRIC 121 was found to be less than that of clone RRISL 211 (Fig. 1). As gs is mediated by changes in photosynthesis, a reduced photosynthetic capacity could be one of the reasons for the lower stomatal conductance of clone RRIC 121. The intercellular CO2 concentration (Ci) showed a gradual decrease with increasing light intensity in both clones. This was because of increased utilization of CO_2 for photosynthesis, which is stimulated with increasing light intensity. The observed reduction of Ci during the initial stages of light intensity increase indicates that stomatal opening was not rapid enough to supply CO_2 to meet the increased demand from increased photosynthesis. Therefore, stomatal limitation was evident in the clones used in the present study during the initial part of light intensity increase. However, it was also observed that the initial reduction of Ci stabilized as light intensity increased beyond a certain point. This could be due to the stomatal limitation decreasing or due to the photosynthetic capacity saturating with increasing light intensity or both. This is in agreement with the model proposed by Von Caemmerer and Farquhar [10]. Nugewela [3] also reported that higher CO2 assimilation rates are associated with higher stomatal conductance and lower internal CO2 concentrations. This is broadly in agreement with the observations of the present study. In both clones, transpiration rates in the top canopy layer were lower in the tapped treatment as compared to the untapped treatment. This may be due to the reduced turgor pressure in the xylem vessels as a result of tapping. This reduction of transpiration rates in the top canopy layer under tapping is possible, because a higher turgor pressure is essential to transfer water to the upper layers of the canopy. Therefore, the reduction of turgor pressure due to tapping would be felt most, in the transpiration rates of top canopy layer. Furthermore, the inter-treatment variation pattern of the transpiration rate was largely similar to that of the gsat. This shows that transpiration was largely under stomatal control. The temperature optimum for A in higher plants varies among species and is closely related to the ambient temperature of the growth environment [11,12]. In both clones, leaf temperature (Tleaf) decreased until the light intensity (LI) reached around 200 μ mol m^{-2} s^{-1} and gradually increased with increasing LI, irrespective of the tapped and untapped treatments

(Fig. 4). The initial reduction was due to loss of energy from leaf surface for increased transpiration. However, with increasing light intensity, the energy loads on the leaves increases and consequently increasing leaf temperature. Clone RRISL 211 showed a higher leaf temperature than RRIC 121 under tapping (Fig. 4). It is evident that Amax and the temperature at which it is achieved vary with the genotype. Temperature determines the rates of component biochemical steps of the photosynthetic process through its effects on the kinetics of the reactant molecules and enzymes. In both tropical and temperate environments, within genotypically and environmentally determined limits, higher temperature increases the specific activity of photosynthetic machinery and hence increases the Amax [13]. Furthermore, the water use efficiency (WUE) of clone RRISL 211 was higher than that of clone RRIC 121 under tapping (Fig. 5). This was primarily because of the greater photosynthetic rates of RRISL 211, rather than lower transpiration rates. The highest leaf-air vapour pressure deficit (lvpd) was shown in the leaves of the upper canopy and lowest in the bottom canopy layer (Fig. 6). This is probably because of decreasing wind speed with increasing canopy depth which allowed accumulation of water vapour at lower levels of the canopy [14].



(a)



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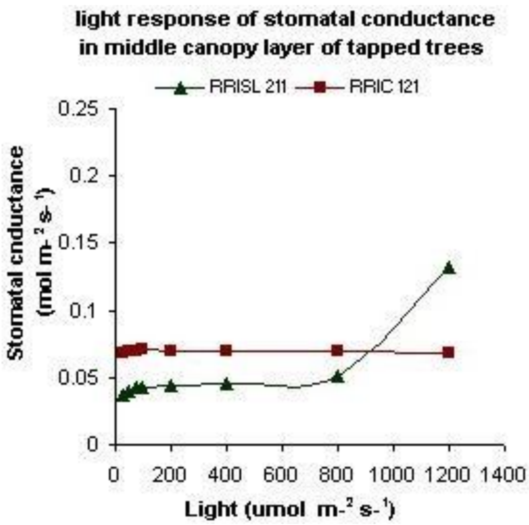

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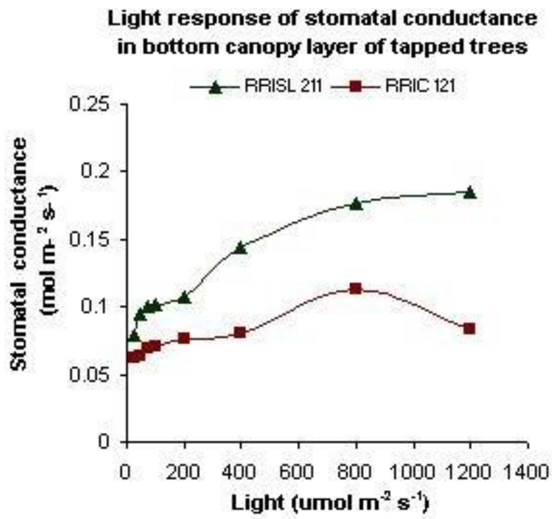


(d)

Fig. 1. The light response of overall mean stomatal conductance (a) and stomatal conductance of the two clones at different canopy layers (b, c and d) for the tapped trees. Each point is the mean of four observations made from four randomly selected plants

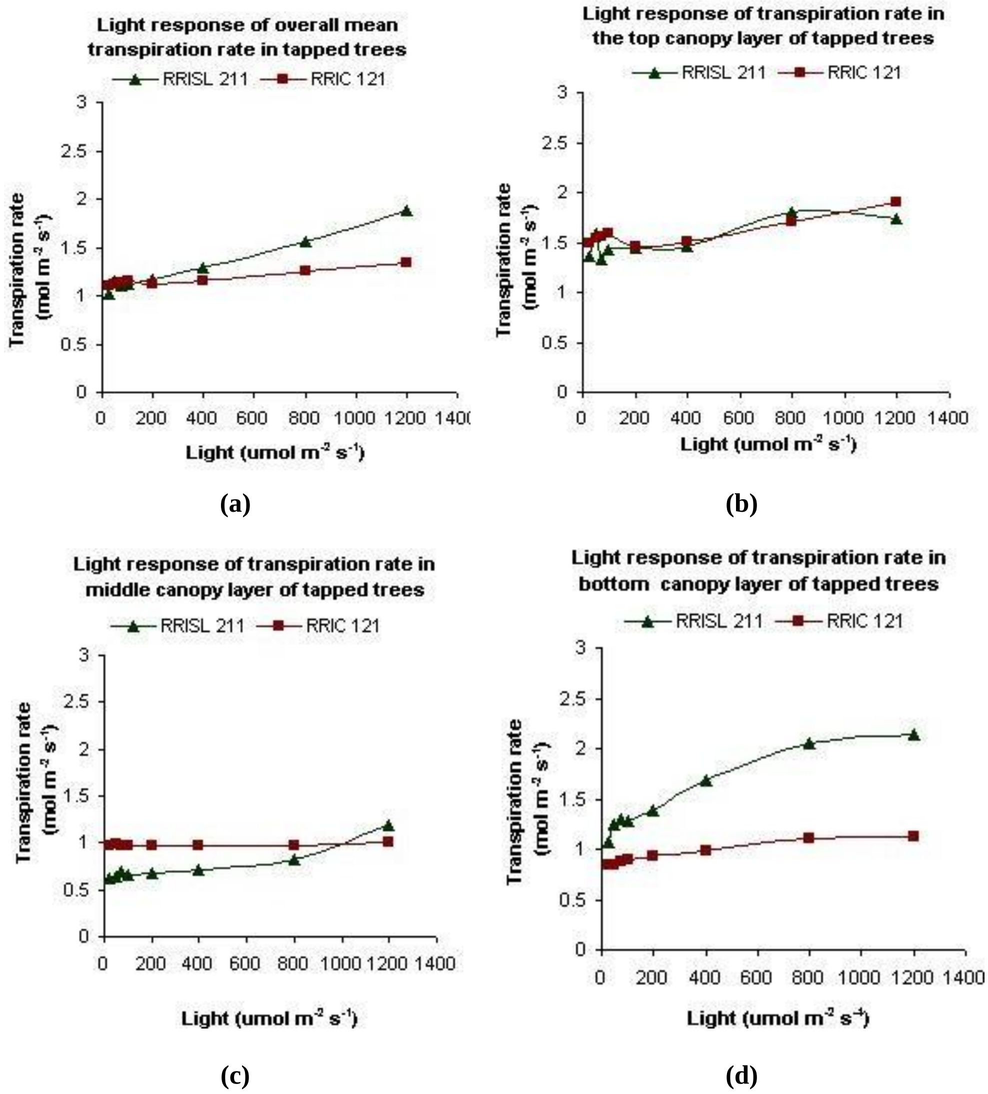
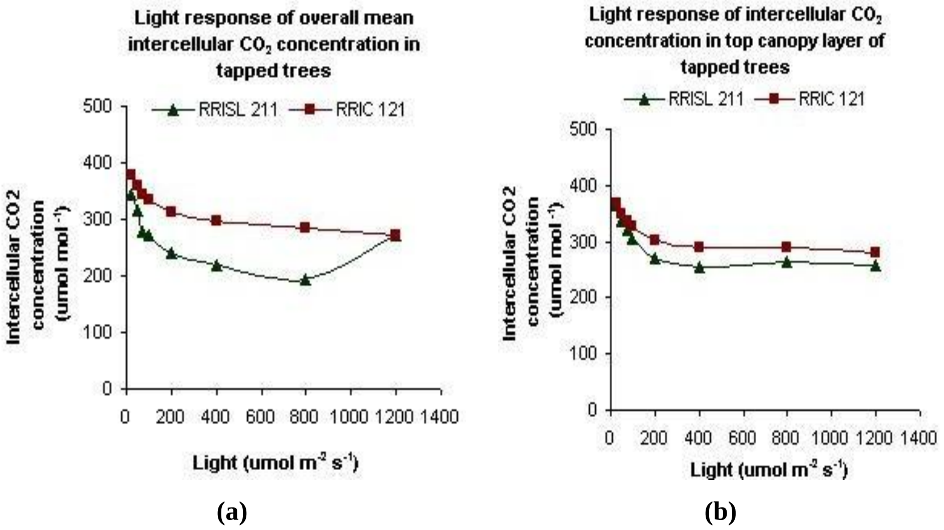
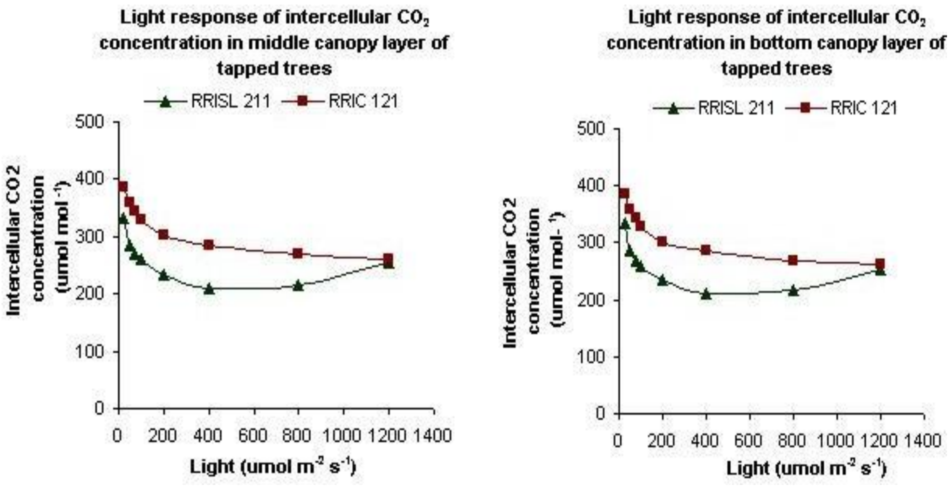


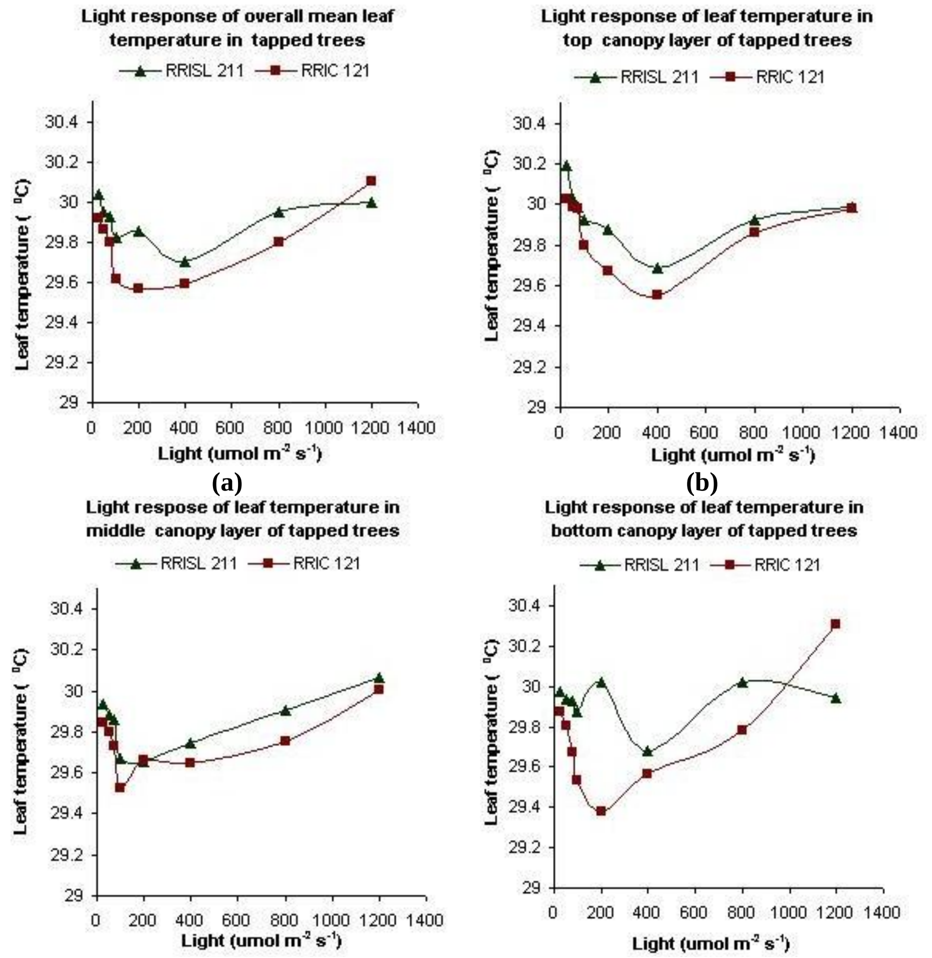
Fig. 2. The light response of overall mean transpiration rate (a) and transpiration rate of the two clones at different canopy layers (b, c and d) for the tapped trees. Each point is the mean of four observations made from four randomly selected plants





(c) **(d)**

Fig. 3. The light response of overall mean intercellular CO_2 concentration (a) and intercellular CO_2 concentration of the two clones at different canopy layers (b, c and d) for the tapped trees. Each point is the mean of four observations made from four randomly selected plants



(c) **(d)**

Fig. 4. The light response of overall mean leaf temperature (a) and leaf temperature of the two clones at different canopy layers (b, c and d) for the tapped trees. Each point is the mean of four observations made from four randomly selected plants

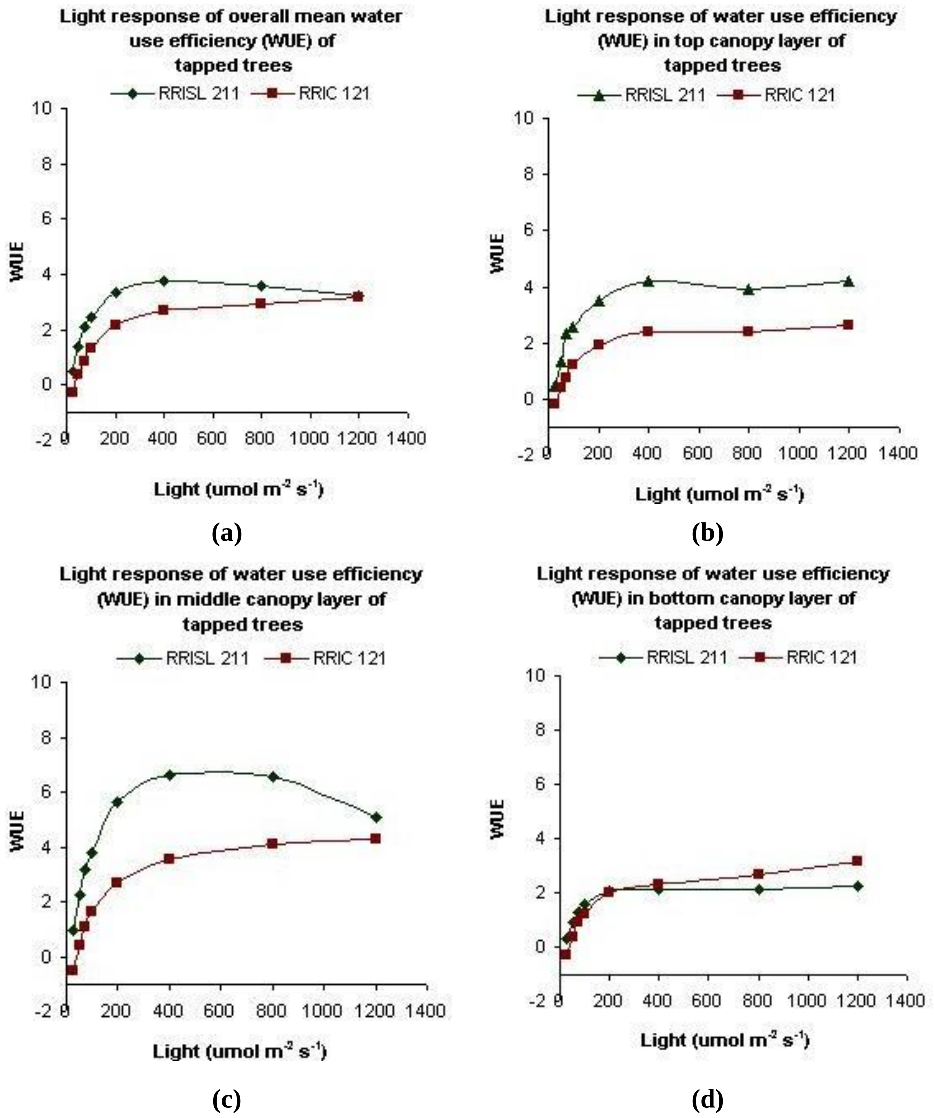
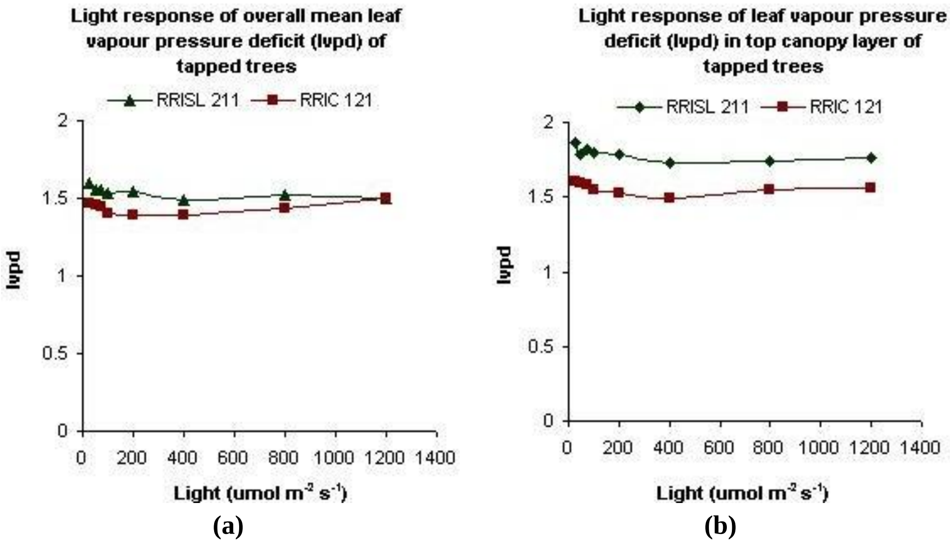
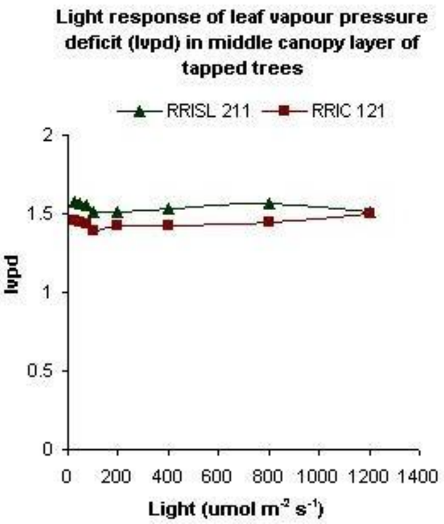
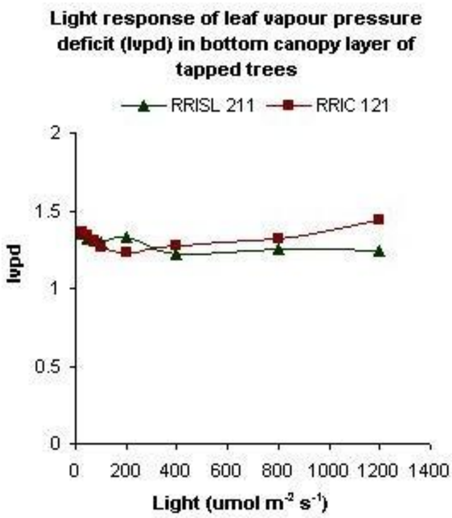


Fig. 5. The light response of overall mean water use efficiency (a) and water use efficiency of the two clones at different canopy layers (b, c and d) for the tapped trees. Each point is the mean of four observations made from four randomly selected plants





(c)



(d)

Fig. 6. The light response of overall mean leaf-air vapour pressure deficit (a) and leaf-air vapour pressure deficit of the two clones at different canopy layers (b, c and d) for the untapped trees. Each point is the mean of four observations made from four randomly selected plants

5. CONCLUSION

The positive latex yield response to WUE and lvpd of top leaves indicates that, in top leaves when lvpd increases transpiration also increases accordingly. Clone RRIC 121 showed a greater contribution from the middle layer to overall canopy photosynthesis while RRISL 211 showed a lower contribution. This could have been due to the more open canopy architecture in clone RRIC 121 or maintenance of a higher A in low light levels.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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