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Assessing Growth and Physiological Parameters of Young *Hevea brasiliensis* to Identify Adaptable Clones for WL_{1a} Agroecological Region in Sri Lanka

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ABSTRACT

The rubber (*Hevea brasiliensis*) industry plays an important economic role in Sri Lanka. Expansion of rubber cultivation is very important for the increase in production. In general, the performance of a crop varies with the environment and thus the climatic conditions greatly affect the physiological and growth performance of the rubber plant. In this regard, factors such as rainfall, temperature, wind, humidity and characteristics of the soil are extremely important. Rubber requires a considerable period before giving its potential yield and hence, the identification of high yielding clones is a time-consuming process. Therefore, having an approach in identifying elite clones at early stages is important in predicting the yield. In this regard, RRIC 100, RRIC 121, RRISL203 and RRISL 2001 clones were selected in Dartonfield experimental site situated in WL_{1a} agroecological zone. Poly-bagged budded plants were planted in a Randomized Complete Block Design with four replicates and their performance was monitored using vegetative growth parameters and physiological parameters for three years. The results revealed that there was a negative relationship between stomatal conductance and wind speed of four *Hevea* genotypes during the morning and evening periods. Also, the clones other than RRIC 100 had shown a positive relationship between stomatal conductance and air temperature. There was a clear positive relationship between stomatal conductance and relative humidity in RRIC 121, RRIC 100 and RRISL 203 clones during morning and evening times. The stomatal conductance of all four clones was negatively related to soil moisture content. The performance of RRISL 2001 showed better growth among all four clones followed by RRIC 121. With regards to plant physiological status, RRIC 121 and RRISL203 showed better performance in terms of leaf epicuticular wax content and chlorophyll content and hence, they could be more tolerable in dry climates.

Keywords: Agro-ecological zone, Growth parameters, Physiological parameters, Rubber clone.

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INTRODUCTION

Rubber (*Hevea brasiliensis*) is one of the domesticated tree species from the Amazonian rain forest and is very important for producing latex for commercial utility. This industry plays an important role in Sri Lankan economy as a foreign exchange earner, a commodity for value addition, for domestic consumption and also to create employment. Rubber is an economically important crop in the humid tropics, not only as a natural rubber producer but also as a sustainable agroforestry system which is almost equivalent to the native rainforest system (Penot, 2004).

In Sri Lanka, the rubber sector is the third-largest export earner of the country (Anon, 2019a). The area under rubber cultivation is 136,000 hectares, out of which 107,000 hectares are under tapping in Sri Lanka (Ranasinghe *et al*., 2020). Traditional rubber growing areas are located mainly in the Wet Zone of Sri Lanka. It includes Colombo, Galle, Gampaha, Kalutara, Kandy, Kegalle, Kurunegala, Matale, Matara, and Rathnapura Districts.

Rubber was expanded to various areas in Sri Lanka, where annual average rainfall was closer to the range of 2000 to 4000 mm/year. Due to land unavailability in traditional rubber growing areas, rubber has been introduced to nontraditional areas in Intermediate and Dry Zones of Sri Lanka such as Ampara, Moneragala, Mullaitivu and Vavuniya districts (Anon, 2019b). Therefore, the expansion of rubber cultivation in Sri Lanka is timely important specially to improve the livelihood of the people who are directly and indirectly involved in the rubber industry (Ranasinghe *et al*., 2020).

In general performance of a crop varies with the environment. Rubber needs a minimum rainfall of 1,650mm or more with even distribution (without any marked dry season and with 125-150 rainy days per annum) during the year. The maximum temperature required for the cultivation is about 29-32 ^{0}C and the minimum is about 20 ^{0}C. High atmospheric humidity of 80 %, moderate wind and bright sunshine amounting to about 2,000 hours per annum, at the rate of 6 hours per day in all months are the optimum weather conditions for rubber cultivation (Rao and Vijayakumar, 1992).

Plant growth and the geographic distribution of the plants are greatly affected by the environment. Climatic conditions play a vital role in physiological and growth of rubber tree. If any environmental factor is less or more than ideal, it limits plant growth and its performance. Most of the improved varieties have been adapted to the environmental conditions, in which they have been developed (Turk and Tawaha, 2002).

These environmental factors will differently affect different genotypes. It is very important to understand how these factors affect plant growth and development. With a basic understanding of these factors, we may be able to manipulate plants to meet our needs. A large number of high-yielding *Hevea brasiliensis* clones have been developed worldwide (Alika, 1982). Accordingly, the Rubber Research Institute of Sri Lanka (RRISL) developed a number of elite clones for different agroecological regions of Sri Lanka, such as Rubber Research Institute of Ceylon (RRIC) clones, RRIC 100, RRIC 121, RRISL 203 and RRISL 2001. Furthermore, RRISL is continuing its research on developing and evaluating suitable clones for the future.

Rubber needs a considerable period to commence harvesting latex and the trees must be approximately six years old and 50 cm in circumference at 120 cm above the bud union in order to be tapped for latex. Therefore, identification of the high-yielding clones is a time-consuming process. If growers can check the high-yielding clones at the very first stage, it is very important for predicting the yield. Early yield predicting techniques can play a vital role for the rubber industry by saving time and money while improving productivity.

This research was carried out to study the variation of physiological and growth parameters in different *Hevea* clones under the climatic condition of the proposed area to identify highly adaptable clones for particular environmental conditions.

MATERIALS AND METHODS

Location and Experimental Design of the Study

The experiment was carried out at the Dartonfield estate of Rubber Research Institute of Sri Lanka (RRISL), Agalawatta, in Kalutara district which is classified under Low Country Wet

Zone, WL_{1a}. Average annual precipitation is normally more than 2500 mm and has humid tropical climate (Marambe *et al*., 2015). The elevation is about 3 m above the mean sea level. The average temperature is about 28 ^{0}C and the mean relative humidity is 85 % (Anon, 2019c). Four commonly grown Hevea genotypes i.e., RRIC 100, RRIC 121, RRISL 203 and RRISL 2001 were selected for the study.

The experimental design was a randomized complete block design (RCBD) with four replicates and with 12 plants per experimental plot. As per the recommendation of RRISL, planting was done using 2.4 x 8.1 m spacing to give 500 plants per ha density (Attanayaka, 2001). Planting was done during May-June season in 2016. Casualties were supplied during the first two years after planting to maintain the correct planting density but were not been used for assessments. As per the recommendation, rubber fertilizer mixture R/U/ 12:14:14 with N: P: K respectively, was applied at the rate of 275 g per plant per year, and separately, 75 g kieserite was applied per plant per year in the first year of planting. In the second year, 550 g of the same mixture was applied in four splits and instead of kieserite, 150 g dolomite per plant was applied (Anon, 2016).

Assessment of Growth Parameters

Plant height, diameter and leaf area of each plant were measured at three-month intervals for three years after planting. The heights of all plants were measured using a measuring tape, from the tip of the shoot to the ground. Stem diameter was measured using a digital Vernier caliper, at the first inch, first feet and second feet height from the ground level. The diameter of the 12 plants that represented each clone was measured quarterly. Leaf area was measured by using a digital leaf area meter (LI-3000C, Ll-Cor, Nebraska, U.S.A.). Three leaves of the most recently matured leaf whorl were used for the measurements.

Assessment of Physiological Parameters

For plant physiological assessments, three plants were selected randomly from each replicate, hence 12 plants to represent each clone and measurements were taken at every three-month interval for three years after planting. Physiologically matured top-most fully expanded leaves were selected for sampling. Leaf chlorophyll comparison was indirectly measured as soil plant analysis development (SPAD) values using SPAD 502 Plus chlorophyll meter. In each sampling day stomatal conductance was measured between 9:00 am to 10:00 am in the morning and 3.00 pm to 4.00 pm in the evening. Measurements were taken from two healthy leaves avoiding veins using a porometer (AP4 Leaf Porometer).

Detached leaves from each sampling tree were used to estimate relative water content (RWC) and epicuticular wax content. Two leaf discs (core area-58.789 cm^2) were cut from each sampling tree avoiding large veins. Fresh weight, turgid weight and dry weight of each leaf disc were measured and RWC was estimated (Anon, 2016). Three leaf discs (core area - 58.789 cm^2) were cut from leaves of each sampling plant avoiding the midrib. Initially weighed leaf discs were rinsed with chloroform and reweighed after drying. Leaf epicuticular wax content was estimated using the method described by Anon (2016).

Monitoring of Environmental Condition

Soil moisture content was determined on dry-weight basis on the days of collecting plant physiological data. By using portable weather meter (Kestrel 5000), micro environmental parameters (environmental temperature, relative humidity, wind speed, and soil moisture) were also taken in both morning and evening at the time of taking physiological measurements. Soil moisture content was measured on dry-weight basis by the standard gravimetric method.

Statistical Analysis

Data were analyzed using SPSS software. ANOVA was done for each variable followed by a mean separation using Duncan Multiple Range Test at the significance level of 0.05. Pearson correlation was done between stomatal conductance and each environmental variable in order to identify the possible relationships.

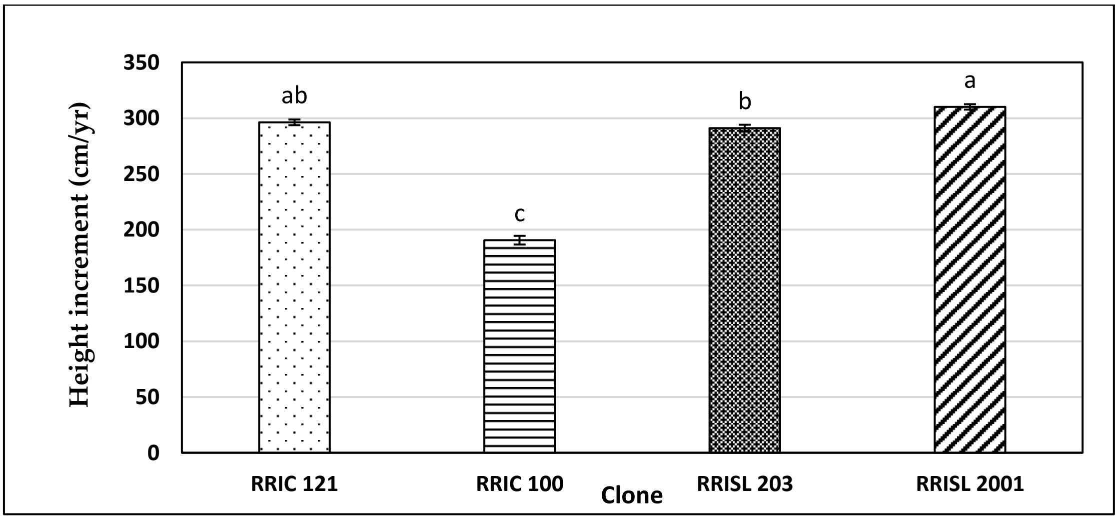
RESULTS AND DISCUSSION

Assessment of Plant Growth Parameters

Height and diameter increment can be considered as important parameters in vegetative growth of crops as those are directly related to crop growth and yield (Amanullah *et al*., 2010).

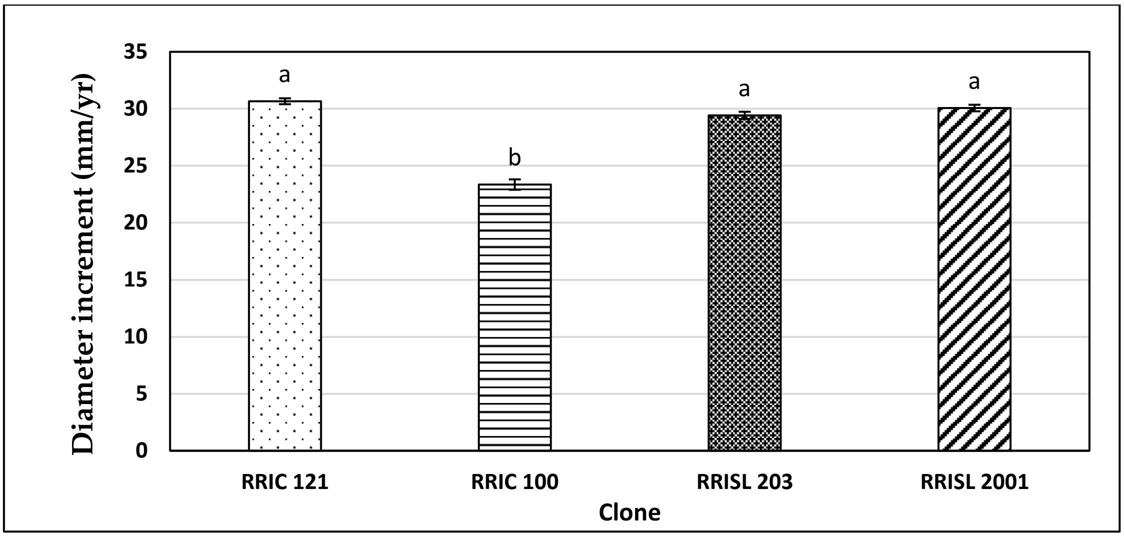
Results showed that there was a significant difference in height increment among the clones at their early growth stage. RRIC 100 had the significantly lowest height increment (190.58 cm/yr ± 7.77) during the study period among the clones tested. The highest height increment was observed in RRISL 2001 (310.06 cm/yr ± 4.83) and RRIC 121 (296.26/yr cm ± 5.109) clones. The height difference between RRISL 203 (291 cm/yr ± 6.08) and RRIC 121 (296.26 cm/yr ± 5.11) had no significant difference (Figure 1).

As shown in Figure 2, analysis of variance (ANOVA) described that the RRIC 100 had the significantly lowest diameter increment (23.36 mm/yr ± 0.91) among the all clones tested during the study period. According to the analysis, other three clones did not show any significant differences among each other in diameter increment. The highest average diameter increment was observed in RRIC 121 (30.66 mm/yr ± 0.53) clone followed by RRISL 2001 (30.07 mm/yr ± 0.58) and RRISL 203 (29.42 mm/yr ± 0).



Means with the same letters are not significantly different at $P < 0.05$

Figure 1. Average annual height increment of different *Hevea* clones over the experimental period of three years



Means with the same letters are not significantly different at P < 0.05

Figure 2. Average annual diameter increment of different *Hevea* clones over the experimental period of three years

In supporting our results, Handapangoda et al. (2017) had also observed similar results with the clone RRIC 121 which had recorded the highest growth rate in the rootstock nurseries (RRIC 100, RRIC 102, RRIC 121, RRISL 203, RRISL 217, RRISL 2001, PB 86 and PB 260) established in Egaloya, Ampara and Moneragala (Intermediate Zone).

The average leaf area of different clones tested in the present study did not show any significant difference, statistically. However, the highest average value was observed in RRISL 203 (121.02 cm^2 ± 9.97) clone followed by RRISL 2001 (114.69 cm^2 ± 4.25) and RRIC 100 (111.73 cm^2 ± 2.77) clones. The lowest leaf area was observed in RRIC 121 (102.99 cm^2 ± 2.92) clone (Figure 3). Leaf area growth determines light interception and is an important parameter in determining plant productivity. However, ascending order of average values was as RRISL 203, RRISL 2001, RRIC 100, and RRIC 121.

Plant Physiological Assessments

Leaf Chlorophyll (SPAD units)

Results indicated that there was a significant difference among chlorophyll SPAD values of young plants of different Hevea clones tested. RRIC 100 had the significantly lowest chlorophyll SPAD units (51.15 ± 0.5) among the all clones tested. The highest chlorophyll SPAD value was observed in RRISL 203 (55.61 ± 0.39) which was not significantly different from RRIC 121 (55.1 ± 0.32). The average leaf chlorophyll SPAD value of RRISL 2001 was 54.16 ± 0 .32 and it was not significantly different from RRIC 121 (55.10 ± 0.32) (Figure 4).

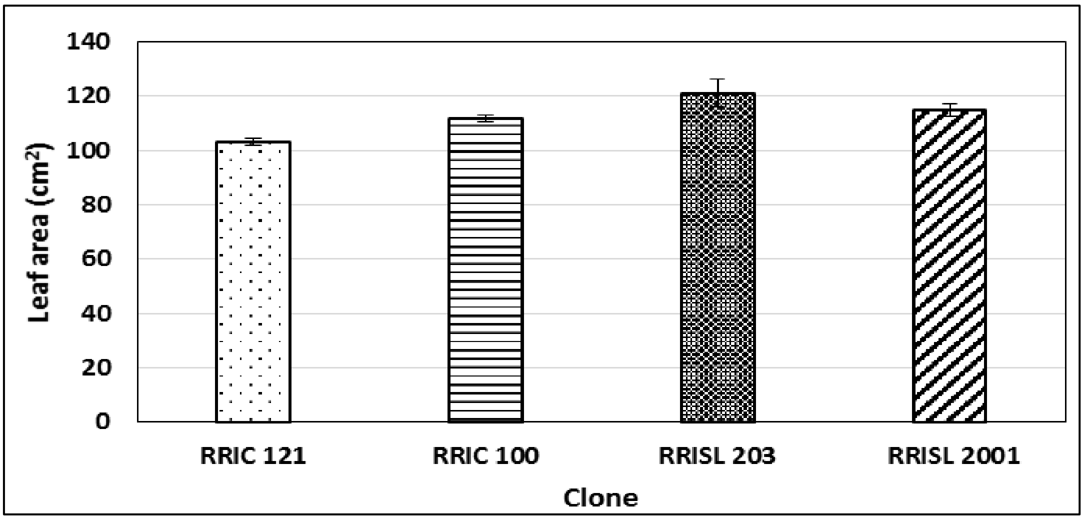
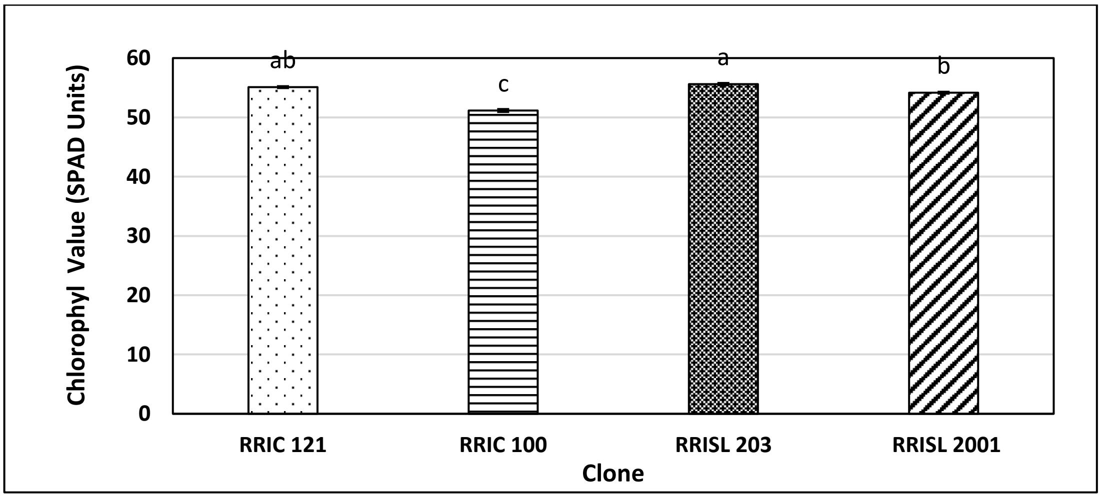


Figure 3. Average leaf area of different *Hevea* clones over the experimental period of three years.



Means with the same letters are not significantly different at $P < 0.05$

Figure 4. Average chlorophyll value of different *Hevea* clones

Considering the chlorophyll SPAD value RRISL 203 and RRIC 121 had performed better among the clones tested.

Relative Water Content

Analysis of variance (ANOVA), showed that there was no significant difference in relative water content (RWC) among different clones tested. However, the highest RWC was observed in RRISL 2001 (89.73 % ± 0.65) clone followed by RRIC 100 (89.16 % ± 0.62) and RRISL 203 (88.94 % ± 0.56) clones. The lowest RWC was observed in clone RRIC 121 (86.97 % ± 1.33) (Figure 5).

Epicuticular Wax Content

A significant difference in epicuticular wax content of the Hevea clones tested has been observed. The RRIC 100 clone showed the lowest epicuticular wax content (33.38 mg/ cm^2 ± 2.64) while RRIC 121 (51.47 mg/ cm^2 ± 2.58) and RRISL 203 (48.89 mg/ cm^2 ± 2.48) clones showed the highest value with no significant difference between these two clones. Epicuticular wax content of clone RRISL 2001 was 41.62 mg/ cm^2 ± 2.34 and it was significantly different in between other clones (Figure 6).

However, environmental conditions may strongly influence the quantity, composition and morphology

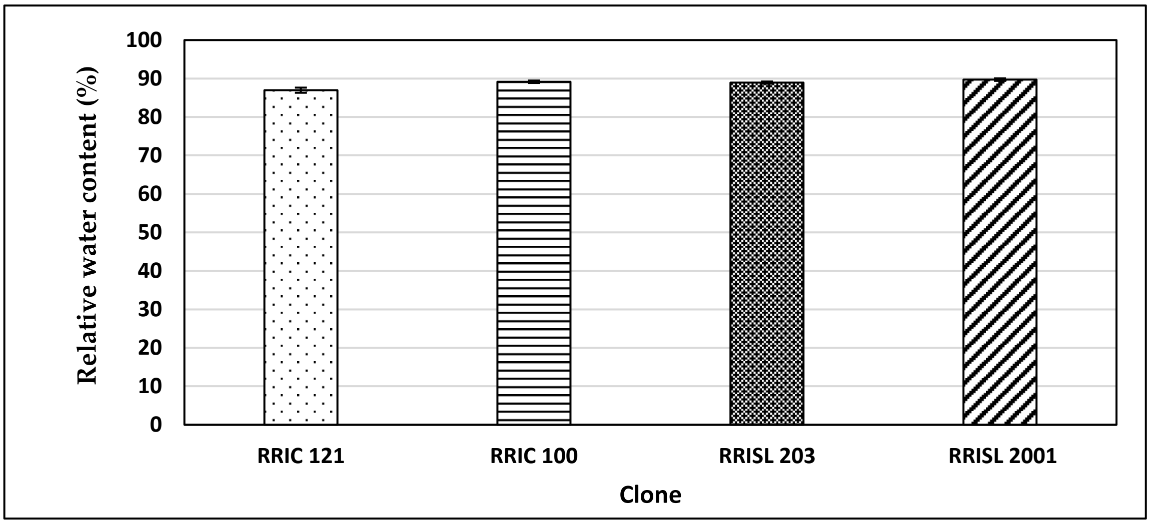


Figure 5. Average relative water content of different *Hevea* clones over the experimental period of three years.

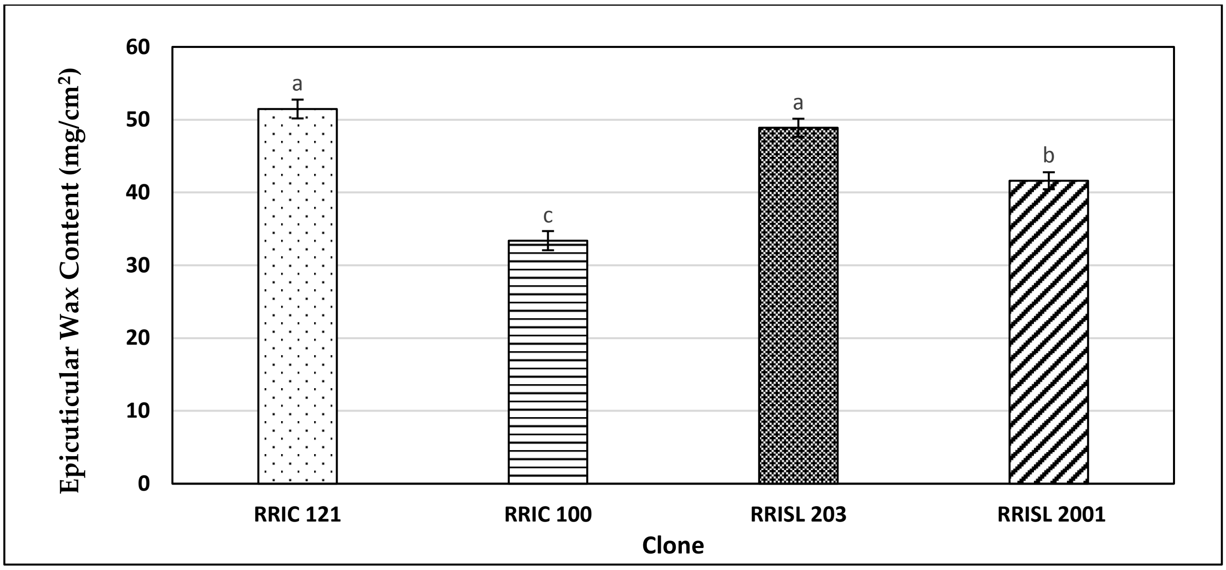


Figure 6. Average epicuticular wax content of different *Hevea* clones over the experimental period over the experimental period of three years.

of waxes covering leaf surfaces (Juniper, 1960; Reed and Tukey, 1982). The epicuticular wax layer protects the aerial parts from insect feeding, probing, or oviposition and this property of the plant cuticle is commonly known as mechanical barriers against insects (Znidarcic, *et al.*, 2008).

Stomatal Conductance

Stomatal conductance of the four different clones was measured during the morning and evening. Analysis of variance (ANOVA), showed that there was no significant statistical difference in stomatal conductance between the morning and evening periods in the four different clones tested. However, RRISL 203 clone showed the highest stomatal conductance (1.60 cm/s ± 0.21) in the morning followed by RRIC 100 whilst RRISL 2001 (1.28 cm/s ± 0.11) showed the lowest value. Stomatal conductance of RRIC 100 and RRIC 121 clones were 1.42 cm/s ±0.16 and 1.36 cm/s ± 0.12, respectively. Similarly, in the evening also RRISL 203 clone showed the highest stomatal conductance (1.40 cm/s ± 0.08) whilst RRISL 2001 (1.18 cm/s ± 0.07) clone showed the lowest average value. Average stomatal conductance of RRIC 121 and RRIC 100 were 1.39 cm/s ± 0.12 and1.18 cm/s ± 0.08, respectively (Figure 7).

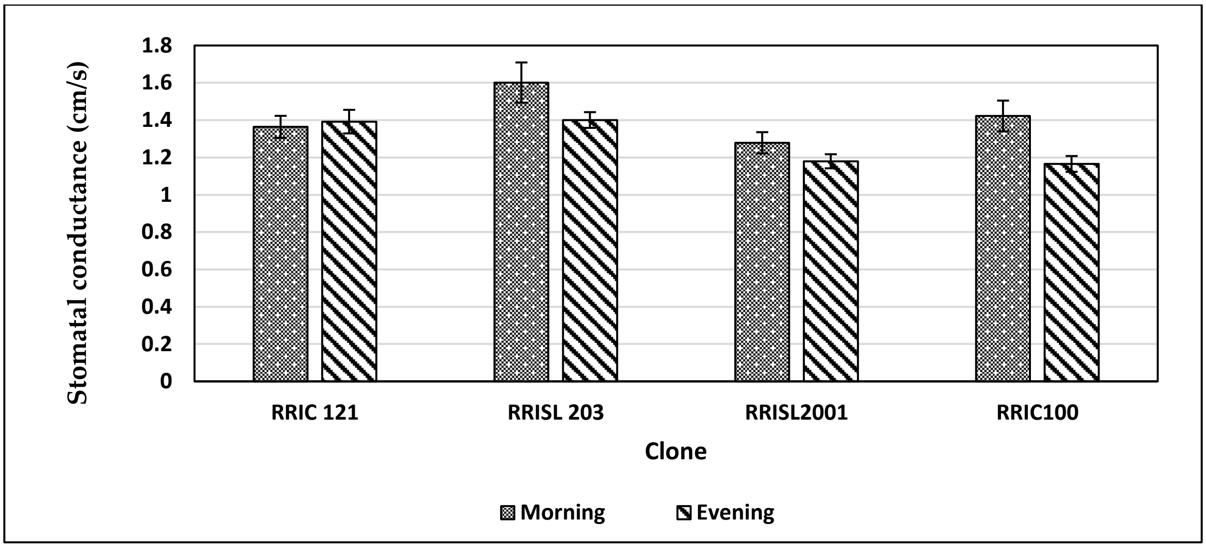


Figure 7. Average stomatal conductance (Morning and Evening) of different *Hevea* clones over the experimental period

Table 1. Correlation of stomatal conductance of different clones with environmental factors

Clone	Pearson correlation coefficient							
	Wind speed (m/s)		Temperature (°C)		Relative humidity (%)		Soil moisture content (%)	
	Morning	Evening	Morning	Evening	Morning	Evening	Morning	Evening
RRIC 100	-0.742	-0.243	-0.052	-0.035	0.287	0.135	-0.196	-0.345
RRIC 121	-0.327	-0.276	0.310	0.280	0.173	0.029	-0.316	-0.284
RRISL 203	-0.284	-0.367	0.248	0.334	0.190	0.122	-0.323	-0.407
RRISL 2001	-0.332	-0.073	0.202	0.284	-0.157	-0.097	-0.399	-0.379

Correlation analysis between stomatal conductance and wind speed of all four clones showed a negative relationship (Table 1). It clearly showed that wind speed is inversely proportional to the stomatal conductance of four *Hevea* genotypes. The stomatal conductance of plants usually decreases with increasing wind speeds (Dixon and Grace, 1984). The higher wind speed usually decreases the boundary layer thickness of the leaf surface, consequently increasing the boundary layer conductance. As the boundary layer conductance increases, CO_2 concentrations both at the leaf surface and in the leaf interior are elevated and cause stomatal conductance to decline (Aphalo and Jarvis, 1993).

Temperature is one of the most variable factors in the environment and it affects many plant physiological processes, yet little is known about its effect on stomatal conductance, especially at high temperatures (Teskey *et al*., 1986). While some evidence suggested that stomatal conductance increased with increasing temperature (Schulze *et al*., 1974; Lu *et al*., 2000; Mott and Peak, 2010), other studies found that temperature had no effect on stomatal conductance (Teskey *et al*., 1986). According to the results of the study, clones other than RRIC 100 had shown a direct positive relationship between stomatal conductance and air temperature. Stomatal conductance values of RRIC 100 had a negative relationship with air temperature (Table 1).

The relative humidity is the amount of water (vapour) in the air relative to the saturation point. Humidity content in the air is a key environmental variable in controlling the stomatal conductance of plant leaves. According to the results, there was a direct positive relationship between stomatal conductance and relative humidity in RRIC 121, RRISL 203 and RRIC 100 clones during morning and evening times. Stomatal conductance values of RRISL 2001 had a negative relationship with relative humidity (Table 1). It is reported that lower humidity leads to stomatal closure, although the transpiration rate may actually increase (Oren *et al*., 1999).

Soil moisture and water stress play a pivotal role in regulating stomatal behavior of plants. Soil moisture exerts a strong control on stomatal conductance (Meszaros et al. 2009). Therefore variability of the stomatal opening is more regulated by the variability of soil moisture than by the other physical variables tested in this study. Plants with adequate soil moisture will normally transpire at high rates because the soil provides the water to move through the plant which may lead to opening of stomata.

However, according to the results of this study, there was a negative or inverse relationship between stomatal conductance and soil moisture of all four clones studied (Table 1).

CONCLUSION

Among the four *Hevea* clones tested, significant differences were observed only in plant height, diameter, leaf chlorophyll content and epicuticular wax content.

According to the growth parameters, RRISL 2001 showed better performances among all four clones followed by RRIC 121. However, the lowest growth performances were shown in RRIC 100.

In plant physiological status, RRIC 121 and RRISL 203 showed better performance in terms of leaf epicuticular wax content and chlorophyll value reflecting a higher tolerance in dry climates. However, RRIC 100 showed the least performances among the tested clones.

There was no statistically significant difference between stomatal conductance at morning and evening periods among four different clones tested. As expected, stomatal conductance of all four clones was negatively related to soil moisture content. Wind speed was inversely proportional to stomatal conductance of four *Hevea* genotypes during morning and evening periods. According to the results, RRIC 100 showed a clear relationship between stomatal conductance and air temperature than other tested clones. There was a clear positive relationship between stomatal conductance and relative humidity in RRIC 121, RRISL 203 and RRIC 100 clones during morning and evening times. Therefore RRISL 203 and RRIC 121 are the best ones among the tested clones to be recommended for WL1a agroecological region in Sri Lanka.

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