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Effect of Rootstock on Growth and Water Use Efficiency of Hevea *during Water Stress*

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The effects of three monoclonal Hevea *rootstocks on growth, water relations parameters, proline content in leaf and water use efficiency of RRIM 901 scion clone during prolonged cycles of water stress were studied. The plants, raised in plastic containers in the glass house, were subjected to cycles of water stress for over a year by irrigating either every alternate day (moderate stress) or once in four days (severe stress). The three rootstocks imparted different stomatal responses to the scion. RRIM 623 rootstock had drought avoidance characteristics as the plants minimised water loss during severe water stress through effective stomatal control in the scion shoot and by having deep rooting characteristics. In contrast, GT 1 rootstock responded to drought by keeping stomata partially opened as soil dehydration intensified allowing leaf photosynthesis to continue. In common with RRIM 623 rootstock, plants on GT 1 rootstock had low pre-dawn leaf water potential and high root: shoot ratio reflecting their deep rooting characteristics. Plants on RRIM 600 rootstock was also very sensitive to water stress but they seem to lack deep rooting characteristics. Plants on GT 1 and RRIM 600 rootstocks tend to accumulate proline in leaves indicating their ability for osmoregulation. Data obtained in this study indicated that plants on GT 1 and RRIM 623 rootstocks are better adapted to prolonged drought than those on RRIM 600 rootstock by virtue of their deep rooting characteristics.*

The availability of water is identified as a major constraint that can limit *Hevea* growth and productivity[1,2] since besides its use for maintenance of normal plant functions, water forms a major component of latex that is removed on a regular basis during tapping. This is especially pertinent in Malaysia where most rubber cultivation is currently being confined to the drier northern part of the peninsula and as more and more *Hevea* cultivation in other parts of the country is being relegated to unproductive marginal areas. Since water uptake occurs *via* the root system, it is envisaged that the use of suitable rootstock would be beneficial in maximising water uptake from the soil for maintenance of growth under water stress. Thus study on the influence of rootstock on water relations parameters of scion is important since under normal circumstances, mineral nutrients and water move by mass flow in response to water potential gradients from root to shoot[3]. In citrus, differences exist between rootstocks in the potential to acquire and transport water and nutrients to scion variety[4 6]. These differences were related to root quantity and distribution and efficiencies in water uptake and transport. Rootstocks have also been shown to influence transpiration rate and crop water use efficiency in peach[7], leaf conductance in apple[8], leaf osmotic potential at full turgor[9] and growth characteristics of grafted plants[10].

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In *Hevea*, monoclonal rootstocks such as GT 1, RRIM 623 and PB 5/51 have been known to have beneficial effect on tree growth and productivity whilst others such as RRIM 600 and PB 260 rootstocks were reported to have poor growth potential[11]. However, there is a dearth of information on the effect of rootstock on water relations of the scion. Limited available data, however, indicated that *Hevea* rootstocks can influence stomatal response of scion clones to water stress; they also differ in their adaptability to moisture availability[12,13]. Indirect evidence for the influence of rootstock on response to water stress was given by Combe and Gener[14] who reported that the use of GT 1 illegitimate seedlings as stocks in Ivory Coast with severe drought improved growth and yield of PR 107 scion compared to those on Tjir 1 rootstock. In contrast, literature on clonal response to drought fared slightly better than those on rootstock response. For instance in Brazil, da Conceicao[15] reported that various clones exhibited different stomatal response to soil water deficit. In India , drought tolerant clones were reported to grow better than those that are less drought tolerant[2].

More detailed information is needed on the response of *Hevea* monoclonal rootstocks to drought particularly in connection with quantity of their root system and how these characteristics are related to field performance of the rootstocks. The objectives of the present study are to compare growth, water relations, proline content in leaves and water use efficiencies of RRIM 901 scion clone grafted on three monoclonal rootstocks during prolonged cycles of drought stress.

MATERIALS AND METHOD

Three monoclonal seedling rootstocks of clones GT 1, RRIM 600 and RRIM 623 grafted with RRIM 901 scion clone were grown in plastic containers on 29 October 1993 when the buddings were at two-whorl stage. Each container (28cm diameter × 60 cm height) containing 34 kg of 1:4 mixture of Muchong series soil and sand incorporated with CIRP was placed on top of a concrete bench in the nursery. To allow root aeration, the side of the containers were drilled with 20 holes of 0.5 cm diameter each. To prevent rain water from percolating through the soil, the containers were covered with transparent polythene sheets tied around the base of the stem. The plants were watered daily and fertilised with kokei slow-release fertiliser every three months. To determine plant biomass at time of planting into plastic containers, five plants of each rootstock per replicate were harvested and dry matter of various plant parts determined after oven drying at 80°C.

Each rootstock was grown under three irrigation regimes : moderate water stress(W_1), severe water stress(W_2) and control (C). For W_1 and W_2 treatments, plants were subjected to continuous water deficit by irrigating with 1.5l water to field capacity every alternate days or once in four days, respectively. Control plants (C treatment) were irrigated daily. The plants were arranged in a randomised complete block design with four replications and three plants per plot.

Measurement of Water Relations Parameters

Leaf photosynthetic rates (PR) and water relations parameters such as leaf diffusive resistance (LDR), stomatal resistance (SC), transpiration rate (TR), leaf-water potential (LWP) and relative water content (RWC) of leaves were measured on healthy, unshaded and recently fully expanded leaves before and after the imposition of water stress. The measurement on three mature leaflets per plant

were carried out on consecutive days with the measurement for each replicate being done on separate days in order to eliminate any day to day variation. Pre-dawn LWP was determined from one leaflet per plant from 0600 h to 0700 h either just before re-irrigation or 24 h after re-irrigation of all treatments to determine the actual level of stress and recovery. With the exception of LWP and RWC which were measured between 0830 h to 0900 h , measurements of PR and other water relations parameters were carried out between 1100 h and 1230 h under natural light when light intensity was at least[16] 1000 μ Em^{-2} s^{-1}. Measurement of LDR, SC and TR were carried out using a steady state diffusion porometer (Li-1600, Li-COR, Nebraska, USA) whilst leaf PR was measured using ADC LDC-3 instrument. For determination of LWP, leaf samples were enclosed in a small plastic bag to minimise moisture loss and excised[17]. Leaf water potential of the wrapped leaves were measured with a pressure chamber (PMS Instrument Company, Corvallis, Oregon, USA) according to the method of Scholander *et al*[18]. The relationship between LWP and LDR were evaluated by correlation analysis to describe the rootstock responses to soil water deficits.

Measurement of Proline Content

Proline content of leaves were determined using the method of Bates *et al*[19]. Leaves from all treatments were sampled either on the same day when the plants were under different state of dehydration by virtue of the soil water regime they were subjected to or on different days when the plants were in similar state of hydration namely before and after re-irrigation. Eight leaf discs , sampled from leaves of each treatment in a replicate, were weighed before floating for 20 h on water. Besides floating on water, leaf discs prepared from leaves sampled when the plants were under different state of dehydration were also floated on polyethylene glycol. The leaf discs were extracted with 10 ml 3% (w/v) sulphosalicylic acid in pestle and mortar. The pooled homogenate was centrifuged at 500 × g for 10 min and the resultant filtrate was boiled with 2 ml glacial acetic acid and 2 ml acid ninhydrin for 1 h before terminating the reaction in an ice bath. After adding 4 ml toluene followed by vigorous shaking, the absorbance of the pink top layer was read at 520 nm using toluene as a blank in a spectrophotometer.

At the termination of the experiment, the plants were harvested and dry matter production and distribution to various plant parts determined after oven drying at 80°C. A leaf sub-sample was taken for leaf area measurement using a LI-COR area meter (Model LI-3000) and specific leaf area (SLA) calculated. This dried leaf sub-sample was ground to 40 mesh size and sent to Utah University, USA for analysis of carbon isotope composition {δ^{13} plant}. Carbon isotope discrimination ratio (CID) which is calculated as $- 8 - (\delta^{13}$ plant)[20] gives an estimate of water use efficiency (WUE) (ratio of carbohydrate accumulation to transpiration) integrated over a period of time[21].

All data were statistically analysed according to a completely randomised block design. Significance of differences between treatment means were assessed using Duncan multiple range test at $P<0.05$.

RESULTS

Water Relations Parameters

Pre-dawn leaf water potential. Pre-dawn LWP measured either before or after re-irrigation are shown in *Table 1*. On both occasions, pre-dawn LWP were significantly affected by rootstock and soil water regime. These

TABLE 1. EFFECT OF ROOTSTOCK AND SOIL WATER REGIME ON PRE-DAWN LEAF WATER POTENTIAL MEASURED BEFORE AND AFTER RE-IRRIGATION

Variable	Before re-irrigation	After re-irrigation
Rootstock		
GT 1	–2.68 a	–2.26 a
RRIM 623	–2.66 a	–2.23 ab
RRIM 600	–2.47 b	–2.15 b
Probability level	***	*
Soil water regime		
Severe water stress (W_2)	–3.66 a	–3.03 a
Moderate water stress (W_1)	–2.52 b	–2.27 b
Control	–1.73 c	–1.42 c
Probability level	***	***

Means with the same letters within columns are not significantly different
*, ***: F-test significant at P<0.05 or 0.001, respectively.

influences were very highly significant (P<0.001) with the exception of the determination made after re-irrigation when the rootstock effect was found to be significant at 5% probability level. There was no significant interaction between rootstock and soil water regime indicating that the variation in pre-dawn LWP between rootstocks is the same at each level of soil water regime. Before re-irrigation, plants grafted on GT 1 and RRIM 623 rootstocks had significantly lower pre-dawn LWP than had RRIM 600 rootstocks. This ranking in predawn LWP between rootstocks was maintained after re-irrigation indicating that during these two phases, RRIM 600 rootstock used significantly less water than did either GT 1 or RRIM 623 rootstocks. Generally, after reirrigation plants recovered their pre-dawn LWP to some extent although these values remained lower than the values of the control.

Before re-irrigation, pre-dawn LWP of plants under the 3 soil water regimes were significantly different from each other with plants grown under severe water stress (W_2) treatment having the lowest pre-dawn LWP whilst the well-watered plants (control) had the highest predawn LWP indicating that they were the least stressed. This difference in pre-dawn LWP between the 3 soil watering regimes were maintained after re-irrigation.

Data of pre-dawn LWP for each treatment were expressed as % of control to estimate recovery from stress. During the two phases (before and after re-irrigation), % predawn LWP was significantly affected by soil water regime and this influence was very highly significant (P< 0.001) *(Table 2)*. However, % pre-dawn LWP was not significantly affected by rootstock. Significant soil water regime X rootstock interactions (P<0.05) occurred before re-irrigation for % LWP. These interactions were caused by RRIM 600 rootstock having higher values than GT 1 rootstock in W_2 treatment but not in W_1 treatment. It is evident

TABLE 2 EFFECT OF ROOTSTOCK AND SOIL WATER REGIME ON % RECOVERY OF PRE-DAWN LEAF WATER POTENTIAL MEASURED BEFORE AND AFTER RE- IRRIGATION

Rootstock	Soil water regime (SWR)	Before re-irrigation	After re-irrigation
GT 1	Control (C)	100	100
	Moderate water stress (W_1)	145	167
	Severe water stress (W_2)	208	219
RRIM 600	Control (C)	100	100
	Moderate water stress (W_1)	146	163
	Severe water stress (W_2)	226	207
RRIM 623	Control (C)	100	100
	Moderate water stress (W_1)	148	153
	Severe water stress (W_2)	216	208
LSD (P<0 05)	SWR	6 3035	14 533
	SWR X Rootstock	11 537	NS
Level of probability	SWR	***	***
	Rootstock	NS	NS
	SWR X Rootstock	*	NS

NS, *, *** F-test non-significant or significant at P<0 05 or 0 001, respectively

that pre-dawn LWP of these two rootstocks under water stress failed to recover fully to control levels within 24 h after re-irrigation with the chances of recovery being more remote in the more severe water stress treatment

Leaf diffusive resistance, stomatal conductance, transpiration rate and photosynthetic rate *Table 3* shows that before imposition of water stress, SC were significantly affected by rootstock (P<0 05) whilst rootstock influence on PR was only marginal (P<0 10) There was however, no difference in LDR between rootstocks GT 1 rootstock produced higher SC resulting in higher PR of leaves than did either RRIM 623 or RRIM 600 rootstock

Water relations parameters measured four weeks after imposition of water stress are shown in *Table 4* Soil water regime affected LDR and TR whilst the effect on SC was only marginal (P< 0 10) Rootstock did not affect any of these water relations parameters LDR was greater in W_2 than in W_1 or C plants and consequently, SC and TR of the former plants were smaller than those of the latter plants

TABLE 3. EFFECT OF ROOTSTOCK ON STOMATAL CONDUCTANCE AND PHOTOSYNTHETIC RATE OF RRIM 901 SCION BEFORE IMPOSITION OF WATER STRESS

Rootstock	Stomatal conductance (cm/s)	Photosynthetic rate (μmole/cm^2/s)
GT 1	0.77 a	0.65 a
RRIM 600	0.67 b	0.61 ab
RRIM 623	0.66 b	0.52 b
Probability level	*	#

Means with the same letters within columns are not significantly different
#, *: F-test indicates significant at P<0.10 or 0.05. respectively

TABLE 4. EFFECT OF SOIL WATER REGIME ON LEAF DIFFUSIVE RESISTANCE, STOMATAL CONDUCTANCE AND TRANSPIRATION RATE OF RRIM 901 SCION AFTER IMPOSITION OF WATER STRESS

Soil water regime	LDR	SC	TR
Severe water stress (W_2)	5.65 a	0.49 b	10.92 b
Moderate water stress (W_1)	2.56 b	0.58 b	13.61 a
Control	2.06 b	0.61 a	14.57 a
Probability level	*	#	*

Means with the same letters within columns are not significantly different
#, *: F-test indicates significant at P<0 10 or 0 05, respectively.
LDR = leaf diffusive resistance; SC = stomatal conductance; TR = transpiration rate

Table 5 shows that 2 weeks after imposition of water stress, PR was significantly affected by soil water regime but not by rootstock. There was only marginal interaction between rootstock and soil water regime (P<0.10). The interaction effect was because severe water stress treatment reduced markedly PR of plants on RRIM 623 rootstock whilst PR of plants on the other 2 rootstocks were not affected by soil water regime. In general, both control and W_1 treatment gave comparable PR of RRIM 901 scion which were significantly higher than that of W_2.

To study rootstock effect on stomatal response to water stress, the relationship between LWP and LDR was determined for each rootstock by correlation analysis. Data were fitted with polynomial equations. Significant and positive relationship between LDR and LWP was only evident for GT 1 rootstock (r^2 = 0.5963*) indicating that stomata on this rootstock tended to open gradually with decreasing LWP. Such relationship was not evident for RRIM 623 and RRIM 600 rootstocks since their LDR values at decreasing LWP were rather erratic; this is mainly attributed to much of stomatal closure

TABLE 5. EFFECT OF ROOTSTOCK ON PHOTOSYNTHETIC RATE OF RRIM 901 SCION AFTER IMPOSITION OF WATER STRESS

Soil water regime (SWR)	Rootstock (R)	Photosynthetic rate (μmole/cm^2/s)
Control (C)	GT 1	0.93
	RRIM 600	0.73
	RRIM 623	0.68
	Mean	0.78
Moderate water stress (W_1)	GT 1	0.65
	RRIM 600	0.88
	RRIM 623	0.85
	Mean	0.79
Severe water stress (W_2)	GT 1	0.68
	RRIM 600	0.71
	RRIM 623	0.38
	Mean	0.59
LSD (SWR) and probabilty level		0.164 *
LSD (R X SWR) and probability level	0.284 #	

Means with the same letters within columns are not significantly different
#, *: F-test indicates significant at P<0.10 or 0.05, respectively

taking place at lower soil water deficit (LWP of about – 6 bars). This indicates that plants on these two rootstocks were more sensitive to changes in LWP than those on GT 1 rootstock.

Leaf Content of Proline

Proline content in leaf discs floated on distilled water and measured when the plants were in variable state of hydration are shown in *Table 6*. Proline content was influenced by soil water regime but not by rootstock. Generally, the highest proline content in leaves was given by W_2 plants whilst there was no difference in proline leaf content between control and W_1 plants. We observed very highly significant (P<0.001) variation between replicates indicating that plant water status was too variable for the rootstock effect to be expressed. After differences in cell hydration were removed by floating leaf discs on polyethylene glycol, there was no difference in leaf proline content either due to rootstock or soil water regime; however, in this analysis significant variation between replicates (P<0.05) were still evident.

The rootstock effect was evident when plant water status among treatments were standardised by doing leaf sampling either before or after re-irrigation[22]. *Table 7* shows that proline leaf content on all rootstocks increased 24 h after re-irrigation and that under

TABLE 6 PROLINE CONTENT IN LEAVES OF RRIM 901 CLONE AS AFFECTED BY SOIL WATER REGIME

Soil water regime	Proline content (μmole/g FW)	
	Leaf disc floated on water	Leaf disc floated on polyethylene glycol
Severe water stress (W_2)	66 8 a	70 78 a
Moderate water stress (W_1)	57 0 b	64 68 a
Control (C)	56 3 b	64 89 a
Probability level	*	NS

Means with the same letters within columns are not significantly different
#, * F-test indicates significant at P<0 10 or 0 05 respectively
LDR = leaf diffusive resistance, SC = stomatal conductance, TR = transpiration rate

TABLE 7 PROLINE CONTENT OF RRIM 901 SCION AS AFFECTED BY ROOTSTOCK AND STATE OF HYDRATION OF PLANTS

Rootstock	Before watering	After watering
GT 1	55 5 a	68 8 a
RRIM 600	50 8 ab	62 4 ab
RRIM 623	45 4 b	54 8 b
Probability level	*	*

Means with the same letters within columns are not significantly different
* F-test indicates significant at P<0 05

both phases, RRIM 623 rootstock consistently gave the lowest proline leaf content whilst the highest proline content of leaves was given by GT 1 rootstock

Growth

Growth and dry matter production of plant parts measured five months after planting in polybags and before imposition of water stress are shown in *Figure 1* RRIM 623 rootstock gave the highest total biomass production whilst the lowest total biomass production was given by GT 1 rootstock The better tree growth given by RRIM 623 rootstock was because both shoot and root growth were improved by the rootstock leading to higher root shoot ratio than given by the other two rootstocks *(Figure 2)* In contrast, poor shoot and root growth of plants on GT 1 rootstock contributed to poor total biomass production *(Figure 1)* Poor growth of RRIM 901 scion grafted on GT 1 rootstock could be attributed to different time of production since these plants were produced about two months later than the other buddings Plants on RRIM 600 rootstock with inter-

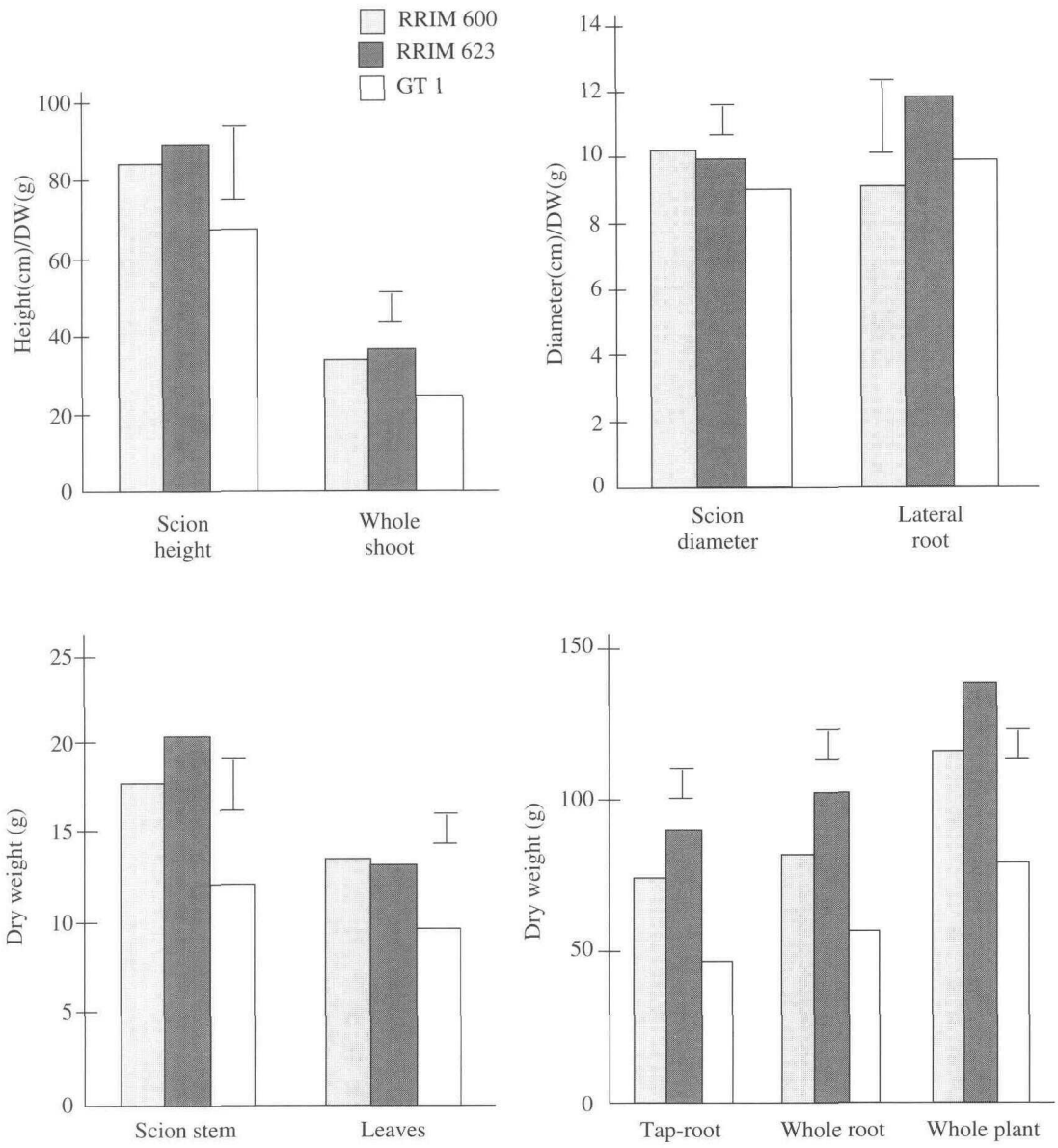

Figure 1. Effect of rootstock on scion height, diameter and dry weights of various plant parts before imposition of water stress. DW = dry weight; vertical bars indicate LSD values (P<0,05).

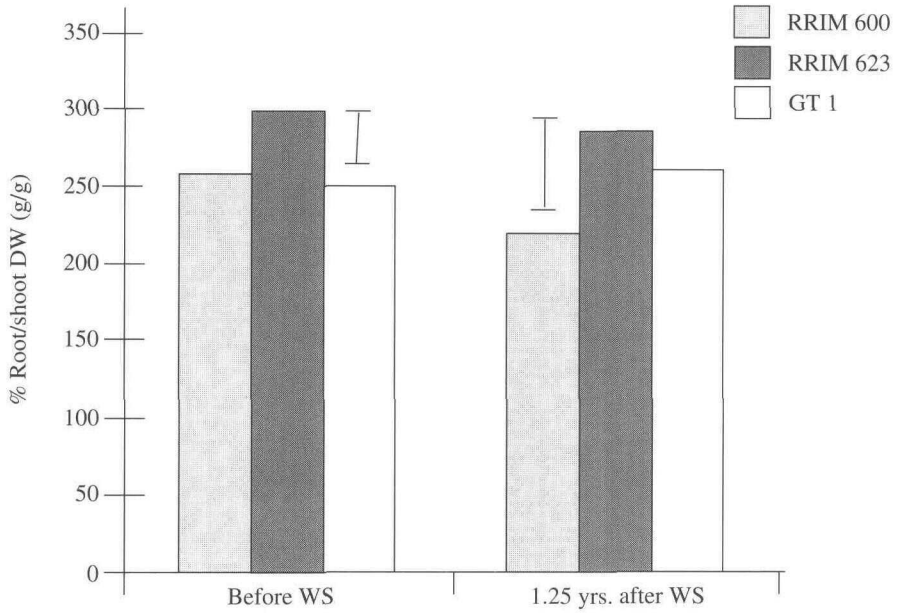

Figure 2. Effect of rootstock on root : shoot ratio before and after imposition of water stress. DW = dry weight, WS = water stress. Vertical bars indicate LSD values (P<0.05).

mediate total biomass production between these two rootstocks had better shoot than root growth, resulting in low root:shoot ratio which were comparable to that given by GT 1 rootstock. In comparison to GT 1 rootstock, RRIM 623 had significantly greater dry weight of lateral and tap-root and a higher proportion of dry matter that went to the root system *(Figures 2–3)*. Both RRIM 600 and RRIM 623 rootstocks had greater proportion of tap-root in the root system than had GT 1 rootstock; however, the reverse is true for the proportion of lateral root in the root system with GT 1 rootstock having a greater potential for production of these root components than did the other two rootstocks *(Figure 3)*. Rootstock had no influence on distribution of dry matter to scion stem. GT 1 and RRIM 600 rootstocks produced comparable proportion of dry matter in leaves which were significantly higher than that given by RRIM 623 rootstock.

Figures 2 and *4–7* show growth and dry matter production of the experimental plants harvested 1.25 years after treatment application. Soil water regime had marked influence on almost all aspects of growth such as height and biomass production whilst rootstock had substantial effect only on distribution of dry matter to shoot and roots and hence root:shoot ratio. There was no significant interaction between soil water regime and rootstock for these growth parameters. Plants on GT 1 and RRIM 623 rootstocks had greater allocation of dry matter that went to the root than to shoot thus resulting in higher root:shoot ratio (*Figures 2* and *4*). In contrast, plants on RRIM 600 rootstock had significantly lower

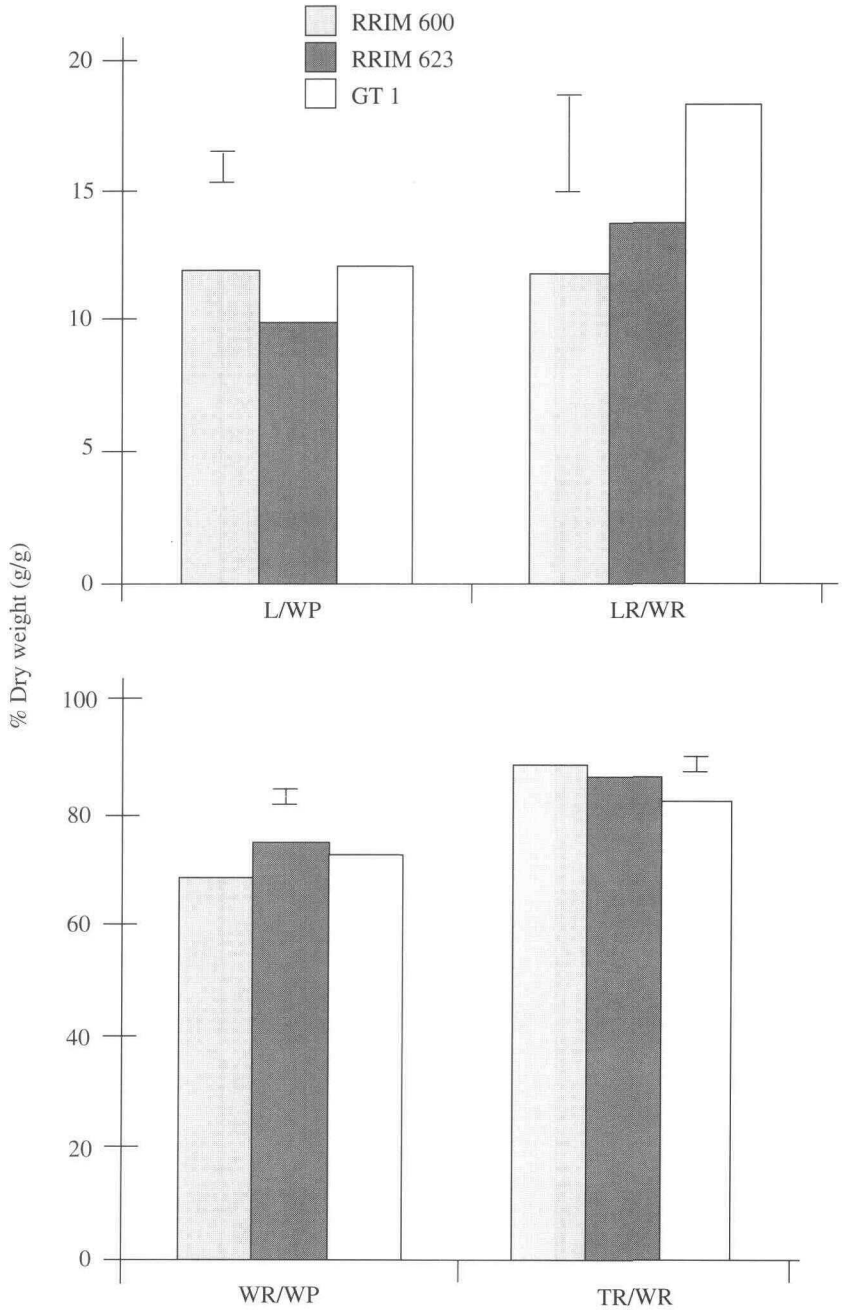

Figure 3. Effect of rootstock on percentage distribution of plant parts before imposition of water stress. L = Leaves; LR = lateral root; WR = whole root; WP = whole plant; TR = tap-root; vertical bars indicate LSD values (P<0.05).

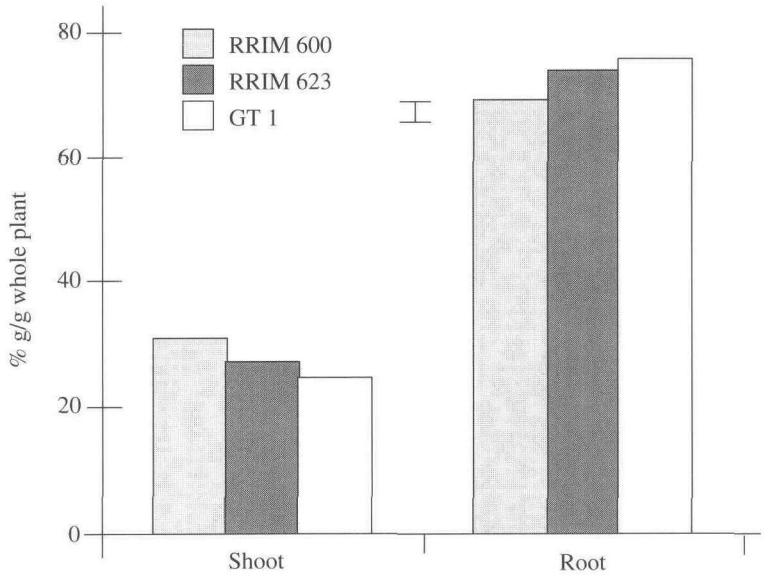

Figure 4. Effect of rootstock on percentage dry weight of shoot and root at 1.25 years after treatment application. Vertical bars indicate LSD values (P<0.05).

root:shoot ratio since dry matter was preferentially allocated to shoot rather than to root growth.

Generally, there was hardly any difference in growth between plants receiving daily irrigation (control) and those irrigated every alternate days (W_1 treatment) whilst substantial reduction in growth was observed for plants receiving irrigation once in 4 days (W_2 treatment) (*Figures 5–6*). Severe water stress reduced growth of whole root and its components (feeder and tap-roots) by about 14% compared to control. However, the effect on various components of scion growth was more variable with leaf area being the most severely affected by severe water stress as it was reduced by about 44% compared to control *(Figure 7)*. Dry weights of scion stem of W_2 treatment were about 79% of the control whilst diameter and height of scion stem were reduced by about 10% and 16%, respectively compared to control *(Figures 5–6)*. The reduction in LA of severely stressed plants resulted in substantially lower specific leaf area (SLA) of these plants which was reduced by about 31% compared to control *(Figure 7)*. The net effect of severe water stress on these growth parameters was a 24 and 17% decrease in total shoot and whole tree dry weight respectively *(Figures 5–6)*.

Carbon Isotope Discrimination Ratio (CID)

CID of RRIM 901 leaves ranged from 17.8‰ to 20.9‰ which were within the range observed for C_3 plants. CID was not significantly affected by either rootstock or soil water regime. There was also no significant interaction between rootstock and soil water regime.

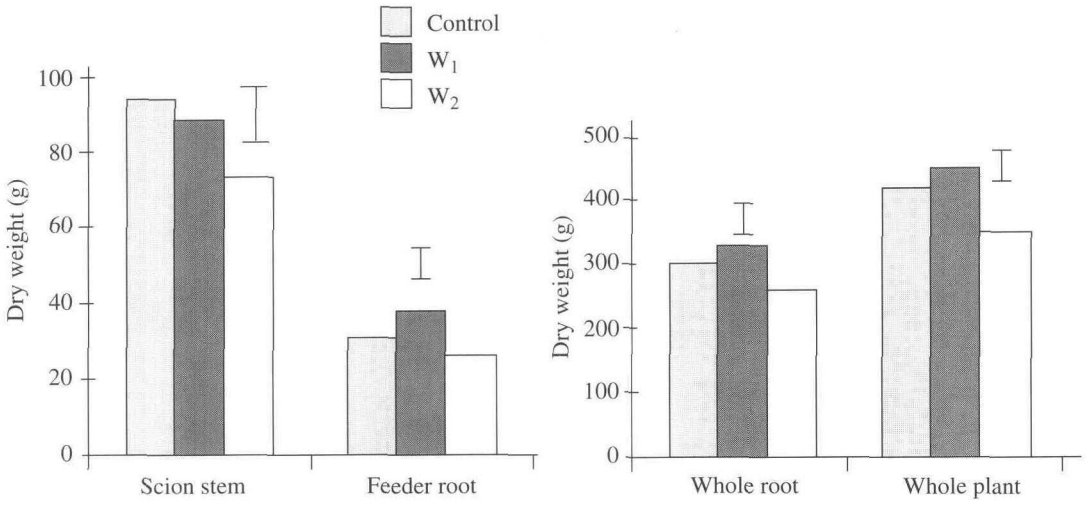

Figure 5. Effect of soil water regime on dry weight of plant parts at 1.25 years after treatment application. W_1 = moderate water stress treatment; W_2 = severe water stress treatment; vertical bars indicate LSD values ($P<0.05$).

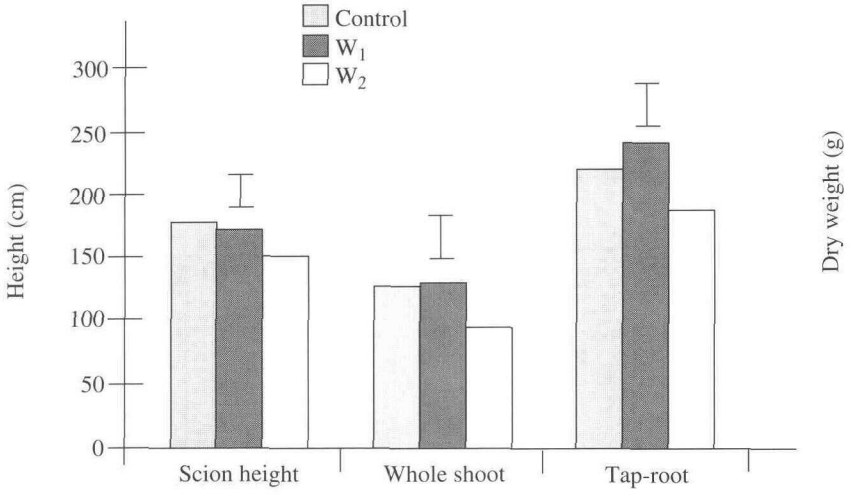

Figure 6. Effect of soil water regime on height and dry weight of whole shoot and tap-root at 1.25 years after treatment application; W_1 = moderate water stress; W_2 = severe water stress; Vertical bars indicate LSD values ($P<0.05$).

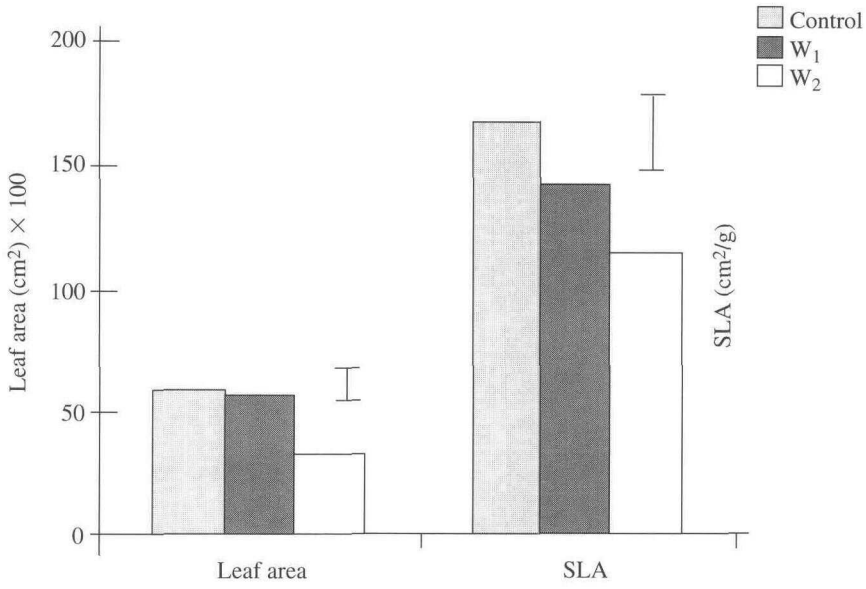

Figure 7. Effect of soil water regime on leaf area and specific leaf area (SLA) at 1.25 years after treatment application. W_1 = moderate water stress; W_2 = severe water stress; vertical bars indicate LSD values (P<0.05).

The relationship between CID of leaves and SLA of scion leaves, whole plant dry weight and distribution of dry matter to various plant parts were examined by correlation analysis to relate CID to photosynthetic capacity of leaves and productivity. CID was significantly and negatively correlated with SLA when data were combined over rootstocks and soil water regime *(Table 8)* although this relationship was rather weak (r^2 = 0.1941*). Subsequently, the regression analysis was conducted on data from either well-watered (C) or combined water stress (W_1 and W_2) treatment combined over rootstocks since the absence of any significant interaction between soil water regime and rootstock indicates that rootstock differences for CID were consistent across the soil water regime. The correlation between CID and specific leaf area was improved for well-watered treatment (r^2 = 0.5072**) but became insignificant under water stressed conditions. This indicates that the specific leaf area *vs.* CID relationship is not stable across the watering treatments.

Correlation analysis on data from all treatments showed significant and positive relationship between CID and whole plant dry weight and percentage dry weight of roots. However, CID *vs.* percentage dry weights of shoots and scion stem correlations were significant and negative *(Table 9)*. These correlations were improved when the analysis was done on water-stress treatments only where r^2 ranged between 0.4614 and 0.6168 but no significant correlations were found for well-watered treatments.

TABLE 8. LINEAR RELATIONSHIP BETWEEN CARBON ISOTOPE DISCRIMINATION RATIO AND SPECIFIC LEAF AREA AVERAGED OVER THREE ROOTSTOCK

Soil water regime	N	Regression equation	R^2
All treatments (control plus water stress)	24	CID = 20.9261 – 0.0106 SLA	0.1941 *
Mild and severe water stress	12	–	0.0266 NS
Control	12	CID = 21.7572 – 0.014726 SLA	0.5072 **

NS, *, ** Correlation coefficient non-significant or significant at $P<0.05$ or <0.01, respectively
CID = Carbon isotope discrimination ratio; SLA = specific leaf area; N = number of samples.

DISCUSSION

Severe water stress caused several changes in many plant processes in *Hevea* buddings including:

- Reduction in growth parameters such as girth and diameter of scion, biomass in scion stem, tap-root, fibrous root, and consequently whole tree dry weight.
- Reduction in the size of assimilatory apparatus such as leaf area and hence SLA.
- Reduction in plant water status as reflected by pre-dawn LWP.
- Slow recovery of plant water status after re-irrigation.
- Increased stomatal resistance to gaseous exchange leading to lower SC, PR and TR of leaves.
- Increased root:shoot ratio.

Many of these physiological responses to water stress are consistent with reports on many other species of woody plants[23–26]. Since the statistical interaction of rootstock and soil water regime was found to be non-significant for most of the determinations, it can be concluded that the rootstock and soil water regime were independent in their effect on these parameters.

Regardless of soil water regime, rootstocks too had pronounced effect on water relations, proline content of leaves and disribution of dry matter to shoot and root, hence root/ shoot ratio. RRIM 901 scion clone tended to have variable stomatal sensitivity to water stress depending on the rootstocks they were grafted onto. Plants on RRIM 623 and RRIM 600 rootstock appear to have drought avoidance characteristics since they responded to water stress by rapid stomatal closure on the onset of water stress, protecting the plants from water loss. Similar response has been reported for many crops such as siratro[26] and tomatoes[27]. In the present study, plants on RRIM 623 and RRIM 600 rootstock attained threshold LWP values (LWP at which stomatal closure begins[28]) at about –5.5 and –6, bars respectively. In contrast, plants grafted on GT 1 rootstock tended to exhibit drought tolerant characteristics as indicated by gradual stomatal closure with decrease in LWP such that the threshold LWP were attained beyond –10 bars.

TABLE 9 LINEAR RELATIONSHIP BETWEEN CARBON ISOTOPE DISCRIMINATION RATIO AND VARIOUS GROWTH PARAMETERS IN EXPERIMENT WS 93

Source of data	Growth parameter (x)	N	Regression equation	r^2	Level of probability
Combined data of SWR	Whole plant DW	25	CID = 15 9350+0 0081 x	0 4440	***
	% shoot DW	25	CID = 21 4442–7 2873 x	0 1962	*
	% root DW	25	CID = 14 1569+7 2873 x	0 1962	*
	% stem DW	25	CID = 22 2217–13 7014 x	0 1929	*
Control treatment	Whole plant DW	12	–	0 2529	#
	% shoot DW	12	–	0 0032	NS
	% root DW	12	–	0 0032	NS
	% stem DW	12	–	0 0277	NS
Water stress treatments	Whole plant DW	13	CID = 15 8747+0 0084 x	0 6036	**
	% shoot DW	13	CID = 22 8965–11 9412 x	0 6168	**
	% root DW	13	CID = 10 9553+11 9412 x	0 6168	**
	% stem	13	CID = 23 9149–22 6413 x	0 4614	*

NS, #, * ** *** Not significant or significant at 0 10, 0 05 0 01 or 0 001 probability levels, respectively

Before imposition of water stress, PR and SC of plants grafted on GT 1 rootstock were significantly higher than those on RRIM 623 rootstock, this ranking in PR persisted during severe water stress *(Table 5)* since stomata of plants on the former rootstock remained open much longer during stress than did those on RRIM 623 rootstock However, PR of plants on RRIM 600 rootstock did not appear to be inhibited despite having effective stomatal control during water stress which would minimise water loss With a potential for continued photosynthesis during stress, plants on GT 1 and RRIM 600 rootstock would thus be able to maintain their productivity during the unfavourable period of growth For plants on RRIM 623 rootstock, the advantage of reduced water loss *via* increased resistances to water vapour movement is offset by the simultaneous reduction in CO_2 assimilation due to the accompanying higher resistances to CO_2 entry into the leaves Despite having better stomatal control which reduces water loss at the expense of C assimilation for photosynthesis, these plants did not turn this advantage to have better WUE than those on the other two rootstocks In peach, seedling rootstock was reported to be less efficient in water use than did clonal rootstocks[7] In black spruce, Blake and Sutton[29] have also reported differences in predawn LWP, SC and PR between rootstocks grown in containers which were related to their ability to adapt to drought

Although water use efficiency as estimated by CID was not significantly affected by both rootstock and soil water regime, CID was found to be associated with SLA *(Table 8)* and biomass of plant parts *(Table 9)* The negative correlation between CID and SLA indicates that treatment with lower CID (greater WUE) had higher SLA (thinner leaves) In peanut, Rao and Wright[30] obtained strong positive correlation between SLA and CID, this

relationship was stronger in location with distinct drought than in wetter location The relationship between CID and % dry weights of shoot and root in the present study suggests that cultivars with high WUE tend to allocate more dry matter to shoot rather than root growth The positive relationship between CID and whole tree biomass suggests that the WUE characteristics in *Hevea* is not influenced by the photosynthetic capacity of the tree[30] This hypothesis is supported indirectly by a significant negative correlation between SLA and ID mentioned earlier Hence it can be concluded that high WUE in *Hevea* would not necessarily lead to improved plant productivity[31]

Proline accumulation in leaves on GT 1 rootstock during severe water stress indicates occurrence of osmotic adjustment, this is yet another drought resistant characteristic exhibited by this rootstock[32] Plants on RRIM 600 rootstock also had a greater tendency to accumulate proline in their leaves unlike those on RRIM 623 rootstock *(Table 7)* This mechanism helps to maintain turgor in the growing regions of shoot and roots[33 34] and hence sustain metabolic activity as water potentials fall Osmotic adjustment also allows stomata to remain partially open[35 36] and net photosynthesis to continue[37] as observed for plants on GT 1 and RRIM 600 rootstocks *(Table 5)* In plants grafted on RRIM 623 rootstock, turgor was probably maintained by stomatal closure on the onset of water stress

Both GT 1 and RRIM 623 rootstock produced lower pre-dawn LWP than did RRIM 600 rootstock and this difference was especially distinct before re-irrigation *(Table 1)* As pre-dawn LWP gives indication of water depletion from deeper soil layer[38], it can be concluded that plants on GT 1 and RRIM 623 rootstock experienced substantial uptake of soil water than did those on RRIM 600 rootstock The greater water removal by plants on the former rootstocks is associated with their larger root system in relation to whole tree biomass as compared to those on RRIM 600 rootstock *(Figure 4)* In spruce and jack pine, shoot xylem pressure potential was shown to be related to root system conductivity as evident by elongation of new unsuberised roots[39] In the present study, RRIM 623 rootstock consistently had high root shoot ratio observed before treatment application and this characteristic persisted even after 1 25 years of cyclical water stress *(Figure 2)* GT 1 rootstock also had high root shoot ratio at the termination of the experiment although this characteristic was not obvious at the start of the experiment This discrepancy is most probably explained by a slight check in their early growth, as evident from their poor initial whole plant growth *(Figure 1)* since GT 1 seeds were germinated about 2 weeks later than seeds of the other two rootstocks High root shoot ratio is said to be associated with the development of a massive rootstock and not to large numbers of absorbing roots[2] This conclusion is confirmed by our results which indicate that tap-root constituted between 81%–88 % of whole root biomass *(Figure 3)* Further support came from results of correlation analysis between dry weight of root components (fibrous lateral and tap-root) with dry weight of whole roots which showed that the main bulk of whole root biomass of plants used in our study is contributed mainly by the tap-root (r^2 = 0 9466***) whilst the contribution by fibrous root is small (r^2 = 0 3721***) Hence it can be concluded that both RRIM 623 and GT 1 rootstock have potential to develop into deep and widely spreading root system This would give advantage to the two rootstocks over RRIM 600 rootstock, especially during drought since they would be more effective in exploring soil water resources to meet water requirement of the plants[4] It is expected that plants on RRIM 623 and GT 1 rootstocks would be able to postpone

drought for a longer period due to a higher water uptake than would those on RRIM 600 rootstock High root shoot ratio would also ensure sufficient hormones[40,41] such as cytokinin and gibberelin and carbohydrate reserves in roots[42] available for plant regrowth when moisture conditions improved Our results indicate that root shoot ratio is an inherent characteristics of *Hevea* monoclonal rootstocks since they were not much affected by environmental conditions (no significant soil water regime x rootstock interaction) Similarly, in other tree seedlings such as eucalypt species and loblolly pine, root shoot ratio was also reported to be genetically determined[43,44] as they were not affected by different environmental conditions In apples, Rogers and Vyvyan[45] had also reported differences in size of root system between rootstocks although there was no difference in root depth

In the present study, differences in scion growth due to the three rootstocks were not evident although these rootstocks are known to have substantial effect on scion growth[11] This lack of significant difference could be attributed to the restriction in girthing arising from the use of masking tape to secure the polythene sheet around the base of the stem throughout the duration of the experiment

In the present study, all rootstocks failed to recover to their original pre-dawn LWP within 24 h after re-irrigation *(Table 1)* with plants under severe stress showing the slowest recovery Rodrigues *et al*[46] have also reported that plant-water status of lupin plants under prolonged water stress did not recover to values of well-watered plants Under moderate water stress, slow recovery to control plant water status is attributed to internal redistribution of water to other plant organs and tissues[47] or because the absorbing system had not yet regained its full efficiency after being subjected to water stress[48] However, plants under severe water stress are more likely to suffer injury from dessication such as impairment of water conduction in the vascular system[27] Large xylem tension developed during severe stress was thought to induce cavitation in the xylem leading to increased xylem resistance to water flow[49]

CONCLUSION

In the study reported herein, root production of *Hevea* rootstock is an important factor influencing tree growth and adaptability to drought The three rootstocks are able to influence stomatal response of scion to drought and this falls into two categories drought avoidance (RRIM 623) or drought tolerance (GT 1) Differences in proline leaf content between rootstocks indicate the possible involvement of of osmoregulation in response to water stress which could play a role in the adaptation to drought in GT 1 and to a smaller extent, RRIM 600 rootstock

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