

*Full Length Research Paper*

# Involvement of rootstocks and their hydraulic conductance in the drought resistance of grafted rubber trees

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Improving drought resistance of rubber trees has become a pressing issue with the extension of rubber plantations and the prevalence of seasonal drought. Root system is vital to water and nutrients uptake of all plants, therefore, rootstocks could play decisive roles in drought resistance of grafted rubber trees on a specific scion clone. To investigate the responses of different clone rootstocks and their grafted trees to water stress and find applicable methods for selecting drought resistant rootstocks, seven related parameters and root hydraulic properties of both seeds originated and grafted saplings of PB86, PR107, RRIM600 and GT1 were measured to assess their drought resistance. It was shown that the rootstock drought resistance and root hydraulic conductance may improve the drought resistance of the grafted rubber trees. Among the four clone rootstocks, GT1, which demonstrated more resistant to drought and higher root hydraulic conductance, was comparatively resistant to drought both for the seed propagation seedlings and grafted saplings. In addition, studies on the grafted saplings with different root hydraulic conductance further validated the possibility of selecting drought resistant rootstocks on the basis of rootstock hydraulic conductance using a high-pressure flow meter.

**Key words:** Rubber tree (*Hevea brasiliensis*), rootstock, hydraulic conductance, drought resistance, high-pressure flow meter.

## INTRODUCTION

With the extension of rubber (*Hevea brasiliensis*) plantations to suboptimal regions and due to global climate change, drought incidence was recently boosted in all rubber tree planting countries (Sangsing et al.,

2004a) and large scale severe drought disasters occurred almost every year in recent years in major rubber tree planting areas in China, Thailand and India. Consequently, developing drought-efficient planting materials and identifying reliable and pertinent techniques to improve rubber tree drought resistance have been of great interest to both rubber tree researchers and farmers.

Grafted saplings are almost the only current planting material in all rubber plantations. A grafted rubber tree consists of two different genetic components: a scion elected by breeding program and a rootstock collected from a rubber clone plantation. Normally, selecting a good scion cultivar has been the focus of the research and it is not unusual that several decades are required for a successful selection in order to increase the yield or stress resistance. However, rootstocks which are propa-

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**Abbreviations:** PEG, Polyethylene glycol; RWC, relative water content; REC, relative electrical conductivity; MDA, malondialdehyde; SOD, superoxide dismutase content; Pr, free proline content; SS, soluble sugar content; SP, soluble protein content; HPFM, high-pressure flow meter.

gated by seeds are seldom selected.

Previous studies indicated that rubber trees with good rootstocks which are characterized by 'strong vigor' presented strong growing abilities, high yield and more resistant to stresses. For example, Ng et al. (1981) and Cardinal et al. (2007) claimed that rootstock could significantly promote the growth and yield of the scions for up to 18 or 20%. Ahamad (1999) showed that the rootstocks could alter the growth and water use efficiency of different rubber tree clones, while scions on GT1 and RRIM 623 rootstocks are better adapted to prolonged drought than those on RRIM 600 rootstocks by virtue of their deep rooting characteristic. Sobhana et al. (2001) revealed that interstocks could affect the growth and physiological performance of the grafted rubber trees. Therefore, Cardinal et al. (2007) insisted that unselected seedlings should not be used as rootstocks due to their very low compatibility for dry rubber yield. Nevertheless, how rootstocks affect the grafted rubber trees and how 'strong vigor' rootstocks are selected have not been extensively investigated in the literature, particularly in relation to drought resistance.

The ability of hydraulic system to supply water to the leaves determines the plant water potential and controls plant stomatal conductance, photosynthetic carbon assimilation, growth traits and yield (Sperry, 2000; Tyree, 2003). Furthermore, it is proved that the drought resistance of rootstocks could affect the water relationship and growth performances of grafted mango (Reddy and Singh, 1993), peach (Luis et al., 2006; Solari and Dejong, 2006), pistachio (Gijon et al., 2010), citrus (Rodriguez-Gamir et al., 2010), coffee (Silva et al., 2010) and kiwifruit (Clearwater et al., 2007). Seed propagated rootstock provides grafted rubber trees with a root system that is essential to the water and nutrients absorption. Consequently, the characteristics of root hydraulic conductance could determine the vigor and abiotic stress resistance of the grafted rubber trees on a specific cultivar and thus, act as a promising parameter for selecting drought resistant rootstocks for the prevailing cultivars. However, although it was reported that the hydraulic properties were different from rubber tree clones (Ranasinghe and Milburn, 1995; Sangsing et al., 2004a, b) and could explain their variations in stomatal conductance and growth performances (Sangsing et al., 2004a, b), these results are all on scions. Whether rootstock drought resistance and hydraulic conductance can improve the drought resistance of the grafted rubber trees was not studied.

In this paper, root hydraulic conductance and other seven parameters related to drought resistance on both illegitimated seedlings and grafted saplings were compared to investigate the possibility of selecting drought resistant rootstocks on the basis of rootstock hydraulic conductance. Furthermore, validation was conducted on the categorized grafted saplings of unselecting rootstocks. To our knowledge, this is the first result of selecting drought resistant rootstock on the basis of root

hydraulic conductance with high-pressure flow meter.

## MATERIALS AND METHODS

### Plant materials

#### Characteristics of clones involved in this study (Huang, 2005)

PB86 is an old primary clone developed in Malaysia and has been very popular in China for many years. Its ten-years-average-yield is 1105 kg·ha<sup>-1</sup>·year<sup>-1</sup>. However, it is not resistant to wind and cold weather.

PR107 is an old primary clone selected in Indonesia and its ten-years-average-yield is 1470 kg·ha<sup>-1</sup>·year<sup>-1</sup>. It is suitable for ethrel stimulation tapping and windy area.

RRIM600 is a high yielding clone evolved by the Rubber Research Institute of Malaysia with the parents of Tjir1×PB86. It has been extensively cultivated previously in Indian and Chinese rubber plantations for its fast growing rate before tapping. Its ten-years-average-yield is 1710 kg·ha<sup>-1</sup>·year<sup>-1</sup> and it is the main rootstock source of China.

GT1 is a primary clone developed in Indonesia and widely used in Indonesia and Yunnan Province of China. Its ten-years-average-yield is 1396 kg·ha<sup>-1</sup>·year<sup>-1</sup> and it has been identified as a drought and cold resistant clone. However, its initial yield is relatively low and is not suitable for windy areas.

CATAS7-33-97 is evolved by Rubber Research Institute of China with the parents of RRIM600×PR107. It is widely planted now in the rubber plantations of Hainan Province of China for its high and steady yield of 1959 kg·ha<sup>-1</sup>·year<sup>-1</sup> from ten years measurements.

### Different clone seedlings

The fresh, full seeds of PB86, PR107, RRIM600 and GT1 were collected from rubber tree seed collection area in Hainan. After sterilizing, the seeds were sowed on sand beds. When the first whirl leaves were in stable stage, the seedlings of similar heights and growth performances were culled and cultured with complete Hoagland solution in a glass greenhouse for 60 to 65 days. In the period of water culture, the Hoagland solution was exchanged once every two weeks and was aerated every day.

At the end of the solution cultured, the seedlings were transplanted to the Hoagland solution with different concentrations of PEG6000 to simulate water stress conditions. The concentrations of PEG6000 were 0, 5, 10, 15 and 20%, and the corresponding water potentials were approximately 0, -0.1, -0.3, -0.4 and -0.6 (MPa), respectively (Michel and Kaufmann, 1973). The seedlings were cultured for about 24, 36 and 48 h, then their stable leaves in the second whirl were used for measuring the physiological parameters related to drought resistance, that is, leaf relative water content (RWC), relative electrical conductance (REC), free proline accumulation (Pr), content of soluble sugar (SS), content of soluble protein (SP), content of malondialdehyde (MDA) and the activity of superoxidisedismutase (SOD). At the same time, the roots were also sampled to measure root hydraulic conductance and root vigor. Five seedlings in each of the three repetitions were measured in all measurements.

### Grafted saplings with different clone rootstocks

PB86, PR107, RRIM600 and GT1 seeds were collected from the seed collection area and were cultivated in the plastic bags for about three months. When two whirls of leaves were stable, CATAS7-33-97 buds were grafted on the seedlings that had similar heights and growth performances. The grafted saplings were then

cultivated to be the poly bagged saplings. After the grafting, saplings were cultured roughly for another four months until there were two whorls of stable leaves. Then they were cultured in a greenhouse with complete Hoagland solution mentioned earlier for 7 days as described earlier. After that, half of them were treated with 20% PEG6000 for 48 h to simulate the water stress. At the end of the treatments, the drought resistance related physiological parameters were examined again on both treated and untreated saplings.

### Grafted saplings with mixed unselected rootstocks

Poly bagged grafted saplings were collected from a nursery of Rubber Research Institute, Chinese Academy of Tropical Agricultural Sciences. The scions were all CATAS7-33-97. The rootstocks of the saplings were derived from mixed unknown clone seeds as the conventional culture process in nurseries. 120 poly bagged grafted saplings with two whorls of stable leaves that had similar heights and growth performances were initially sampled. After the root hydraulic conductance was measured, three groups of saplings were selected as the materials for further study. There were 20 plants in each of the three groups on the basis of their whole root hydraulic conductance, namely, high whole root hydraulic conductance (HRHC), middle whole root hydraulic conductance (MRHC) and low whole root hydraulic conductance (LRHC). Later on, after each group of the saplings was cultured in greenhouse with the complete Hoagland solution for 7 days, half of them were treated with 20% PEG6000 for 48 h. At the end of the treatments, the drought resistance related physiological parameters were examined again on both PEG treated and untreated saplings.

## Methods

### Root vigor

Root vigor was determined by 2,3,5-triphenyltetrazolium chloride (TTC) method (Yan et al., 2010) with little modification. Briefly, root apex (0.5 g) was harvested and incubated at 37°C in 10 ml 0.4% TTC solution and 10 ml 0.1 M phosphate buffer for 1 h, then 2 ml 1 mM sulfuric acid were added to terminate the reaction. Afterward, root tissue was taken and homogenized in 5 ml ethyl acetate with a little of quartz sand with a mortar and pestle. The extract was put in a 10 ml volumetric flask and then the volume was left to settle. The absorbance of the extract was then assayed with a UV-Vis spectrophotometer (UV1102, Shanghai) at 485 nm and the root vigor was calculated according to standard curve.

### Root hydraulic conductance

For un-grafted seedlings, root hydraulic conductance was measured in a pressure chamber (Soil Equipment Co, USA) as described by Javot et al. (2003) with some modifications. Root system detached from the stem was inserted into a container filled with the complete Hoagland solution in the pressure chamber. The cut stump was put carefully through the soft plastic washer of the metal lid and sealed onto the pressure chamber. The balance pressure ( $P_0$ ), which was the ex-pressure when the sap exuded initially, was determined first. Then the pressure was increased from  $P_0$  (MPa) to  $P_0 + 0.5$  (MPa) with an increment of 0.1 MPa. Under each pressure, when the flow rate was stabilized (about 5 min), the exuded sap ( $V$ ,  $m^3$ ) was collected for 5 min. The collections were repeated at least three times at an interval of 1 min. The weight of the exuded sap was determined using an analytical balance with an accuracy of 0.1 mg. After the measurements, the roots were dyed with 0.2 mM methylene blue solution and the whole roots surface

areas ( $S$ ,  $m^2$ ) were determined by using a WinRHIZO root image analysis system (Regent instruments Inc. Canada). The flow rate  $J_v$  ( $m \cdot s^{-1}$ ) was calculated by  $J_v = V/(S \times t)$ . Root hydraulic conductance,  $L_{pr}$  ( $m \cdot s^{-1} \cdot MPa^{-1}$ ), was determined from the slope of the regression line by plotting  $J_v$  against hydrostatic pressure, that is,  $L_{pr} = \Delta J_v / \Delta P$ .

For the grafted saplings, root hydraulic conductance was determined on the intact saplings with high-pressure flow meter (HPFM, Dynamax, USA) under the "transient mode" (Bogeat-Triboulot et al., 2002). Firstly, a narrow ring of bark at the top of the rootstock stump was removed under water to improve the seal tightness. Subsequently, the rootstock stump apex was kept under water and mounted to the HPFM. After that, the sapling was kept in poly bag and subjected to an injection pressure increase from 0 to 0.5 MPa. Whole root hydraulic conductance,  $K_r$  ( $mmol \cdot s^{-1} \cdot MPa^{-1}$ ), was the slope of the regression line of rootstock water flow over the applied pressure.

### Leaf relative water content

The stable leaves in the second whorl of the seedlings were chosen in the measurement. After measuring the fresh weight (FW, g), the leaves were dipped in deionized water for more than 24 h to get the saturated fresh weight (SFW, g), then the leaves were killed for 1 h at a temperature of 105°C and dried at a temperature of 80°C in an oven for 24 h to get the dry weight (DW, g). The RWC of the leaves were calculated by the function of  $RWC = (FW - DW) / (SFW - DW) \times 100$  (Sharma et al., 2011).

### The relative electrical conductivity

The REC represents the leaf membrane injury degree of the trees under water stress. In this study, It was calculated by measuring the leaf plasma electrical conductivity with electrical conductivity meter (EC214, Hanna Instrument, Italy) before and after the leaf discs were boiled according to Sharma et al. (2011).

### The activity of superoxide dismutase

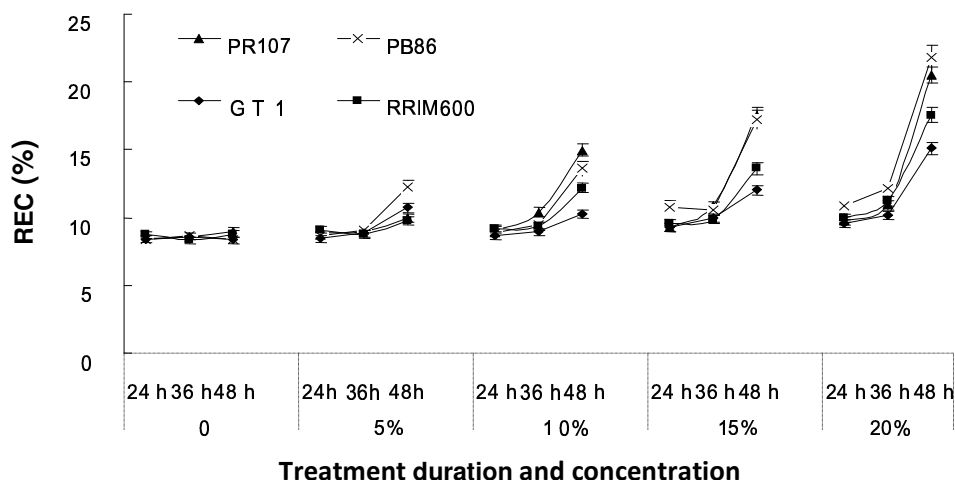
The nitroblue tetrazolium reduction method as described by Sharma et al. (2011) was employed to determine the activity of leaf SOD.

### Contents of malondialdehyde, free proline, soluble sugar and soluble protein

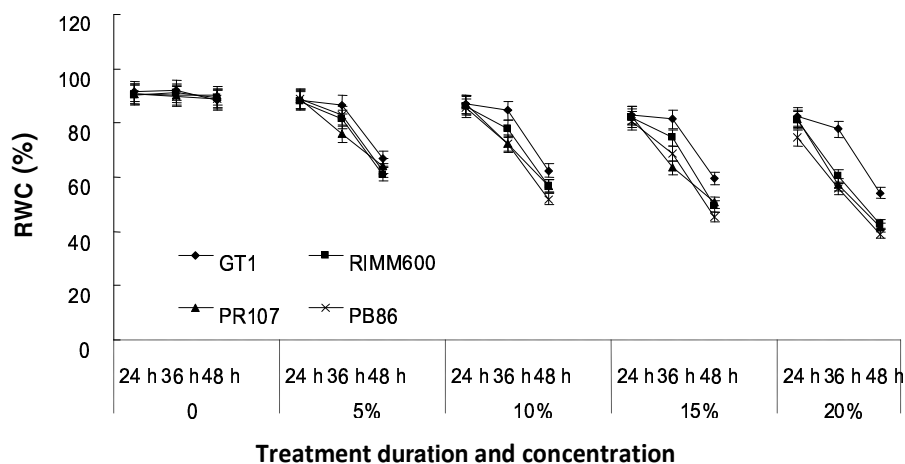
The content of MDA was examined with thiobarbituric acid colorimetry method developed by Kramer et al. (1991); the content of free proline ( $P_r$ ) was determined by using triketohydrindene hydrate colorimetry method as described by Sharma et al. (2011); the content of soluble sugar was measured with anthrone colorimetry method as reported by Watanabe et al. (2000) and the content of soluble protein was determined by using Coomassie brilliant blue G250 staining method proposed by Jones et al. (1989).

### Assessing drought resistance with subordinate function

The subordinate function of  $U_{ij} = (X_{ij} - X_j^{\min}) / (X_j^{\max} - X_j^{\min})$  was used to evaluate the drought resistance of the seedlings. In the function,  $U_{ij}$  represents the subordinate value of the parameter  $j$  for the clone  $i$ ;  $X_{ij}$  is the value of the parameter  $j$  for the clone  $i$ ;  $X_j^{\min}$  and  $X_j^{\max}$  is the minimum and maximum value of parameter  $j$  measured in all clones separately. In the calculation, when there is



**Figure 1.** Variation of membrane injury rate of *H. brasiliensis* seedlings under PEG drought stress.



**Figure 2.** Variation of leaf relative water content of *H. brasiliensis* seedlings under PEG drought stress.

a negative correlation between the parameter and drought resistance, the function will be changed to  $U_{ij} = 1 - (X_{ij} - X_j^{\min}) / (X_j^{\max} - X_j^{\min})$  (Zeng et al., 2010).

#### Statistics analysis

All measurements mentioned earlier were repeated three to five times and the data were analyzed using Microsoft Excel 2003 and DPS6.55 software.

## RESULTS

### Variation of several drought resistance related physiological parameters in the different Hevea clone seedlings

Figures 1 to 7 show that variation in all parameters

examined for the four clone seedlings had a similar trend when they were subjected to different PEG concentration (0, 5, 10, 15 and 20%) and treatment duration (24, 26 and 48 h). With prolonged water stress in a specific PEG concentration, the relative electrical conductance (Figure 1), MDA accumulation (Figure 3), soluble sugar (Figure 4) and free proline accumulation (Figure 6) tend to increase, while the relative water content (Figure 2), soluble protein (Figure 5) and the activity of SOD (Figure 7) decreased. There were also some discrepancies of these parameters among clones, which indicate that different clone seedlings adopt different strategies in response to drought stress. For example, the significant higher leaf RWC, free proline, soluble sugar and SOD and lower REC and MDA in GT1 seedlings than those in others, showed that GT1 had a higher osmotic adjustment and drought tolerant ability than the other three clone

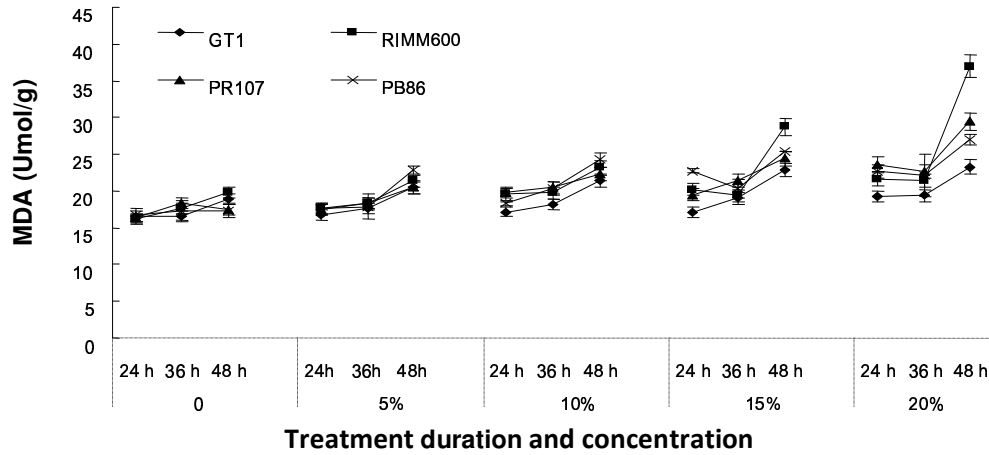


Figure 3. Variation of leaf MDA content of *H. brasiliensis* seedlings under PEG drought stress.

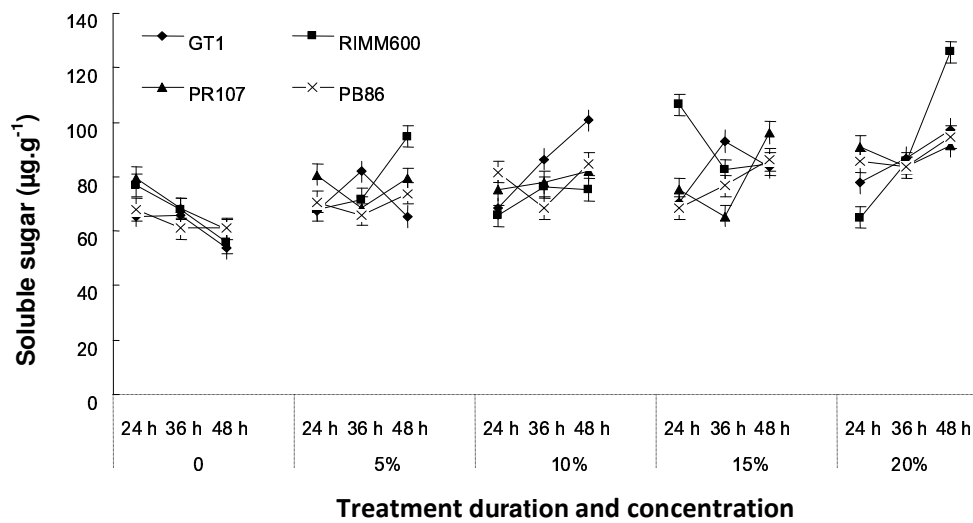


Figure 4. Variation of leaf soluble sugar content of *H. brasiliensis* seedlings under PEG drought stress.

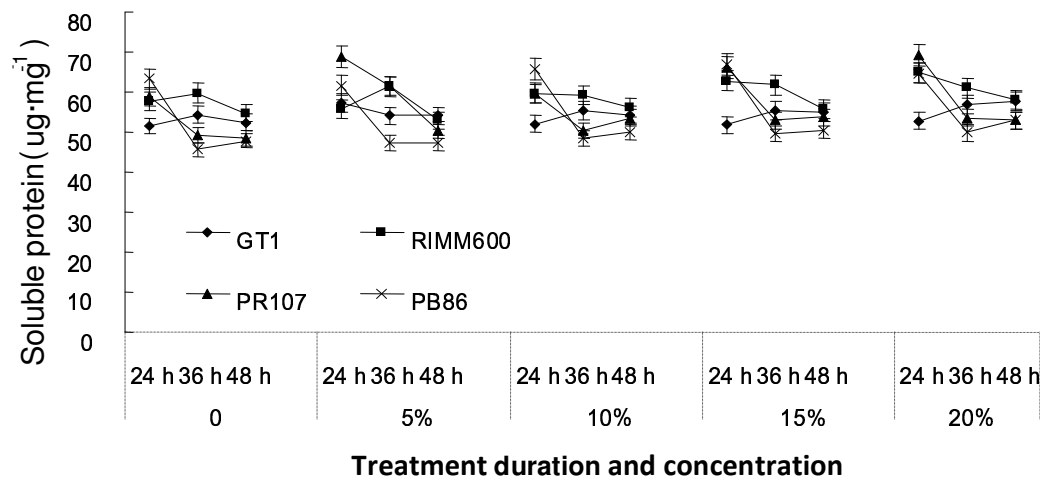


Figure 5. Variation of leaf soluble protein content of *H. brasiliensis* seedlings under PEG drought stress.

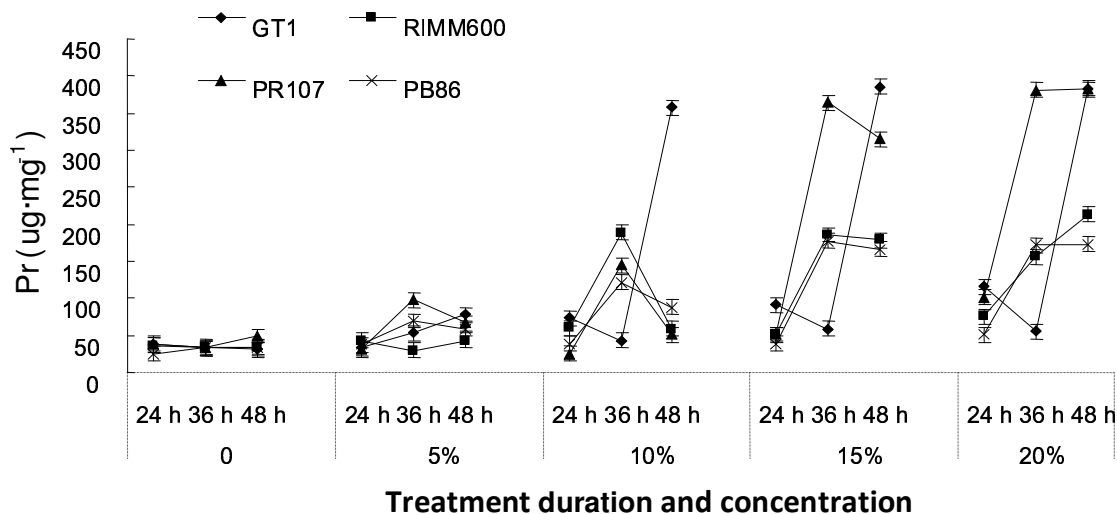


Figure 6. Variation of leaf free proline content of *H. brasiliensis* seedlings under PEG drought stress.

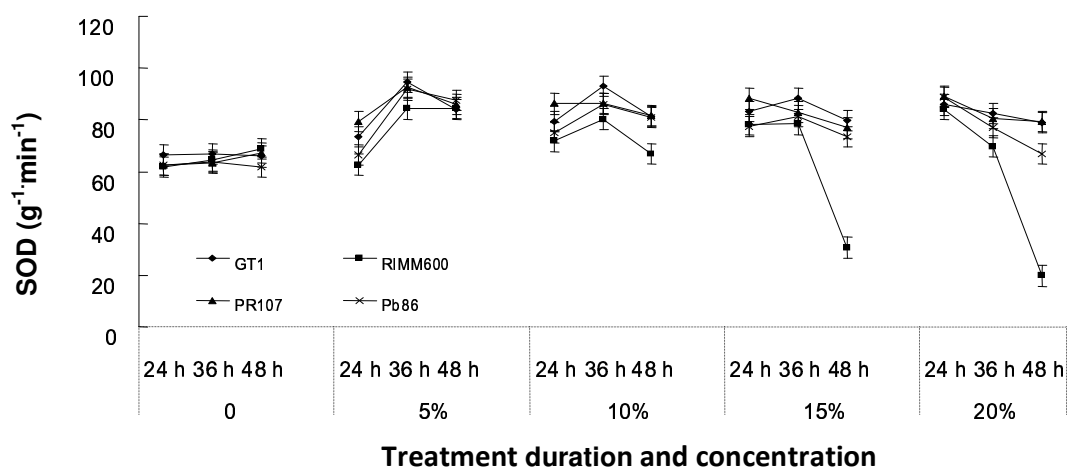


Figure 7. Variation of leaf SOD activity of *H. brasiliensis* seedlings under PEG drought stress.

seedlings. In addition, it is depicted that the drought resistant parameters of the four clones exhibited the most dissimilarity when they were treated with 20% PEG for 48 h. Therefore, the subsequent comparison could be conducted under this treatment.

### Comprehensive evaluation of drought resistance of the different clone seedlings

In order to evaluate the drought resistance of the four clone seedlings, subordinate function values of every clone subjected to drought were calculated after the seedlings were treated with 20% PEG for 48 h (Table 1). It was indicated that the drought resistance of these four clone seedlings was in the sequence of GT1>PR107>RRIM600>PB86.

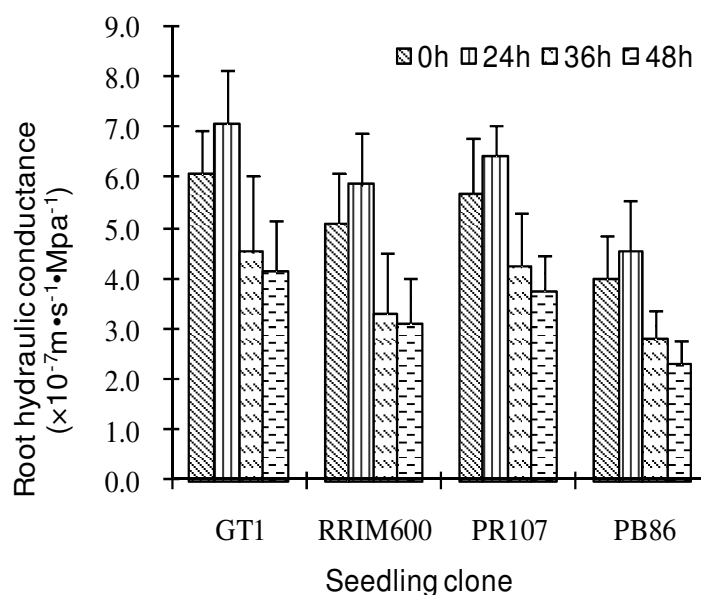
### Root hydraulic conductance and root vigor of the different clone seedlings

Figure 8 reveals that the root hydraulic conductance of the four clone seedlings exhibited similar variation dynamic under PEG simulated water stress. The root hydraulic conductance increased at the early stage of the water stress but decreased gradually with further water stress.

The initial root water conductance of GT1, PR107, RIMM600 and PB86 seedlings was 6.11, 5.09, 5.71 and 4.0 ( $\times 10^{-7} \text{m}\cdot\text{s}^{-1}\cdot\text{MPa}^{-1}$ ), respectively. Although there are some deviations in the initial root water conductance and the sequence is the same to rootstock drought resistance, no significant difference was found among the four clone seedlings. However, when the four clone seedlings were subjected to water stress for 24 h, their

**Table 1.** Subordinate function value of the seven parameters for the four clone seedlings.

Parameter	GT1	RIMM600	PR107	PB86
REC	1.0000	0.6306	0.1899	0.0000
RWC	1.0000	0.2461	0.1640	0.0000
MDA	1.0000	0.0000	0.5490	0.7306
SS	0.1707	0.0000	1.0000	0.0906
SP	0.9730	1.0000	0.0000	0.0674
Pr	0.9928	0.1917	1.0000	0.0000
SOD	0.9961	0.0000	1.0000	0.7902
Average	0.8761	0.2955	0.5576	0.2398
Sequence	1	3	2	4

**Figure 8.** Variation of whole root hydraulic conductance for the different clone seedlings under PEG drought stress.

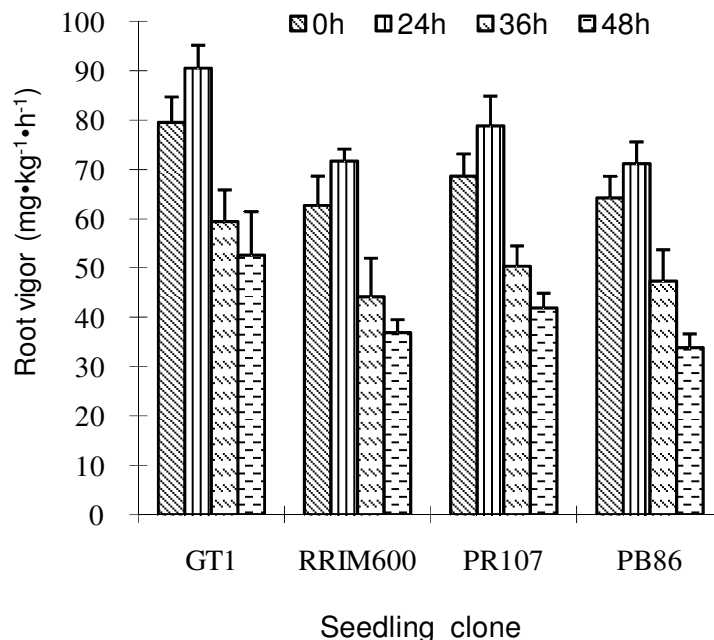
root hydraulic conductance increased to 7.096, 6.429, 5.871 and 4.0139 ( $\times 10^{-7} \text{m} \cdot \text{s}^{-1} \cdot \text{MPa}^{-1}$ ), respectively and the multiple comparisons revealed that the hydraulic conductance of GT1, PR107, RRIM600 seedlings was significantly higher than that of PB86 seedlings ( $P < 0.05$ ). In addition, it was noted that the decrease rate of whole root hydraulic conductance between 24 and 48 h PEG treatment was PB86(49.29%)>RRIM600(46.86%)>PR107(41.71%)>GT1(41.65%), which is also consistent to the drought resistance sequence of the four clone seedlings.

Correspondingly, root vigor of the four clone seedlings demonstrated a similar trend to the whole root hydraulic conductance under water stress (Figure 9). Root vigor could explain the whole root hydraulic conductance with a positive linear correlation (Figure 10). Based on the root hydraulic conductance and root system vigor plus their decreasing degree under PEG stress, it is concluded that

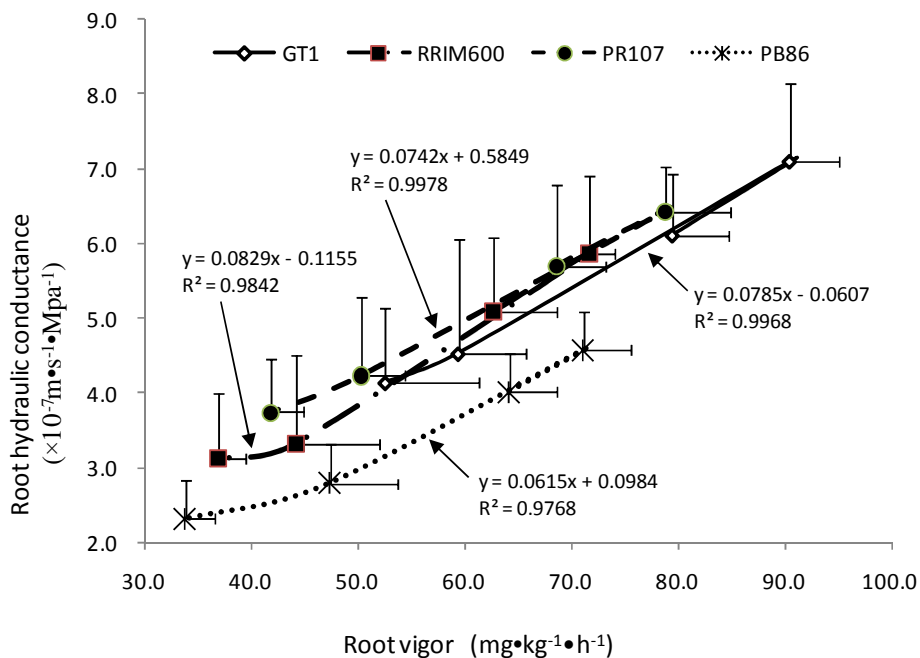
GT1 seedlings are again comparatively resistant to drought for its higher and less decreased root system vigor and hydraulic conductance under stress. For other clone seedlings, the order of drought resistance was PR107 > RRIM600 > PB86.

#### Drought resistance of the scions grafted on different clone rootstocks

In order to investigate the effect of rootstock drought resistance and hydraulic conductance on the grafted saplings, rubber clone of CATAS 7-33-97 was grafted on PB86, PR107, RRIM600 and GT1 seedlings and seven parameters of the scions were measured (Table 2). It is revealed that 20% PEG treatment for 48 h could inflict water stress on the grafted rubber saplings. Although the scions are the same, saplings with different clone rootstocks



**Figure 9.** Variation of root vigor for the different clone seedlings under PEG drought stress.



**Figure 10.** Correlations of whole root hydraulic conductance and root vigor of the four clone seedlings. Note: Regression functions and square correlation coefficients were presented for the four clones correspondingly.

had diverse responses to water stress. Furthermore, the drought resistance responses of the grafted saplings were determined by their rootstocks in most cases. The subordinate function values calculated on the PEG

treated saplings (Table 3) showed that the scions grafted on drought resistant rootstocks demonstrated more resistance to water stress, which is also in the same sequence as the rootstock hydraulic conductance under



**Table 2.** Drought resistance related parameter variation of CATAS7-33-97 grafted on different clone rootstocks.

Parameter	Control				PEG (48 h)			
	RRIM600	PR107	PB86	GT1	RRIM600	PR107	PB86	GT1
REC	3.24±0.02 <sup>bcAB</sup>	1.44±0.00 <sup>cB</sup>	6.88±0.02 <sup>aA</sup>	4.83±0.03 <sup>abAB</sup>	87.00±0.01 <sup>aA</sup>	86.98±0.13 <sup>abA</sup>	72.04±0.09 <sup>bAB</sup>	55.24±0.05 <sup>cB</sup>
RWC	93.65±0.07 <sup>abA</sup>	98.54±0.00 <sup>aA</sup>	78.18±0.15 <sup>bA</sup>	97.19±0.01 <sup>aA</sup>	84.02±0.02 <sup>abAB</sup>	72.65±0.04 <sup>aA</sup>	76.91±0.23 <sup>bcB</sup>	90.94±0.07 <sup>cB</sup>
SP	87.75±25.83 <sup>cC</sup>	167.39±15.69 <sup>aA</sup>	132.15±1.70 <sup>bB</sup>	149.86±3.26 <sup>abAB</sup>	164.00±13.08 <sup>bAB</sup>	180.29±2.64 <sup>aA</sup>	117.46±5.41 <sup>cC</sup>	160.80±5.33 <sup>bB</sup>
SS	152.25±2.37 <sup>aA</sup>	150.89±3.00 <sup>aA</sup>	118.29±6.67 <sup>cC</sup>	141.28±4.44 <sup>bB</sup>	233.77±4.95 <sup>aA</sup>	235.83±9.68 <sup>aA</sup>	182.65±8.33 <sup>bB</sup>	186.12±5.81 <sup>bB</sup>
MDA	17.40±1.22 <sup>aA</sup>	16.85±0.36 <sup>aA</sup>	17.51±0.90 <sup>aA</sup>	17.58±1.38 <sup>aA</sup>	24.01±0.40 <sup>aA</sup>	23.38±1.17 <sup>bB</sup>	27.70±1.39 <sup>bB</sup>	18.85±0.63 <sup>cC</sup>
Pr	62.82±10.37 <sup>aA</sup>	14.25±2.95 <sup>cB</sup>	22.68±5.86 <sup>cB</sup>	46.38±10.11 <sup>bA</sup>	46.85±5.40 <sup>bB</sup>	88.69±8.90 <sup>aA</sup>	20.13±3.85 <sup>dC</sup>	37.37±0.73 <sup>cB</sup>
SOD	90.06±3.97 <sup>aA</sup>	28.52±9.69 <sup>bB</sup>	83.30±7.04 <sup>aA</sup>	84.80±10.18 <sup>aA</sup>	14.26±1.23 <sup>cC</sup>	75.05±16.00 <sup>aA</sup>	79.17±11.71 <sup>aA</sup>	37.90±4.48 <sup>bB</sup>

The values are mean ± SD; different lower-case and capital letters in the same line represent the significant difference at  $p < 0.05$  and  $p < 0.01$  respectively.

**Table 3.** Subordinate function value of CATAS7-33-97 grafted on the four clone rootstocks.

Parameter	GT1	RRIM600	PR107	PB86
REC	1.0000	0.0000	0.0005	0.4708
RWC	1.0000	0.6213	0.2330	0.0000
MDA	1.0000	0.4164	0.4880	0.0000
SS	0.0652	0.9612	1.0000	0.0000
SP	0.6898	0.7407	1.0000	0.0000
Pr	0.9807	0.3897	1.0000	0.0000
SOD	0.3642	0.0000	0.9364	1.0000
Average	0.7285	0.4470	0.6654	0.2101
Sequence	1	3	2	4

water stress.

### Drought resistance of saplings with different root hydraulic conductance

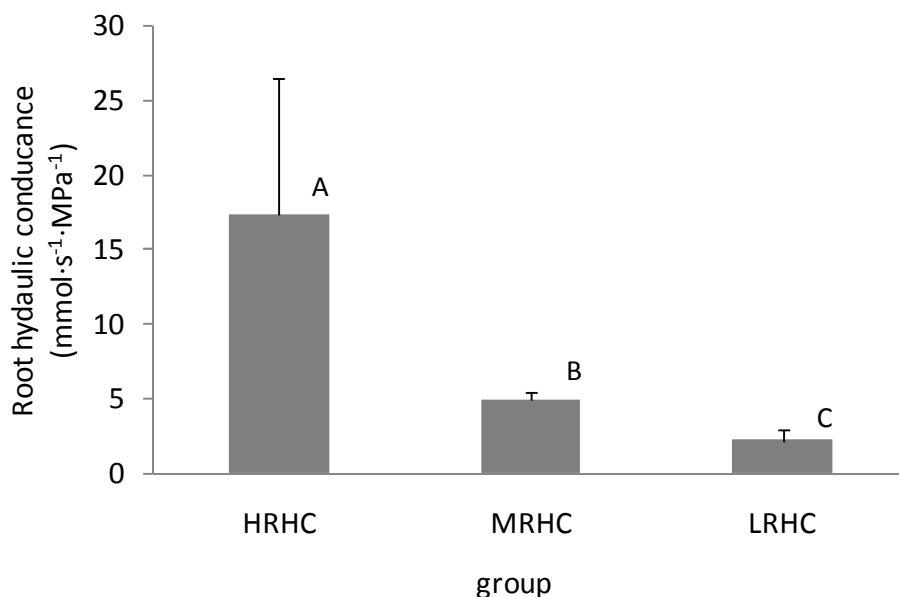
Further experiments were conducted on the mixed unknown clone rootstock saplings. Whole root hydraulic conductance was measured with high-pressure flow meter on 120 poly bag cultured grafted saplings. After that, 60 plants were selected and divided into three groups on the

basis of the whole root hydraulic conductance, namely high whole root hydraulic conductance group, middle whole root hydraulic conductance group and low whole root hydraulic conductance group. As shown in Figure 11, the whole root hydraulic conductance of the three groups had a highly significant difference ( $P < 0.01$ ). When the seven drought resistance related parameters of the grafted saplings were measured, they exhibited similar trend to different drought resistant rootstocks (Table 4), that is, different group exhibited different extent of alteration

responding to water stress; high whole root hydraulic conductance group tended to be the drought resistant rootstock. This result is further confirmed from the subordinate functional evaluation (Table 5).

### DISCUSSION

At present, rubber seeds are mainly used for producing rootstock. Some comparisons have been conducted on various illegitimated seedling



**Figure 11.** Whole root hydraulic conductance of different groups. Note: HRHC represents high root hydraulic conductance group; MRHC represents middle root hydraulic conductance group; LRHC represents low root hydraulic conductance group; different capital letters at the top of the bars mean significant difference at  $p < 0.01$ .

**Table 4.** Drought resistance related parameter variation of grafted CATAS7-33-97 with different root hydraulic conductance rootstocks.

Parameter	Control			PEG (48 h)		
	HRHC	MRHC	LRHC	HRHC	MRHC	LRHC
REC	1.43±0.02 <sup>ba</sup>	5.15±0.02 <sup>abA</sup>	3.25±0.01 <sup>aA</sup>	57.44±0.05 <sup>bB</sup>	83.32±0.02 <sup>aA</sup>	77.76±0.12 <sup>aA</sup>
RWC	96.18±0.01 <sup>aA</sup>	94.67±0.02 <sup>abA</sup>	92.35±0.01 <sup>ba</sup>	91.00±0.03 <sup>aA</sup>	80.34±0.01 <sup>bB</sup>	72.27±0.03 <sup>cb</sup>
SP	157.63±13.01 <sup>aA</sup>	112.34±6.69 <sup>cC</sup>	132.40±1.67 <sup>bB</sup>	161.84±4.70 <sup>aA</sup>	162.92±14.23 <sup>aA</sup>	80.37±11.92 <sup>bB</sup>
SS	142.81±4.55 <sup>bB</sup>	152.03±2.05 <sup>aA</sup>	117.98±5.59 <sup>cB</sup>	194.59±8.89 <sup>bB</sup>	233.50±2.80 <sup>aA</sup>	183.00±5.71 <sup>cb</sup>
MDA	18.35±1.32 <sup>aA</sup>	17.76±1.66 <sup>aA</sup>	17.97±1.94 <sup>aA</sup>	19.01±0.55 <sup>cC</sup>	24.39±0.50 <sup>bB</sup>	26.58±1.06 <sup>aA</sup>
Pr	47.20±5.09 <sup>aA</sup>	37.37±0.90 <sup>bB</sup>	20.34±3.46 <sup>cC</sup>	63.00±9.44 <sup>aA</sup>	44.36±7.85 <sup>bB</sup>	23.47±5.02 <sup>cC</sup>
SOD	82.18±7.19 <sup>ba</sup>	90.81±3.24 <sup>aA</sup>	82.93±4.96 <sup>ba</sup>	36.40±4.48 <sup>bB</sup>	42.03±6.77 <sup>bB</sup>	79.92±10.78 <sup>aA</sup>

The values given are mean ± SD; different lower-case and capital letters in the same line represent the significant difference at  $p < 0.05$  and  $p < 0.01$  respectively. HRHC, high whole root hydraulic conductance; MRHC, middle whole root hydraulic conductance; LRHC, low whole root hydraulic conductance.

**Table 5.** Subordinate function value of grafted CATAS7-33-97 with different root hydraulic conductance rootstocks.

Parameter	REC	RWC	SP	SS	MDA	Pr	SOD	Average	Sequence
HRHC	1.0000	1.0000	0.9869	0.2294	1.0000	1.0000	0.0000	0.7452	1
MRHC	0.0000	0.4306	1.0000	1.0000	0.2892	0.5285	0.1293	0.4825	2
LRHC	0.2149	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000	0.1736	3

clones as rootstocks. It is reported that the rootstock can significantly affect the girth, yield, water use efficiency and growth physiology of the rubber tree scions (Ahamad, 1999, 2001; Cardinal et al., 2007). Therefore, selecting good rootstock is vital to improve the yield and abiotic resistance of the prevailing clones. Due to

rootstock originated root system being acted as the substantial organ of water and nutrients absorption, rootstock, as a critical part, determines the drought resistance of the grafted rubber trees. Ahamad (1999; 2001) showed that scions grafted on RIM901, RRIM-623 and GT1 rootstocks could be benefited from their deep

rooting characteristics and impart low pre-dawn leaf water potential, high root and shoot ratio of the grafted trees. However, scions on RRIM-600 rootstocks seem to lack deep rooting characteristics and were more sensitive to drought. In addition, scions on RRIM623 rootstock could minimize water loss through effective stomata closure. By contrast, scions on GT1 rootstock could keep the stomata partially opened by proline accumulation triggered osmoregulation under severe water stress. These results indicate that different clone rootstocks have different responses to water stress and can bestow various drought resistant strategies on the scion. In this study, it is confirmed that GT1 seedlings exhibited higher proline accumulation under stress. This may facilitate the osmoregulation and therefore, impart higher water content to the leaves of seedlings. Nevertheless, higher root vigor and whole root hydraulic conductance measured in this study possibly also contributed to GT1 adaptation to the drought environment. In addition, the drought resistant sequence of the scions grafted on the four clone rootstocks is consistent to the drought resistance of the four clone rootstock seedlings, indicating that the drought resistance of a specific scion clone could be improved by the drought resistant rootstock. Among the four clones, GT1 was the best drought resistant rootstock for CATAS7-33-97 scion.

Water status of a tree under stress is an important factor determining the tree drought resistance. The equilibrium of plant water is moderated by the leaf evaporation and root uptake (Steudle, 2000). High root hydraulic conductance can ensure a sufficient water supply to the leaves and condition the water balance of the plant. However, although numerous studies have showed that specific rootstock can affect the shoot water potential and growth rate (Agele and Cohen, 2009; Gijon et al., 2010; Luis et al., 2006; Nardini et al., 2006; Rodriguez-Gamir et al., 2010; Solari and Dejong, 2006; Trifilo et al., 2007) and is suitable for different water regimes (Ozden et al., 2010), the effect of rootstock hydraulic conductance on whole plant hydraulic conductance and water balance is controversial. Luis et al. (2006) found that the rootstock hydraulic conductance of peach trees is positively related to scion to rootstock ratio. Clearwater et al. (2004) suggested that the xylem water potential of kiwifruit is compromised with the rootstock hydraulic conductance. Rodriguez-Gamir et al. (2010) also showed that citrus rootstock hydraulic conductance was positively correlated to whole plant transpiration. However, Nardini et al. (2006) and Olien et al. (1986) argued that although rootstocks have different ability in conducting water to the scion, the scion's hydraulic conductance and tree size was not determined by rootstock. Solari et al. (2006) claimed that the effect of rootstock hydraulic conductance on peach scion growth was differed with field conditions and even can be negated in specific environments.

In this research, it was found that the comprehensively

evaluated drought resistance of the four clone seedlings was consistent with their whole root hydraulic conductance and could be explained by their root vigor. In addition, the response of rootstock hydraulic conductance was altered with the water regime. Significant difference was not identified among the four clones possibly due to the seedlings been all sexually reproduced and the standard deviations been too large. Moreover, when the same clone scions were grafted onto the four clone seedlings, the drought resistance of the grafted rubber trees was determined by their rootstocks which are different in whole root hydraulic conductance. Furthermore, it was shown that the grafted trees with high root hydraulic conductance tended to be more adaptive to water stress. These results suggest that whole root hydraulic conductance or root vigor may reflect the drought resistance of the rootstock seedlings and can be used for selecting drought resistant rootstocks of rubber trees, at least for CATAS7-33-97 scion. Therefore, the conclusion validates the viewpoint of Jackson et al. (2000) that root system properties alone may comprise a key element of plant sensitivity to drought stress. However, these results were only on the PEG simulated water stress. The root morphology, stomatal conductance, transpiration, photosynthesis and growth performance of the grafted rubber trees were not studied. Therefore, further confirmation shall be done in the future.

Rootstocks can confer specific attributes on the trees (Cohen et al., 2007). With the extension of rubber plantations to suboptimal area and the increase of drought incidences, selecting root 'vigor' rootstock to improve the yield and abiotic stress tolerance of the scions has been a concern. Unlike other orchard trees such as citrus, apple, cherry and grapevine which have commercial producing rootstocks, rubber tree rootstocks are mostly from sexually reproduced seedlings with high heteromorphosis. Therefore, how to select drought resistant grafted saplings for drought plantations promptly without injuring the budding is worthwhile trying. In this study, we have examined the relationship between the whole root hydraulic conductance and the diameter of the rootstocks and scions; however, no correlation has been identified (data not presented). The HPFM is a rapid and easy instrument that can be used to measure whole root hydraulic conductance in field and in laboratory (Bogeat-Triboulot et al., 2002). After the budded rubber trees were pruned, the whole root hydraulic conductance can be measured with HPFM intact. The results presented in this study validated the possibility of using HPFM in selecting rubber tree rootstock. However, further research should be conducted on selecting rootstocks with HPFM.

## Conclusions

In this study, we find that the rootstock drought resistance and root hydraulic conductance could improve the drought

resistance of the grafted rubber trees; GT1 was the best drought resistant rootstock for CATAS7-33- 97 scion among the four clone rootstocks. In addition, we proposed, to our knowledge for the first time, that high-pressure flow meter is likely a promising method for selecting drought resistant rubber tree rootstocks intact.

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