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PRELIMINARY RESULTS OF LATEX YIELD PROFILE AND GROWTH PERFORMANCE OF TISSUE CULTURE DERIVED *Hevea brasiliensis* TREES

Nor Mayati Che Husin[*1] and Abd. Razak Ahmad[2]

[1]Genetic Transformation Section, Genetic Resources and Improvement Unit, Plant Production and Development Division, RRIM Experimental Station, Malaysian Rubber Board, 47000, Sg. Buloh, Selangor

[2]Crop Management Unit, RRIM Kota Tinggi Research Station, Batu 3 ½ Jalan Mawai, 81900, Kota Tinggi, Johor

**Email: normayati@lgm.gov.my*

ABSTRACT

The performance of virgin basal panel (BO-1) of RRIM 2025 tissue culture (TC) derived mother plant (MP) and their bud-grafted generation (V1) trees were tested in comparison to Glenshiel (Gl 1) TC trees, RRIM 600 TC trees (mother plant and V1), conventional bud grafted of normal RRIM 2025 (NB) and control (seedlings RRIM 2025). Normal tapping at virgin bark (BO-1) half spiral day 3 (½S d3) was implemented and the trees were tapped nine to ten times per month. The latex yield profile for three yielding periods namely low yielding period (LYP) (Mar 2016 – May 2016), medium yielding period (MYP) (Jun 2016 – Sep 2016) and high yielding period (HYP) (Oct 2016 – Jan 2017) were recorded. The tree productivity for LYP (39 g/tapping/tree), MYP (40 g/tapping/tree) and HYP (38 g/tapping/tree) were not significantly different ($P = 0.5233$). Overall, tree productivity was the highest ($P < 0.0001$) for RRIM 2025V1 (54 g/tapping/tree), and the lowest tree productivity was recorded for RRIM 600V1 (25 g/tapping/tree). Generally, monthly productivity and land productivity differed between LYP, MYP and HYP with the highest apparently recorded in LYP followed by MYP and HYP. The land productivity was also significantly different between clones, with the highest recorded for RRIM 2025V1 at 1626 kg/ha/yr, while the lowest was recorded for RRIM 600V1 at 760 kg/ha/yr. Clear bole volume showed significant increase from 2016 to 2017, at average increment of 36 %. Generally, the clones tested were classified into two groups namely high performance and low performance group.

Keywords: Clear bole volume, land productivity, laticifers, somatic embryogenesis, tree productivity, virgin panel

INTRODUCTION

Tissue culture derived RRIM 2025 has the potential to serve as feedstock for both conventional and forest plantings. In the RRIM planting recommendation 2003, land productivity of RRIM 2025 was estimated at 2700 kg/ha/yr (Masahuling et al. 2007). In another trial, the yield of 10 year-old RRIM 2025 tapped with half spiral day 3 (½ S d3) tapping intensity (eight to ten tapping per month) with stimulant enhancement (SEN) solutions was estimated at 2396 kg/ha/yr, albeit with high incidence (22%) of white root disease (Zulkefly et al. 2010). Consequently, RRIM 2025 was omitted from the latest planting recommendation to give way to superior clones. Owing to its vigorous growth and high clear bole volume (CBV), this clone is favoured in forest plantings particularly for its characteristic high timber yield (Nurmi-Rohayu et al. 2015). This notion is supported by the 11 million RRIM 2025 planting materials supplied by Malaysian Rubber Board (MRB) formerly known as Rubber Research Institute of Malaysia (RRIM) to rubber growers throughout Malaysia, from 2012 to 2014 (Sharifah-Nurhafizah et al. 2015).

The first *Hevea* plant regenerated through somatic embryogenesis was developed in 1977 by research group from China Academy of Tropical Agriculture Science (CATAS) (Anon 1997, Chen et al. 1979). The RRIM established tissue culture (TC) research as an alternative propagation method for *Hevea* clones. The first anther culture plantlet of GI 1 clone was obtained in 1980 (Wan Abd. Rahman et al.

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1982), followed by RRIM 600 in 1990 (Hafsah and Wan Abd. Rahman 1995) and success of RRIM 2000 series TC which was achieved in the early 2000s. In the 1990's, this technology found a new purpose as a platform for genetic transformation of rubber trees to produce foreign proteins of interest (Arokiaraj et al. 2004, Sunderasan et al. 2016). Successful regeneration of *Hevea* TC plants, however, is clone-media specific and their survival is largely dependent on environmental attributes. Many factors are at play in acclimatisation, including the balance in microclimatic changes, optimal lighting and optimum nutrient supply that are essential for hardening of the plants and successful transition from *in vitro* to outside environment (Nor-Mayati 2015). Apart from G1 1, the clone of choice for tissue culture and genetic transformation, RRIM 2025 has also shown promising regeneration potential (Nor-Mayati 2015). As this clone is favoured by growers, it could well be genetically transformed to further improve specific agronomic traits, leaving other characteristic unaltered (Arokiaraj et al. 2004). Thus, recent tissue culture efforts were aimed at increasing embryogenesis (> 10%) and reducing contamination (< 50%), in preparation for a higher survivability of the regenerated plants.

In this study, latex yield performance of the RRIM 2025 TC trees, G1 1 TC trees, RRIM 600 TC trees and vegetatively propagated RRIM 2025 were evaluated. Normal ½S d3 tapping on BO-1 panel was employed to evaluate latex yield in three different yielding periods namely low yielding period (LYP), medium yielding period (MYP) and high yielding period (HYP); growth in terms of clear bole volume (CBV) was also investigated.

MATERIALS AND METHODS

Study plot establishment

The initial TC experiments of RRIM 2025 (performed in year 2000) have yielded a total of 224 plantlets (11.8%) designated as RRIM 2025 tissue culture mother plant (MP), from which 15 trees were cultivated with RRIM 600 seedlings rootstock and successfully transplanted on 25th March 2004. First copy of these trees were obtained by bud-grafting of the buds from their vegetative branches resulted for 210 new generation 1 (V1) trees. Transplanting of V1 trees were in two phases consisted of 78 trees on 18th April 2004 and 132 trees on 30th June 2004. The trial plot was also planted with 40 trees of Gl 1MP, 29 trees of RRIM 600MP and 24 of RRIM 2025 seedlings (non-TC) trees (Figure 1). Replacement (also vegetatively propagated normal RRIM 2025) trees were only planted whenever the original planted trees were dead, thus there were differences in ages of the trees and year planted.

A total of 318 plants were transplanted in Field 118 (0.64 ha), Pelepah Division, RRIM Kota Tinggi Research Station (SPKT), Johor (Figure 1A). The planting distance was 6.09 m x 3.65 m. Soil was Jerangau series, classified as Typic Hapludox, very fine, kaolinitic, isohyperthermic (USDA Soil Taxonomy) and Rhodix Ferralsol (FAO/UNESCO Legend). The Jerangau series is characterised as dark brown, granular and block, fine size, deep, high water penetration and medium nutrient balance. It is highly suitable for crops planting such as oil palm, cocoa and rubber (DOA 1993). Weeds were controlled with Roundup® (Monsanto) containing glyphosate and fertiliser applications were carried out once a year applied by spreading 720 g per tree at 1 m from the tree trunk at inter-rows. Compound fertiliser comprising nitrogen (N):phosphorus (P):potassium (K):magnesium (Mg) (12:12:17: 2) was used. Open tapping was started in January 2016, and data collection was started on March 2016. Average girth during first tapping for each clone was shown in Table 1. Rainfall data was recorded by the localised installed weather station.

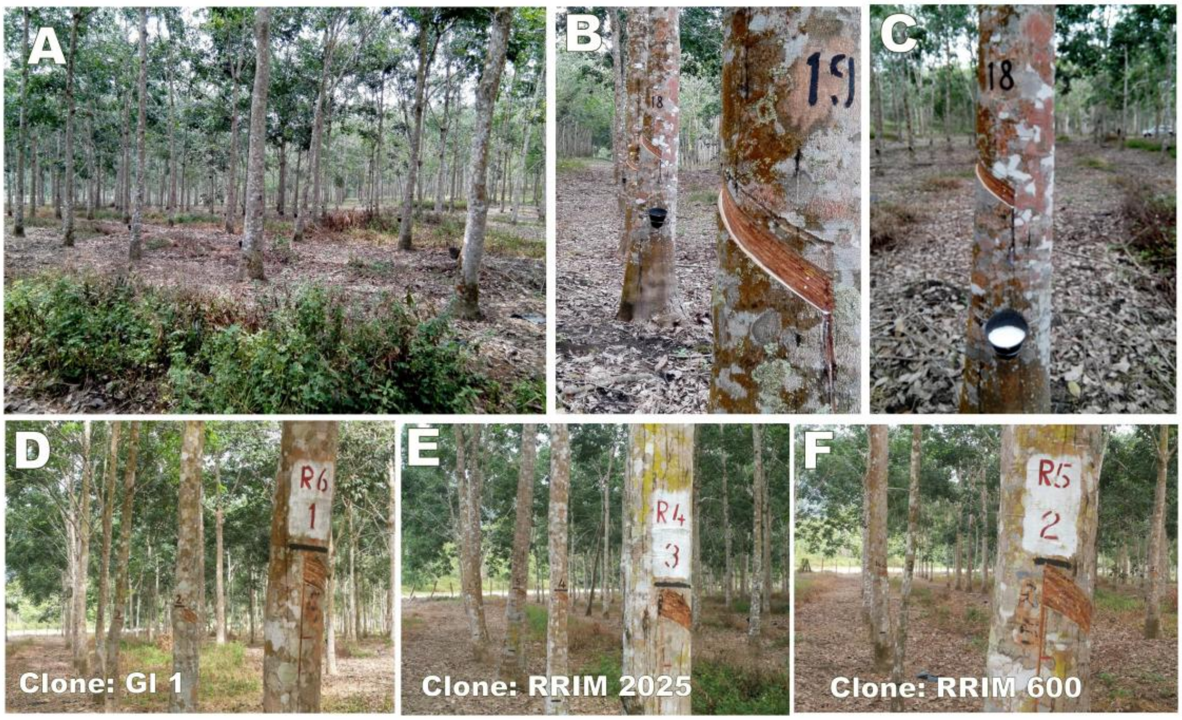

Figure 1. *Hevea* TC trees planted in Field 118 at the Pelepah Division, SPKT, Johor (A-C) comprising Gl 1 (D), RRIM 2025 (E), and RRIM 600 (F).

Table 1. Girth measured on April of three years duration for each clone tested

	Average girth size (cm)		
Clones	2015	2016	2017
Control	80	80	80
Gl 1MP	49	50	50
Replacement	62	62	63
RRIM 2025MP	75	77	77
RRIM 2025NB	68	69	69
RRIM 2025VI	71	71	72
RRIM 600MP	47	49	50
RRIM 600V1	50	54	54

Latex yield measurement

A total of 249 tappable trees in a single replicate were evaluated as experimental units. They were control (21 trees), Gl 1MP (30 trees), replacement (20 trees), RRIM 2025MP (13 trees), RRIM 2025NB (31 trees), RRIM 2025V1 (110 trees), RRIM 600MP (15 trees) and RRIM 600V1 (8 trees). Tapping was on virgin bark (BO-1) using half spiral tapped at every three days interval (BO-1 ½S d3) tapping system (Figure 1B). The trees were tapped nine to ten times a month. Latex yield collections continued for up to 2.30 hours after tapping, followed by recording of fresh weight and dry weight of the cup lump. Profile

yield data for each yielding period namely LYP (Mar-May 2016), MYP (Jun-Sep 2016) and HYP (Oct 2016-Jan 2017) (Mohd Akbar 2006) were calculated. Tree productivity is expressed as g/t/t (g/tapping/tree), which is employed to calculate monthly yield performance (kg/ha/mth) and yearly yield performance (kg/ha/yr).
The tree productivity was calculated as:

$$\text{g/t/t} = \frac{\text{total dry weight (i.e. ½ of fresh weight)}}{\text{total number of tapping days in a month}}$$

Monthly productivity was calculated as:

$$\text{Monthly productivity} = \frac{\text{g/t/t x number of tappings x stand per hectare (i.e. 297 days)}}{1000}$$

Land productivity was calculated as: Monthly tapping x 12 months.

Growth performance measurement

Growth performance in terms of clear bole volume (CBV) increment was recorded during the first quarter every year. CBV was calculated following Ramli et al. (1995):

$$\text{CBV } (m^3) = \pi/12\ [(D1 + D2)^2 - (D1 \times D2)] \times t$$

Where,
π = 3.142
D1 = diameter at 60 cm from ground,
D2 = diameter at 150 cm from ground,
t = [(tan D1 x distance of observer from the tree) + (tan D2 x distance of observer from the tree)].

**The bole height was measured using a clinometer*

Statistical analysis

Experimental design was Complete Randomised Design (CRD) and data were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) program of Statistical Analysis System (SAS®, SAS Institute Inc., Cary, NC, USA) release 9.2. Wherever the data was found significantly different by ANOVA, the means of treatment variables were compared by least significant difference (LSD) test at $P \leq 0.05$. Graphs were plotted using SigmaPlot 12.0.

RESULTS AND DISCUSSION

Tree productivity and yield productivity

Evaluation of monthly yield profile, showed that there were three yielding periods in one year namely low (LYP), moderate (MYP) and high (HYP) yielding periods. The parameters subjected to change every year, and yield pattern can be used to estimate the onset of yielding periods. The yield profile of each yielding period is markedly diverse. Elsewhere, inconsistency has been observed in terms of absolute yield, duration and the percentage of yield per yielding phase compared to the annual total yield, even from the same tapping panel (Mohd Akbar 2006).

LYP was in accordance with the wintering period, where it started from the onset of the period with a sign of leaves yellowing until the end of the period when the tree productivity started to increase (Figure 2A). During LYP that commenced in February (March in this study) until May, old leaves were shed, followed by the formation of new red leaflets that later gained their optimum size but with less chlorophyll content. The total land productivity during the period was 1252 kg/ha/yr with an average monthly productivity of 104 kg/ha/mth (Table 2).

When MYP began, it was marked by an increase in the chlorophyll content in the newly developed leaves and the canopy reached its optimum density; this period normally occured between June and September. The land and productivity decreased to 1240 kg/ha/yr with an average monthly productivity of 103 kg/ha/mth. Average of land productivity during the period was on par with LYP but plummeted in September. At this phase, tree productivity decreased rapidly, which was in contrast tree productivity recorded for RRIM 901 in another trial (Mohd Akbar 2006). While maturing, leaves would lead to stable photosynthesis and enable the trees to recover nutrient starvation during LYP, it was still too early to draw correlation of our results with that reported elsewhere.

Table 2. Latex yield measurement for field planted *Hevea* TC trees

Parameters	Means		
	Tree productivity (g/t/t)	Monthly productivity (kg/ha/mth)	Land productivity (kg/ha/yr)
	45.7 ± 0.58***	115.2 ± 1.49***	1381.5 ± 17.82***
$F_{probability}$	<.0001	<.0001	<.0001
CV	61.41	62.91	62.92
Period			
LYP	38.5 ± 1.44ns	104.4 ± 3.72***	1251.7 ± 44.68***
MYP	39.9 ± 1.25ns	103.3 ± 3.23***	1239.8 ± 38.81***
HYP	38.1 ± 1.27ns	88.3 ± 3.27***	1059.0 ± 39.22***
$F_{probability}$	0.5233	0.0007	0.0007
Clone			
RRIM 2025V1	53.5 ± 0.81***	135.5 ± 2.10***	1625.7 ± 25.24***
Control	51.5 ± 1.88***	130.3 ± 4.85***	1562.9 ± 58.15***
RRIM 2025NB	51.5 ± 1.52***	130.6 ± 3.92***	1567.3 ± 47.06***
Replacement	44.5 ± 1.88***	112.8 ± 4.85***	1353.1 ± 58.15***
RRIM 2025MP	29.6 ± 2.36***	76.2 ± 6.09***	913.5 ± 73.08***
RRIM 600MP	27.7 ± 2.21***	70.2 ± 5.72***	841.7 ± 68.64***
Gl 1MP	27.6 ± 1.51***	70.4 ± 3.89***	843.8 ± 46.75***
RRIM 600V1	25.1 ± 3.82***	63.4 ± 9.86***	759.8 ± 118.28***
$F_{probability}$	<.0001	<.0001	<.0001

****** Significant at P = 0.05, ns = not significant*

Meanwhile, HYP is the duration of four months started from October until January of the following year. During HYP, the tree productivity, the sucrose level and chlorophyll content are considered optimum and stable until the onset of the next wintering season (Mohd Akbar 2006). In this study, the tree productivity generally decreased to 38 g/tapping/tree compared to LYP (39 g/tapping/tree) and MYP (40 g/tapping/tree) (Table 2), with a slight increase in the first month followed by marginal increment thereafter (Figure 2A). The slight increase could be an indicator of stabilisation of physiological factors including photosynthesis that has led to stable leaves and latex nutrient content.

Based on the results obtained in this study (Tables 2 and 3, Figure 2), yield productivity of tissue culture and normal trees can be classified into two groups i.e. higher performance group represented by RRIM 2025V1, control (RRIM 2025 seedlings trees), RRIM 2025NB and replacement, and low performance group represented by RRIM 2025MP, RRIM 600MP, Gl 1MP and RRIM 600V1. During LYP, the highest tree productivity recorded for higher performance group was observed highest for RRIM 2025V1 (52.1 g/tapping/tree) and the lowest tree productivity was recorded for low performance group, RRIM 600V1 (20 g/tapping/tree) (Table 3). During MYP, RRIM 2025NB gave the highest tree productivity (53.2 g/tapping/tree) and RRIM 2025MP showed the lowest g/t/t (26.3 g/tapping/tree). Meanwhile during HYP, highest tree productivity was recorded for RRIM 2025V1 (55.4 g/tapping/tree), and low performance group RRIM 600V1 produced lowest tree productivity (22.5 g/tapping/tree) (Table 3, Figure 2A). The monthly and land productivity were higher during MYP and decreased significantly during HYP (Figures 2B, C). Both yield productivities were also significant ($P = < 0.0001$) between clones (Table 3). In general, the low performance group particularly RRIM 600 gave tree productivity reading below 30 g/tapping/tree (Table 2, Figure 2) and below 40 g/tapping/tree throughout three yielding periods (Table 3). Overall, the projected monthly yield productivity throughout three yielding periods for this group remained under 100 kg/ha/mth and land productivity was recorded below 1000 kg/ha/yr (Table 3, Figure 2). However, further monitoring of yield data is necessary to draw a valid comparison with other reports such as by Masahuling et al. (2007).

Tree productivity (g/t/t) and number of tapping days, are among two crucial parameters that have always been manipulated to achieve targeted higher land productivity. In such a case, the deleterious factors such as wash out during raining days and reduced number of tappable trees due to bark/panel dryness are always of issues in field evaluation. Thus, prevention approaches had been introduced including the usage of rain-guarding devices and reduce tapping intensities. However, throughout this study, rainfall was recorded at 2199 mm in 2016 and 2741 mm (max at 574 mm in January only) in 2017 and only one day wash out had occurred which was in August (MYP). Average rainfall during this study was LYP (706 mm), MYP (945 mm) and HYP (898 mm). The amount of rainfalls showed no correlation to the yield. Number of tappable trees also did not reduce during this first year tapping evaluation. It is postulated that the age at open tapping of the trees may have negative effects on the yield data. However, to get a clear picture of the tissue culture trees performance, a longer evaluation period is required.

Land productivity, latex diagnosis such as dry rubber content (drc) and total solid content (tsc) are among the parameters used by the industry to estimate the targeting yield in the following years. However, in this study, measurement of drc and tsc was only planned from year 2 onwards. Clonal differences, soil series, topography, rainfall pattern, seasonal fluctuation, frequency of manuring, number of tapping days, age, tapping panels, latex harvesting technique and skilled tappers are amongst the major attributes of yields performances. Tapping systems alone are unable to optimise the production of latex. Agronomic input also affects the trees condition, and thereby evaluation of leaves and bark nutrients statuses should also be determined.

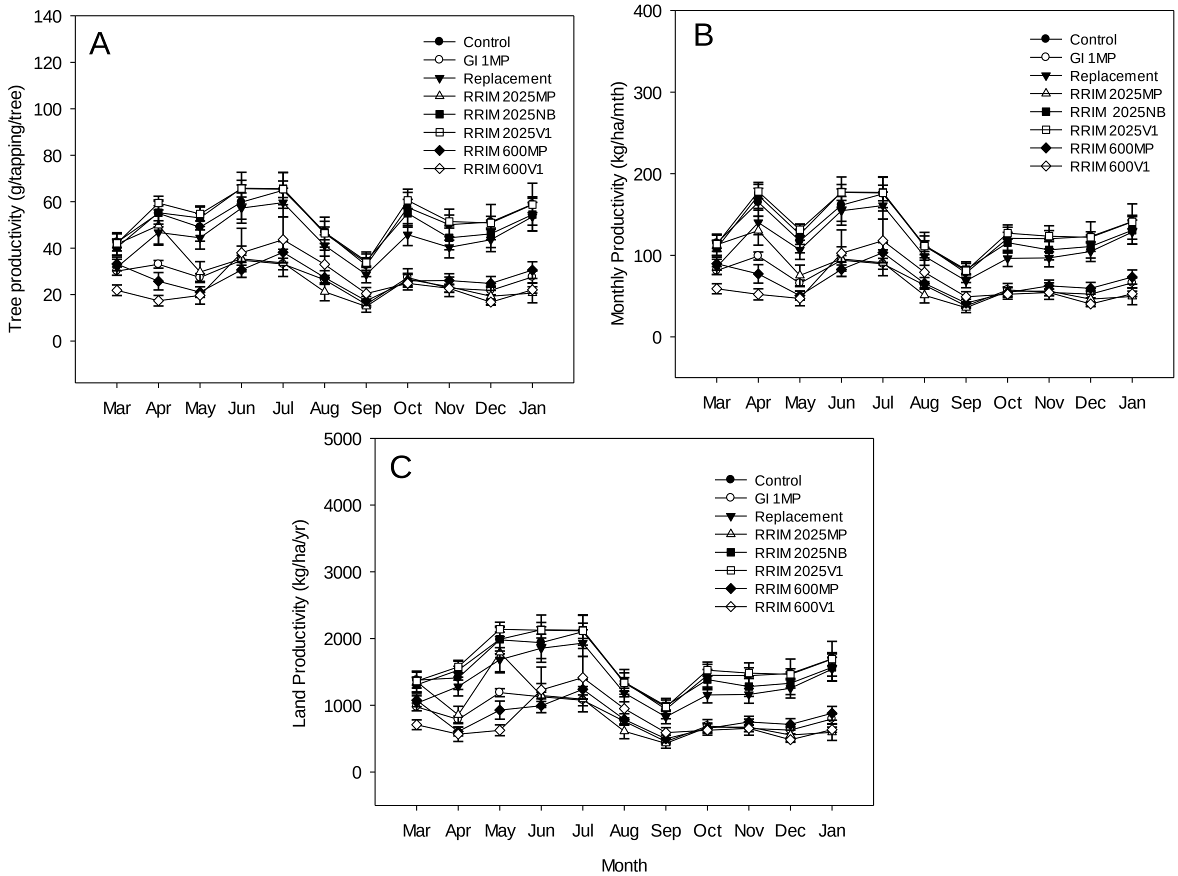

Figure 2. Tree productivity, g/t/t (A) and latex yield profiles (B and C) for *Hevea* TC trees during LYP (Mar-May 2016), MYP (Jun-Sep 2016) and HYP (Oct 2016-Jan 2017)

Higher latex yield also influence by number of laticifer and its formation (Gomez, 1982). The laticifers in *Hevea* sp. involved in latex production and localised in the secondary phloem of the trunk. Its distribution and ring number are varies in different clones. There are few factors influencing the differentiation of liticifer ring *viz*; genetic mark-up, environmental conditions and chemical application (Hao and Wu, 2000). Mechanical tapping induced formation of laticifer in the area of injury, however the tapping activity alone not significantly increase laticifer formation in the systemic area. Thus latex exploitation using stimulants such as ethephon (2-CEPA), SEN (sucrose base stimulant), and MORTEX (oil palm and ethephon base stimulant) were proven to increase latex flow accelerate induction of laticifer differentiation and increase the number of laticifer rings 2 to 3 times higher than un-stimulated Hevea trees (Hao and Wu, 2000). In order to increase yield productivity in this trial, exploitation technology using ethylene-base stimulants mentioned above should be implemented. Since clonal responses to the stimulant are also different, the diverse effect shall be expected.

Table 3. Tree productivity and yield productivity in three different yielding periods namely LYP, MYP and HYP during 2016 to 2017 for different clones of TC *Hevea* trees planted in F118, Pelepah Division, SPKT, Johor

Parameters	Tree productivity (g/t/t)			Monthly productivity (kg/ha/mth)			Land productivity (kg/ha/yr)		
Clones	LYP 2016	MYP 2016	HYP 2016	LYP 2016	MYP 2016	HYP 2016	LYP 2016	MYP 2016	HYP 2016
RRIM 2025V1	52.1±1.3***	52.8±1.3***	55.4±1.4***	141.1±3.6***	136.4±3.5***	128.4±3.2***	1692.0±42.6***	1637.2±41.5***	1540.8±38.2***
RRIM 2025NB	50.4±2.4***	53.2±2.5***	50.1±2.5***	136.6±6.6***	137.4±6.4***	115.9±5.9***	1638.3±78.9***	1648.6±77.3***	1391.1±70.5***
Control	48.9±2.9***	51.1±3.1***	54.5±3.2***	132.5±8.1***	131.9±7.9***	126.4±7.4***	1589.4±97.5***	1582.8±95.4***	1516.6±89.2***
Replacement	41.0±2.9***	46.7±3.1***	45.9±3.2***	110.9±8.1***	120.8±7.9***	106.6±7.4***	1331.2±97.5***	1449.7±95.4***	1278.5±89.2***
RRIM 2025MP	38.3±3.8***	26.3±3.9***	22.5±3.9***	104.9±10.3***	68.3±10.1***	52.0±9.2***	1257.7±123***	819.7±21.2***	623.7±110***
Gl 1MP	30.3±2.4***	27.6±2.5***	24.9±2.5***	82.2±6.6***	71.2±6.4***	57.7±5.9***	984. 7±78.9***	854. 5±77.3***	692.2±70.5***
RRIM 600MP	26.8±3.5***	28.4±3.6***	26.8±3.7***	72.6±9.6***	73.3±9.4***	62.4±8.6***	870.2±115***	879.5±112***	749.5±103***
RRIM 600V1	20.0±6.1***	33.9±6.2***	21.7±0.4***	52.8±16.6***	87.2±16.4***	50.1±14.9***	633.5±199**	1046.3±195***	599.7±178***
$P_{Probability}$	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
CV	52.79	61.02	62.66	52.89	61.63	62.74	52.93	61.63	62.74

****Significant at P = 0.05*

Clear Bole Volume

Clear bole volume (CBV) significantly increased from 2016 to 2017, with an average of 35.95% increment. The CBV in 2016 ranged from 0.0686 to 0.2791 m^3, while in 2017 it ranged from 0.0980 to 0.3825 m^3. Average CBV increment in 2017 was significant ($P < 0.0001$) at 0.2733 m^3 as compared to 0.2107 m^3 in 2016 (Table 4). At this stage, the CBV increment was disassociated with the grouping of high/low yield performance aforementioned above.

Table 4. Mean CBV between 2016 and 2017 for *Hevea* tissue culture trees at F18, Pelepah Division, SPKT Johor

	CBV		CBV increment (%)
	2016	2017	
Clone			
Control	0.2791 ± 0.03***	0.3825 ± 0.03***	48.60 ± 5.33ns
RRIM 2025V1	0.2501 ± 0.01***	0.3159 ± 0.01***	32.34 ± 2.36ns
RRIM 2025MP	0.2462 ± 0.03***	0.3169 ± 0.04***	31.92 ± 6.61ns
RRIM 2025NB	0.2080 ± 0.02***	0.2718 ± 0.02***	33.77 ± 4.03ns
Replacement	0.1850 ± 0.03***	0.2455 ± 0.03***	39.05 ± 5.33ns
GL1MP	0.1173 ± 0.02***	0.1568 ± 0.03***	37.61 ± 4.28ns
RRIM 600MP	0.0992 ± 0.03***	0.1376 ± 0.04***	41.60 ± 6.15ns
RRIM 600V1	0.0686 ± 0.05ns	0.0980 ± 0.06ns	45.00 ± 10.7ns
Mean	0.2107	0.2733	35.95
$F_{Probability\ (n=242,\ 241)}$	<.0001	<.0001	0.1582
CV	56.19	50.62	66.24

**** Significant at P = 0.05, ns = not significant*

CONCLUSION

This study had found that the tissue culture *Hevea* trees planted in the area of 0.64 ha in F118 Pelepah Divison, SPKT can be classified into two groups which were high tree productivity and high yield performance group comprising RRIM 2025V1, normal budding RRIM 2025NB, control and replacement, and the low tree productivity and low yield performance group represented by RRIM 2025 tissue culture mother plant (MP), RRIM 600MP, RRIM 600V1 and Gl 1MP. The results presented in this report were for the first year evaluation of newly open tapped trees, and further evaluation of latex yield and additional parameters as well as implementation of suitable latex harvesting technologies will contribute more information regarding rubber tree performance.

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