

**Full Paper**

**Effects of different container structures on growth and root architecture of rubber (*Hevea brasiliensis*) rootstock seedlings**

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**Abstract:** This study evaluates the effect of container structures on the growth and root architecture of rubber (*Hevea brasiliensis*) rootstock seedlings. The container structures and planting materials used were cylindrical plastic tubes, size 7.5 cm × 35 cm without plastic rods, and with two or four plastic rods glued inside, filled with a mixed medium of topsoil : coir dust : rice husk ash (1:1:1), and polybags, size 7.5 cm × 35 cm, filled with topsoil alone (control). The rubber seedlings' growth were measured above-ground and below-ground at 4, 6 and 8 months. The results showed that the rubber seedlings reached a suitable size for budding at 6 months with optimum seedling growth. The cylindrical plastic tubes with two and four plastic rods glued inside produced a good root architecture characterised by a high number of strong vertically oriented roots, an increase in the distributed root percentage in the upper root zone and also a decrease in branch roots based on both the root dry weight and root spiralling in the lower root zone, resulting in fewer circling roots.

**Keywords:** *Hevea brasiliensis*, planting container structures, rootstock, shoot growth, branch root distribution

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## INTRODUCTION

Rubber (*Hevea brasiliensis*) is an important industrial-economic crop in Thailand. Rubber plantations in Thailand are distributed in all regions with an estimated total planting area in 2016 of 3.73 million ha, mostly in the southern part (2.33 million ha), the north-east (0.78 million ha), the central (0.41 million ha) and the north (0.21 million ha) [1]. Rubber planting materials can be in the form of budded stumps or young budded seedlings in polybags. Suitable rootstock seedlings of high yielding varieties for budding take 6-8 months to reach a diameter of 0.9-2.5 cm [2]. Various factors may affect rootstock propagation or rootstock quality such as seed quality, planting material and container structure [3]. Using different planting materials influences the growth of durian [4] as well as rubber rootstock seedlings [5]. The container structure may also influence root growth [6,

7]. Containers with vertical ribs inside have been found to prevent root spiralling [8] and produce more vertical roots [9]. Amoroso et al. [10] reported that field elm seedlings in smooth-sided containers have the highest percentage of deformed roots. In addition, the container type affects seedling survival after transplanting in the field [11]. Therefore, the application of suitable planting materials and containers seems to influence the growth of plants and promote strong root growth and also affect the success of plant establishment after transplanting, which is often linked to root health [12, 13]. Detailed study on the root distributions in the upper, middle and lower part of root zones and root circling of rubber rootstock has not been conducted so far. The objective of this research is to investigate the effect of different container structures on the growth and root architecture of rubber seedlings for using as quality rootstock.

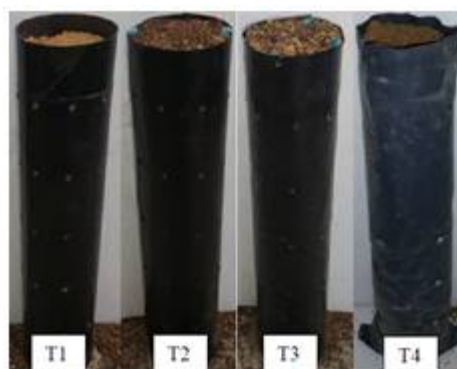
## MATERIALS AND METHODS

### Plant Materials

Seeds of the rubber clone, RRIM 600, were collected from Songkhla province during September 2016 and germinated. Two weeks after germination, the seedlings were transplanted and grown in growing media in different container types (Table 1 and Figure 1). All the rubber seedlings received a water-soluble NPK fertiliser (15:15:15) monthly at a dosing rate of 1 kilogram per 100 litres. Irrigation was delivered via an individual drip release to each plant, three times a day, viz. morning, midday and evening, throughout the duration of the study. The average day and night temperatures in the glasshouse during the experiment period were 37.2°C and 25.5°C respectively. The average relative humidity was 79.7%.

**Table 1.** Container types and planting materials used in the experiment

Treatment	Container structure and planting material
T1	Cylindrical plastic tube, size 7.5 cm × 35 cm, filled with topsoil: coir dust:rice husk ash (1:1:1 by volume)
T2	Cylindrical plastic tube, size 7.5 cm × 35 cm, with two 4-mm-thick plastic rods glued inside and filled with topsoil: coir dust: rice husk ash (1:1:1 by volume)
T3	Cylindrical plastic tube, size 7.5 cm × 35 cm, with four 4-mm-thick plastic rods glued inside and filled with topsoil: coir dust: rice husk ash (1:1:1 by volume)
T4	Polybags, size 7.5 cm × 35 cm, filled with topsoil 100% (control)



**Figure 1.** Container types and planting materials used in the experiment

### **Growing Media Analysis**

The chemical characteristics of the growing media, viz. their pH, electrical conductivity (EC), organic matter (OM), organic carbon (OC), cation exchange capacity (CEC), carbon to nitrogen ratio (C/N ratio), total nitrogen (N) and available phosphorus (P), potassium (K) and magnesium (Mg), were tested at the Central Laboratory of the Faculty of Natural Resources, Prince of Songkla University. The average weight of growing medium in each container was monitored monthly throughout the 8 months of the experiment.

### **Vegetative Growth of Rubber Seedlings**

The data for plant growth characteristics, i.e. shoot height, stem diameter (at 5 cm above soil surface), number of compound leaves and chlorophyll content in the rubber plant leaves, were recorded every month for eight months. For the determination of the chlorophyll content, compound leaves were cut into four small leaf discs with an area of 1 cm<sup>2</sup> per disc using a cork borer, and placed in 3 mL of *N,N*-dimethylformamide and kept in darkness for 24 hr. Then the chlorophyll extract was measured using a spectrophotometer (Pharmacia Biotech Ultrospec 3000 UV/VIS, USA) at 647 and 664 nm. The equation used to calculate the chlorophyll content was : Chlorophyll content =  $(7.04 A_{664} + 20.27 A_{647})$ , expressed in mg/cm<sup>2</sup> [14]. In addition, seedlings at the 4-, 6- and 8-month stages were analysed for chlorophyll by a nondestructive method using a chlorophyll meter (SPAD-502, Minolta, Japan). Compound leaves were taken on opposite side of the midrib of each leaflet and then averaged. The reading values from the chlorophyll meter with growth stage of seedlings could be observed in this study.

### **Root Length and Biomass of Seedlings**

Seedlings aged 4, 6 and 8 months were cut at the root collar and taproot length and circling root length were measured. The seedling shoot (including stem and leaves) and circling roots were dried separately at 80°C for 24 hr for biomass estimation [15]. The shoot and root dry weight and root-to-shoot ratio was also determined by root/shoot = total root dry weight/total shoot dry weight [16]. The circling roots were weighed and averaged for each plant. The growing containers were cut into three equal parts : upper, middle and lower, at depths of 0-11, 12-22 and 23-34 cm respectively, to find the root mass distribution in each part.

### **Statistical Analysis**

The experiment was designed based on a completely randomised design and means were compared using Duncan's multiple range test with the significance of results tested at  $P < 0.05$  and  $P < 0.01$  with four replications per treatment (three plants per replication for vegetative growth evaluation and one plant per replication for root length measurement and biomass analysis).

## **RESULTS AND DISCUSSION**

### **Chemical Properties of Media**

The pH, EC, OM, OC, CEC, C/N ratio, total N and available P, K and Mg of the planting materials (topsoil (control) and topsoil: coir dust: rice husk ash in a ratio of 1:1:1 by volume) were analysed before and after planting and the results are shown in Table 2. The results show that the growing medium consisting of a mixture of topsoil, coir dust and rice husk ash has higher OM (5.76%), OC (3.99%) and CEC (2.32 meq per 100 g) before planting compared to topsoil alone.

The mixed growing medium also provides various nutrient elements in abundance with total N, available P, K and Mg of 0.06, 624.40, 1,248.00 and 113.40 mg per kg respectively (Table 2). Noguera et al. [17] reported that addition of coir dust provides relatively high P and K and in the present study the mixed medium is found to have better properties after growing the plants for 8 months compared to topsoil (Table 2). Chanthai [18] reported that coir dust and rice husk ash improve the properties of the growing media by increasing their water holding capacity and CEC, which is beneficial to nutrient adsorption [19].

**Table 2.** Chemical properties of media before and after planting for 8 months

Property	Before		After	
	Topsoil	Topsoil:coir dust: rice husk ash (1:1:1)	Topsoil	Topsoil: coir dust: rice husk ash (1:1:1)
pH (1:5)	4.72	6.94	3.94	4.67
EC ( $\mu$ S/cm)	17.66	635.50	259.50	134.20
OM (%)	2.74	5.76	1.03	5.43
OC (%)	0.91	3.99	0.61	3.16
CEC (meq/100 g)	0.53	2.32	3.46	6.96
C/N ratio	13.25	38.67	12.20	35.11
Total N (mg/kg)	0.04	0.06	0.05	0.09
available P (mg/kg)	5.77	624.40	202.45	342.88
K (mg/kg)	20.34	1,248.00	84.11	164.94
Mg (mg/kg)	12.54	113.40	11.17	57.52

### Weight of Planting Materials

The initial and post-planting weights of the four treatments, T1-T4, are shown in Table 3. Increases in planting material weight occur after planting the seedlings, which is mainly due to the growth increment of the seedlings and the accumulation of moisture both in the plant structure and in the planting media. The same trend is obtained as before planting: T1-T3 containers are lighter by 30.53-37.17% compared to the control. Jaenicke [20] reported that addition of organic components improve the properties of the growing media especially by reducing the weight of the planting material, thus saving transportation costs, particularly for highland rubber growing.

**Table 3.** Weights of planting materials

Treatment	Initial weight (before planting)		Weight after planting during 8 months	
	(kg)	(%)	(kg)	(%)
T1	0.97c	(44.25) †	1.42b	(37.17) †
T2	1.02bc	(41.38)	1.52b	(32.74)
T3	1.05b	(39.66)	1.57b	(30.53)
T4 (Control)	1.74a		2.26a	
F-test	**		**	
CV (%)	3.21		14.59	

Note: \*\* Difference significant at  $p < 0.01$ ; † Figures in parentheses are percentage weight decrease compared to control. Means in each column with the same letters are not significantly different.

### Shoot Height and Stem Diameter

Changes in seedling shoot height and stem diameter 4, 6 and 8 months after transplanting are shown in Table 4. The shoot heights are not significantly different among treatments at 4, 6 and 8 months after transplanting. After 8 months they are in the range of 157.87-183.17 cm. There are no significant differences in stem diameter among the treatments at 4 and 6 months after transplanting. However, at 8 months old, there is a significant difference in T2 (15.82 mm) and T3 (15.88 mm) compared to the control T4 (13.79 mm). Most of the plants reach a suitable size for budding (0.9-2.5 cm diameter) [2] within 6 months after planting. The data suggest that T2 and T3 provide rootstock seedlings of more suitable size for good quality budding.

**Table 4.** Average shoot heights and stem diameters of rubber rootstock seedlings in different containers

Treatment	Shoot height (cm)			Stem diameter (mm)		
	4 months	6 months	8 months	4 months	6 months	8 months
T1	86.48	137.00	177.22	8.31	11.71	14.99ab
T2	100.34	140.90	177.72	8.49	12.00	15.82a
T3	84.44	147.00	183.17	8.57	11.72	15.88a
T4 (Control)	96.75	131.03	157.87	8.09	11.37	13.79b
F-test	ns	ns	ns	ns	ns	*
CV (%)	7.84	9.13	8.23	10.74	5.54	5.31

Note: ns= not significant; \* Difference significant at  $p < 0.05$ .

Means in column with the same letters are not significantly different.

### Number of Leaves and Chlorophyll Content

There are no significant differences among the treatments in the number of leaves at 4, 6 and 8 months after transplanting (Table 5). At 8 months after planting, the seedlings of T1, T2 and T3 have chlorophyll contents of 41.93, 41.58 and 45.44 mg/cm<sup>2</sup> respectively, which are higher than control (T4) as shown in Table 5. This might be due to a greater accumulation of plant nutrients, especially N and Mg, and their better adsorption in the mixed growing material compared to topsoil alone (Table 2). Chanthai [18] reported that the addition of coir dust to planting media reduces N and K losses through leaching. N and Mg are essential constituents of chlorophyll necessary for photosynthesis [21].

**Table 5.** Average numbers of leaves and chlorophyll content of rubber rootstock seedlings in different containers

Treatment	Number of leaves per plant			Chlorophyll content (mg/cm <sup>2</sup> )		
	4 months	6 months	8 months	4 months	6 months	8 months
T1	31.92	55.92	75.83	41.80	37.15	41.93a
T2	35.00	56.00	78.25	41.10	37.43	41.58a
T3	35.17	51.94	73.39	45.73	37.94	45.44a
T4 (Control)	30.75	48.43	64.83	38.22	36.74	36.93b
F-test	ns	ns	ns	ns	ns	*
CV (%)	27.30	18.75	23.95	13.60	18.01	6.14

Note: ns= not significant; \* Difference significant at  $p < 0.05$ .

Means in column with same letters are not significantly different.

### Shoot Dry Weight, Root Dry Weight and Plant Partitioning

Table 6 shows that the container structure has no effect on the shoot or root dry weight of 4-month-old rubber rootstock seedlings. At 8 months after transplanting, T1, T2 and T3 have higher average shoot and root dry weights than those of the control (T4). A similar result was found in *Shorea balangeran* seedlings planted in a growing medium of 80% topsoil: 20% sawdust compost [22]. The root-to-shoot ratio, which indicates the plant partitioning and balance between the above-ground and underground parts of the seedling, is not significantly different among the treatments at 4, 6 and 8 months. Othman et al. [23] previously reported that the root-to-shoot ratio of rubber rootstock seedlings range between 0.31-0.65. An adequate root system improves root adsorption of water and nutrients from the planting media for photosynthesis and growth of the seedling [16, 24].

**Table 6.** Average shoot dry weights, root dry weights and root-to-shoot ratios of rubber rootstock seedlings in different containers

Treatment	Shoot dry weight (g)			Root dry weight (g)			Root-to-shoot ratio		
	4 months	6 months	8 months	4 months	6 months	8 months	4 months	6 months	8 months
T1	13.58	44.48a	110.93a	4.00	18.84a	33.56a	0.29	0.42	0.30
T2	14.01	48.43a	112.98a	5.38	19.79a	41.08a	0.38	0.41	0.36
T3	16.36	49.72a	117.43a	5.42	22.75a	42.98a	0.33	0.46	0.37
T4 (Control)	11.72	34.06b	85.54b	3.74	9.67b	31.62b	0.32	0.28	0.37
F-test	ns	*	**	ns	*	*	ns	ns	ns
CV (%)	27.46	11.31	11.44	24.44	23.85	13.24	28.01	30.81	23.05

Note: ns= not significant; \*, \*\* Difference significant at  $p < 0.05$  and  $0.01$  respectively  
Means in column with the same letters are not significantly different.

### Root Distributions in Upper, Middle and Lower Part of Root Zones

Table 7 shows the percentage of branch roots in the upper, middle and lower parts of each container (Figure 2). Treatments T2 and T3 have the highest concentrations of branch roots (41.43% and 37.82% respectively) in the upper part. The branch root distribution ranges between 20.51-24.98% and is not significantly different among the treatments in the middle zone. In contrast to the upper zone, the lower root zone of T4 and T1 has the highest branch root percentage of 48.86 and 50.51% respectively while T2 and T3 have lower branch root percentages. The results indicate that different container structures result in different root architectures or branch root distributions of the rubber rootstock seedlings. An increase in branch roots in the lower root zone leads to circling roots, which represents a serious root defect contributing to instability and poor root health and retards further growth of the seedling [25].

### Taproot Length and Root Circling of Rubber Rootstock Seedlings

The container structures have no effect on the taproot length of the seedlings at 4, 6 and 8 months after transplanting (Table 8). However, the container structures influence the circling root length and circling root dry weight of the 8-month-old seedlings. T4 and T1 containers have longer circling roots and higher circling root dry weights than those found in T2 and T3 containers (Table 8 and Figure 3). Mckee [26] reported that vertical ridges in the container wall reduce the development of roots in a spiral pattern and also promote downward vertical root growth. These lead to more lateral root growth at the upper part of the root zone, resulting in lower circling roots in

the bottom part of the root zone (Table 7). Circling roots cause long-term tree growth problems in the field such as instability, stunted or lower growth rate and lower survival rate [10, 27-30].

**Table 7.** Branch root distribution in different root zones (upper, middle and lower parts) of rubber rootstock seedlings in different containers at 8 months after transplanting

Treatment	Branch root distribution (% dry weight) in different root zones		
	Upper	Middle	Lower
T1	28.48b	21.01	50.51a
T2	41.43a	20.51	38.06b
T3	37.82a	24.98	37.21b
T4 (Control)	28.97b	22.17	48.86a
F-test	*	ns	*
CV (%)	6.89	13.94	9.74

Note: ns = not significant; \* Difference significant at  $p < 0.05$ .

Means in column with the same letters are not significantly different.



**Figure 2.** Root growth characteristics of rubber rootstock seedlings at 8 months after transplanting in different container structures: (A) T1; (B) T2; (C) T3; (D) T4

(sr = spiraling root, vr = vertical root, cr = circling root, rup = root at upper part, rmp = root at middle part and rlp = root at lower part)

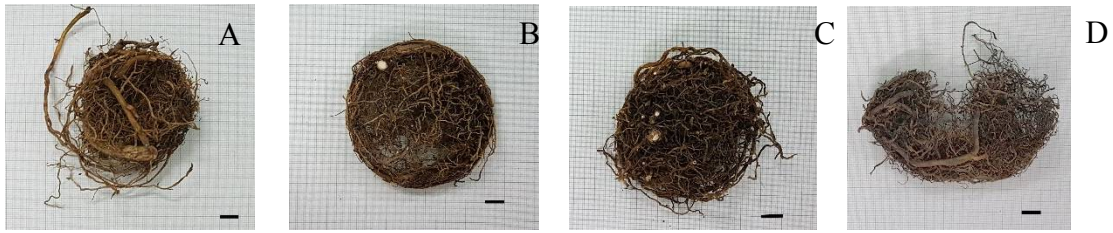
**Table 8.** Average circling root lengths and circling root dry weights of rubber rootstock seedlings in different container structures

Treatment	Taproot length (cm)			Circling root length (cm)			Circling root dry weight (g)		
	4 months	6 months	8 months	4 months	6 months	8 months	4 months	6 months	8 months
T1	39.18	46.66	50.27	7.88	15.17	20.93ab	0.28	1.03	2.76b
T2	39.16	43.60	50.10	7.00	10.93	15.70b	0.21	0.95	2.63b
T3	38.60	44.65	50.55	6.17	12.15	18.40b	0.24	0.58	2.53b
T4 (Control)	36.13	43.00	54.30	9.13	13.50	24.93a	0.14	0.61	3.12a
F-test	ns	ns	ns	ns	ns	*	ns	ns	*
CV (%)	9.46	10.09	12.85	39.83	32.75	11.07	29.96	30.58	6.62

Note: ns = not significant; \* Difference significant at  $p < 0.05$ .



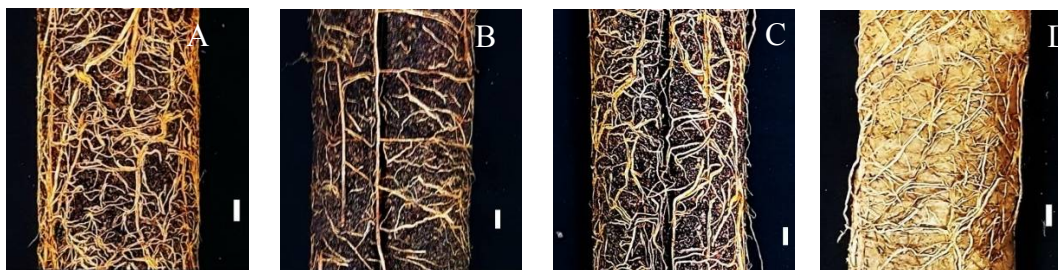
Means in column with the same letters are not significantly different.



**Figure 3.** Circling root characteristics of rubber rootstock seedlings at 8 months after transplanting in different container structures: (A) T1; (B) T2; (C) T3; (D) T4 (Bar indicates 1 cm.)

### Characteristics of Root Growth

Figure 4 shows root growth characteristics of rubber rootstock seedlings at 8 months after transplanting in different container structures. The root growth characteristic in polybags and smooth wall cylindrical plastic tubes (T4 and T1) is non-directional (or spiral) while the roots of the seedlings in cylindrical plastic tubes with two or four internal vertical plastic rods (T2 and T3) obviously have more downward growth along the vertical rods. These vertical rods in the container wall promote vertical root growth, reduce root spiralling and stimulate branch roots. Less spiral roots benefit future root growth and enhance the establishment of the seedling in the field owing to a more efficient root adsorption capacity [8, 31].



**Figure 4.** Root growth characteristics of rubber seedlings at 8 months after transplanting in different container structures: (A) T1; (B) T2; (C) T3; (D) T4 (Bar indicates 1 cm.)

### CONCLUSIONS

Growing containers consisting of cylindrical plastic tubes with two and four vertical rods glued inside and filled with a medium of topsoil: coir dust: rice husk ash at a ratio of 1:1:1 by volume enhance the shoot and root growth of rubber rootstock seedlings, which produce an increased percentage of branch roots in the upper root zone and a decreased percentage in the lower root zone. Circling roots and root spiralling also decrease.

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