

Effect Of Various Types And Size Of Container On Growth And Root Morphology Of Rubber (*Hevea Brasiliensis* Mull. Arg.)

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Abstract: Evaluation of the effect of container types and size currently used by nursery rubber growers is important to reduce the problem of dieback and causalities during transplanting of seedlings. Assessment of the planting material is essential for replanting exercise to meet its objective of early growth and optimum yield in the later stage of the plants. The study was conducted to evaluate the impact of various container sizes on the growth of rubber seedlings. Containers of different volumes 600 ml 710 ml, and 900ml and polybag 15 x 20 cm as control evaluation was used. All the containers except polybag greatly support plant stem diameter. Only the biggest container 900 ml maintained higher leaf area index (LAI). Plant biomass like total fresh and dry weight and the root-shoot ratio (RSR) were significantly different in the same container. Plants grown in this container recorded higher nitrogen content which could be considered suitable for rubber seedlings. Root length of the plants was greatly influenced by the container (900 ml) and significantly higher than those in other containers. Root average diameter was positively affected except for the plants grown in the poly-bag. This suggests that the polybag and the remaining container sizes inhibited the roots and this could have contributed to the poor growth and some of the morphological traits. Consequently, a large container size, 900 ml had resulted in a better and vigorous growth of the seedlings and it could be ideal pot size for use in raising rubber seedlings of *H. brasiliensis*.

Keywords: container size; immature rubber; *Hevea brasiliensis*; root morphology

1 INTRODUCTION

Rubber (*Hevea brasiliensis*) producing countries traditionally use polybag as a major container for seedlings due to its several advantages when compared to direct planting especially with the planting of budded stumps. The advantages include reduced immaturity period, facility to cull out weaklings in the nursery, growth uniformity and relatively reduced causality. Despite all these advantages, many growers have reported significant drawbacks such as less lateral roots and coiling of the taproot. A plant which has both roots patterns damaged finds it difficult to attain its normal growth as the root is distorted and strangling in subsequent years or throughout its lifespan.

Many researchers have reported the adverse effect of this damage which includes poor drought tolerance and wind fastness [1] and the root damage begins when it rooted reach the lower end of the bag. Another demerit of polybag planting is the difficulties in the movement of the plants from nursery site to the field; there is possible severe damage to root and soil core of this planting activities. Root trainers (scientifically designed plastic containers) are being considered as an alternative to promote healthy root growth and prevent damage during planting. It is labour-saving, eco-friendly and cost-effective, especially for commercial use. Better morphological plant traits such as height and stem diameters of plants grown in root trainers or plastic containers than those of polybags had equally been reported [2]. The use of root trainers in rubber nursery is becoming well-known in many rubber producing countries. This container is made from polypropylene. It has a different holding capacity of 600-800 cm³. Though crops especially those grown under shelter or greenhouse use different types and the containers are widely varies in volumes. Nutrients and water availability for the plant growth are determined by the size of a container. Container size has become an issue of interest in many experiments and it has occasionally received attention especially in plant biology related fields [3]. Most of the available studies centred on the growth of vegetables [4]. Most scientific literature pays little attention to the pot size of tree seedlings and it is not frequently reported in the materials and methods of tree crops related publications. Interestingly, it has become an issue of interest in the recent time as a poor growth response to plants is attributed to root deformation [5] Small container sizes are being accepted by many growers because it is easy to handle when transporting to the field during transplanting due to reduced weight even with the plant. The amount of soil or substrate required is less compared to large containers. However, plant experiences an impediment to root growth especially for the seedlings as many plant species exceed 1 m in length, consequently goes beyond the dimensions of many containers used [6]. Soilless medium containing proportions of both organic and inorganic like coir pith, rock phosphate, and compost is used to fill the container [7]. It reduces some of the

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problems like pests and diseases associated with the use of soil and reduced use of soil fumigants is equally possible [8]. Air contact of the root tips, self-pruning, and well-aerated of this planting medium helps the plants to experience little stress and these subsequently leads to the emergence of a significant quantity of well-developed lateral roots without deformation as previously reported when the plants are grown in polybags [9]. Due to its different advantages in rubber nursery, it may possibly be the best choice of rubber seedlings planting materials in years to come [10]. The objective of this study is to evaluate the effect of different sizes of container on morphological traits of *Hevea* seedlings and to determine the container which relatively shows a better support for plant growth.

2 MATERIALS AND METHODS

2.1 Root trainers, planting materials and treatments

Four container sizes were evaluated with immature rubber in this experiment comprises of a newly modified container similar to the commercial plastic container "root trainer" used for rubber seedlings. The plastic was locally sourced and generally known as plastic bottle water painted in a black colour to reduce effect of sunlight. It is 900 ml in volume, 40 cm length and 80 mm. The second size is a container with 710 ml, 30 cm, 60 mm. The third container was a commercially designed root trainer which has 600ml volume, 26 cm length and 74 mm. It is a black plastic container with straight ridge downward of different circumferences. The shapes of the containers were similar with a rounded hole at the end to allow air and water drainage. A Polybag size 15 x 20 cm and 4.5 kg of Oxisol soil was filled per poly-bag designated as control evaluation. Bare rooted budded stumps of RRIM 3001 were sourced from Liman Plantation Sdn Bhd. The rubber budded stick was planted in different sizes of the containers and polybag designated as the control. The plastic containers were filled with soilless medium and polybags were filled with Oxisol soil. The experiment was conducted in field No. 2 of the Faculty of Agriculture, Universiti Putra Malaysia. The budded stumps were planted in the containers according to rubber industry standard procedure. The experiment was arranged in Randomized Complete Block Design (RCBD) with five replications under a rain shelter. Irrigation of the plants was done using a sprinkler irrigation system. The plants were watered overhead twice at the beginning of the experiment and later changed to once a day when the soilless medium was observed to be saturated. Three weeks after planting and after the emergence of leaves from scions, a mixture of fertilizer solution and fungicides were applied. Bayfolan 5 mL, MZ – 45 2 g and Brightconil 75 WP 2 g in 800 ml of water were sprayed as foliar spray two times weekly (Malaysia Rubber Board, 2009).

2.2 Plant growth and some plant physiological parameters

Stem diameter of the plants was measured using a digital veneer calliper (Model 500–196, Mitutoyo Products, America). This was measured 10 cm from the surface of the soil/media respectively. The leaf chlorophyll content was measured non-destructively on the fourth leaf using a Minolta-502 dual wavelength chlorophyll (SPAD) meter (Minolta Co. Ltd, Osaka, Japan). It is a portable held hand device with an inbuilt meter usually clamped around the leaf to be measured.

Measurements were taken three times for each leaf sample. An average figure was calculated from a single measurement on each side of the main vein, at the distal part of the leaf. A press roller flattens any curled edges and feeds the leaves properly between the transparent belts before they enter the scanner, ensuring the full area was represented in each measurement. Leaves are ejected from the scanning bed after they are measured. Thereafter the reading was recorded. Leaf area index (LAI) was equally measured and calculated.

2.3 Foliar and Root Nutrients Analysis

The Foliar analysis was carried out according to the rubber industry foliar sampling techniques which specify four basal leaves from the first sub-terminal whorl for the leaf samplings (Noordin, 2012). The leaves were oven-dried at 50°C for 48 – 72 hours. A 0.25 g of the weighed ground dried samples were put into digestion tube. Then 5 ml concentrated sulphuric acid (H₂SO₄) was added, shaken and left for about 2 hours to adsorb moisture. Thereafter, the mixture digestion tubes were placed in the digestion block at the temperature 450°C in the Fume Chamber for approximately 45 minutes. The digestion tubes were removed and allowed to cool, after which, 2 ml of hydrogen peroxide (H₂O₂) was added and the heating process was repeated in the Fume Chamber. After the stipulated heating period, the sample in the tube became colourless. The solution was left to cool and later diluted with distilled water to make up 100 ml. The samples were analyzed for N, P, and K using Auto Analyzer, while Mg was analyzed with Atomic Absorption Spectrophotometer (Perkin-Elmer, Model AAS 3110).

2.4 Measurement of Root morphology

At the end of the experiment, after the last harvest, root morphological traits were examined. Roots were gently separated from the growing and containers, washed thoroughly with water to remove excess medium. The roots were spread in a transparent plastic tray in a thin layer of water and analyzed for image data. Root morphologies were measured using WinRHIZO pro software (Epson Perfection V700 Photo, Regent Instrument Inc. Canada). The following data were collected root length, average diameter, root surface area, and a number of tips.

2.5 Data analysis

All data were analyzed with analysis using SAS statistical software Package (Version 9.1). The Least Significant Difference (LSD) was used to compare treatment means at the 0.01 and 0.05% probability levels.

3 RESULTS

Results showed that container size significantly influenced plant chlorophyll content as shown in figure 1a. The values recorded in the plants grown in the biggest container size 900 ml indicated the highest chlorophyll content 327.8 and it was significantly different $p < 0.05$ from container size 600 ml 255.58, 710 ml 253.04, and polybag plants 225.72 which is commonly used in rubber nursery. Also, plants grown in the biggest container size 900 ml significantly ($p < 0.01$) maintained a higher leaf area index at 10.18 m²m⁻² over time and significantly different $p < 0.05$ from plants grown in container size 600 ml (3.32 m²m⁻², 710 ml 2.32 m²m⁻² and polybag 6.17 m²m⁻² (Figure 1b). There was a significant difference ($p < 0.05$) which indicated that the plants grown in container size 900 ml

4.21mm and 600 ml 4.44mm were significantly different from plants grown in polybag 3.04 mm (Figure 1c). This could have been due to growth inhibition caused by the bag limiting the plant's water and nutrients uptake for the plant part development.

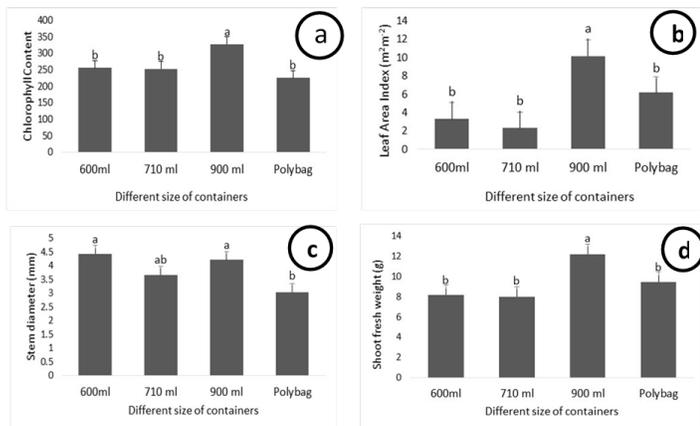


Figure 1a–d. Effect of different size of containers on chlorophyll content, leaf area index, stem diameter and shoot fresh weight.

Mean values followed by the same letter on each figure are not significantly different at $P < 0.05$, based on least significant difference test (LSD). Vegetative traits like the shoot fresh weight, total fresh weight, total dry weight and root: shoot ratio (RSR) were significantly influenced by container size as shown in figure 1c. Shoot fresh weight (SFW) of the plants grown in the biggest container volume recorded the highest value 12.22 g/plant of SFW and significantly different from plants grown in other container sizes 600 ml, 710 ml and polybag which recorded 8.18 g/plant, 8.02 g/plant, and 9.46 g/plant respectively. Similarly, greatest total fresh weight (TFW) was related to the biggest container size 900 ml at (22.86 g/plant) and significantly different $p < 0.05$ from plants grown in other container sizes 600 ml, 710 ml and polybag which recorded 12.99 g/plant, 600 ml 13.68 g/plant and polybag 13.14 g/plant as shown in figure 2a. The same scenario was observed with regards to total dry weight (TDW) whereby the same container size 900 ml recorded the highest value 4.87 g/plant and significantly different $p < 0.05$ from plants grown in other container sizes figure 2b. Noticeably, similar values were recorded with no significant differences $p > 0.05$ among other container sizes as 701 ml 3.56 g/plant, 600 ml 3.51 g/plant and polybag 3.39 g/plant respectively. Furthermore, the results indicated that the root-shoot ratio of the plants was significantly influenced $p < 0.05$ with a highest value 0.34 recorded in the plants grown in the biggest container volume 900 ml indicating a significant relationship between shoot and root biomass in this container compared to other containers which recorded closer values whereby 701 ml recorded 0.22, 600 ml 0.15 and polybag 0.20 (Figure 2c). Root length as one of important root morphology was shown in figure 2d. The results showed that root length of plants grown in biggest container 900 ml significantly increased 2711.7 cm and significantly different from plants grown in container size 600 ml (1627.9 cm), 710 ml (1591.9 cm), and polybag plants (1089.6 cm). The effect of the various container sizes was presented in figure 5.

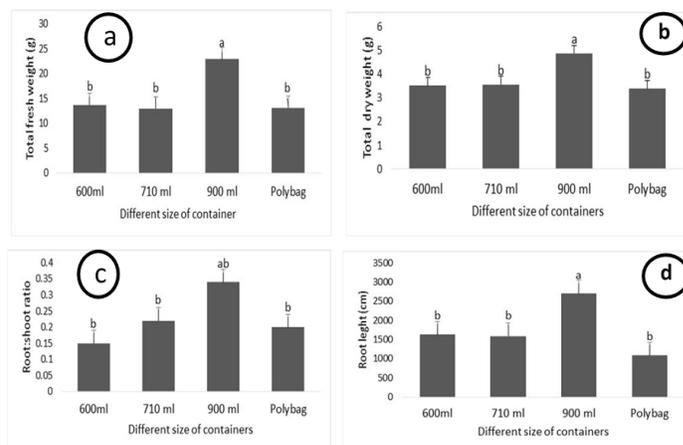


Figure 2a–d. Effect of different size of containers on total fresh weight, total dry weight, root: shoot ratio and root length

Mean values followed by the same letter on each figure are not significantly different at $P < 0.05$, based on least significant difference test (LSD).

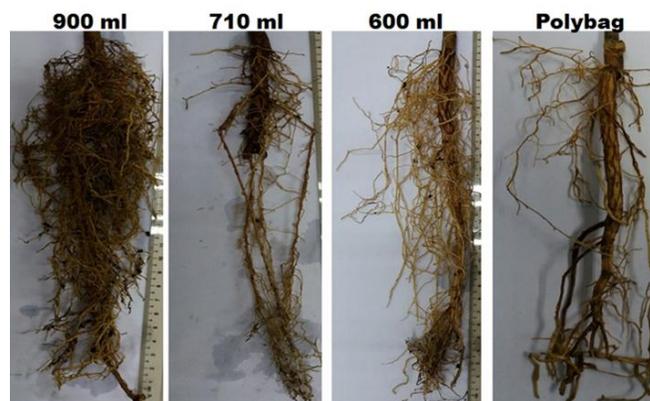


Figure 5. Effect of different size of root trainer on roots of rubber seedlings

Some of the major nutrient elements required by rubber were analysed and presented in figure 3a - d. The results showed a higher concentration of leaf nitrogen content in the plants grown in container size 900 ml 3.90 % and significantly different from N concentration in plants grown in other container sizes whereby 600 ml 710 ml, and the polybag recorded 2.11 %, 2.82 % and 2.22 % N concentration respectively (Figure 3a). The concentration of N in the plants grown in the biggest container size could be related to the best vegetative growth of leaves as previously shown in total fresh weight and total dry weight biomass of the plants which had indicated an availability of the nutrient for the plant's growth due to adequate space for nutrient release. Higher concentration of leaf potassium content was recorded in plants grown in the smallest container size 600 ml at 0.82 % and significantly different $p < 0.05$ from the leave of plants grown in 900 ml 0.60 %, 710 ml 0.64 % and polybag 0.69 % (Figure 3b).

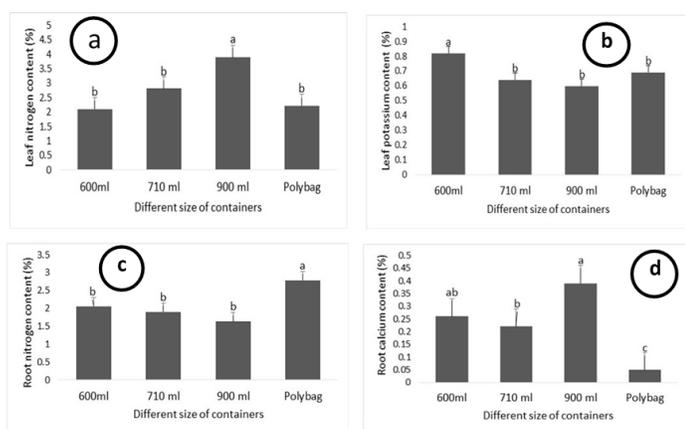


Figure 3a –d. Effect of different size of containers on leaf nitrogen content, leaf potassium content, root nitrogen content and root calcium content

Mean values followed by the same letter on each figure are not significantly different at $P < 0.05$, based on least significant difference test (LSD). The results indicated a concentration of nitrogen in plants grown in polybags at 2.79 % in the plant roots and it was significantly different $p < 0.05$ from the plants grown in 600 ml (2.06 %), 710 ml (1.91 %), and 900 ml (1.64 %) as shown in figure 3c. There was a significant difference at $p < 0.01$ in the concentration of Calcium content in the root of the plants grown in container size 900 ml (0.39 %), 710 ml and the polybag (Figure 3d).

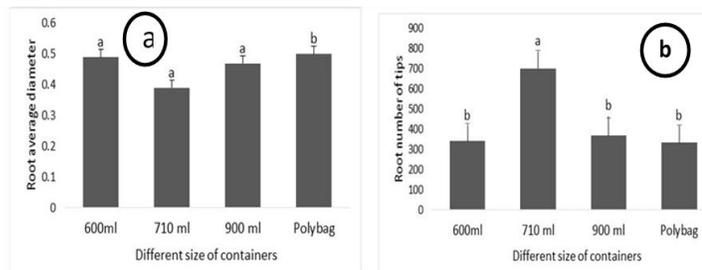


Figure 4a – b. Effect of different size of containers on leaf nitrogen content, leaf potassium content, root nitrogen content and root calcium content.

Mean values followed by the same letter on each figure are not significantly different at $P < 0.05$, based on least significant difference test (LSD). There was a significant effect of container size on root average diameter except for the plants grown in the polybag. Plants grown in 900 ml (0.47 mm), 710 ml (0.39 mm) and 600 ml (0.49 mm) polybag were significantly different $p < 0.05$ from plant grown in polybag Figure 4a. On the other hand, root number of tips were significantly affected by the container size 710 ml (70196) and significantly different at $p < 0.05$ from root tips of plants grown in container size 900 ml, 600 ml and polybag Figure 4b.

5 DISCUSSION

Many studies had been conducted on the effect of container size or shape especially on planting stocks quality of vegetables and ornamentals plants. A relationship between seedling growth and container size has been generally

established [11]. But there are limited studies on the effects of container size on nursery trees like rubber (*Hevea brasiliensis*). In this study, different size of containers showed significant effects on growth of rubber budded stumps. Morphological traits especially fresh and dry biomasses of plants, as well as some root morphological traits, were significantly affected by various container sizes especially the biggest container size. This shows that containers used could impact rubber seedling growth. These results were supported by [12] who observed that biomass yield could increase by at least 43% under an increased size of container or times two of the small pot size because plants enjoy a favourable photosynthetic rate in relation to leaf area. The results indicated that various container sizes especially the biggest size significantly supported plant scion stem diameter and significantly different from plants grown in other container sizes. This is in agreement with a study conducted by [13] who reported that container size increases plant stem diameter depending on species of the tree seedlings and growing conditions. However, stem diameter has been identified as an essential part of rubber plant because it helps to determine the extent of plant maturity. It also determines the ability of the plant to support the extended crown of leaves [14]. This study is supported by findings by [15] who reported that containers of various sizes have advantages, however, the use of small pot size has been basically attributed to the resource control like the use of little soil and water. Appropriate delivery of the plant quality is equally possible where small or medium pot sizes are used [16]. However, biological constraints are one of the disadvantages, when small containers are used for research [12]. Also, in this study, the biggest container size significantly maintained high chlorophyll content than plants grown in smallest or smaller container size. Physiology of the plant grown in other containers might have been affected due to root restriction. This is supported by [17] who observed that when the root is negatively affected, it could cause physiology imbalanced like in the transpiration, stomatal and chlorophyll content. This could be attributed to internal water deficit due to the poor release of water. The results equally showed that only plants grown in the biggest container size recorded higher leaf area index (LAI). [18], reported that bigger container filled with nutrient-rich growing medium promotes CO_2 enrichment and significantly increases it during plant growth than smaller containers. Thereby, enhance gas exchange such as photosynthetic, stomatal and other leaf vegetative traits provided humidity and water stress factors are eliminated. More so, reduction of plant canopy could occur due to small container size due to root restriction. The results indicated a significant influence of the container size on plant biomass production like the fresh and dry weight was significantly influenced by the biggest container volume used in this study. Plant vegetative traits increase is correlated with bigger container size [19]. The biggest container volume significantly affected shoot fresh weight (SFW). This is supported by [20] who reported that plant vegetative weight like the shoot and root biomass increases in large container size than the smaller pot size because the former continuously supports plant growth while retardation may occur in small pot size as the growth increases. In addition, [21] observed that when compared to the small container sizes, crops like Red Sunset red have been successfully grown in large containers with better growth traits. However, [22] noted that species of plant responds differently with container size especially with regards

to biomass distribution while in some species, it rather remains constant as the container changes. More so, in predicting the plant morphological development index and suitability of the seedlings for out planting, plant biomasses such as root dry weight, root/shoot ratio have been recommended in various studies for different plant species [23]. The same biggest container size maintained significant of the plant's total fresh and dry weight of plant biomasses. This could have been due to enough space provided by the bigger container for adequate root growth compared to smaller or medium containers which might have cause root restriction. [24], reported from the studies conducted on Avocado that bigger container maintains plant growth noticeable in leaf area, fresh and dry weight accumulation. This could due to increase in leaf conductance and CO₂ assimilation rate [25]. Root restriction by container size could lead to drastic reduction of plant dry matter yield but poor nutrients have not been frequently reported [26]. The results indicated only plants grown in the biggest container maintained significant root-shoot interaction noticeable in root: shoot ratio. This could be attributed to the adequate release of water and nutrients by root to shoot system due to unrestricted roots. This is in agreement with [27] on studies conducted on Peach *Prunus persica* (L.) Batsch showed that the plant experience rapid growth when a bigger container was used due to the internal regulation of root including the release of growth substances. Therefore, root restriction caused by small container negatively affects root-shoot interaction and overall plant growth while plants grown in bigger container apparently enjoy the existence of an equilibrium between water usage and plant growth increment moderated by the container capacity. However, [28], noted that root/shoot ratio would not be negatively affected by a container volume, where rooting volume is well coordinated by root and shoot growth. Furthermore, results of this study showed the significant concentration of N and K in leaves of plants grown in biggest and smallest plastic container size. On the other hand, McConnaughay [29] reported that irrespective of container size, high nutrient concentrations could occur with the help of CO₂ inducement because various container aids CO₂ responses of plant noticeable in total biomass. On the other hand, the results indicated the high significant concentration of N concentration in roots of plants grown in polybag while Ca concentration was noticed in the root of the plants grown in the biggest plastic container and smallest container. The N concentration in polybag could be attributed to the soil used. [30], reported that poor release of water due to compaction or drought could be related to the poor release of nutrients. Thus, nutrient elements like nitrogen could be held in the soil or plant root. Its negative effect is noticed in poor photosynthetic rate noticeable in plant shoot biomass. Therefore, it appeared that the N was held in the root of the plants grown in the polybag due to inhibitory activity of the roots as soilless nutrient availability did not translate into other vegetative growth of the plants grown in the polybag. Effective nutrient supply in the nursery is important to aid maturation and vegetative growth of rubber seedlings, as well as reduce cost of production [31]. Most importantly it is needed for immature rubber to boost its vegetative growth and yield in later stage [32]. The results indicated that only biggest container size significantly affected roots length of the plants. This could have been due to the depth of the container among other characteristics. [33], reported that root system could be

determined by container depth especially rooting length, and thus determines plant growth vigour. All the containers except polybag significantly impact root average diameter. Reduction of the root in small containers and bag could be contributed by poor growth factors like poor water drainage in the bag. This is supported by [34] who reported that water dries out quickly in the small container due to its reduced total water holding capacity and its extreme levels increase root mass fraction (RMF).

6 CONCLUSIONS

The newly introduced biggest container size 900ml remarkably supported plant growth and morphological traits of the rubber (*Hevea brasiliensis*), most importantly the growth traits like stem diameter and leaf area index (LAI). Biomass production like total fresh and dry weight and the root-shoot ratio (RSR) significantly improved under for the plants grown in the biggest container size. The container dimensions allow extensions of different root patterns as the plant's growth progresses. However, improvement of the container may be required from time-to-time for standardization and for further use in the commercial nursery. Noticeable, the bag (control) used in the study which is commonly used encouraged strangulation and distortion of the plant roots. The lateral roots penetrated the bag earlier (after two months) and grew out which could lead to root causalities when moving the plant from nursery site to the field and this causes the retarded growth of plants and its severe levels may cause the complete death of the plants.

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