Syafiqah Nabilah, S.B. *et al*, International Journal of Advances in Agricultural Science and Technology,
Vol.5 Issue.6, June- 2018, pg. 1-8 **ISSN: 2348-1358**
Impact Factor: 6.057
NAAS Rating: 3.77

VEGETATIVE PROPAGATION USING STEM CUTTINGS AND MICROSCOPIC CHARACTERISTICS OF *CHROMOLAENA ODORATA*

Syafiqah Nabilah, S.B., Farah Fazwa, M. A., Norhayati, S., Masitah, M. T., Mohd Zaki, A.

Plant Improvement Program, Forestry Biotechnology Division, Forest Research Institute Malaysia, 52109 Kepong, Selangor, syafiqah@frim.gov.my

Abstract

Stem cuttings of *Chromolaena odorata* obtained from wild stock plants were planted in open mist propagation system. Each stem was divided into three parts i.e. upper, middle, bottom. The length of each cutting was 11 cm. The base of each cutting was treated with commercial powdered hormone, Seradix 2 (0.2 % IBA). These cuttings were planted in cleaned river sand medium for seven weeks. Results showed that cuttings taken from the top part of stem produced significantly higher rooting percentage (90%) and root length (11.47 cm) than those of middle and basal part of stem. The stem anatomy showed the presence of vascular bundles increase from upper to bottom parts of cuttings. However, it does not significantly affect the rooting formation in *Chromolaena odorata* as the upper part gave good rooting performance compared to others. The upper part of the stem is recommended to be used as cutting materials for future planting stocks production.

Keywords: vegetative propagation, root length, rooting percentage, planting stock production

1. Introduction

Chromolaena odorata L. (Siam weeds) is commonly known as pokok kapal terbang in Malaysia belongs to family Asteraceaea. This plant is native in North America and has been introduced to South America, Tropical Asia, West Africa and parts of Australia (Mc Fadyen and Skarratt, 1996). This weed has a minimum 10 years life span which grows 2-3 meter in height with straight, pithy, brittle stem that branch readily. The leaves are arrowhead-shaped that grow in opposite pairs along the stems and branches (Sirinthipaporn and Jraungkoorskul, 2017). The roots are narrow and fibrous and generally reach 0.3 km in depth (Chakraborty et al., 2011).

It was reported that *C. odorata* is an aggressive competitor that suppresses young plantation, agriculture crops and grows on other vegetations (Azmi, 2002). However, there was research showing that the species has positive contribution to the agricultural sector. The leaves of *C. odorata* are used as an ingredient for formulating animal feeds especially in rabbit's diet where the nutrient profile is similar to a concentrated feed (Bamikole et al., 2014). The leaves are also claimed to have high nutritive values and have potential to be used as protein supplements to ruminants (Apori et al., 2000).

Traditionally, fresh leaves or a decoction of *C.odorata* have been used by tropical countries for the treatment of leech bite, soft tissue wounds, burn wounds and skin infection (Phan et al., 2001). Scientific studies on the effects of *C. odorata* leaf extract towards wound healing had been conducted and it shows positive results (Vaisakh and Pandey, 2012). The leaves extract can also inhibit the growth of microorganisms in the wounds and help in forming new tissue cells (Phan et al., 1998).

This plant has great potential to be commercialized in pharmaceutical industry. In consequence, researchers from Plant Improvement Programme, Forest Research Institute Malaysia (FRIM) have started the breeding

studies on this plant for future planting stocks production. The present experiment was carried out to observe the effects of different stem cutting position on rooting of *C.odorata*. The finding from this study can be used as guideline in mass propagation of this plant in future.

2. Materials and Methods

Stem cuttings

An experiment using stem cuttings of *C. odorata* was carried out in nursery of FRIM in April 2017. The stems were collected from abandoned area within FRIM region. Each stem was cut into three portion, i.e. top, middle and bottom. The length of each cutting was 11 cm. The base of the cutting was treated with commercial powdered hormone, Seradix 1 (0.1% IBA). A total of 90 cuttings were used and they were arranged randomly in 3 blocks with 30 cuttings per block. These cuttings were planted in cleaned river sand in a propagation tray. Observation on cuttings was made weekly and the experiment was terminated at week 7 since most of them had rooted. Variables collected were the number of cuttings rooted, dead and root length. Data were subjected to analysis of variance using a statistical package for social study (SPSS) version 22.0 International Business Machines Corporation, New York, USA). This was followed by Duncan Multiple Range Test (DMRT) to see the effect of different cutting part on rooting. The results of the analysis were considered significant when $P < 0.05$.

Microscopic identification

The fresh specimen used in this study was collected at FRIM, Kepong, Selangor, Malaysia. The stem specimens collected were fixed in AA (70% ethanol: 30% acetic acid in ratio of 1:3) for 48 hours before further procedure was carried out. The stems were sectioned using sliding microtome and stained using Safranin and Algian Green. The specimens were then dehydrated in an alcohol series (50%, 70%, 90%, 95% to absolute ethanol) and mounted using Euparal. The samples were placed in an oven at 50ºC for nearly 2 weeks. The slides were photographed using Cell^B software.

3. Results and Discussion

Propagation through stem cuttings

Analysis of variance on variables taken seven (7) weeks after planting showed that there was significant difference among the positions of cuttings. The cuttings taken from upper showed higher percentage of rooted cutting and higher survivality than those of middle and bottom part as presented in Table 1. For root length, upper part showed the highest measurement (11.47±1.37cm) while bottom part showed poor performance of root length (4.02±0.91 cm).

Table 1: Effect of cutting position on rooting ability of *Chromolaena odorata* 7 weeks after cutting

Cutting position	Rooted cuttings (%)	Unrooted cuttings (%)	Dead cuttings (%)	Mean root length±SEM (cm)
Upper	90.0a	NA	10.0b	11.47±1.37a
Middle	63.3b	NA	36.7a	6.61±1.30b
Bottom	60.0b	NA	40.0a	4.02±0.91b

Means followed by the same letters in the same column are not significantly different at $P<0.05$. SEM: Standard error of means.

The difference in rooting percentages with the position of cutting could be due to the different degree of juvenility along the stem. This assumption is based on the diameter of cuttings where the top cutting has significantly smaller diameter and more juvenile than the middle and bottom parts (Figure 1). The juvenility of stem reduces from top to bottom thus give different response on the rooting percentage. This finding is in line with Otiende et al. (2016) study on the effects of cutting

position (top, middle and bottom) of *Rosa hybrida* rootstocks. Porlingis & Therois (2015) they also found that juvenile cuttings rooted faster and in higher percentages as compared to adult cuttings of leafy olive.

Leaf retention could also be a factor that influences the rooting capacity of cuttings. As in Figure 1 (b), the top part has leafy stem cuttings compared to middle and bottom with leafless cuttings. The leaves function as an auxin holder which translocated the auxin to the cutting base and allowing the production of carbohydrates by photosynthesis (Hartmann et al., 2002; Bordin et al., 2005), and acting as a trigger to the developmental process of rhizogenesis (Robert & Friml, 2009). Bona and Biasi (2010) also found the root length and fresh weight of *Lavandula dentata* L. were statistically greater with 2/3 of leaf retention as compared to the leafless cutting. However, excessive leaf area left on the cutting should be avoided as it can cause dehydration on cuttings and affect the rooting formation (Hartmann et al., 2002; Lima et al., 2006).

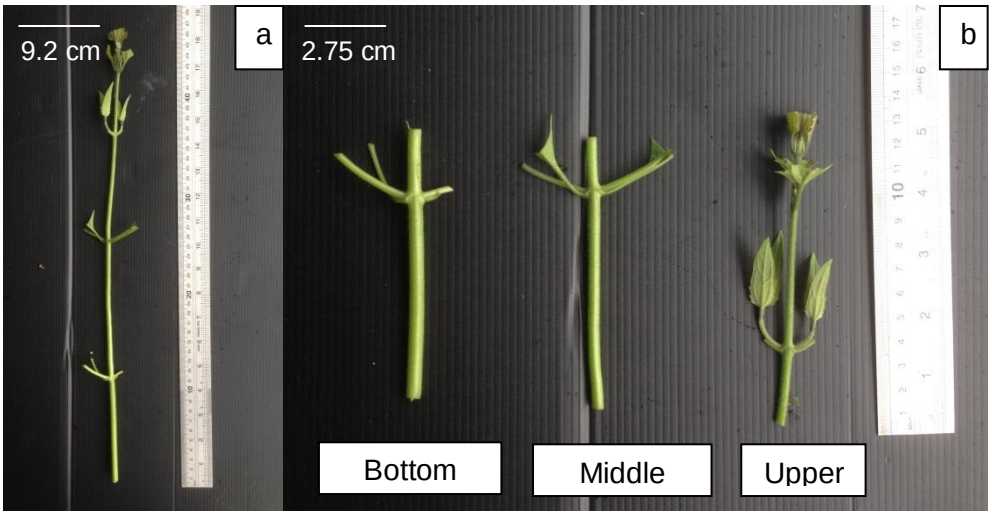

Figure 1: *Chromolaena odorata* L. a) Stem b) top, middle and bottom cuttings

Microscopic study of *Chromolaena odorata*

Table 2 showed the microscopic characteristics comparison between upper, middle and bottom layers of *C. odorata* stem cutting. The presence of vascular bundles, pith and parenchyma cells increase from upper to bottom layers of stem. Whereas, the trichome cells reduced from upper to bottom layer as shown in Figure 2 (c), Figure 3 (d) and Figure 4 (c).

Table 2: Microscopic characteristics comparison of three layers of *C. odorata* stem

Characteristics	Upper layers	Middle layers	Bottom layers
Vascular bundles	*ca.* 26-27 of vascular tissues	*ca.* 33-34 of vascular tissues	ca. 39-40 of vascular tissues
Pith	*ca.* 30-32 of layers	*ca.* 33-34 of layers	*ca.* 35-40 of layers
Parenchyma cells	2-4 layers	5-6 layers	6-9 layers

ISSN: 2348-1358
Impact Factor: 6.057
NAAS Rating: 3.77

Microscopic Characteristic of Kapal terbang Upper Stem

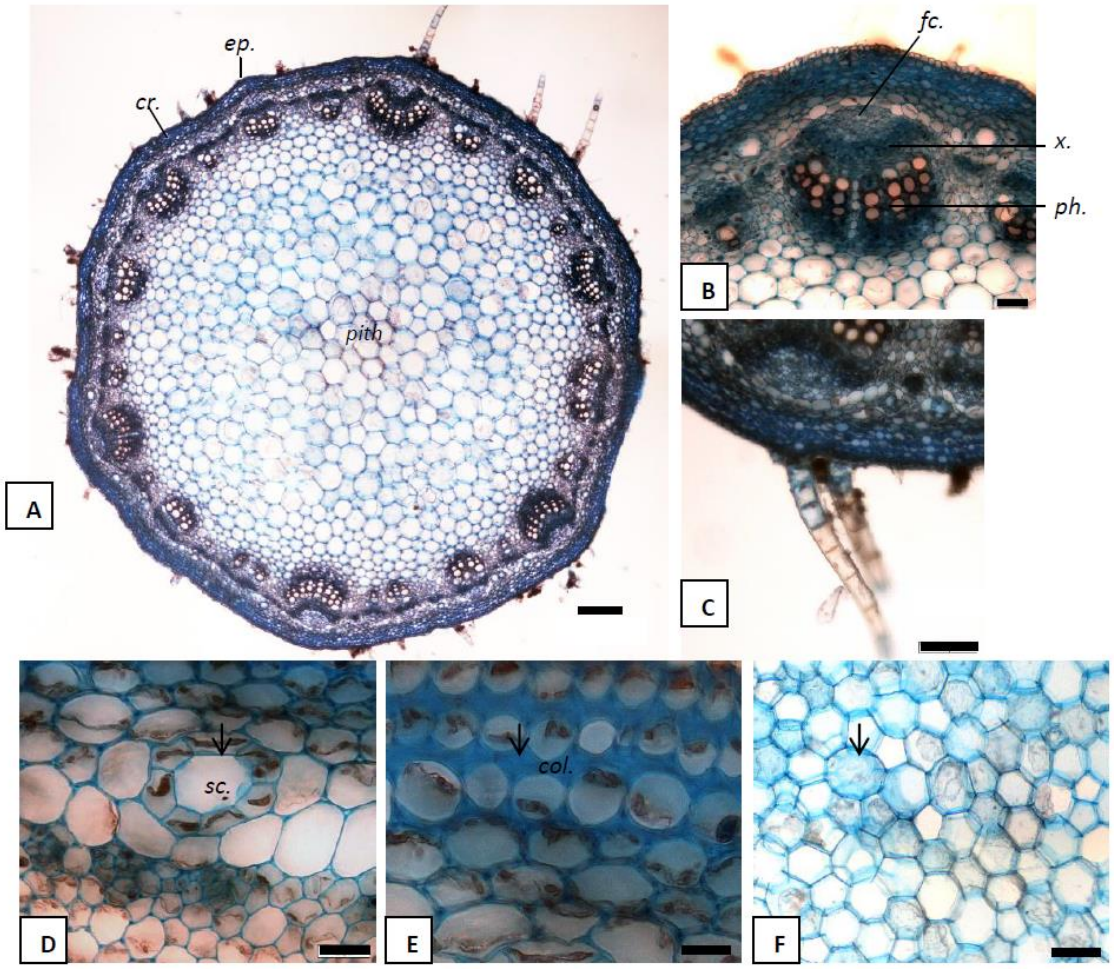

Figure 2 Cross section of Kapal terbang upper stems
(A) CS of upper layers of stem; circular shaped, showing of epidermal cells (*ep.*), separated vascular bundles with closed system, *ca.* 30-32 layers of pith and cortex layer (*cr.*) with collenchyma cells (*col.*), parenchyma cells in 2-4 layers and sclerenchyma cells occur as bundle caps outside phloem. **(B)** Vascular tissue; xylem (*x.*) and phloem (*p.*) and fibre cap (*fc.*) **(C)** Simple, uniseriate trichomes. **(D)** Secretory cells (*sc.*). **(E)** Collenchyma cells (*col.*). **(F)** Mucilage cells (arrow). [CS - Cross section, (*ep.*) – epidermal cell, (*cr.*) - cortex, (*sl.*) - sclerenchyma cells, (*fc.*) – fibre cap, (*x.*) – xylem, (*ph.*) – phloem, (*sc.*) – secretory cells, (*col.*) – collenchyma cells and (*mc.*) – mucilage cells]. Scales bar: A = 200 µm; B = 500 µm; C = 100 µm and D, E, F= 20 µm.

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Microscopic Characteristic of Kapal terbang Middle Stem

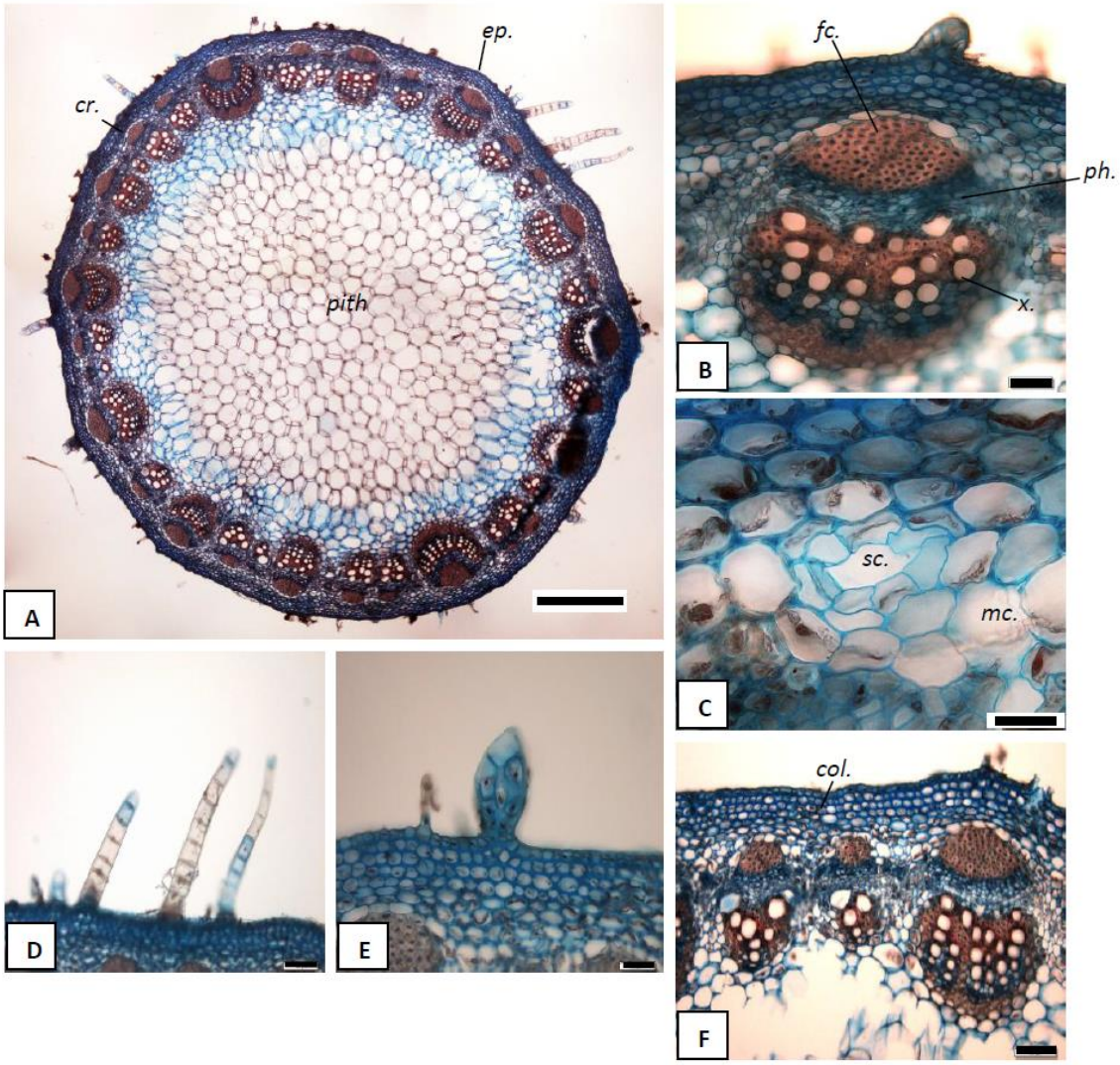

Figure 3 Cross section of Kapal terbang middle stems
(A) CS of middle layers of stem; circular shaped, showing of epidermal cells (*ep.*), separated vascular bundles with closed system, *ca*. 30-33 layers of pith and cortex layer (*cr.*) with collenchyma cells (*col.*), parenchyma cells in 5-6 layers and sclerenchyma cells occur as bundle caps outside phloem. **(B)** Vascular tissue; xylem (*x.*) and phloem (*p.*) and fibre cap (*fc.*) **(C)** Secretory cells (*sc.*) and mucilage cells (*mc.*). **(D)** Simple, uniseriate trichomes. **(E)** Glandular trichome. **(F)** Collenchyma cells (*col.*). [CS - Cross section, (*ep.*) – epidermal cell, (*cr.*) - cortex, (*sl.*) - sclerenchyma cells, (*fc.*) – fibre cap, (*x.*) – xylem, (*ph.*) – phloem, (*sc.*) – secretory cells, (*col.*) – collenchyma cells and (*mc.*) – mucilage cells]. Scales bar: A = 500 µm; B, E = 50 µm; C = 20 µm and D, F = 100 µm.

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Microscopic Characteristic of Kapal terbang Bottom Stem

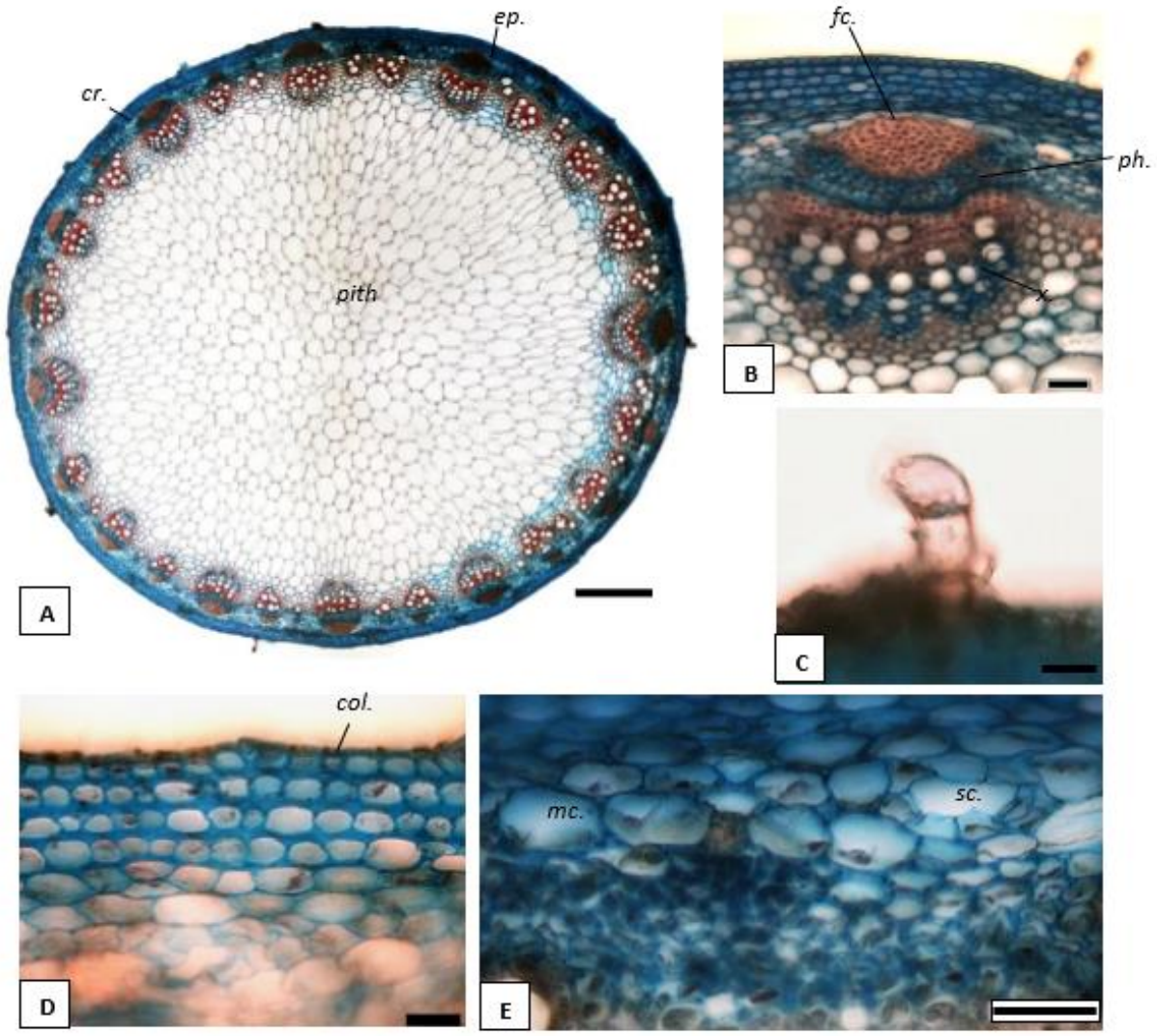

Figure 4 Cross section of Kapal terbang bottom stems
(A) CS of upper layers of stem; circular shaped, showing of epidermal cells (*ep.*), separated vascular bundles with closed system, *ca*. 35-40 layers of pith and cortex layer (*cr.*) with collenchyma cells (*col.*), parenchyma cells in 6-9 layers and sclerenchyma cells occur as bundle caps outside phloem. **(B)** Vascular tissue; xylem (*x.*) and phloem (*p.*) and fibre cap (*fc.*) **(C)** Simple, uniseriate trichome. **(D)** Collenchyma cells (*col.*). **(E)** & **(F)** Secretory cells (*sc.*) and mucilage cells (*mc.*). [CS - Cross section, (*ep.*) – epidermal cell, (*cr.*) - cortex, (*sl.*) - sclerenchyma cells, (*fc.*) – fibre cap, (*x.*) – xylem, (*ph.*) – phloem, (*sc.*) – secretory cells, (*col.*) – collenchyma cells and (*mc.*) – mucilage cells]. Scales bar: A = 500 µm; B, E = 50 µm and C, D = 20 µm.

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4. Conclusion

Results from this experiment showed that planting materials of *C. odorata* can be propagated from cuttings taken from propagules. This technique is suitable and useful in areas where planting materials are lacking and they can be made available by cutting one propagule into several pieces. The technique will also be more useful for production of elite clone in future.

Acknowledgement

Authors would like to thanks Forest Research Institute Malaysia for providing research grant *Pemantapan Bank Germplasma (Rancangan Malaysia Ke 11*), Ummu Hani Badron (Biology Resources Program, FRIM) and Plant Improvement Program for making this research a success.

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ISSN: 2348-1358
Impact Factor: 6.057
NAAS Rating: 3.77

A Brief Author Biography

Sayfiqah Nabilah Samsul Bahari – A research officer at Forest Research Institute Malaysia (FRIM). She graduated from Universiti Teknologi MARA (UiTM) Shah Alam, with Master of Science. Her current research interests include plant breeding, plant tissue culture and plant bioactivity.

Dr. Farah Fazwa Md Ariff – A senior research officer at Forest Research Institute Malaysia (FRIM). She is the Head of Herb and Tree Improvement Branch, FRIM. She received her PhD from Universiti Kebangsaan Malaysia (UKM). Her current research interests include genetics, plant improvement, plant breeding and plant propagation.

