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Suitable Maturity Stage, Type of Cuttings and Potting Media for Vegetative Propagation of *Pogostemon heyneanus* Benth

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Abstract *Pogostemon heyneanus* Benth. (Lamiaceae) is an industrially important aromatic crop extensively cultivated in Asian countries as the main ingredient of perfume, high valued cosmetic and an array of ayurvedic products. Eventhogh it has high potential as an industrial crop, commercial cultivation in Sri Lanka is hampered due to lack of authentic, superior quality planting materials and optimized protocol for mass propagation of *P. heyneanus*. Therefore, in the present study an attempt has been made to investigate the optimum protocol for mass propagation of authentic, superior quality variety of *P. heyneanus* in order to ensure the commercial scale cultivation in Sri Lanka. Two types of cuttings (single and double nodal) at three maturity stages (*i.e*. softwood, semi-hardwood and hardwood) were obtained from healthy mother plants and planted in poly bags filled with top soil: sand: compost ratios of 1:1:1, 1:1:2 and 1:2:1. Number of leaves, number of branches, length of branches, height and the spreading of plants were recorded at 2 weeks intervals. Data were analysed and the highest plant height (52.9 ± 2.1 cm), length of branches (82.2 ± 5.6 cm), number of leaves (32.3 ± 2.6), number of branches (4.6 ± 1.5) and spreading of plant canopy (28.5 ± 1.7 cm) were observed in hardwood double nodal cuttings planted in potting medium 1:1:1 ratio. It is thus suggested to use hardwood double nodal cuttings as planting material and top soil 1: sand 1: compost 1 as potting medium for propagation of *P. heyneanus* in commercial scale cultivation.

Keywords: *Lamiaceae, maturity stage, Pogostemon heyneanus, potting media, vegetative propagation*

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1. Introduction

Pogostemon heyneanus Benth. (Lamiaceae) is a large, straggling undershrub found from Western to Southern in India, Malay Peninsula, Phillippine Island and Sri Lanka which is cultivated to extract fragrant patchouli oil. It is known as *Gan-Kollankola* or *Kollankola* in Sinhala and *Pataraka*, *Sughandaka* in Sanskrit [1]. Leaves of the plant contain sweet smelling oil which is accredited for a number of medicinal and cosmetic applications [20]. The dry leaves of patchouli on steam distillation yield an essential oil called the of patchouli oil [2].

Patchouli oil is one of the important natural essential oils used to give a base and lasting character to a fragrance in perfumery industry [3,4]. Patchouli essential oil has characteristic heavy, woody, earthy and camphoraceous odour and it has long lasting fixative properties [5]. The oil is widely used in the manufacturing of soaps, scents, body lotions and detergents [21]. Patchouli oil is a secondary metabolite produced by this plant contains a unique and complex composition of more than 24 different sesquiterpenes. The sesquiterpene patchoulol (patchouli alcohol) is the key element and is the most important factor responsible for the typical patchouli aroma [21]. The patchouli essential oil is also well known for its medicinal properties such as anti-inflammatory, anti-depressive, diuretic, anti-emetic, anti-bacterial and anti-fungal activities [6,7]. In aromatherapy, it is used to calm nerves, relieve depression and stress [22]. Fibrinolytic and anti thrombotic [23,24] activity of this essential oil is also been reported. It is estimated that Patchouli Alcohol has neuroprotective [25], anti-influenza [26] and anti-inflammatory activities [27]. Recently, it has reported that PA has anti-inflammatory activities in macrophage and colorectal cancer cells [28]. Essential oil of patchouli has demonstrated insecticidal activity [8] including repellence against mosquito species [9]. Dry patchouli leaves have also been found to possess moth repellent properties and therefore, are used to scent wardrobes and protecting clothes especially woolens from insects damage [29]. As there is no synthetic substitute available to replace the oil of Patchouli, its value and position in the perfumery market are further enhanced [30]. Among all the essential oil yielding plants, Patchouli (*Pogostemon cablin* Benth.) is considered to have tremendous business potential [21,31].

Patchouli is usually propagated by stem cuttings [32]. Meristemtip culture for mass production of patchouli was successfully carried out which resulted in pathogen free plantlets for cultivation in the fields [35] and results were promising with significant increases in leaf biomass and essential oil yield. Clonal propagation and shoot meristem culture were also reported in patchouli [32]. Volkhovskaya (1968) [33] first reported rooting of patchouli leaves and subsequent development of shoots. Selvarajan and Madhava Rao (1981) [34] reported only rooting but no shoot formation. Vasantha Kumar and Narguda (1987) [36] reported shoot formation in leaf cuttings but subsequent growth was slow and took 80-140 days for plantlet to reach optimum size suitable for transplanting. Further, leaf with a portion of petiole was used and shoot development occurred after callus formation in the cut end of petiole. This resulted in delayed shoot initiation and low success in plantlet production [32].

Since Patchouli oil possess an array of diversified therapeutic and other uses, there is a high potential for commercial scale cultivation of *P. heyneanus* for its fragrance and other therapeutically benefits.

Having understood this enormous necessity, present study was undertaken to develop a simple, cost effective vegetative propagation protocol for mass propagation of *P. heyneanus.*

2. Materials and Methods

2.1. Location

Experiment was carried out in the experimental plots and the laboratory of the Department of Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila (NWP) from March to July 2014. The experimental site was situated in the Low Country Intermediate-Zone (IL_{1a}), at an elevation of 25 m above mean sea level (Panabokke, 1996). The average annual temperature, relative humidity and solar intensity of the experimental area are 31^0C, 78% and 85 kilo lux respectively. The photo period of the area is 8 hours.

2.2. Experimental Design and Treatments

Experimental design was a three factor factorial in Complete Randomized Design (CRD). Three factors were potting medium (three potting media), maturity stage (three maturity stages) and type of cuttings (two types of cutting). There were 18 treatment combinations and 30 plants were treated with each treatment combinations.

2.3. Planting Materials

Single and double nodal stem cuttings with three different maturity stages were obtained from six months old, well maintained, healthy *P. heyneanus* mother plants planted at university research plots.

2.4. Preparation of Stem Cuttings

Vigorously grown healthy shoots were selected from mother plants to obtain cuttings. Two types of stem cuttings (*i.e*. single node and double node) at three different maturity stages (*i.e*. softwood, semi-hardwood and hardwood) were obtained from selected shoots and cuttings were kept in bucket of water until planting.

2.5. Preparation of Bags with Potting Medium

Gauge 150, transparent polythene bags (10cm × 15cm) were filled with three different potting media as follows.

Medium 1 – Top soil 1: Sand 1: Compost 1
Medium 2 – Top soil 1: Sand 1: Compost 2
Medium 3 – Top soil 1: Sand 2: Compost 1

The top soil was taken from the university field where the land suitability class was Low Country Intermediate-Zone (IL_{1a}) in Sri Lanka. The compost fertilizer was provided from the research station near the university premises which consisted of poultry manure, cow dung, plant residuals and house litter in the equal ratios.

2.6. Planting and Aftercare Operations

Pre-prepared single and double nodal stem cuttings with three different maturity stages were planted separately in the poly bags having three different potting media arranged in 18 treatment combinations.

Immediately after planting shade was provided and watering was done twice a day. Plants were exposed to the direct sunlight gradually and shade was completely removed one month after planting. Each and every plant was maintained with similar conditions to minimize environmental variation.

2.7. Growth Parameters

Number of leaves, number of branches, length of branches, height and the spreading of plant were recorded as the growth parameters.

2.8. Statistical Analysis

Statistical comparison of mean values was performed by General Linear Model (GLM) of ANOVA followed by Turkey Multiple Range Test using Minitab 15 version and presented as means ± SD with 95% confidence level.

3. Results and Discussion

Present study describes the effect of maturity level of cuttings, potting medium and the type of cuttings (single or double nodal) on propagation of *P. heyneanus* by means of number of leaves, number of branches, length of branches, height and spread of plant. Results revealed that cuttings which were obtained at different maturity stages survived and produced shoots. Vegetative propagation offers several advantages compared to the sexual propagation, among them the homogeneity of plant material, crop and sub-products, rate of plant development and productivity since healthy, superior adult stock plants are used and the clonal propagation of the plant material increases the chances of obtaining higher quality essential oil [18]. As shown in Table 1 and Table 2, the interaction effect of maturity level, type of cuttings and medium directly affect on the number of leaves, length of branches, height and the spread of plant except the number of branches (P=0.013) at 0.05 significant levels.

3.1. Number of Leaves

Significantly higher numbers of leaves were observed in double nodal cuttings irrespective of type of medium and maturity stage of cuttings. Moreover, cuttings in all three maturity stages planted in Top soil 1: Sand 1: Compost 1 (M1) medium (32.3 ± 2.6) and Top soil 1: Sand 1: Compost 2 (M2) medium (33.6 ± 2.3) demonstrated the higher number of leaves than M3 medium which contained Top soil 1: Sand 2: Compost 1 ratio (Table 1).

Table 1. Mean values of number of leavesves, number of branches and length of branches of *Pogostemon heyneanus* in each treatment combinations at eight weeks after planting

Maturity stage	Potting medium	Types of cutting	No of leaves	No of branches	Length of branches (cm)
Softwood	M1	SN	$16.3^{f} \pm 1.7$	$2.1^{bcde} \pm 0.3$	$31.5^{h} \pm 4.1$
		DN	$24.5^{b} \pm 2.6$	$4.3^{a} \pm 2.3$	$6.0^{i} \pm 3.9$
	M2	SN	$18.9^{def} \pm 2.7$	$2.1^{cde} \pm 0.2$	$36.3^{fgh} \pm 7.9$
		DN	$20.3^{cde} \pm 1.6$	$3.4^{abcd} \pm 2.$	$7.4^{i} \pm 7.7$
	M3	SN	$15.4^{f} \pm 2.9$	$1.8^{de} \pm 0.4$	$33.2^{gh} \pm 5.0$
		DN	$22.8^{bcd} \pm 1.8$	$2.3^{bcde} \pm 2.0$	$6.5^{i} \pm 9.9$
Semi-hardwood	M1	SN	$18.3^{ef} \pm 2.0$	$2.1^{cde} \pm 0.2$	$37.8^{efgh} \pm 6.7$
		DN	$25.1^{b} \pm 3.0$	$3.7^{ab} \pm 1.3$	$59.5^{b} \pm 5.7$
	M2	SN	$6.0^{g} \pm 6.0$	$0.8^{e} \pm 0.8$	$8.1^{i} \pm 9.0$
		DN	$25.5^{b} \pm 2.8$	$4.3^{a} \pm 1.8$	$58.1^{b} \pm 9.4$
	M3	SN	$15.7^{f} \pm 2.3$	$1.9^{de} \pm 0.3$	$34.5^{gh} \pm 5.8$
		DN	$23.3^{bc} \pm 2.7$	$3.3^{abcd} \pm 1.4$	$53.7^{bc} \pm 12.9$
Hardwood	M1	SN	$22.8^{bcd} \pm 4.4$	$2.3^{bcde} \pm 0.4$	$47.2^{cd} \pm 4.3$
		DN	$32.3^{a} \pm 2.6$	$4.6^{a} \pm 1.5$	$82.2^{a} \pm 5.6$
	M2	SN	$23.4^{bc} \pm 5.0$	$2.2^{bcde} \pm 0.5$	$46.8^{cde} \pm 6.3$
		DN	$33.6^{a} \pm 3.0$	$4.3^{a} \pm 0.9$	$44.3^{def} \pm 6.5$
	M3	SN	$19.3^{def} \pm 2.3$	$2.1^{cde} \pm 0.2$	$32.3^{gh} \pm 6.6$
		DN	$23.3^{bc} \pm 2.0$	$3.5^{abc} \pm 0.6$	$41.3^{defg} \pm 5.4$

Mean values followed by the same letters are not significantly different at 0.05 significant level; M_1 = Medium 1 (1:1:1); M_2 = Medium 2 (1:1:2); M_3 = Medium 3 (1:2:1); SN - Single Nodal; DN - Double Nodal.

Garbuio *et al.* (2007) also observed the importance of leaf retention as leafy patchouli (*Pogostemon cablin*) cuttings showed a greater rooting percentage than leafless cuttings. Presence of the higher number of leaves in double nodal cuttings than single nodal cuttings is probably due to higher number of branches and assimilates presence in double nodal cutting. Leaves are the main source of carbohydrates in the cuttings (12) and they also provide the physiological requirements to stimulate rooting apart from the supply of carbohydrate.

3.2. Number of Branches

In the present study there was a variation in number of branches with the treatment combinations (Table 1). Significantly higher number of branches resulted in double nodal cuttings than the single nodal cuttings in all treatment combinations irrespective of type of maturity stage and media. This result may be due to having more growing points for the development of axillary buds in double nodal cuttings than the single nodal cuttings.

Hardwood cuttings planted in three media resulted higher number softwood and semi-hardwood cuttings. When compared with all other treatment combinations, highest number of branches observed in double nodal hardwood cuttings in top soil 1: sand 1: compost 1 (M1) potting medium (4.6 ± 1.5). Auxin hormone is mainly responsible to develop the apical meristem of a plant and inhibits the outgrowth of axillary buds. Auxin is mainly synthesized in the shoot apex in young leaves [14]. Removal of the shoot apex leads to the release of dormant axillary buds below it to form branches [13]. This might be a reason to develop more new shoots from the hardwood cuttings in the experiment due to the absence of apical dominance.

3.3. Length of Branches

As shown in Table 1, there was significant variation in length of branches in all treatment combinations. The significantly higher length of branches (36.3 cm ± 7.9) observed in softwood single nodal cuttings. This might be due to formation of vigorous root system in softwood cuttings compared to hardwood cuttings. The hormonal control of signals associated with root development especially auxin is known [15]. Auxins induced ethylene synthesis may play a role in adventitious root initiation and the associated increase in cellulase activity [16].

Similar observation was reported by previous studies [10] for softwood cuttings of *Camelia sinensis*. The highest length of branches (82.2 cm ± 5.6) resulted in hardwood double nodal cuttings planted in M1 medium.

Hardwood and semi-hardwood double nodal cuttings in all potting media exhibited the higher length of branches than single nodal cuttings. As far as maturity stage of cutting is concerned, softwood cutting has apical dominance which retards the growth of axillary buds and accelerates the growth of epical shoot and hence it induces the faster growth of shoot. The leaf age and maturity play the crucial role in adventitious root growth and shoot development of a cutting [13]. Similarly higher length of branches in double nodal hard wood and semi hardwood cuttings may be due to emergence of more new branches.

3.3. Height of Plant

The vertical length of main shoot was measured as the height of plant. Significantly different height of plants was observed in all treatment combinations (Table 2). Significantly higher plant height was observed in shoots developed in double nodal cuttings than single nodal cuttings in all treatment combinations irrespective of types of media and maturity stage (P = 0.05). When compared to all treatment combinations, hardwood double nodal cuttings in top soil 1: sand 1: compost 1 (M1) potting medium demonstrated significantly highest plant height (52.9 cm ± 2.1).

Table 2. Mean values of plant height plant spread of *Pogostemon heyneanus* in each treatment combinations at eight weeks after planting

Maturity stage	Potting medium	Types of cutting	Height of plant (cm)	Spread of plant (cm)
Softwood	M1	SN	$21.3^{hi} \pm 3.6$	$20.3^{fgh} \pm 2.0$
		DN	$38.8^{cd} \pm 4.8$	$24.2^{bcde} \pm 1.9$
	M2	SN	$23.0^{gh} \pm 4.1$	$20.4^{fgh} \pm 2.6$
		DN	$34.6^{de} \pm 4.7$	$23.3^{def} \pm 2.1$
	M3	SN	$26.6^{fgh} \pm 4.2$	$20.4^{fgh} \pm 2.1$
		DN	$42.2^{bc} \pm 4.4$	$23.7^{cdef} \pm 1.5$
Semi-hardwood	M1	SN	$23.7^{gh} \pm 5.0$	$19.5^{gh} \pm 2.2$
		DN	$40.1^{c} \pm 1.7$	$26.7^{abcd} \pm 1.9$
	M2	SN	$6.7^{j} \pm 6.9$	$8.1^{i} \pm 8.2$
		DN	$45.5^{b} \pm 4.3$	$27.8^{ab} \pm 2.1$
	M3	SN	$25.3^{gh} \pm 3.4$	$19.3^{gh} \pm 2.9$
		DN	$38.7^{cd} \pm 5.1$	$27.2^{abc} \pm 2.2$
Hardwood	M1	SN	$23.2^{gh} \pm 3.5$	$20.2^{fgh} \pm 2.6$
		DN	$52.9^{a} \pm 2.1$	$28.5^{a} \pm 1.7$
	M2	SN	$26.3^{fgh} \pm 2.0$	$20.1^{fgh} \pm 3.2$
		DN	$28.1^{fg} \pm 4.3$	$22.2^{efg} \pm 1.4$
	M3	SN	$16.7^{i} \pm 4.3$	$16.8^{h} \pm 2.7$
		DN	$31.5^{ef} \pm 4.2$	$20.4^{fgh} \pm 2.3$

Mean values followed by the same letters are not significantly different at 0.05 significant level; M_1 = Medium 1 (1:1:1); M_2 = Medium 2 (1:1:2); M_3 = Medium 3 (1:2:1); SN - Single Nodal; DN - Double Nodal.

Elongation of cuttings facilitates intercept more sunlight to the leaves by reducing the mutual shading. Height is a major determinant of plant's ability to compete for light [37]. The higher number of leaves and more light interception in double nodal cuttings would produce more assimilates through photosynthesis to support stem elongation than the single nodal cuttings. Source leaf photosynthesis is the main source of carbon skeletons required for cellular processes and growth of cuttings [13]. In addition, the greater carbohydrate reserves in double nodal cuttings than single nodal cuttings would provide additional support for stem development in double node cuttings. Minimum percentage of mother leaves was observed in the single nodal cuttings. This can be a consequence of the low availability of carbohydrate reserve tissue, and higher content of ABA [19].

3.4. Spread of Plant

In the present study the canopy diameter of plants was taken as the measure of the spreading. There was significantly different variation in spreading of plant in all treatment combinations (Table 2). Higher plant spreading was also recorded in double nodal cuttings than single nodal cuttings in all treatment combinations. Hardwood double nodal cutting in top soil 1: sand 1: compost 1 (M1) potting medium had maximum spreading of canopy (28.5 cm ± 1.7) when compared to all other treatment combinations (Table 2). Presence of higher spreading of shoots developed from double nodal cuttings might be due the availability of much higher branches in double nodal cuttings than the single nodal cuttings.

This study revealed that hardwood double nodal cutting planted in M1 medium (top soil 1: sand 1: compost 1) shows better growth performances than other treatment combinations. Results of the present study are in agreement with previous studies [11] who reported the suitability of top soil 1: sand 1: compost 1 as the best medium for vegetative propagation of *P. heyneanus*. It is thus recommended to use hardwood double node cuttings as planting materials and top soil 1: sand 1: compost 1 as potting medium for propagation of *P. heyneanus* in commercial scale cultivation.

4. Conclusions

Present study demonstrated the effect of stem cutting type, maturity stage and potting medium concerning vegetative propagation of *P. heyneanus*. Hardwood double nodal stem cuttings was the most appropriate type of stem cutting for vegetative propagation of *P. heyneanus* as it gives the best results in all growth parameters tested. Potting medium top soil 1: sand 1: compost 1 is the most suitable potting medium for propagation of *P. heyneanus*. It is thus suggested to recommend hardwood double nodal cuttings as planting materials and top soil 1: sand 1: compost 1 as suitable potting medium for propagation of *P. heyneanus* in commercial scale plant production.

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