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Short Communication

Vegetative Propagation of Rare Tree Species for Forest Restoration

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ABSTRACT

Nine rare tree species were selected for investigating their suitability for cuttings: 1) *Crypteronia paniculata* Bl. var. *paniculata*, 2) *Diospyros coaetanea* Flet., 3) *Gardenia sootepensis* Hutch., 4) *Haldina cordifolia* (Roxb.) Rids., 5) *Ilex umbellulata* (Wall.) Loesn., 6) *Mesua ferrea* L., 7) *Rothmania sootepensis* (Craib) Brem., 8) *Schoutenia glomerata* King ssp. *peregrine* (Craib) Roekm. & Hart., and 9) *Scleropyrum pentandrum* (Dennst.) Mabb. Five separate experiments were run to test the effect of various treatments; i) concentrations and forms of rooting hormones, ii) node positions iii) fungicide, iv) leaf area, and v) propagation media. None of these treatments were successful in producing viable planting stock in sufficient quantities, although limited success was achieved with *Shoutenia glomerata*. The best treatment was no hormone treatment (control), which produced the highest relative performance score (86.1%). It required almost 10 months from collecting cuttings to potting of rooted cuttings.

Keywords: cuttings, FORRU, rare tree species, rooting.

1. INTRODUCTION

Northern Thailand has undergone considerable forest loss. According to the Royal Forest Department [1], northern Thailand's forest cover declined from 68.54% in 1961 to 53.09% in 2006. Forest restoration programs have been implemented to degraded forest. This activity requires large-scale production of planting stock of a wide range of indigenous forest tree species. However, many of these species are difficult to propagate from seeds. Therefore, vegetative propagation from cuttings is one potential way to produce planting stock of such species.

The aim of this research was, therefore, to investigate how to produce planting stock of rare tree species in northern Thailand by propagation from cuttings using a non-mist propagation system.

2. EXPERIMENT

Nine tree species, which are rare or threatened with extirpation from northern Thailand, and could not previously be grown from seeds [2], were chosen for propagation by cuttings. Medium-sized twigs or vigorous juvenile stem, were randomly selected from

many mother trees for genetic diversity at Doi Suthep-Pui National Park, Chiang Mai, Thailand and taken to FORRU nursery, where they were watered thoroughly before propagation.

2.1 Experiment 1: Effect of Auxins

2.1.1 Cutting Preparation

The juvenile stems of all nine species were cut into heel shape and 10-20 cm long with node and leaf on the cuttings. Leaves were trimmed transversely by about 30-50% depending on leaf area to reduce water loss through transpiration. To prevent fungal infection, the prepared cuttings were immersed in solution of the fungicide Benomyl (0.3 g/ 1,000 cm^3) for 10 minutes [3].

2.1.2 Auxin Preparation

Different concentrations and formulations of auxins were used as treatments. The auxin solutions were prepared individually by dissolving IBA or NAA in 95% ethanol then following by distilled water in a 1:1 ratio. For combination treatment, IBA and NAA were mixed together before using. Thus, five treatments were obtained; Control (no auxin treatment), Seradix® (powder containing IBA 3,000 ppm), IBA solution 3,000 ppm, IBA solution 8,000 ppm, and the mix of IBA:NAA 5,000:2,500 ppm.

Leafy stem cuttings of all nine species were treated with rooting hormone treatments after immersed in fungicidal solution. For solution treatments, the base 5-10 mm of each cutting was dipped into the testing solution for 10 minutes. For Seradix®, quick drip and only a single layer with powder hormone were applied to the cutting base.

2.1.3 Experimental Design

A total of 300 leafy stem cuttings (12 cuttings × 5 treatments × 5 replicates) was tested and arranged in Complete Randomized Design (CRD) of each tree species.

Non-mist propagator system, which proved successful in the propagation of many tree species [3-5], was applied by using big clear plastic bags. After applied rooting hormone treatments, the cuttings were inserted in the rooting medium in small black plastic bags which consisted of sand and rice husk charcoal (1:1). The plastic propagation bags were added with 1,000 cm^3 of water when originally prepared and tied closed which created circulating condensation and high humidity inside [3]. The bags were shaded in the nursery under black shade netting and checked weekly for tears and water level. Dead cuttings and dried leaves were removed from the bags to prevent diseases.

Cuttings were moved out of clear plastic bags and transplanted after roots could be seen. The potting mixture consisted of forest soil, peanut husk, and coconut husk (2:1:1) [6]. Potted plants were shaded in the nursery under black shade netting for two weeks. Then, the plants were moved out of the nursery and placed under full sunlight.

2.2 Experiment 2: Effect of positions in stem

Three parts; top part, middle part, and base part, leafy stem cutting of *Rothmania sootepensis* were taken sequentially down the stem and their leaf areas trimmed as described in Experiment 1. Three auxin treatments were used, including control, Seradix®, and IBA 8,000 ppm, since these treatments produced high number of cuttings with new shoots resulted from a preliminary determining in Experiment 1. Moreover, number of replication per treatment was decreased from five to three due to cutting source constraint.

2.3 Experiment 3: Effect of Fungicides

Four treatments; Control (no fungicide), Benomyl, Captan, and red lime were used with cuttings of *Ilex umbellulata*. Benomyl and Captane were prepared in solution form by

dissolving Benomyl (0.3 g/1,000 cm^3) or Captan (2 g/1,000 cm^3) in water. For the solutions, cuttings were immersed in the solutions for 10 minutes before application of Seradix®. For the red lime treatment, cuttings were treated with Seradix® before coating with red lime at each cutting base. For each treatment; there were three replicates combination in a CRD, with 15 cuttings in each replicate.

2.4 Experiment 4: Effect of Leaf Area

Crypteronia paniculata were tested with three leaf area treatments; leafless, half leaves (half of its original size), and full leaves (whole leaf area). All cuttings were treated with Benomyl solution as detailed in Experiment 1. Then, cuttings were treated with Seradix®, inserted in the cutting container, and arranged according to the statistical method as explained in Experiment 3.

2.5 Experiment 5: Effect of Rooting Medium

Cuttings of *Mesua ferrea* were prepared to test with four different rooting medium treatments; sand, sawdust, a mixture of sand and rice husk charcoal (1:1), and a mixture of sand, rice husk charcoal, and coconut husk (1:1:1). All prepared cuttings were treated with Seradix® before being placed into different rooting media and arranged according to the statistical method described for Experiment 3.

2.6 Data Analysis

Data collected when roots became visible were calculated as percentages and the mean number of cuttings surviving, rooting, shooting, and rooting with shooting, together with mean numbers and lengths of roots and shoots per cutting. These results were analyzed to test for significant differences among the treatments for each species by ANOVA (one-way analysis of variance), followed by least significant difference (LSD) tests if differences were detected at $P<0.05$. Moreover, Relative Performance Score (RPS) was calculated for comparing cuttings performance within species as follow [3]:

To determine effects of potting after removal from clear plastic bags, the number of surviving plants and height of plants were measured every 30 days to evaluate percentages of survival plants and relative growth rate of height (RHGR). Moreover, environmental conditions played an important role for propagation systems. Every aspect was measured thoroughly in this experimental study.

$$RPS = 50 \times \left[\frac{TrtS}{MaxS} + \left\{ \left(\frac{TrtNR}{MaxNR} + \frac{TrtNS}{MaxNS} + \frac{TrtRL}{MaxRL} + \frac{TrtSL}{MaxSL} \right) \times 0.25 \right\} \right]$$

where: Trt = the mean value for each individual treatment
Max = largest mean value among treatment
S = percentage of cuttings surviving with roots and shoots
NR = mean number of roots RL = mean root length
NS = mean number of shoots SL = mean shoot length

3. RESULT AND DISCUSSION

3.1 Environmental Conditions

The plastic propagator created higher relative humidity inside; over 85%, while the air temperature at FORRU's nursery ranged from 21-26°C. The air temperature in plastic bag propagator was mostly higher than in the rooting medium, which ranged from 22-28°C and 21-24°C, respectively. For successful

rooting of leafy cuttings, it has been suggested that a high relative humidity (more than 80%) must be maintained around cuttings [3], daytime air temperature should be 21-27°C, and medium temperature should be 25-32°C [7]. Moreover, the air temperature should be lower than medium temperature because if air temperature is not controlled or is too high, most of the stored food in cutting stems would be rapidly utilized for shoot development and thus root development would be hampered [7]. In contrast with this study, the rooting medium was always cooler than the air surrounding the cuttings. To maintain high air humidity in the propagator system, some water was added at the base of the plastic bags to avoid water stress in the cuttings. This may result in low temperature in rooting medium. Moreover, during weekly checking, it was necessary to shake the plastic bags to make them transparent. Therefore, some water fell to the bottom of the plastic bags, possibly resulting in low temperature in rooting medium.

3.2 Vegetative Propagation

Only one species, *Schoutenia glomerata* in Experiment 1 produced roots. This species could be potted after 283 days from collection of leafy stem cuttings. Chemical treatments had no significant effect on the success of cutting propagation of this species. Without chemical treatments, a highest of 8.3% of cuttings survived with both roots and shoots. Calculation of RPS ranked the control as the most effective treatment (Table 1). Applying the results obtained during the study reported here, the production on 100 *Shoutenia glomerata* trees for planting would require preparation of 723 leafy stem cuttings (assuming 8.3% survival with both roots and shoots). Other treatments should be tried to accelerate rooting and increase survival of cuttings with both roots and shoots.

Table 1. Cutting results and relative performance score of *Shoutenia glomerata.*

Treatment	Cuttings surviving with roots and shoots		Vigour					Total[8]
	%[1]	Survival score[2]	No roots[3]	No shoots[4]	Root length[5]	Shoot length[6]	Vigour score[7]	
Control	8.3	50.0	1.9	0.7	5.2	0.9	36.1	86.1
Seradix®	5.0	30.1	2.8	0.8	8.4	1.1	48.1	78.2
IBA 3,000 ppm	5.0	30.1	3.2	0.8	4.5	0.6	38.4	68.5
IBA 8,000 ppm	0.0	0.0	1.0	0.0	7.8	0.0	15.2	15.2
IBA:NAA 5,000:2,500 ppm	5.0	30.1	2.8	0.4	8.6	1.0	41.1	71.2

[1] % of cuttings surviving with roots and shoots
[2] calculated of survival score
[3] mean number of roots per cutting
[4] mean number of shoots per cutting
[5] mean length of root per cutting (cm)
[6] mean length of shoot per cutting (cm)
[7] calculated of vigour score
[8] total performance score

With the methods used in this study, none of the other eight species tested with different treatments produced roots. The cuttings developed brown leaves, rapidly wilted and

followed by stem collapse. Cuttings must retain leaves for successful root initiation and development, for supply of auxins and nutritional factors [7]. Further experiments should be carried out to test other propagation methods to produce planting stock of rare tree species.

3.3 Plant Growth of *Shoutenia Glomerata*

Since only this species produced strong roots development for potting, all 34 plants were measured for percentages survival and RHGR for 5 months. The number of surviving plants continually decreased from the 1st month to the 5th month. By 5th month, only 20 plants (58.8%) remained alive. RHGR (cm) was 73.6 %/year.

During tree production to restore forest, all trees must reach a plantable size (about 50 cm high) in the planting season. Also, it is not efficient to keep trees in nursery more than one year [6]. However, from the results, it is not possible to produce viable planting stock in less than one year, because roots emerging and relative growth rate were very slow. Potted plants would have to be kept further in the nursery for 67.7 months or at least five years before they would be ready for planting. It is suggested that further research for this species is needed, especially in terms of time consuming and applying fertilizer utility.

4. Conclusions

Cuttings by using clear plastic bags as a simple non-mist propagator system with various treatments, including auxin applications, fungicides, leaf areas, rooting media, and positions in the stem was not suitable for the rare tree species tested in this study as the percentage of survival was very low. Moreover, the relative growth rate of potted plants was very slow. As a result, it is not possible to mass produce quality planting materials within one year for forest restoration and for species conservation.

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