Indian Journal of Plant Sciences ISSN: 2319–3824(Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jps.htm
2015 Vol. 4 (4) October-December, pp.30-34/Saradha and Samydurai
Research Article

EFFECT OF DIFFERENT ROOTING MEDIA AND PLANT GROWTH HORMONES ON ROOTING OF CRITICALLY ENDANGERED SPECIES, *UTLERIA SALICIFOLIA* LEAFY STEM CUTTINGS: A CONSERVATION EFFORT

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ABSTRACT
Utleria salicifolia (Periplocaceae) is an endangered medicinal plant species. It is commonly known as Mahali kizhangu. This study aimed to investigate the effect of different rooting media and plant growth hormones on the formation of rooting of stem cuttings. The basal portions of cuttings were dipped into 250, 500, 1000, 2000 and 2500 ppm of IBA (Indole-3-butyric acid) and IAA (Indole-3-acetic acid) respectively. After which the cuttings were planted on different rooting media (Coir pith, vermiculite and forest soil). Treatments were arranged in randomized complete block design with three replications and 20 cuttings/treatment were used. Data on percent rooting and other root and shoot growth parameters were collected and analyzed using analysis of variance (ANOVA). Results revealed that the significant effect ($p \leq 0.5$) was recorded in IBA with coir pith on rooting percentage and growth performance of leafy stem cuttings. The highest rooting (92) percent, root number (17.1) and average root length was 16.9cm observed significantly in the growth hormone IBA (2000 ppm) with the rooting media containing coir pith. 96 percentage of survival rate was observed in rooted leaf cuttings in the same treatment after acclimatized and transplanting in polybags. In conclusion rooted cuttings can be used successfully for obtaining healthy, uniform plantations of *U. salicifolia* for large-scale, biomass production and tree planting programmes.

Keywords: *Utleria Salicifolia, Periplocaceae, Rooting Media, Growth Hormones*

INTRODUCTION
Utleria salicifolia Bedd. Ex. Hook. F. (Periplocaceae) is a branched shrub endemic to South Western Ghats of peninsular India. It is widely distributed in Anamalais of Tamil Nadu, Nelliampathy and Marayaoor forests of Kerala (Radhakrishnan *et al*., 1998). It is mainly located in rocky substances. It is commonly known as Mahali kizhangu. Malasar, Kadar and Muthuvam tribes are used decoction of its tubers to treat debility due to tuberculosis, skin diseases and bleeding due to ulcer (Radhakrishnan *et al*., 1998). It is known to have antiulcer (Rao *et al.,* 2004) hepatoprotective (Remya *et al.,* 2010) and antiinflammatory (Shailasree *et al.,* 2012), activities. *U. salicifolia* is under great threat due to over exploitation by local tribal people and traditional healers for its medicinal value. Its conservation status has been variously assessed as Endangered (Walter and Gillett, 1997; MoEF, 2010) Critically Endangered (FRLHT, 2014), Owing to these threats and the growing importance of medicinal plants, the conservation of these valuable genotypes is imperative. Modern propagation method like clonal propagation is the most essential for this species to meet the demand and to conserve it as well. The present experiment was investigated to observe the effect of hormone and rooting media on the rooting of *U. salicifolia* leafy stem cuttings.

MATERIALS AND METHODS
Collection of Plant Material
The experiments were carried out inside the polyhouse conditions. Macropropagation was established in the nursery garden, Department of Botany, Nirmala College for Women, Coimbatore, Tamilnadu. The

leafy stem cuttings were collected from Anaimalai Reserve Forest Range, Coimbatore, Tamilnadu. The cuttings were excised in the morning and brought to the laboratory in polythene bags kept in cold box in order to prevent desiccation, during the first week of February 2014.

Shoot and Root Formation

The leafy stem cuttings of 20-25cm length were obtained from healthy (one year old plant), normal stem tip growth; very early in the morning when the plant is fully turgid with a sharp sterilized thin-bladed pocket knife. It was surface sterilized with 0.1% Bavistin (a systemic fungicide; containing 5% Carbendazim from BASF India Ltd., Mumbai, India) for 5 minutes at room temperature. The basal end of the cuttings was treated with different hormone concentrations before being planted into the media. They were dipped 12 hours in the different concentrations (250, 500, 1000, 2000 and 2500 ppm) of root hormones, Indole butyric acid (IBA) and Indole-3-acetic acid (IAA) to a depth of about 2.5mm. The plant cuttings were then planted in root drainer tubes (17cm by 16cm) filled with sterilized coir pith, vermiculite and forest soil separately. In each treatment 20 cuttings were applied and untreated cuttings were treated as control. After planting, the cuttings were watered using a watering can and subsequently twice a day (morning and evening). A bi weekly assessment was carried out on the cuttings starting two weeks after planting. At each assessment, the length of shoot, percentage of rooting, root length, number of roots per rooted cutting and survival of rooted cuttings were recorded until the end of the experiment. After 40 days of incubation in polyhouse condition leafy stem cutting were relaxed from over temperature and humidity. The rooted cuttings were gently lifted and transplanted into pots.

Statistical Analysis

The experimental design was a completely randomized design (CRD) with three replications. The data was analyzed using one-way ANOVA was applied to evaluate ($p<0.05$) was calculated significant differences in the studied parameters in the different treatments.

RESULTS AND DISCUSSION

Results

The results of the rooting parameters of *U. salicifolia* leafy stem cuttings are showed in the Table 1, 2 and 3. The higher rooting percentage (92%) was obtained in Indole butyric acid in the rooting media containing coir pith. However, IAA treated cuttings gave lower rooting percentage (32.8%) compared with IBA treated cuttings. There is no rooting response was observed in control. There were clearly differences in rooting response in the Indole-3-butyric acid (IBA) and Indole-3-acetic acid (IAA) treated and untreated cuttings. The maximum number of roots per shoot cutting 17.1 and 5.6 root/cuttings was observed in the treatment with 2000ppm of indole butyric acid (IBA) and indole acetic acid (IAA) respectively while, untreated leafy stem cuttings could not produced rooting (Table 1). The poor rooting numbers 5.2 and 1.4 root/cuttings in IBA and IAA respectively in the lower concentration of 250 ppm.

The highest shoot length 35cm and 27.9 cm long was recorded in 2000 ppm and 2500 ppm of IBA in the rooting media containing forest soil inoculated cuttings followed by 15.9cm length was observed in coir-pith media in the same IBA concentration and also 14.1cm of shoot length was observed in IAA with coir-pith media. The highest root length was observed in coir-pith media inoculated cuttings (16.9cm) treated with 2000ppm IBA followed by 1000 ppm of IBA with coir pith media. Good survival rate was recorded (92%) in 2000 ppm IBA treated cuttings with Coir pith media (Table 3). Thus the results revealed that the 2000ppm concentration of IBA with Coir pith rooting media is most suitable for the better clonal propagation of *U. salicifoalia*. Moreover, better responses under IBA were in conformity with the reports of its effectiveness as compared to several naturally occurring auxins in promotion of adventitious roots (Hartmann and Kester, 1983).

Discussion

The effect of growth hormone and rooting media on adventitious root formation in leafy stem cuttings of *U. salicifolia* was examined for the first time. A successful propagation was achieved by using the rooting hormone IBA (Indole-3-butyric acid). IBA have been reported to be more effective in inducing rooting in stem cutting of *Taxus baccata* (Nandi *et al.*, 1996, 1997) and *Cedrus deodara* (Nandi *et al.*, 2002).

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Table 1: Showing the ability of rooting percentage of *Utleria salicifolia* leafy stem cuttings on different growth hormones and rooting media

S. No	Growth hormones concentration in ppm	Control	Rooting response in percentage					
			IBA (Indole-3-butyric acid)			IAA (Indole-3-acetic acid)		
			Coir-pith	Vermiculite	Forest soil	Coir-pith	Vermiculite	Forest soil
1	250	0.0	31.3±0.54^{e}	12.8±0.64^{e}	12.3±0.53^{d}	18.3±0.32^{e}	10.2±0.27^{e}	9.6±0.26^{cd}
2	500	0.0	42.7±0.09^{d}	18.4±0.65^{d}	13.5±0.21^{c}	19.9±0.32^{d}	11.1±0.24^{d}	10.9±0.23^{c}
3	1000	0.0	73.2±0.32^{c}	25.5±0.27^{c}	20.2±0.16^{b}	26.4±0.16^{c}	14.5±0.21^{b}	12.4±0.25^{b}
4	2000	0.0	92.6±0.43^{a}	30.2±0.68^{a}	21.4±0.20^{a}	30.3±0.14^{b}	15.9±0.19^{a}	14.2±0.26^{a}
5	2500	0.0	85.7±0.24^{b}	29.3±0.29^{b}	21.0±0.83^{ab}	32.8±0.13^{a}	13.7±0.25^{c}	12.0±0.24^{b}

Mean ± Standard Deviation in a column followed by a same letters are not significantly (P<0.05) different accordingly DMRT by ANOVA

Table 2: Shoot and rooting growth potential of *Utleria salicifolia* leafy stem cuttings inoculated on different concentrations of plant growth regulators and rooting media

S. No.	Growth hormones	Concentration (ppm)	Control	Shoot length (cm)			Root length (cm)			No. of root		
				CP	V	FS	CP	V	FS	CP	V	FS
1.	**IBA**	250	0.0	5.8±0.06^{hi}	5.6±0.06^{fg}	5.4±0.06^{g}	8.7±0.08^{d}	4.4±0.03^{d}	4.1±0.03^{c}	5.2±0.04^{e}	4.1±0.02^{d}	3.6±0.02^{c}
2		500	0.0	7.4±0.07^{f}	6.4±0.08^{f}	6.2±0.07^{f}	10.4±0.13^{c}	5.1±0.04^{c}	4.4±0.03^{c}	7.9±0.08^{d}	6.8±0.05^{c}	4.4±0.03^{b}
3		1000	0.0	9.1±0.09^{d}	7.4±0.09^{e}	17.1±0.43^{c}	13.1±0.18^{b}	5.8±0.06^{c}	5.6±0.06^{ab}	12.4±0.16^{c}	8.3±0.09^{b}	6.2±0.04^{a}
4		2000	0.0	15.9±0.38^{a}	12.3±0.17^{a}	35.0±0.76^{a}	16.9±0.25^{a}	8.5±0.09^{a}	6.3±0.07^{a}	17.1±0.29^{a}	9.5±0.13^{a}	6.9±0.05^{a}
5		2500	0.0	15.0±0.37^{ab}	8.4±0.11^{d}	27.9±0.68^{b}	12.4±0.19^{bc}	7.5±0.08^{b}	6.0±0.05^{a}	16.3±0.2^{ab}	9.1±0.12^{a}	5.9±0.04^{ab}
6	**IAA**	250	0.0	6.5±0.07^{gh}	6.1±0.07^{f}	6.1±0.07^{f}	6.3±0.07^{e}	6.2±0.05^{bc}	3.7±0.04^{d}	1.4±0.01^{h}	2.7±0.02^{f}	2.5±0.02^{d}
7		500	0.0	6.8±0.08^{g}	6.3±0.08^{f}	6.5±0.07^{f}	6.9±0.07^{e}	6.5±0.05^{bc}	4.3±0.05^{c}	3.6±0.02^{fg}	3.1±0.02^{e}	3.0±0.02^{c}
8		1000	0.0	8.5±0.09^{e}	10.7±0.17^{c}	7.4±0.08^{e}	8.2±0.09^{d}	7.3±0.08^{b}	5.5±0.06^{ab}	4.2±0.02^{f}	3.8±0.04^{de}	3.6±0.03^{c}
9		2000	0.0	14.1±0.36^{b}	11.4±0.18^{ab}	9.0±0.10^{d}	9.8±0.12^{cd}	8.1±0.09^{ab}	5.7±0.06^{ab}	5.6±0.03^{e}	4.7±0.04^{d}	4.2±0.03^{b}
10		2500	0.0	12.7±0.31^{c}	10.2±0.19^{c}	9.01±0.10^{d}	7.5±0.08^{de}	7.2±0.06^{b}	5.0±0.04^{ab}	4.9±0.03^{f}	4.1±0.03^{d}	3.8±0.02^{c}

Mean ± Standard Deviation in a column followed by the same letter(s) are not significantly (p<0.05) different accordingly DMRT by ANOVA. IBA- Indole-3-butyric acid; IAA - Indole-3-acetic acid; CP – Coir pith; V – Vermiculite; FS – Forest soil

Table 3: Survival rate of *Utleria salicifolia* leafy stem cuttings inoculated on different concentrations of plant growth regulators and rooting media

S. No.	Growth regulators	Conc. (ppm)	Control	Survival rate (%)		
				Coir pith	Vermiculite	Forest soil
1.	**IBA**	250	0.0	46±0.43^{gh}	24±0.25^{g}	17±0.64^{h}
2		500	0.0	68±0.64^{d}	26±0.63^{fg}	21±0.14^{f}
3		1000	0.0	79±0.24^{bc}	34±0.74^{d}	25±0.23^{de}
4		2000	0.0	92±0.53^{a}	42±0.71^{c}	32±0.28^{b}
5		2500	0.0	80±0.18^{b}	45±0.25^{a}	37±0.17^{a}
6	**IAA**	250	0.0	25±0.25^{j}	17±0.28^{h}	12±0.12^{j}
7		500	0.0	36±0.28^{i}	24±0.34^{g}	15±0.32^{i}
8		1000	0.0	52±0.75^{f}	27±0.36^{f}	19±0.16^{g}
9		2000	0.0	64±0.74^{e}	32±0.70^{e}	26±0.11^{d}
10		2500	0.0	47±0.63^{g}	43±0.17^{b}	29±0.30^{c}

Mean ± Standard Deviation in a column followed by the same letter(s) are not significantly (p<0.05) different accordingly DMRT by ANOVA. IBA- Indole-3-butyric acid; IAA - Indole-3-acetic acid

The application of IBA may have an indirect influence by enhancing the speed of translocation and movement of sugar to the base of cuttings and consequently stimulate rooting (Haissig, 1974). The rooting efficiency observed in *U. salicifolia* showed satisfactory result within one month of planting inside the polyhouse conditions. The influence of auxin in promoting adventitious root formation through their ability to promote the initiation of lateral root and enhancing the transport of carbohydrates to the cutting base is well documented in several studies (Leaky *et al.*, 1982; Davis, 1988; Hartmann *et al.*, 1997; Mamo Kebede *et al.*, 2013).

The differential root regeneration capacities of different growth hormones individually or in combination might depend on their respective capacities for the regeneration and elongation of roots (Ghosh and Basu, 1974). The large number of root primordia induced by the root promoting hormones act as effective metabolic sinks, drawing on the nutritional reserves of the cuttings for their growth and development (Das *et al*., 1996). Vegetative propagation using 2000 ppm IBA showed highest rooting percentage in the rooting media comprising coir pith compared with IAA other media.

The greatest rooting percentage, root number and length were exhibited by basal cuttings of *U. salicifolia* treated with 2000ppm IBA in the rooting media coir pith. According to Hartmann *et al.*, (2002), one of the best rooting promoters is the IBA due to its fast auxin activity and an enzymatic system of fairly slow destruction.

Waman *et al.*, (2015) reported that coir pith is the most congenial substrate for silk banana multiplication. Zeinab and Hossein (2014) also reported that IBA treatments significantly increased rooting percentage compared with the control in *Hibiscus rosa-sinensis.* Vegetative propagation of *Paris polyphylla* important medicinal plant using various soil composition with different concentration and combinations of IBA and GA_3 (50, 100 and 150 mg/L) treatments. The 100mg/l GA_3 and IBA showed highest sprouting and rooting percentage along best composition of 3:2:1 soil: loam: sand (Kavita *et al*., 2015).

Conclusion

In conclusion the current study revealed that the application of auxin and rooting media showed considerable effect on propagation of *U. salicifolia.* Among the treatments Indole butyric acid at 2000 ppm with the rooting media coir pith achieved higher percentage of rooting, number of roots, root length and survival rate. The plants are ready to planting in wild habitat at 30-40 days after planting in the nursery condition. The technique will be more useful for production of elite clones.

ACKNOWLEDGEMENT

The first author graciously acknowledges the financial support given by University Grants Commission, Hyderabad (Grant No. F. MRP-5676/15 (SERO/UGC)) to carry out the work.

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