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Effects of Exogenous Growth Regulators on Direct and Indirect Micropropagation of *Corbichonia decumbens* (Forssk.) Exell (Molluginaceae)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors KK and VB designed the study and corrected the manuscript. Author RP wrote the first draft of the manuscript. Authors GU and VC carried out micropropagation experiment, collected the literature and identified the species of plant. All authors read and approved the final manuscript.

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ABSTRACT

Corbichonia decumbens (Forssk.) Exell is a medicinally important plant belonging to the family Molluginaceae. In this study we have standardized a protocol for both direct and indirect micropropagation. Callus was induced from the leaf, node and internode explants on Murashige and Skoog (MS) medium containing 6 benzylaminopurine (BAP 4.44 µM) in combination with naphthalene acetic acid (NAA 5.37 µM). 7.33 shoots were observed from callus while 32.16 shoots were observed during sub culture. In nodal culture 11.16, 34.16 number of shoots were obtained in

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initiation and subculture respectively on the MS medium supplemented with BAP (4.44 µM) and TDZ (2.27 µM). Regenerated shoots were rooted on MS medium along with NAA (16.05 µM) and activated charcoal (2 g/l). The rooted *in vitro* raised plantlets were acclimatized in shade house and successfully transferred to normal environment with higher survival rate (90.21%). The supplementation of growth hormones BAP and NAA enhanced the multiple shoot formation in direct and indirect micropropagation. Root formation is at the maximum in NAA and activated charcoal combination. Hence, the direct and indirect micropropagation of *C. decumbens* is more effective, reliable, reproducible and commercially viable than the conventional methods.

Keywords: Corbichonia decumbens; micropropagation; callus culture; acclimatization.

1. INTRODUCTION

Medicinal plants are the greatest source for generating novel drugs compounds - phytomedicine for treatment of diseases [1]. In developing countries about 70% of population relies on traditional medicine for their primary health care needs [2]. Worldwide increasing the demand of medicinal plants, leading to an over exploitation, unsustainable harvesting and loss of habitat has been leading to virtual decimation of several medicinal plant species in the wild. The biotechnological tools are important to select, multiply and conserve the critical genotypes in plants. Most of the medicinal plants either do not produce seeds or seeds are too small and do not germinate in soils. *In vitro* propagation of plants holds tremendous potential for the production of high quality plant-based medicines and conservation of medicinal plants [3].

Molluginaceae family comprises about 100 species, and was previously included in the larger family Aizoaceae. They are commonly known as stone plants or carpet weeds. *Corbichonia decumbens* (Family: Molluginaceae) is a prostrate, glabrous, succulent and annual weed found throughout India. Distribution of this plant in Africa, W. Asia, and W. Pakistan also found. It is found in rocky, sandy and dry hot areas up to 1000 m alt. This plant is used as medicine for kidney and gonorrhea [4]. This plant have pharmacological properties like antioxidant [5], antiinflammatory [6], antiulcer, [7], antimicrobial [8] and antinociception [9]. Due to over exploitation of the plant for medicinal purpose, the population may decline and distribution may be restricted. To our knowledge there is no report on micropropagation studies on this medicinal plant. Keeping the aforesaid view the current study was focused on the exogenous effect of growth regulators on direct and indirect micropropagation of *Corbichonia decumbens* an important medicinal plant.

2. MATERIALS AND METHODS

2.1 Plant Material and Disinfection

Corbichonia decumbens (Forssk.) Exell was collected from Thamaraikkarai, Burgoor hill, Erode district Tamilnadu. The identity was confirmed with the authentic specimen deposited in Madras Herbarium (MH), Southern Circle Botanical Survey of India, Coimbatore. Voucher specimen was deposited in the College herbarium. Healthy explants like node and leaves were selected and washed thoroughly under running tap water for 15 min to wash off the dirt and microbes present on the surface. The explants were cut (1-2 cm) separately and they were washed with Tween 20 detergent solution for 10 min. After, they were thoroughly washed under running tap water until the traces of Tween 20 was removed. Remaining steps of surface sterilization was carried out under aseptic conditions in Laminar air flow chamber. The shoots were then subjected to 70% ethanol treatment for 30 sec and again washed with sterilized double distilled water at least three to four times. After washing with sterilized double distilled water, surface sterilization was done with mercuric chloride (0.12%w/v $HgCl_2$) solution for 3 min and rinsed four to five times with sterilized double distilled water. Sterilized explants were inoculated on medium for direct and indirect micropropagation.

2.2 Culture Media and Conditions

The basal medium consisted of MS mineral salts and vitamins [10] with 3% sucrose (Hi Media, India) solidified with 0.8% (w/v) agar was used for the culture. For callus induction and shoot initiation MS medium supplemented with 2.22 µM to 13.32 µM of benzylaminopurine (BAP) and 5.37 µM of naphthalene acetic acid (NAA) was used. Nodal explants were cultured on MS medium with 2.22 µM to 13.32 µM of BAP and

5.37 µM of TDZ and 50 mg/l adenine sulphate (AS). *In vitro* raised shoots were cultured on MS medium fortified with NAA (5.35 µM to 26.75 µM) with 2 g/l of activated charcoal for rooting. The pH of all media was adjusted to 5.8 with NaOH solution (1 N) or HCl solution (0.1 N) before autoclaving at 121°C for 20 min under pressure of 1.6±0.1 kg. cm^2. All the cultures were incubated under 50 µ mol^2 s^{-1} light (Lux) provided by cool white florescent tube lamps at a photoperiods of 16h at 25±2°C and 70-80% humidity.

2.3 Callus Initiation and Shoot Multiplication

For the induction of callus, leaf disc, internode and nodal explants were cultured in the culture bottle with cap (150 ml) containing 30 ml of MS medium supplemented with growth regulators BAP and NAA at different concentrations for rapid callus induction and shoot initiation (Table 1). Twenty five explants were used for each culture and the experiment was repeated thrice. The percent of explants responding for callus formation were recorded after 35 days. The callus was sub cultured at a regular interval of 20-25 days for callus multiplication as well as shoot induction. For all experiments the control was the MS basal medium without growth regulators.

2.4 Shoot Initiation and Multiplication

The nodal explants were cultured on MS medium supplemented with plant growth regulators like BAP, TDZ and adenine sulphate in combinations for rapid shoot organogenesis (Table 2). Twenty five explants were used for each culture. The percent of explants responding for shoot formation were recorded after 35 days. Subculture was carried out at the regular interval of 20-25 days by using *in vitro* raised shoots. After a period of 20 -25 days the average number of shoots per explant on initiation and after 35-40 days average number of shoot per explant in subculture were evaluated.

2.5 Rooting of *in vitro* Shoots

Regenerated plantlets from *in vitro* raised multiple shoots (both from callus and nodal culture) were separated and individual shoots (3-4 cm) were transferred to MS medium consisting of 5.35 to 26.75 µM of NAA and 2 g/l of activated charcoal for root induction. All the cultures were incubated under the same conditions as during plantlets regeneration. After a month, the percentage of plantlets inducing root, mean number of roots per shoot, mean root length were evaluated.

2.6 Acclimatization

The well-developed plantlets were removed from the culture bottles and washed with tap water to remove the agar trace and dipped in fungicide for few minutes. Then the plantlets were planted on to net pot contains different types of potting substrates (Table 4). However, the effects of these substances were tested on the acclimatization. Twenty plantlets were cultured per substrate and the experiment was repeated thrice. All the net pots were incubated under shade house with 90% shade net for 25 days. The pots were watered at two days interval under shade house condition. After 25 days, the percentage of survival was calculated.

3. RESULTS

3.1 Callus Culture

MS medium supplemented with BAP and NAA combination was tested against various explants like leaf disc, node and internode on callus induction followed by shoot induction. All the three explants showed good response in induction of calli (Data not shown). These callus were green and friable in nature. Among the three explants, the callus produced by the leaf explants was green, friable and nodular in nature and also in high volume (Figs. 1 A & B).The higher percentage of callogenesis (91.00) was obtained with 4.44 µM BAP and 5.37 µM NAA. The days taken for callus induction is very less (9 day) when compared to other combinations (Table 1). The regeneration of shoot on the callus was observed on the MS medium containing BAP (4.44 µM) and NAA (5.37 µM), which was used for callus induction. The percentage of callus inducing shoots and the average number of shoot explants in initiation and subculture varied according to the concentration of BAP and NAA. The higher percentage of shoot induction (95) and (75.5) were obtained respectively with BAP (4.44 µM), NAA (5.37 µM) and BAP (6.66 µM), NAA (5.37 µM) combination (Table 1). The average number of shoots per explant in initiation was maximum (7.33) in the callus induction medium. Sub culturing of the callus on the same medium (BAP 4.44 µM and NAA 5.37 µM) induced the maximum multiple shoot formation (32.16) when compared to the other combinations (Figs. 1 D, E & F).

3.2 Nodal Culture

The multiple shoot cultures were obtained by cultivating nodal explants on MS medium containing BAP (4.44 µM) TDZ (5.37 µM) and AS (50 mg/l). The highest shoot proliferation intensity was 3.16 shoots per primary explant (Fig. 2 B). But the combination with the lowest shoot proliferation was BAP (13.32 µM) TDZ (5.37 µM) and AS (50 mg/l) with 1.21 shoots per explant (Table 2). The number of shoots obtained from primary explant could be increased by dividing microshoots in single node segments. Segmentation was very effective and shoot proliferation intensity increased to 34.16 in the same media composition (BAP 4.44 µM, TDZ 5.37 µM, and AS 50 mg/l) (Figs. 2 C, D).

3.3 Rooting

Both direct and indirect micropropagated shoots were transferred to MS medium with different concentrations of NAA along with or without activated charcoal (2 g/l). The highest percentage of rooting was observed in MS medium containing NAA (16.06 µM) and activated charcoal (2 g/l) (Fig. 2). The rooting percentage, (70.33) and mean number of roots per shoot (3) and mean root length (6.18) was recorded on that medium (Table 3).

Fig. 1. *Corbichonia decumbens*-callus induction and shoot formation form leaf explant

Table 1. Effect of MS medium and different concentrations of BAP and NAA on Callogenesis and shoot formation from leaf of *Corbichonia decumbens*

S. no	MS medium BAP µM/l	NAA	% of callogenesis	Days taken for callus induction	Nature of the callus	% of callus inducing shoots	Average number of shoots per explant	Average number of shoots per explants on subculture
1	2.22	5.37	73.16 ±2.56	14	Green friable	73.50±2.17	4.83±0.47	22.33±1.72
2	4.44	5.37	91.00±2.88	09	Green friable	95± 2.23	7.33±0.63	32.16±1.40
3	6.66	5.37	76.10±3.83	11	Green friable	75.5±2.80	5.01±0.60	23.16±1.16
4	8.88	5.37	62.33±1.92	13	Friable and dark green	59.33±1.99	4.00±0.51	16.50±1.33
5	11.10	5.37	56.16±3.39	14	Friable and dark green	54.23±1.08	2.83±0.30	13.50±0.99
6	13.32	5.37	44.50±2.18	13	Friable and dark green	45.10±4.67	2.16±0.32	08.83±1.01
7	Basal medium	-	-	-	-	-		

Table 2. Effect of BAP, TDZ and adenine sulphate on initiation and multiple shoot induction from nodal explants of *Corbichonia decumbens* cultured on MS medium

S. no	MS medium BAP µM/L	TDZ µM/L	Adenine Sulphate (mg/l)	Days taken for shoots induction	% of explants inducing shoots	Average number of Shoots/explant Initiation	Average number of Shoots/ explants on subculture
1	2.22	2.27	50	14	90.16±2.72	6.0±0.36	20.83±1.40
2	4.44	2.27	50	09	99.2±0.37	11.16±0.47	34.16±2.07
3	6.66	2.27	50	11	81.83±1.85	7.33±0.33	18.50±1.17
4	8.88	2.27	50	13	60.33±2.81	5.10±0.25	14.16±1.13
5	11.10	2.27	50	14	52.16±4.33	3.16±0.30	9.33±0.66
6	13.32	2.27	50	13	20.00±1.41	2.21±0.051	6.16±0.94
7	Basal medium	-	-	-	-	-	-

Table 3. Effect of NAA and activated charcoal on root formation of *Corbichonia decumbens* in MS medium

S. no	MS medium +NAA (µM)	Activated charcoal (g/l)	% of plantlets producing root	Mean number of roots/shoot	Mean root length (cm)
1	5.37	-	43.42	2.16±0.47	2.36±0.69
2	10.74	-	51.66	3.16±0.04	3.66±0.51
3	16.05	-	63.83	3.22±0.42	5.15±0.69
4	21.40	-	24.16	1.30±0.36	3.20±0.63
5	26.75	-	10.16	1.16±0.13	1.66±0.75
6	5.37	2	46.53	2.00±0.63	2.59±0. 75
7	10.74	2	54.72	2.33±0.61	4.00±0.63
8	16.05	2	70.33	3.66±0.51	6.18±0.45
9	21.40	2	27.21	1.16±0.75	2.00±0.89
10	26.75	2	12.11	0.80±0.83	1.85±0.69
11	Basal medium	-	-	-	-

Table 4. Effects of substrates on the acclimatization of plantlets regenerated multiple shoots of *Corbichonia decumbens*

S. no	Planting substrates	No. of plants transferred	No. of plants survived	Survival (%)
1	Vermicompost	50	37	74.13
2	Red soil + Vermicompost	50	45	90.21
3	Red soil + sand+ Decomposed coir waste	50	34	68.30
4	Hardening media (decomposed coir waste: garden soil: vermiculite)	50	36	72.66

3.4 Hardening

The regenerated plantlets from both direct and indirect micropropagation were transferred on different substrates and acclimatized inside the shade house with fogger system. Four substrates examined, the percentage survival of the plantlets was highest (90.21) in red soil and vermicompost in the ratio of 1:1 followed by vermicompost alone (74.13). Partially hardened plantlets from this media, was transferred to the plastic pots containing red soil, sand and compost in the ratio of 1:1:1 (Figs. 2 E, F).

4. DISCUSSION

Traditional medicinal system, have been used over three thousand years. Allopathic medicines, causes lot of side effects when compared to indigenous medicines. Traditional healers claim that their medicine is cheaper and more effective. So these indigenous medicines are preferred by many people. Due to over exploitation and less germination the medicinal plants, become endangered. Plant biotechnology plays an important role in the mass multiplication through direct and indirect micropropagation for conservation. In the present study excellent amount of green, friable, and nodular callus was induced from leaf explants cultured on BAP and NAA combination. But in *Glinus lotoides* (Molluginaceae) BAP along with 2, 4-D induces greenish brown color callus [11]. The callogenic effect of BAP and NAA observed in the present study was in consonance with other reports [12-13]. The multiple shoot formation (32.16 shoots/ explant) was observed in both same callus induction medium (BAP 4.44 µM and NAA 5.37 µM) and fresh medium. Increasing in the concentration of BAP decreases the multiple shoot formation as well as the percentage of callus inducing shoots. In shoot proliferation effects of BAP+NAA observed in the present study was in consonance with *Ceropegia pusilla* [12].

The nodal cuttings cultured on MS medium supplemented with BAP, TDZ and AS were budded after 9 days with higher percentage 99.2±0.37 (BAP4.44 µM, TDZ 5.37 µM and AS 50 mg/L) and initiated more number of shoots in initiation (11.16±0.47) as well as subculture (34.16±2.07). Comparable results have been showed in *Caralluma diffusa* [14]. Where as in *Mollugo nudicaulis* BAP alone, without TDZ induces multiple shoot formation [17]. The stimulating effect of BAP on initiation and multiple shoot formation has been demonstrated

earlier for several medicinal plants like *Caralluma diffusa,* [14], *Boucerosia truncato-coronata,* [15] *Ceropegia pusilla* [16] and *Vitex negundo* [17]. In the present study the lower concentration of TDZ along with BAP shows more effective result. These results are comparable to the results of *Caralluma diffusa* [14]. The type and concentration of cytokinin supplemented in the media was the reason for shoot proliferation. Generally the tropical plants require 16h photo period for their growth. So, in the present study also the cultures were incubated under 16 h photo period. BAP with TDZ and AS shows the best result compared to other combinations (Table 2). The efficiency of BAP in *in vitro* bud breaking and shooting has been successfully reported in Molluginaceae species *M. nudicaulis* [18]. However in this species and also at genus level very scanty reports are available regarding the direct and indirect micropropagation. Adenine sulphate stimulates somatic embryogenesis and caulogenesis, induces the proliferation of axillary shoots in shoot cultures and promotes adventitious shoot formation indirectly from calli or directly from explants and when it is associated together with cytokinin such as BAP

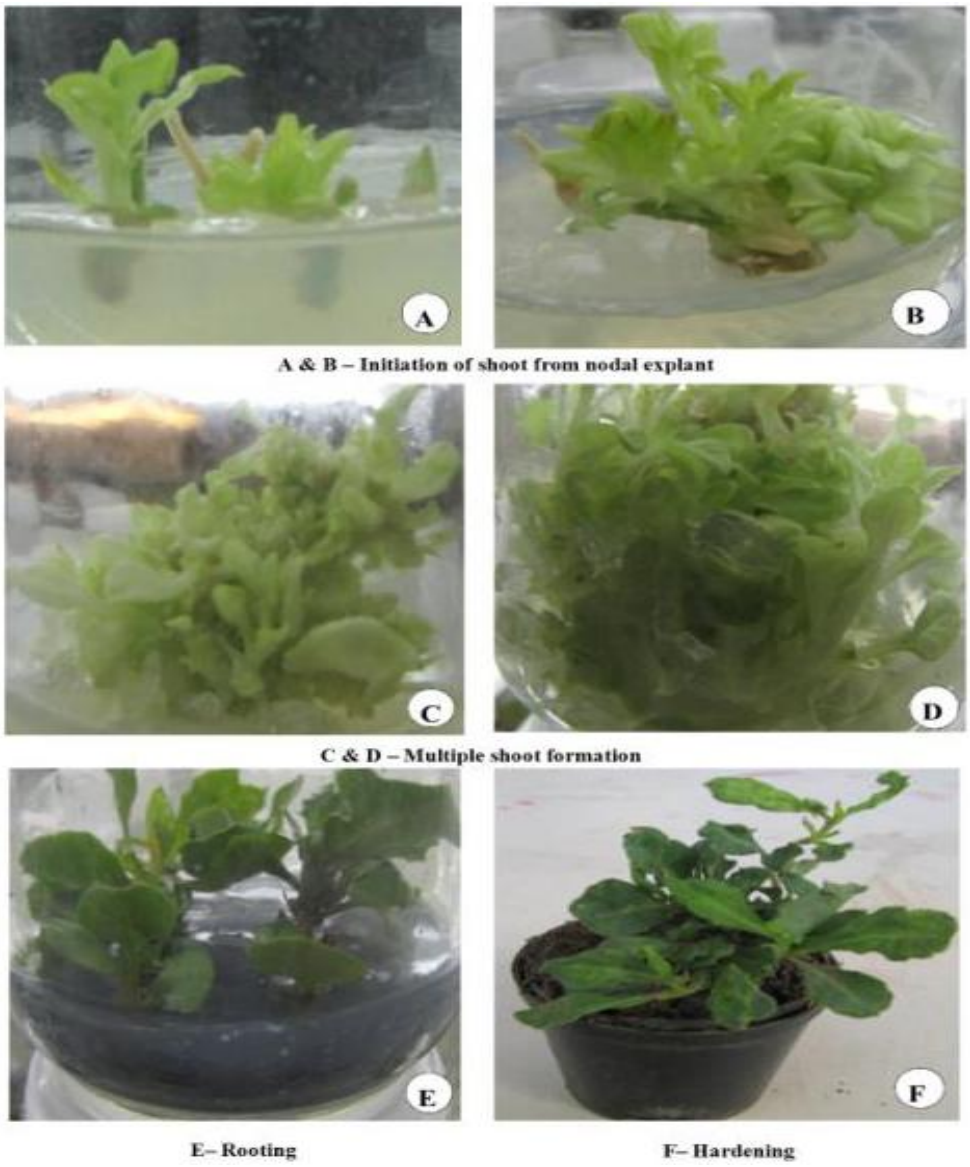

Fig. 2. *Corbichonia decumbens*- multiple shoot formation, root induction and acclimatization form nodal explants

or kinetin [19]. Present study also confirms the addition of adenine sulphate induces shoot proliferation. The *in vitro* regenerated shoots were transferred to MS medium supplemented with different concentrations of NAA and activated charcoal for root induction. Root formation occurs from the basal cut end of the shoots. The highest percentage of (70.33) rooting was observed in MS medium containing NAA (16.05 µM) and activated charcoal (2 g/l) (Table 3). In contrary, the rooting was observed in MS medium containing IBA (1 mg/l) in *M. nudicaulis* [18]. But in *B. truncato-coronata* the NAA promoted efficient root formation [15]. The well-developed plantlets were acclimatized inside the shade house with 90% shade net in selected planting substrates (Table 4). After primary hardening the plantlets were transferred to plastic pots for secondary hardening. The survival percentage was higher (90.21) in red soil with vermicompost in the ratio of 1:1.

5. CONCLUSION

Direct and indirect organogenesis of *Corbichonia decumbens* was established in this study from nodal cuttings and leaf callus in the presence of BAP + TDZ and BAP+ NAA. Plantlets were regenerated fully, when these growth regulators were used. Root formation was induced on regenerated plantlets in the presence of NAA. Around 91% of regenerated plantlets survived when acclimatized in a mixture of red soil with vermicompost. Hence, the direct and indirect micropropagation of *C. decumbens* is more effective, reliable, reproducible and commercially viable than the conventional methods.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Iwu MW, Duncan AR, Okunji CO. New antimalarials of plant origin. Perspective on new crops and new uses. In: Janck J. editor, VA ASHS Press, Alexandria. 1999; 457-462.
2. Antwi S, Martey ON, Donkor K, Nii-Ayitey Okine LK. Anti-diarrhoeal activity of *Blighia sapida* (sapindaceae) in rats and mice. J Pharmacol Toxicol. 2009;4:117-125.
3. Murch SJ, Krishna Raj S, Saxena PK. Tryptophan is a precursor for melatonin and serotonin biosynthesis in *in-vitro* regenerated St. John's wort (*Hypericum perforatum* L. cv. Anthos) plants. Plant Cell Rep. 2000;19:698-704.
4. Katewa SS, Galav PK. Traditional herbal medicines from Shekhawati region Rajasthan. Indian Journal of Traditional Knowledge. 2005;4(3):237-245.
5. Uma G, Balasubramaniam V, Jagathes Kumar S. Evaluation of the antioxidant activity of total phenolic and flavonoid content in *Corbichonia decumbens* (Forsk.) (Molluginaceae). International Journal of Pharmaceutical, Chemical and Biological Sciences. 2014;4(1):159-163.
6. Uma G, Balasubramaniam V, Jagathes Kumar S. *In-vivo* screening of antiinflammatory activity in methanolic extract of *Corbichonia decumbens* (forsk.) Using various animal models of paw oedema. International Journal of Pharmacy and Pharmaceutical Sciences. 2014; 6(1):146-148.
7. Uma G, Balasubramaniam V, Jagathes Kumar S. Assessment of anti-ulcer activity of *Corbichonia decumbens* Forssk. Methanolic extract by aspirin plus pyloric ligation model. International Journal for Pharmaceutical Research Scholars. 2014; 3(I-1):757-761.
8. Uma G, Jagathes Kumar S, Balasubramaniam V. Preliminary phytochemical screening and antimicrobial activity of *Corbichonia decumbens* Forssk. (Molluginaceae). Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2013;4(3):1098-1103.
9. Uma G, Balasubramaniam V, Jagathes Kumar S. *In-vivo* screening of Antinociception activity in methanolic extract of *Corbichonia decumbens* (Forssk.). International Journal of Herbal Medicine. 2013;1(3):61-63.
10. Murashige T, Skoog F. A revised medium from rapid growth and bioassays tobacco tissue cultures. Plant. Physiol. 1962;15: 473-497.

11. Baga Ram, Navdeep S, Bains. Antimicrobial activity of flavonoids from *in vitro* tissue culture and plant parts of two plant species of Western Rajasthan. Indian J. Pharm. Biol. Res. 2014;2(2):18-21.
12. Prabakaran R, Sasikala T, Kalimuthu K. Regeneration of shoots from callus of *Ceropegia pusilla* wight and Arn. British Biotechnology Journal. 2013;3(3):416-423.
13. Beena MR, Martin KP, Kirti PB, Molly Hariharan. Rapid *in vitro* propagation of medicinally important *Ceropegia candelabrum.* Plant Cell Tissue and Organ Culture. 2003;72:285–289.
14. Kalimuthu K, Kalaiyarasi K, Prabakaran R, Sasikala T. *In vitro* propagation of *Caralluma diffusa* (Wight) N.E.Br. British Biotechnology Journal. 2014;4(2):164-172.
15. Kalimuthu K, Prabakaran R, Sasikala T. *In vitro* propagation of *Boucerosia truncato-coronata:* An endemic, medicinally important plant. International Journal of Biotechnology and Research. 2013; 3(3):53-60.
16. Kalimuthu K, Prabakaran R. Micropropagation of *Ceropegia pusilla* - medicinally important plant. J. of Trop. Med. Plants. 2013;14 (1):37-42.
17. Das S, Kanungo V, Naik ML, Sanju S. *In vitro* regeneration of *Vitex negundo* L. A medicinal shrub. Plant Cell Biotechnol. Mol. Biol. 2005;6:143-146.
18. Nagesh KS, Shanthamma C. Micropropagation and antioxidant activity of *Mollugo nudicaulis* Lam. Journal of Medicinal Plants Research. 2011;5(6):895-902.
19. Van Staden J, Zazimalova E, George EF. Plant growth regulators II, Plant propagation by tissue culture: Cytokinins, their analogues and antagonist. In George E F, Hall M, De Kleck GJ (eds) Academic press, Netherland. 2008;205-226.

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