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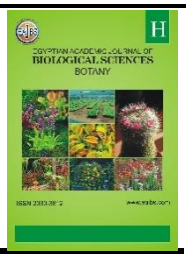

In vitro Propagation, Caulogenesis, and Tuberization of *Ceropegia woodii* Plants

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ABSTRACT

Ceropegia woodii is a flowering plant in the genus *Ceropegia* L. which is one of the largest plant groups in the family Asclepiadaceae (Apocynaceae). The aim of this study is to develop an efficient protocol for the rapid *in vitro* micropropagation, caulogensis and *in vitro* tuberization of *Ceropegia woodii* Plants via enhanced axillary bud proliferation from single nodal explants cultured on full strength MS medium, 30g/l sucrose, 4g/l gelrite augmented with various concentrations of plant growth regulators as BA, NAA, IBA and their combinations. In general, the present study concluded that the best results for the initiation stage were recorded on MS medium fortified with NAA and BA at 2.00 and 0.50 mg/l, respectively. Meanwhile, supplemented medium with BA and NAA at 1.00 and 0.50 mg/l, respectively, gave rise to the best results for the multiplication stage. Regarding the rhizogenesis stage, the best results were recorded when the neoformed shoots of the multiplication stage were divided singly and cultured on MS medium plus IBA and NAA at 1.00 and 0.50 mg/l, respectively which led to the highest mean number of roots formed per propagule. For callus induction, the leave segments were cultured in to MS medium supplemented with different concentrations of BA and NAA. Meanwhile, supplemented medium with BA and NAA at 1.00 and 0.50 mg/l, respectively, gave rise to the best results for caulogensis stage. Concerning the percentage of explants formed callus and callus size, the best results obtained from BA and NAA at 0.50 mg/l and 2.00 mg/l, respectively for callus formation percentage, with high and intensive size and compact green colour and for callus size formed per propagule. The tuber induction medium was based on1/2 strength MS fortified with different combinations of sucrose and BA, gelled using 4 gm/l gelrite. Concerning The highest mean value of *in vitro* tuberization percentage, a number of *in vitro* formed tubers, highest mean of *in vitro* formed tubers weight and diameter was recorded at 8.00 mg/l BA and sucrose at 60g/l. Neoformed plantlets were acclimatized *ex vitro* and *in vivo* vigorously in a mixture of perlite and peatmoss at (1:1) or (2: 1) or (3: 1) or (2:3) and (3:3) consecutively, in addition to fixed volume (1 portion) of sand; which resulted in the highest mean value of survival percentage/plant (100%) and showed true-to-type plants *ex vitro*.

INTRODUCTION

The genus Ceropegia L. is one of the largest plant groups in the family Apocynaceae (APG III, 2009). The 357 species of highly succulent stapeliads and four lineages of the 141 species of Brachystelma R.Br. ex Sims are nested within the 219 species of Ceropegia L., which show large diversity in habit, habitat, flower architecture, and ecological adaptations, according to recent phylogenetic reconstructions within the Ceropegieae (*Apocynaceae-Asclepiadoideae*). (Bruyns 2003; Murthy *et al*. 2012a; Bruyns *et al*., 2015). *Ceropegia spp*. is known by many names, including lantern flower, Christensen, parasol flower, parachute flower, bushman's pipe, snake creeper, wine-glass vine, rosary vine, and necklace vine flower., (Yadav, 1996; Quattrocchi, 2000). Several species have medicinal uses, while others have been domesticated and are used as ornamentals in homes. (Reynolds 2006; Chavan *et al*. 2011b).*Ceropegia woodii* is a flowering plant in the genus *Ceropegia*, native to South Africa, Swaziland, and Zimbabwe. It is sometimes treated as a subspecies of the related *Ceropegia linearis*, as *C. linearis* subsp. *woodii* (Bruyns *et al*., 2015, Chavan *et al*., 2018). Common names include chain of hearts, collar of hearts, String of hearts, rosary vine, hearts-on-a-string, and sweetheart vine. The species name honors John Medley Wood (1827-1915), who collected native African plants after he retired from the East Indian Merchant Service. And the current conservation status of *Ceropegia woodii* is Least Concern (LC) (Foden & Potter 2005; Murthy *et al*., 2012a). It shows a variety of morphological properties and growing in cultivation such as non-succulent or leafless succulent climbers, erect herbs and rarely subshrubs, the familiar *Ceropegia linearis subsp. woodii* is a creeper with heart shaped variegated leaves. The variegation in the leaves is not caused by a lack of chlorophyll, but by the presence of a space between the epidermis and the tissues underneath. The variegation of *C. L. woodii* is more intense if the plant is grown in shadier conditions. Climbing forms of *C. woodii* are also common in the wild - even the occasional shoot of the 'houseplant' variety climbs. Some of these climbing forms just develop a large caudex, without growing any additional tubers. (Ansari, 1984; Meve and Liede-Schumann, 2007; Albers *et al*. 1991). The status of many Ceropegia species has been influenced by increasing human and livestock populations (Phulwaria *et al*., 2013). Ceropegias are overexploited due to the pharmaceutically important alkaloid ‘cerpegin’ and now the genus as a whole is under serious threat (Nayar and Sastry, 1987; Yadav and Kamble, 2006). They were classified on the basis of Carbohydrate residue is attached by an acetal linkage at carbon atom 1 to a non carbohydrate residue. *Ceropegia spp* has revealed the presence of volatile oil and terpenes, in C. *woodii*, 41 peaks were isolated, of which 24 compounds were identified. Of the total volatile matter, 70.73% was terpenes, 5.82% was taxanes, and 1.52% was ketones.64 (Huang *et al*. 2007; Xue *et al*. 2010).

Conventionally, lantern plants are propagated through the seeds and tubers nevertheless, low viability, dormancy, and poor seed germination limit their large-scale propagation (Yadav and Kamble, 2006; Srinivasarao *et al*., 2010). The root tubers of the many lantern flowers are a store house of starch, gum, sugars, fats, albuminoids, crude fiber and other valuable phytoconstituents which are regularly utilized in conventional Indian Ayurvedic drug preparations for treating diarrhea and dysentery. Ceropegias are highly-prized for their medicinal properties, edible tubers, and ornamental flowers. Owing to their ornamental potential, some species are cultivated as horticultural crops (McNew 2002; Hodgkiss2004; Reynolds 2006). Starchy tubers of the many Ceropegias are liable to fungal infections and thus decay of tubers may be a major problem in their cultivation and maintenance (Yadav and Kamble, 2006; Nautiyal *et al*. (2009). The tubers of Ceropegias are edible and contain starch, sugars, gum, albuminoids, carbohydrates, fats, and crude fiber (Mabberly1987; Jain and Defillips 1991).

The hallmark features of lantern flower species are their attractive and decorative flowers and leaves. The flowers of most species are morphologically unique; possess great diversity in corolla size, form and coloring patterns, flower design, corona organization and mechanisms for illumination of necessary organs (Chavan *et al*. 2018). Hence, this group of plants has created middle of attention from botanists, horticulturalists, gardeners and succulent enthusiasts. Newly, some species are domesticated as houseplants, used as ornamentals and marketable in Europe (Hodgkiss, 2004; Reynolds, 2006; Chavan *et al*., 2011a).

MATERIALS AND METHODS

The experiments regarding the effect of different concentrations of certain growth regulators and their combinations on micropropagation and tuberization of *ceropegia woodii* plantlets using nodal segments as explants were conducted in the Plant Tissue Culture Laboratory of The Faculty of Agriculture Saba Basha, Alexandria University, during the period of 2018 and 2019.

Plant Materials:

1. Explant Preparation:

The explant materials were picked up from healthy mother plants grown in the greenhouse of Antoniades Botanical Garden, Horticultural Res. Inst., Agric. Res. Center, Alexandria, Egypt. The collected material were brought to the laboratory to process, leaves were removed and washed after that to be ready for sterilization and tissue culture manipulation.

2. Explant Sterilization:

The shoot explants from cuttings were washed thoroughly in the water, using liquid soap for 30 min., and then the excised explants were placed under running tap water for 90 minutes then dipped in 70% ethanol for 15 sec. After pretreatment with ethanol, the explants were washed with double distilled water twice to lower the toxic effect of ethanol. Nodal segments of only (2cm) long which contained a single node were then surface sterilized with concentration of mercuric chloride ($HgCl_2$) at 0.1% (v/v) with a few drops of wetting agent "Tween-20" (surfactant agent) for fifteen minutes. A similar procedure was repeated, but the explants were immersed in the concentration of sodium hypocholrite solution (NaOCl) at 30%. After the surface sterilization of explants with mercuric chloride and sodium, hypocholrite solution was decanted and the explants were washed with sterile double distilled water for four times, so as to lower the toxic effects of $HgCl_2$ and NaOCl and became ready for culturing.

***In vitro* Experimental Stages**:

1. **Initiation Stage**:

During this stage explants were cultured on solidified Murashige and Skoog medium (1962) solidified with gelrite (3g/l). The pH of the tested media was adjusted to 5.7 before adding gelrite, and then sterilized autoclaving at 121°C for 20 min., then explants were cultured into the given MS medium which contained different concentrations of cytokinin (BA at four concentrations: 0.00 (nil), 0.25, 0.50, and 1.00 mg/l, in combinations with auxin (NAA) at five concentrations 0.00 (nil), 0.5, 1.00, 2.00, and 4.00 mg/l.

2. **Multiplication Stage**:

In this stage the neoformed propagules of the initiation stage were sectioned into single leaflets nodes (*ca.* 1 cm). The excised nodal cuttings explants of the different positions were cultured, randomly, on multiplication media which supplemented with BA at five concentrations: 0.00 (nil), 0.50, 1.00 2.00, and 4.00 mg/l, in combinations with NAA at four concentrations: 0.0 (nil), 0.25, 0.50 and 1.00 mg/l.

3. Rhizogenesis:

The obtained shoots from the multiplication stage were, individually, cultured on a rooting medium, contained two types of auxins were tested, Indole-3-Bytric acid (IBA) at four concentrations: 0.00 (nil), 0.50, 1.00 and 2.00 mg/l, in combinations with NAA at four concentrations: 0.0 (nil), 0.25, 0.50 and 1.00 mg/l.

4. Callus Formation:

The obtained shoots from the initial explants were used for the induction of callus. The leaves were excised from shoots and cut into (0.5 cm^2) segments, more or less. The explants were cultured onto MS medium supplemented with different concentrations of BA at four concentrations; 0.00 (nil), 0.125, 0.250 and 0.500 mg/l, in combinations with NAA at four concentrations; 0.0 (nil), 0.5, 1.0 and 2.0 mg/l. The percentage of callus formation, size of callus, percentage of direct organogenesis, number of shoots and number of roots per propagule were recorded after 35 days in culture. Callus size was determined, macroscopically, and for statistically convenience, the minus or plus symbols were converted to a numerical code as follows: (-), 0; (+), 1; (++), 2; (+++), 3; (++++), 4. These symbols refer to no callus, low, moderate, high, and intensive callus formed per propagule, respectively.

5. *In vitro* Tuberization:

The tuber induction medium was based on1/2strength MS nutrients fortified with different combinations of four concentrations of sucrose (0.00 or nil, 30, 60 and 90 gm/l) and five levels of BA (0.00, 1.00, 2.00, 4.00 and 8.00 mg/l), gelled using 4 gm/l gelrite. The combination led to 20 medium combinations used for tuberization stage. Tubers and roots started to develop epigeally at the nodes and the end of the plants within 30 days and they are ready for harvesting after 60 days.

Acclimatization of Neoformed Plantlets:

The plantlets produced from the rooting stage was washed gently out of solidified medium under running tap water, followed by immersing them into fungicide for 25 sec. They were, then, transplanted *ex vitro* in small plastic pots (6 cm) plastic pots contained a combination of an autoclaved mixture of the perlite (0, 1, 2 and 3 volume) and peatmoss at (0, 1, 2, 3 volume) each; and one constant volume of washed and autoclaved sand. Then, they were arranged in a factorial experiment and finally placed in transparent plastic bags (*ex vitro*), to maintain high relative humidity at (RH) 85% and 27±1°C, for hardening-off. However, the tested pots with different media were rearranged, randomly, weekly within the same plot to avoid the experimental error. Ten days later, the plastic bags were perforated for gaseous exchange, then transferred into the plastic house (*in vivo*) and continued for further hardening. After four weeks, the plastic bags were removed and the acclimatized plantlets were watered, as needed, and fertilized weekly with mineral fertilization (20:20:20) at 0.5g/l.

Experimental Design And Statistical Analysis:

All the experiments carried out during this study were designed as factorial experiments layout in completely randomized design (Gomez and Gomez, 1984). Recorded data were analyzed statistically using the analysis of variance technique (ANOVA) and means were compared by L.S.D tests (Steel *et al*., 1997) and significance was determined at $p \leq 0.05$.

RESULTS AND DISCUSSION

1. Initiation Stage of *Ceropegia woodii:*

Data presented in Table (1) and Figure (1) Exhibit that both applied growth regulators (BA and NAA) and their combinations exerted significant effects on the initiation stage

characters of *Ceropegia woodii* single node explants grown *in vitro*. Concerning the main effect of BA on the studied characters, i.e. numbers of the shoot, shoot length, leaflets, nodes and roots formed per propagule, in general, the highest mean values were always recorded at (0.50 mg/l) BA in the culture medium, but the lowest ones were noticed at either the absence of BA or at 1.00 mg/l for the number of shoots, shoot length, the number of leaflets and nodes number formed per propagule.

Table 1: Effect of different levels of NAA and BA (mg/l) and their combinations on the initiation stage of *Ceropegia woodii* cultured *in vitro* for 35 days.

Characters	BA Levels (mg/l)	NAA levels (mg/l)					Mean (BA)	Significance		
		0.0	0.5	1.0	2.0	4.0		NAA	BA	NAA X BA
(a) Mean number of shoots formed/propagule:										
	0.0	1.11	1.33	2.11	3.11	1.33	1.80	**	**	**
	0.25	2.67	2.78	3.67	4.11	3.67	3.38			
	0.50	3.67	4.44	4.67	5.33	3.89	4.40			
	1.00	1.56	2.00	2.11	2.89	1.33	1.98			
Mean (NAA)		2.25	2.64	3.14	3.86	2.56				
L.S.D. (0.05)								0.45	0.41	0.91
(b) Mean shoot length (cm)/propagule:										
	0.0	2.11	2.59	3.14	3.77	2.39	2.80	**	**	**
	0.25	3.14	4.09	5.28	7.40	4.92	4.97			
	0.50	3.02	5.40	6.60	9.78	7.19	6.40			
	1.00	2.92	3.31	4.49	6.24	3.14	4.02			
Mean (NAA)		2.80	3.85	4.88	6.80	4.41				
L.S.D. (0.05)								0.69	0.62	1.39
(c) Mean number of leaflets formed/propagule:								**	**	*
	0.0	4.89	7.56	8.22	9.11	7.33	7.42			
	0.25	7.56	9.56	12.22	9.33	7.78	9.29			
	0.50	6.44	8.89	11.78	14.67	11.33	10.62			
	1.00	6.00	8.67	8.67	10.89	6.44	8.13			
Mean (NAA)		6.22	8.67	10.22	11.00	8.22				
L.S.D. (0.05)								1.13	1.01	2.26
(d) Mean number of nodes formed/propagule:								**	**	**
	0.0	2.44	3.78	4.44	5.11	3.67	3.89			
	0.25	3.78	5.00	5.56	6.22	3.89	4.89			
	0.50	3.44	4.78	5.89	8.00	5.67	5.56			
	1.00	3.44	4.67	4.33	6.00	3.67	4.42			
Mean (NAA)		3.28	4.56	5.06	6.33	4.22				
L.S.D. (0.05)								0.44	0.47	0.88
(e) Mean number of roots formed/propagule:								**	**	**
	0.0	0.00	2.67	3.11	0.44	0.22	1.29			
	0.25	0.56	1.56	1.78	0.11	0.56	0.91			
	0.50	0.11	0.33	0.56	0.33	0.22	0.31			
	1.00	0.22	0.44	0.11	0.11	0.00	0.18			
Mean (NAA)		0.22	1.25	1.39	0.25	0.25				
L.S.D. (0.05)								0.49	0.44	0.98

L.S.D. (0.05) = Least significant difference test at 0.05 level of probability.
*, **: Significant or highly significant**.**

Fig. 1: Initiation stage of *Ceropegia woodii* single nodal grown *in vitro* on MS medium augmentedwith BA at 0.50 mg/l and NAA at 2.00 mg/l over 35 days.

Regarding the main effect of NAA tested levels on the above-mentioned traits, commonly, the highest mean values were always recorded at 2.00 mg/l of NAA in the culture medium, and the lowest ones were noticed at its absence from the cultured medium (0.00 mg/l) or at (4.00 mg/l), except the mean number of roots formed per propagule. Concerns the interaction between levels of both factors under the study the BA at 0.25 and NAA at 1.00 mg/l led to the highest mean values of the studied characters in general. On the other hand, the lowest mean values of the above-mentioned characters were recorded at the highest levels of both factors (i.e. NAA and BA at 4.00 and 1.00 mg/l, each in turn). On the other side, the number of roots showed the highest mean values at 1.00 mg/l NAA added in the culture medium, but the lowest ones were noticed at its absence or highest level (4.00 mg/l).

The above-mentioned results, generally, indicated that increasing the mean values of the studied characters was concomitant with the high BA levels. BA has been the most efficient cytokinin for multiple shoot induction. Structural stability and quick metabolization of BA by plant tissue in comparison to other synthetic cytokinins are the reasons for the supremacy of BA for shoot induction. BA also causes the production of natural hormones such as zeatin within the plant tissue causing enhanced shoot production (Ahmad *et al*. 2013; Malik *et al*. 2005). Previously published reports suggest that lower concentrations of NAA in combination with BA play a crucial role in the regeneration of plants, such as *Habenaria edgeworthii* (Giri *et al*. 2012), *Aconitum violaceum* (Mishra *et al*. 2012) and *Ceropegia* spp. (Chavan *et al*. 2018).

This finding could be attributed to the mode of action of auxin (NAA) within cultured tissues which is capable of controlling various distinctive processes such as cell growth and elongation (George *et al.*, 2008).

On the other extreme, the high concentration of NAA used affected well the root formation of *Ceropegia woodii* in vitro. This might be due to the role and mode of action of auxin for their abilities to enhance root formation, as stated by George *et al*., (2008) and Waseem *et al*., (2011). Concerns to the interaction between both growth regulators at 0.50 BA and 2.00 mg/l NAA, expressed significant effects on the various tested traits.

Effectiveness of various PGRs (BAP, IBA, NAA) with MS medium were tested the physiological effects and recommended a combination of BAP (2.5mg l^{-1}) and NAA (0.5mg

l^{-1}) for the initiation of a maximum number of shoots in the primary as well as in subsequent subcultures of *C. pusilla* Kalimuthu and Prabakaran (2013d, 2013e). The highest shoot length and maximum quantity of shoots when shoot tip explants were cultured on MS medium supplemented with BA (8.88 µM) and NAA in the grouping. Similarly, singular supplementation of BAP (1.0 µM) was found appropriate for shoot multiplication in *C. bulbosa* (Balakrishnan *et al*., 2015; Dhir and Shekhawat, 2013, 2014a). An efficient in vitro propagation protocol was developed with phytochemical profiling for *Ceropegia media*. Callus cultures with 1 µM (BAP) and 1 µM 2,4-D. Conversion into plantlets was attained only from tissue culture-derived seedling leaf explants (Pandey *et al*., 2020).

2. Multiplication Stage of *Ceropegia woodii*:

Results in Table (2) and Figure (2) Describe the effect of various levels of both growth regulators (BA and NAA) and their combinations on the studied characters of *Ceropegia woodii*. Concerning the mean number of shoots, the mean value of shoot length, the number of leaflets and the number of nodes formed per propagule, BA levels had a highly significant effect on this trait. The highest mean value was recorded at 1.00 mg/l BA, but, the least response was observed with the absence of BA. On the other hand, NAA had a highly significant effect on the given traits. Meanwhile, the interaction between both growth regulators exerted a highly significant effect. However, the combination of BA and NAA, at 1.00and 0.50 mg/l respectively, resulted in the highest mean value.

Table 2: Effect of different levels of BA and NAA (mg/l) and their combinations on the multiplications stage of *Ceropegia woodii* cultured *in vitro* for 35 days.

Characters	NAA Levels (mg/l)	BA levels (mg/l)					Mean (NAA)	Significance		
		0.0	0.5	1.0	2.0	4.0		BA	NAA	BA X NAA
(a) Mean number of shoots formed/propagule:								**	**	**
	0.0	2.67	2.78	3.33	3.22	2.22	2.84			
	0.25	2.67	3.22	4.67	3.89	3.44	3.58			
	0.50	2.56	4.44	7.67	5.33	3.78	4.76			
	1.00	3.00	4.11	5.89	3.89	2.89	3.96			
Mean (BA)		2.72	3.64	5.39	4.08	3.08				
L.S.D. (0.05)								0.64	0.57	1.35
(b) Mean shoot length (cm)/propagule:								**	**	**
	0.0	2.28	3.16	4.30	3.99	2.91	3.33			
	0.25	3.14	4.09	8.63	7.86	4.92	5.73			
	0.50	3.02	5.40	11.47	9.49	7.94	7.46			
	1.00	3.56	3.96	7.48	7.08	3.76	5.16			
Mean (BA)		3.00	4.15	7.97	7.10	4.88				
L.S.D. (0.05)								0.86	0.77	1.73
(c) Mean number of leaflets formed/propagule:								**	**	*
	0.0	6.67	9.56	10.89	10.00	9.33	9.29			
	0.25	8.00	9.33	13.56	10.44	9.78	10.22			
	0.50	7.56	10.44	15.78	13.33	11.11	11.64			
	1.00	7.11	9.56	12.00	11.33	9.11	9.82			
Mean (BA)		7.33	9.72	13.06	11.28	9.83				
L.S.D. (0.05)								0.84	0.75	1.68
(d) Mean number of nodes formed/propagule:								**	**	**
	0.0	3.33	4.78	5.44	5.00	4.67	4.64			
	0.25	4.00	4.67	6.78	5.22	4.89	5.11			
	0.50	3.78	5.22	7.89	6.67	5.56	5.82			
	1.00	3.56	4.78	6.00	5.67	4.56	4.91			
Mean (BA)		3.67	4.86	6.53	5.64	4.92				
L.S.D. (0.05)								0.42	0.37	1.84
(e) Mean number of roots formed/propagule:								**	**	**
	0.0	0.00	0.22	0.11	0.00	0.00	0.07			
	0.25	0.44	0.78	1.11	0.33	0.00	0.53			
	0.50	4.67	2.22	1.33	0.56	0.22	1.80			
	1.00	4.33	2.44	1.00	0.33	0.11	1.64			
Mean (BA)		2.36	1.42	0.89	0.31	0.08				
L.S.D. (0.05)								0.73	0.65	1.45

L.S.D. (0.05) = Least significant difference test at 0.05 level of probability.

*, **: Significant or highly significant.

Fig. 2: Multiplication stage of *Ceropegia woodii* explants grown *in vitro* on MS medium augmented with BA at 1.00 mg/l and NAA at 0.50 mg/l over 35 days.

The interaction between both BA and NAA at 1.00 and 0.50 mg/l, respectively, resulted in the highest mean value. With respect to the number of roots formed per propagule, both growth regulators had a highly significant effect on the given trait. The main effect of BA showed that MS-basal medium free of BA, gave the highest mean value. In case of NAA main effect, augmenting basal medium with 1.00 mg/l of it brought about the maximum mean value of the number of roots formed per propagule. While, the combinations between BA at 0.00 mg/l, and NAA at 50 or 1.00 mg/l led to the formation of the highest mean number of roots.

It could be inferred from the above results that BA at 1.0 mg/l, was the optimal concentration for better performance of this particular hormone. Whereas, any above or lower deviation from this concentration of that growth regulator the propagules showed poor performance. These results could be brought about to the mode of action of cytokinins on stimulation both cell division and promotion growth of axillary shoots in plant tissue culture as, also, found by George *et al*. (2008), and Trigiano and Gray (2000).

Likewise, this might be due to the mode of action of auxin (NAA) at the above-mentioned level within cultured tissues may enhance, control various distinctive processes such as cell growth and elongation (George and Sherrington, 1984). Wilkins (1989), additionally, stated that auxin-induced number of responses which involved cell division, cell enlargement, protein and nucleic acids synthesis which are concomitants of auxin-induced growth and changes in wall plasticity of plant cell and increase the apical dominance as there are essential and rapid processes involved in growth and elongation. It is known that an average number of leaves per shoot and number of nodes is one of the important growth factors and is an indirectly proportional relationship to the length of the shoot, and if the shoot length increases, the number of leaves and number of nodes increases as well (Waseem *et al*., 2009). Furthermore, BA considers as an antagonist for rhizogenesis and in favour of stimulates cell division, stimulates morphogenesis (shoot initiation/bud formation) in tissue culture and stimulates the growth of lateral buds-release of apical dominance. (Salisbury and Ross 1992; Davies 1995) with respect to the combinations between both growth regulators between BA and NAA led to significant effects on the studied traits.

The combined treatments of cytokinins and auxins also support shoot multiplication rates than the individual treatments of the BAP and NAA. The addition of IBA along with BAP or 2iP significantly boosted the multiplication rate, (Chavan *et al*., 2014b; Adsul *et al*., 2019) resulted in the development of a quick in vitro multiplication protocol for an endemic, critically endangered and medicinally important *C. noorjahaniae* via enhanced axillary bud proliferation from nodal explants. BAP alone at 2.0 mg l^{-1} was responsible for the production of the maximum number of shoots. Similarly in *C. fimbriifera*, (Desai *et al*., 2014) reported the singular supplementation of BAP (3.0mg l^{-1}) for direct shoot organogenesis from nodal explants (Chavan *et al*., 2015). However, Shete (2014) reported the BAP (1.6 mg l^{-1}) and IAA (0.2 mg l^{-1}) were found suitable for multiple shoots formation from apical shoot tips of *C. bulbosa* var. bulbosa. The highest number of shoots for *C. candelabrum* was reported when BAP (1.5 mg l^{-1}) and KN (1.0 mg l^{-1}) were in corporated in the media. In more recent studies (Binish *et al*., 2014), Balakrishnan *et al*. (2015) reported the singular supplementation of BAP (1.0 mg l^{-1}) produced the greatest quantity of shoots in *C. juncea.*(Chavan *et al*., 2011a), found that the efficiency of various in vitro propagation approaches for an endemic and endangered *C. attenuata*. among various in vitro propagation approaches, quick shoot multiplication from nodal explants was established using varying concentrations and combinations of PGRs. BAP (13.31 μM) alone served best for the direct shoot organogenesis. In vitro regeneration procedure was developed for threatened and medicinally important *C. pusilla* by Kondamudi and Murthy (2011). Avinash *et al*., (2019) Found that nodal buds of *Ceropegia mohanramii* cultured on MS medium supplemented with BAP (2.0 mg/l) along with (IBA, 0.5 mg/l) resulted in the production of the maximum number of shoots (17.1 ± 1.2).

3. Rooting Stage of *Ceropegia woodii*:

Results in Table (3) and Figure (3) Showed that the applied auxin levels exerted a highly significant effect on the studied characters of *Ceropegia woodii* and the interaction between IBA at 1.00 mg/l and NAA at 0. 50 mg/l resulted in the highest mean value of number of roots formed /propagule. Concerning, the main effect of IBA tested levels on the mean value of shoot length, the mean number of leaflets and nodes, showed that IBA at 1.00 mg/l, led to the highest mean values of the above-mentioned traits. On the other hand, NAA main effect disclosed that augmenting MS-basal medium with 0.50 mg/l of it, gave the highest mean values of the above-mentioned traits. However, the interaction between both added levels of IBA and NAA at 1.00 and 0.50 mg\l respectively resulted in the highest mean values of the given traits. On the other side, the main effect of NAA indicated that supplying MS-basal medium with NAA at 0. 50 mg/l recorded the highest mean value of the number of roots.

The obtained results showed that the used auxins (*viz*. NAA and IBA), in general, produced the best results in almost all studied traits. These results could be explained on the basis that auxin-induced number of responses which involved cell division, cell enlargement, protein and nucleic acids synthesis which are concomitants of auxin-induced growth and changes in wall plasticity of plant cell and increase the apical dominance as there are essential and rapid processes involved in growth and elongation (Wilkins, 1989). The inferior effect of NAA on the root number may be due to the reason that NAA is more persistent than IBA, remains present in the tissue and may block further development of root meristemoids (De Klerk *et al*., 1997).

Regarding the mean value of callus formation percentage formed per propagule, results showed that IBA had a highly significant effect on the given trait at 2.00 mg/ l (50%). On the other side, the main effect of NAA indicated that supplying MS-basal medium with NAA at 1.00 mg/l recorded the highest mean value of callus formation percentage (100%).

The interaction between IBA at 1.00 or 2.00 mg/l and NAA at 1.00 mg/l, gave the highest mean value of the given trait (78% and 100%) respectively.

The synergistic effects of IBA and NAA were reported in *C. bulbosa* var. bulbosa; however, IBA (2.0 mg l^{-1}) produced maximum rooting instead of NAA Britto *et al*. (2003). In vitro rhizogenesis for *C. bulbosa* achieved by adding 0.5 mg l^{-1} IAA into the MS medium. Interestingly (Shete 2014, Adsul *et al*., 2019) reported that In vitro regenerated shoots of *Ceropegia mohanramii* were transferred to one-half MS medium fortified with singular supplementation of auxins, where IBA (1.5 mg/l) served optimal for the production of a maximum number of roots (5.7 ± 0.6). Reddy *et al*. (2014) evaluated the in vitro rooting responses in recently described *C. pullaiahii* and reported the highest rooting on IBA supplemented medium.

Table 3: Effect of different levels of IBA and NAA (mg/l) and their combinations on the rooting stage of *Ceropegia woodii* cultured *in vitro* for 35 days.

Characters	NAA Levels (mg/l)	IBA levels (mg/l)				Mean (NAA)	Significance		
		0.0	0.5	1.0	2.0		IBA	NAA	IBA X NAA
(a) Mean number of shoots formed/propagule:							**	**	**
	0.0	1.00	1.33	2.22	1.56	1.53			
	0.25	1.00	1.67	2.56	2.22	1.86			
	0.50	1.22	1.89	3.56	1.78	2.11			
	1.00	1.11	1.33	1.44	1.11	1.25			
Mean (IBA)		1.08	1.56	2.44	1.67				
L.S.D. (0.05)							0.34	0.34	0.68
(b) Mean shoot length (cm)/propagule:							**	**	**
	0.0	1.90	2.96	3.17	2.17	2.55			
	0.25	2.07	3.93	4.67	3.44	3.53			
	0.50	2.14	3.54	4.11	2.44	3.06			
	1.00	2.12	2.44	3.09	1.98	2.41			
Mean (IBA)		2.06	3.22	3.76	2.51				
L.S.D. (0.05)							0.43	0.43	0.87
(c) Mean number of leaflets formed/propagule:							**	**	**
	0.0	4.44	6.44	7.78	6.89	6.39			
	0.25	8.67	12.89	15.56	9.78	11.72			
	0.50	11.33	16.00	15.33	9.78	13.11			
	1.00	10.22	11.11	10.89	9.11	10.33			
Mean (IBA)		8.67	11.61	12.39	8.89				
L.S.D. (0.05)							1.36	1.36	2.73
(d) Mean number of roots formed/propagule:							**	**	**
	0.0	0.00	0.56	1.22	1.00	0.69			
	0.25	1.89	2.78	4.89	2.67	3.06			
	0.50	3.78	5.33	6.11	4.22	4.86			
	1.00	2.44	3.44	2.33	0.67	2.22			
Mean (IBA)		2.03	3.03	3.64	2.14				
L.S.D. (0.05)							0.67	0.67	1.34
(e) Mean of callus formation percentage/propagule:							**	**	**
	0.0	0%	0%	11%	22%	8%			
	0.25	0%	0%	11%	33%	11%			
	0.50	0%	0%	11%	44%	14%			
	1.00	0%	11%	78%	100%	47%			
Mean (IBA)		0%	3%	28%	50%				
L.S.D. (0.05)							0.16	0.16	0.33

L.S.D. (0.05) = Least significant difference test at 0.05 level of probability.

*, **: Significant or highly significant.

Fig. 3: Rooting stage of *Ceropegia woodii* shoots grown *in vitro* on MS medium plus IBA at 1.00 mg/land NAA at 0.50 mg/l over 35 days.

Chavan *et al*. (2013b) reported the in vitro rooting of *C. panchganiensis*, the shoots showed a better rooting response in IBA supplemented medium. The highest incidence of root formation (96 ± 1.9%), number of roots (9.3 ± 0.9), and root length (3.6 ± 0.5 cm). maximum rooting percentage (92%), and a maximum number of roots (10.3 ± 0.9) for *C. evansii* was achieved with ½ MS medium fortified with IBA (1.0 mg l−1) after 28 days (Chavan *et al*. 2015 and 2018).

4. Callus Formation Experiment:

Data presented in Table (4) and Figure (4) Exhibit that both applied growth regulators (BA and NAA) and their combinations exerted significant effects on the studied characters of *Ceropegia woodii* using leaf explants.

Concerning the percentage of explants formed callus and callus size, the main effect of BA declared that the different levels of BA at 0.250 or 0.500 mg/l, had a significant effect on these traits (64% and 67%) for callus formation percentage, (2.11 and 2.25) with high and intensive size and compact green colour for callus size formed per propagule respectively, compared to the control treatment which didn't show any callus formation. On the other side, the presence of NAA at either 1.00 or 2.00 mg/l, gave the highest callus percentage (89% and 100%) and the highest callus size (2.75 and 3.67) respectively. Meanwhile, the interaction between BA and NAA at any applied levels except nil levels had a significant effect on the given traits.

Regarding the percentage of direct organogenesis and number of shoots formed per explants, the main effect of BA revealed that providing the MS medium with BA at either 0.125 or 0.250 or 0.500 mg/l, gave the highest percentage of direct organogenesis (from 28% to 36%) and the highest number of shoots formed per explants (from 0.33 to 0.75) respectively. On the other side, the main effect of NAA at either 1.00 or 2.00 mg/l, gave the lowest percentage of direct organogenesis (6% and 0%) and the lowest number of shoots formed per explants (0.06 and 0.08) respectively, declared that the presence of NAA into culture medium led to inhibition of direct organogenesis, while the absence of it gave the highest percentage of this trait (67%). With respect to the interaction between BA at either

0.25 or 0.50 mg/l, and the absence of NAA resulted in the highest percentage (100%) of direct organogenesis per explants and highest number of shoots (2.33) formed per explants. Respecting the number of roots formed per propagule, the main effect of the different levels of BA at 0.125 or 0.25 mg/l had a significant effect on this trait (0.83 and 0.97) and the absence of BA from the culture medium resulted in the lowest number of roots (0.56). On the other hand, the main effect of NAA showed that there was a directly proportional relationship between NAA levels and the mean number of roots. However, the highest value was recorded due to the presence of NAA at 1.00 mg/l in culture medium. The interaction between BA at 0.250 mg/l and NAA at 1.00 mg/l brought about the highest number of roots per propagule.

Table 4: Effect of different levels of BA and NAA (mg/l) and their combinations on the caulogenesis stage of *Ceropegia woodii* cultured *in vitro* for 35 days.

Characters	NAA Levels (mg/l)	BA levels (mg/l)				Mean (NAA)	Significance		
		0.0	0.125	0.250	0.50		BA	NAA	BA X NAA
(a) Percentage of explants formed callus/propagule:							**	**	**
	0.0	0%	0%	0%	0%	0%			
	0.50	0%	0%	56%	67%	31%			
	1.00	67%	89%	100%	100%	89%			
	2.00	100%	100%	100%	100%	100%			
Mean (BA)		42%	47%	64%	67%				
L.S.D. (0.05)							0.12	0.13	0.25
(b) Percentage of direct organogenesis formed/propagule:							ns	**	**
	0.0	0%	67%	100%	100%	67%			
	0.50	33%	44%	33%	44%	39%			
	1.00	22%	0%	0%	0%	6%			
	2.00	0%	0%	0%	0%	0%			
Mean (BA)		14%	28%	33%	36%				
L.S.D. (0.05)							0.26	0.26	0.53
(c) Callus size/propagule:							**	**	**
	0.0	0.00	0.00	0.00	0.00	0.00			
	0.50	0.33	0.67	1.67	2.00	1.17			
	1.00	2.33	2.67	3.00	3.00	2.75			
	2.00	3.22	3.67	3.78	4.00	3.67			
Mean (BA)		1.47	1.75	2.11	2.25				
L.S.D. (0.05)							0.23	0.23	0.47
(d) Mean number of shoots formed/propagule:							*	**	**
	0.0	0.00	0.67	1.67	2.33	1.17			
	0.50	0.33	0.67	0.67	0.67	0.58			
	1.00	0.22	0.00	0.00	0.00	0.06			
	2.00	0.33	0.00	0.00	0.00	0.08			
Mean (BA)		0.22	0.33	0.58	0.75				
L.S.D. (0.05)							0.37	0.37	0.74
(d) Mean number of roots formed/propagule:							ns	**	**
	0.0	0.00	0.00	0.00	0.00	0.00			
	0.50	0.67	1.00	0.44	0.33	0.61			
	1.00	1.11	2.33	3.44	2.44	2.33			
	2.00	0.44	0.00	0.00	0.00	0.11			
Mean (BA)		0.56	0.83	0.97	0.69				
L.S.D. (0.05)							0.41	0.41	0.82

L.S.D. (0.05) = Least significant difference test at 0.05 level of probability.

*, **: Significant or highly significant.ns: not significant

Fig. 4: Caulogensis stage of *Ceropegia woodii* leaf segments and shoots regenerations grown *in vitro* on MS medium augmented with BA at 0.5 mg/l and NAA at 2.00 mg/l over 35 days.

The above-mentioned results could be attributed to the role of exogenous cytokinin in enhancing plant cell division. It has been suggested that cytokinins might be required to regulate the synthesis of proteins involved in the formation of new cells (Pasternak *et al.*, 2002). Regarding the main effect of auxin, it represents the important growth regulator for the induction of callus in the majority of angiosperms (Roy and Banerjee, 2003). NAA acts as an auxin to induce cell division and enlargement at low concentrations. Cell enlargement has been associated with an increase in activities of autolytic and synthetic enzymes, which affect cell wall plasticity and synthesis of new wall materials (Cleland, 1981). Hence, a balance between auxin and cytokinin growth regulators is most often required for the formation caulogenesis and subsequent events (Johri and Mitra, 2001).

Phulwaria *et al*. (2013) focused their attention on the efficacy of several explants for callus induction and observed the highest efficiency of epicotyl during the establishment of cultures and further shoot regeneration in *C. bulbosa*. Callus was induced on MS medium containing 1.0 mg l^{-1} of 2, 4-D, while the addition of BAP (0.5 mg l^{-1}) was essential for the proliferation of callus. In *C. pusilla* (Kalimuthu *et al*., 2014b) found that a combination of BAP and NAA was required for callus induction and morphogenesis from stem explants. Prabakaran *et al*. (2013) evaluated several concentrations and combinations of PGRs for callus initiation and succeeding shoot organogenesis in *C. pusilla*. They suggested the supplementation of BA and NAA was essential for callus induction and morphogenesis.). An efficient indirect shoot organogenesis protocol developed for *C. thwaitesii* by Muthukrishnan *et al*. (2015b), reported the comparative account among internode and leaf explants and highlighted the utility of internode over leaf explants. Chavan and Ahire (2016) developed an efficient callus induction and subsequent plant regeneration for multiplication and conservation of medicinally important *C. noorjahaniae*.

5. *In vitro* Tuberization:

Results in Table (5) and Figure (5) Describe the effect of various levels of BA and sucrose and their combinations on the studied characters of *in vitro* tuberization of *Ceropegia woodii*. Concerning the mean value of *in vitro* tuberization percentage per propagule, BA levels had a highly significant effect on this trait. The highest mean value was recorded at 8.00 mg/l BA, but, the least response was observed with the absence of BA. On the other hand, sucrose had a highly significant effect on the mean value of *in vitro* tuberization percentage per propagule. Meanwhile, the interaction between both factors exerted a highly

significant effect. However, the combination of BA and sucrose, at 8.00 mg/l and 60g/l respectively, resulted in the highest mean value. Respecting the mean value of the number of *in vitro* formed tubers, mean of *in vitro* formed tubers weight and mean of *in vitro* formed tubers diameter per propagule both factors and their interactions exerted highly significant effects on the given traits. In case of BA main effect, augmenting the culture medium with BA at 8.00 mg/l, brought about the highest mean value.

On the other side, the main effect of sucrose cleared a proportional relationship between the given trait and sucrose levels; whereas, the level of sucrose at (60 g/l) gave the maximum mean value of the given traits. However, the interaction between both added levels of BA and sucrose at 8.00 mg/l and 60g/l, respectively, gave the highest mean value on the given traits.

It could be inferred from the above results that BA at 8.0 mg/l, was the optimal concentration for better performance of this particular hormone. These results could be brought about to the mode of action of cytokinins on stimulation both cell division and promotion growth of the tuber formation as observed by Badoni and Chauhan (2010) and Islam *et al*. (2008). The cytokinin alone, and in combination with auxins were reported to increase the frequency of *in vitro* tuberization in a number of yam species, (Uranbey, 2005). Le (1999) revealed that BAP alone had a significant effect on microtuber diameter and fresh weight as compared to its combination with Kn. The BAP and other cytokinins were found to stimulate the tuberization process (Hussey and Stacey, 1984). Zakaria *et al*. (2008) supported the high concentrations of BAP and its role in the induction of microtubers.

Table 5: Effect of different levels of BA (mg/l) and sucrose g/l and their combinations on the *in vitro* tuberization stage of *Ceropegia woodii* cultured *in vitro* for 60 days.

Characters	Sucrose Levels (g/l)	BA levels (mg/l)					Mean (Sucrose)	Significance		
		0.0	1.0	2.0	4.0	8.0		BA	Sucrose	BA X Sucrose
(a) Mean of *in vitro* tuberization percentage/propagule (%):										
	0.0	0%	0%	0%	0%	0%	0%	**	**	**
	30	0%	0%	11%	22%	44%	16%			
	60	0%	22%	56%	78%	100%	51%			
	90	0%	0%	0%	0%	0%	0%			
Mean (BA)		0%	6%	17%	25%	36%				
L.S.D. (0.05)								0.15	0.13	0.30
(b) Mean number of *in vitro* formed tubers /propagule:										
	0.0	0.00	0.00	0.00	0.00	0.00	0.00	**	**	**
	30	0.00	0.00	1.11	2.00	2.33	1.09			
	60	0.00	0.89	1.67	2.78	5.89	2.24			
	90	0.00	0.00	0.00	0.00	0.00	0.00			
Mean (BA)		0.00	0.22	0.69	1.19	2.06				
L.S.D. (0.05)								0.77	0.70	1.54
(c) Mean of *in vitro* formed tubers weight /propagule:								**	**	**
	0.0	0.00	0.00	0.00	0.00	0.00	0.00			
	30	0.00	0.00	1.96	4.18	5.47	2.32			
	60	0.00	2.70	3.45	5.84	9.54	4.31			
	90	0.00	0.00	0.00	0.00	0.00	0.00			
Mean (BA)		0.00	0.68	1.35	2.51	3.75				
L.S.D. (0.05)								1.35	1.14	2.55
(d) Mean of *in vitro* formed tubers diameters /propagule:								**	**	**
	0.0	0.00	0.00	0.00	0.00	0.00	0.00			
	30	0.00	0.00	1.12	1.65	2.76	1.10			
	60	0.00	1.62	2.16	2.41	2.74	1.79			
	90	0.00	0.00	0.00	0.00	0.00	0.00			
Mean (BA)		0.00	0.40	0.82	1.02	1.38				
L.S.D. (0.05)								0.47	0.42	0.94

L.S.D. (0.05) = Least significant difference test at 0.05 level of probability. *, **: Significant or highly significant.

Fig. 5:*In vitro* tuberization stage of *Ceropegia woodii* shoots grown *in vitro* on MS medium augmented with BA at 8 mg/l and sucrose at 60 g/l over 60 days.

A known fact is that cytokinins are synthesized in the roots and play a vital role in cell divisions. The auxins had the rooting activity. Both these activities resulted in the meager growth of the shoots and vice versa with roots. Hence, the roots will become more active when compared to shoots. Maximum incidence of microtubers production was achieved with the seedling apical buds grown on MS medium containing BAP (6 mg l^{-1}) and sucrose (6%, w/v) in studied lantern flower species, *C. hirsuta, C. lawii, C. maccannii, C. oculata* and *C. sahyadrica* (Pandit *et al*., 2008). Microtubers formation in some Ceropegia genotypes viz. *C. jainii, C. bulbosa* var. bulbosa, and *C. bulbosa* var. lushii was examined by culturing both individual and multiple shoot clusters on half-MS medium containing 4% sucrose with KN (46.5 µM) and BA (22.2 µM) (Patil,1998). Chavan *et al*. (2013b) reported that shoots established to the medium having BAP (17.74 µM) and sucrose (175 mM) resulted in the highest (85%) of microtuber formation response. Murthy *et al*. (2010a, 2012a) treated the cultures with BAP (13.32 µM) along with the combination of NAA (2.68 µM) and achieved 84% of microtuber formation frequency. Kondamudi and Murthy (2011) also reported the in vitro tuber formation in *C. pusilla* on MS medium supplemented with BA. From the sections of aerial parts, differential responses can be achieved in a shorter period (Pandey *et al*., 2020). Mentioned that in vitro tuberization was achieved from tissue culture derived seedling leaf (TCDSL) with BAP and Naphthalene acetic acid (NAA).

6. *Ex vitro* and *in vivo* Acclimatization of *Ceropegia woodii:*

Data presented in Table (6) and Figure (6) Exhibit that both applied mixtures of perlite and peatmoss (v/v) and their combinations, in addition to fixed volume (1 portion) of sand on acclimatization of neoformed plantlets of*Ceropegia woodii* grown *ex vitro* for four weeks.

Concerning the average survival percentage per plant, perlite had a highly significant effect on this trait. The highest mean value was recorded at levels (1v/v) (81%). Also, peatmoss had a highly significant effect on the given traits was recorded at levels (3v/v) (92%). Meanwhile, the interaction between peatmoss and perlite exerted a highly significant effect. However, the combination of peatmoss and perlite at either (1:1) or (2: 1) or (3:1) or (2:3) and (3:3), respectively, resulted in the highest mean value (100%).

Respecting the average number of neoformed shoots per plant and plant height, peatmoss and perlite mixture and their interactions exerted significant effects on the given trait. In case of perlite main effect at (1 v/v), brought about the highest mean value (1.67 and 5.66), respectively. On the other side, peatmoss had a highly significant effect on the number of neoformed shoots/plant and plant height per plant at (3 v/v) (2.00 and 5.64), respectively. However, the interaction between both added levels of perlite and peatmoss at (1:2), resulted in the highest mean value (2.33 and 16.00), respectively.

Table 6: Effect of different levels of peat moss and perlaite (v: v) and their combinations on the acclimatization stage of *Ceropegia woodii* cultured *in vitro* for 30 days.

Characters	Peat Levels (v:v)	Perlaite levels (v:v)				Mean (Peat)	Significance		
		0.0	1.0	2.0	3.0		Perlaite	Peat	Perlaite X Peat
(a) Mean number of neoformed shoots:							**	**	**
	0.0	0.00	0.67	0.67	0.33	0.42			
	1.00	1.78	1.89	1.22	0.67	1.39			
	2.00	1.78	2.33	1.89	1.44	1.86			
	3.00	1.67	1.78	2.22	2.33	2.00			
Mean (Perlaite)		1.31	1.67	1.50	1.19				
L.S.D. (0.05)							0.30	0.30	0.61
(b) Mean plant height (cm):							**	**	**
	0.0	0.00	3.18	3.01	3.01	2.30			
	1.00	4.20	6.44	5.29	3.28	4.80			
	2.00	3.30	7.33	7.07	4.86	5.64			
	3.00	4.12	5.69	5.28	6.74	5.46			
Mean (Perlaite)		2.91	5.66	5.16	4.47				
L.S.D. (0.05)							0.86	0.79	1.63
(c) Mean number of neoformed leaves:							**	**	**
	0.0	4.44	6.44	7.78	6.89	6.39			
	1.00	8.67	12.89	15.56	9.78	11.72			
	2.00	11.33	16.00	15.33	9.78	13.11			
	3.00	10.22	11.11	10.89	9.11	10.33			
Mean (Perlaite)		8.67	11.61	12.39	8.89				
L.S.D. (0.05)							1.33	1.33	2.65
(d) Mean of servaival percentage %:							**	**	**
	0.0	0%	22%	22%	11%	14%			
	1.00	67%	100%	56%	22%	61%			
	2.00	67%	100%	56%	44%	67%			
	3.00	67%	100%	100%	100%	92%			
Mean (Perlaite)		50%	81%	58%	44%				

L.S.D. (0.05) = Least significant difference test at 0.05 level of probability.
*, **: Significant or highly significant.

Concerning the number of newly formed leaves (true leaves) the highest mean value for the perlite was at (2 v/v) on the other hand, peatmoss recorded the highest mean value at (2 v/v). The mixture which contained both perlite and peatmoss recorded the highest mean value at either (1:1 v/v) or (1:2 v/v) and (2:2 v/ v) respectively, resulted in the highest mean value (16.00)

In this respect, material as peatmoss is one of the most important constituents of media due to its capacity in affecting plant growth either indirectly or directly. Indirectly, improves the physical conditions of media by enhancing aggregation, aeration (8%) and water retention (77%), thereby creating a suitable environment for root growth (Sensi and

Loffredo, 1999). On the other hand, perlite is known to have a moderate capacity to retain water (38%) and provide aeration (25%) and its neutral pH and the fact that it is sterile and weed-free. Hence, it is ideal for use in container growing substratum. Also, it is known that perlite decreases the bulk density of the soils and increases the porosity (Abido, 2016).

Survival was observed when plantlets were transferred to pots containing a potting mixture of sterile soil, sand and coco peat (Chavan *et al*., 2013b). Similarly, in vitro rooted plants of *C. evansii* and *C. noorjahaniae* were transferred to sterile soil, sand and coco peat for their ex vitro establishment with (90% and 85%) of survival rate, respectively (Chavan *et al*., 2015; Chavan *et al*., 2014b; Chavan and Ahire, 2016). Decomposed coir waste, perlite and compost were a planting substrate for successful acclimatization of *C. pusilla* was reported twice by the same research group (Kalimuthu and Prabakaran, 2013d; Prabakaran *et al*., 2013). Rooted plantlets of *Ceropegia mohanramii* were transferred to sterile planting substrate (a mixture of sand and coco peat 1:1) for hardening. Initially, the plantlets were covered and kept in the laboratory conditions for 2 weeks recorded the highest mean value percentage of plant survival (Avinash *et al*., 2019). The successful acclimatization of *Ceropegia media* (58%) was attained after two months resulting in normal phenotype in pots (Pandey *et al*., 2020).

Fig. 6: Acclimatization stage of *Ceropegia woodii* shoots grown on rooting medium of peatmoss andperlite at (1:1, v/v) over 30 days.

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ARABIC SUMMARY

الإكثار المعملى الدقيق، تكوين الكالس و تكوين الدرنات فى نباتات السيروبيجيا

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نبات السيروبيجيا وودى هو نبات مزهر من جنس السيروبيجيا وهو أحد أكبر المجموعات النباتية في العائلةالدفلية .و كان الهدف من هذه الدراسة هو تطوير بروتوكول فعال للإكثار الدقيق المعملى وتكوين الكالس والدرنات في المختبر لنباتات السيروبيجيا وودى عن طريق تشجيع نمو البراعم الإبطية الموجودة فى القطع البرعمية الساقية المفردة و المزروعة على بيئة موراشيج وسكوج كاملة القوة و التى تحتوى على 30 جم / لتر سكروز و 4 جم / لتر جيل رايت للتصلب و مدعمة بتركيزات مختلفة من منظمات نمو النبات مثل البنزيل ادنيين وحامض النفثالين اسيتك اسيد واندول بيوتريك اسيد. بشكل عام ، خلصت الدراسة الحالية إلى أن أفضل النتائج لمرحلة التنشئه تم تسجيلها على بيئة موراشيج وسكوج مدعمة بـحامض النفثالين اسيتك اسيد والبنزيل ادنيين عند 2.00 و 0.50 مجم / لتر بالتتابع. في حين أن البيئة المضاف إليها بنزيل ادنيين وحامض النفثالين اسيتك اسيدعند تركيز 1.00 و 0.50 مجم / لتر على التوالي أعطت أفضل النتائج لمرحلة التضاعف. فيما يتعلق بمرحلة تكوين الجذور ، تم تسجيل أفضل النتائج عندما تم تقسيم البراعم الناتجه من مرحلة التضاعف و زراعتها على بيئة موراشيج وسكوج مدعمة بالإندول بيوتريك اسيد وحامض النفثالين اسيتك اسيدعند 1.00 و 0.50 مجم / لتر بالتتابع ، مما أعطى أعلى متوسط لعدد للجذور المتكونة لكل نبات. لتشجيع نمو الكالس ، تمت زراعة أجزاء من الاوراق في بيئة موراشيج وسكوج مدعومة بتركيزات مختلفة من البنزيل ادنيين وحامض النفثالين اسيتك اسيدعند تركيز 1.00 و 0.50 مجم / لتر على التوالي و أعطت أفضل النتائج لمرحلة تكوين الكالس .بالنسبة للنسبة المئوية للكالس المتكون وحجم الكالس ، فإن أفضل النتائج التي تم الحصول عليها من البنزيل ادنيين وحامض النفثالين اسيتك اسيدعند 0.50 مجم / لتر و 2.00 مجم / لتر على التوالي. لتشجيع نمو الدرنات تم زراعتها على بيئة موراشيج وسكوج بنصف القوة و مدعمة بتركيزات مختلفة من السكروز والبنزيل ادنيين و 4 جم / لتر من الجيل رايت للتصلب. وكانت اعلى قيمة للنسبة المئوية للدرنات المتكونة وعدد الدرنات المتكونة في المختبر و أعلى متوسط لوزن وقطر الدرنات عند تركيز 8.00 ملجم / لتربنزيل ادنيين والسكروز بتركيز 60 جم / لتر. تم أقلمة النبيتات حديثة التكوين بقوة خارج المختبر في خليط من البيرلايت والبيتموس فى خلطات نموكان أفضلها عند (1: 1) أو (2: 1) أو (3: 1) أو (2: 3) و (3: 3) على التوالي ، بالإضافة إلى حجم ثابت (جزء واحد) من الرمل المعقم ؛ و التى أعطت أعلى متوسط لنسبة البقاء على قيد الحياة / نبات (100٪).