Research Article

Plant growth and plantlet regeneration from *Clerodendrum colebrookianum* Walp. leaf

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Abstract

The *Clerodendrum colebrookianum* Walp. leaf explants were cultured on Murashige and Skoog (MS) medium, supplemented with synthetic α-naphthaleneacetic acid (NAA) auxin, 2,4-dichlorophenoxyacetic acid (2,4-D) and indole-3- butyric acid (IBA) at concentration 0, 0.5, 1, 3, 5, 7 and 10 mg/L, then MS medium, supplemented with cytokinin 6-benzyladenine (BA) and kinetin (Kn) at concentration 0, 1, 3 and 5 mg/L. The MS medium with combination of 1 mg/L NAA and 5 mg/L BA (after 4 weeks); MS medium with 1 mg/L NAA, 1 mg/L BA and 1 mg/L 2,4-D (after 8 weeks); and MS medium with 7 mg/L NAA (after 2 weeks) had induced the maximum calluses, shoots, and roots of 23.3 ± 5.3, 4.1 ± 0.6 and 2.6 ± 0.6 per plantlet, respectively.

Keywords: *Clerodendrum colebrookianum* Walp., plant growth regulator

Introduction

Clerodendrum colebrookianum Walp., folk medicinal plant, belongs to the family Verbenaceae. Globally the species is distributed in the Bangladesh, Bhutan, China, India, Indonesia, Myanmar, Nepal, Sri Lanka and Vietnam. *C. colebrookianum* is a perennial shrub and grow up to 4 to 8 ft. height. Stem quadrangular, branches robust and sparsely pubescent with corky internodes. Leaves often 9 inch diameter, opposite, broad-ovate, acute, entire, petiolate, small lateral veins (6-9) with few glands clustered at the petiole and scattered beneath. C. colebrookianum is distinguished by having broadly ovate or cordate leaf blade with large peltate glands or glands on the abaxial surface of the leaf base and corymb thrysoid inflorescence. [1] The genus Clerodendrum L. (Verbenaceae) consisting of 400 to 500 specific and subspecific taxa which are widely distributed in tropical and subtropical regions of the world. [2]

Murashige and Skoog (MS), plant growth medium, was originally formulated by Murashige and Skoog in 1962 to optimize tobacco callus bioassay system for facilitating the study of cytokinins. Since then, it is widely used for micro propagation, organ culture, callus culture and suspension culture. The formulation is a nutrient blend of inorganic salts, vitamins and amino acid. MS medium used in the laboratory for cultivation of plant cell culture, and it became the most suitable for tissue culture of most plant tissue. [3]

Plant growth regulators are important in plant tissue culture since they play vital roles in stem elongation, tropism, and apical dominance. They are generally classified into the following groups; auxins, cytokinins, gibberellins and abscisic acid. Moreover, proportion of auxins to cytokinins determines the type and extent of organogenesis in plant cell cultures. [4]

The present investigation therefore attempts to seek the appropriate protocol for MS medium supplemented with the different plant growth regulator (PGR) in *C. colebrookianum* leaf plantlet regeneration, affecting on callus, shoots and root induction.

Material and Method

The young *C. colebrookianum* leaves were collected and washed in running tap water for approximately 20 min. They were sterilized with 1% mercuric chloride for 5 min, followed by 10% sodium hypochlorite for 5 min and 5% sodium hypochlorite for 3 min, respectively. After that, the explants were washed in sterilized distilled water for 3 times to complete the surface sterilization process. The sterilized leaves were cultured on MS medium (pH 5.7) supplemented with auxin and cytokinin in various concentration, containing 30 g/L sucrose and 7 g/L agar. The cultures were maintained in the culture room at 25 ± 1 ºC and white fluorescent light 17.97 µmol.photons.m^{-2}.s^{-2} for 16 hrs. The number of callus, shoot and root per explant were collected. The study was comprised of 3 experiments.

1. **Callus induced in *C. colebrookianum***

The sterilized leaf was excised 1.5 x 1 cm, and cultured on MS medium supplemented with PGR including α-naphthaleneacetic acid (NAA), 6-benzyladenine (BA) and kinetin (Kn) at concentration 0, 0.5, 1, 3 and 5 mg/L, amount of 20 replicates for 4 weeks.

2. **Shoot induce from callus of *C. colebrookianum***

The callus was excised into 1 × 1 cm, and cultured on MS medium NAA, BA, Kn, indole-3-butyric acid (IBA) and 2,4-dichlorophenoxyacetic acid (2,4-D) at concentration 0 and 1 mg/L, amount of 10 replicates for 8 weeks.

3. **Roots induce from callus of *C. colebrookianum***

The callus with shoots were excised into 1 explant/bottle, and cultured on MS NAA at concentration 0, 1, 3, 5, 7 and 10 mg/L, amount of 5 replicates for 2 weeks.

The mean comparison was analyzed by Duncan's multiple range test (DMRT) at $p \leq 0.05$ using statistical package for social science (SPSS).

Results

In comparison with the control (brown morphogenetic response - **Figure 1 A)**, the maximum calluses (green compact) of 23.6 ± 5.3 per plantlet (after 4 weeks) were induced using 1 mg/L NAA and 5 mg/L BA with 90% survival rate. (**Table 1**)

Table 1 Different PGR and callus formation

PGR (mg/L)			Survival rate	No. per plantlet (mean)
NNA	BA	Kn		
0.5	1	-	70	9.7 ± 4.3[d]
0.5	3	-	75	5.4 ± 1.8[d]
0.5	5	-	65	12.6 ± 2.1[c]
1	1	-	70	15.5 ± 6.1[b]
1	3	-	85	22.7 ± 4.0[a]
1	**5**	**-**	**90**	**23.3 ± 5.3[a]**
0.5	-	1	80	7.9 ± 2.7[ed]
0.5	-	3	80	7.8 ± 3.6[ed]
0.5	-	5	85	8.5 ± 3.4[d]
1	-	1	75	9.1 ± 3.3[d]
1	-	3	85	8.8 ± 4.2[d]
1	-	5	80	7.4 ± 2.5[ed]

In comparison with the control (no shoot), the maximum shoots of 4.1 ± 0.6 per plantlet (after

8 weeks) were induced using 1 mg/L NAA, 1 mg/L BA and 1 mg/L 2,4-D (after 8 weeks). (**Table 2 and Figure 1 B**)

Table 2 Different PGR and shoot formation

PGR (mg/L)					Weight of callus (g)	No. per plantlet (mean)
NAA	BA	Kn	IBA	2,4-D		
1	1	1	-	-	0.3 ± 0.1^{b}	0
1	-	1	-	1	0.7 ± 0.2^{ab}	2.1 ± 0.2^{b}
1	**1**	**-**	**-**	**1**	$\mathbf{1.2 \pm 0.2^{a}}$	$\mathbf{4.1 \pm 0.6^{a}}$
-	1	1	-	1	1.1 ± 0.2^{a}	0
-	1	1	1	-	1.2 ± 0.3^{a}	0

In addition, 8-week calluses were excised into 1 explant/bottle, the maximum roots of 2.6 ± 0.6 per plantlet (after 2 weeks) were induced using 7 mg/L NAA. **(Figure 1 C)** Other concentration (except 5 mg/L, 1.4 ± 0.2 roots per plantlet) had no root growth affect. The complete plantlet was shown. (**Figure 1 D**)

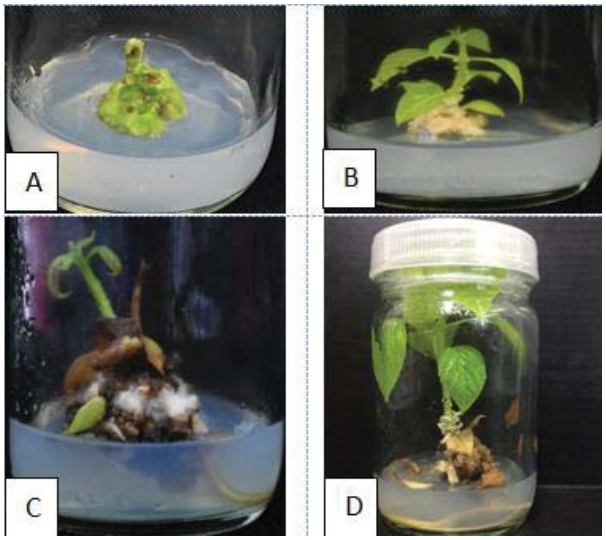

Figure 1 (A) green compact callus; (B) shoot formation; (C) root formation; (D) complete plantlet

Discussion

The appropriate PGR concentration of NAA and BA provided the initial callus and shoot formation, while NAA at specific concentration had potential root formation. The effectiveness is similar to a study in India, with the survival rate of the plantlets under ex vitro condition was 80%. [5] However, the experiment does not get through the final success rate of field planting. The study may be concluded that it is highly effective method of propagation of *C. colebrookianum*, and probably reliable tool for genetic engineering studies.

References

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