

In Vitro Propagation of a Sporadic Medicinal Plant *Cleome monophylla* L

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Abstract

A sporadic medicinal plant *Cleome monophylla* L. comes under the family Capparidaceae selected for the *in vitro* regeneration. The leaf explants showed differential responses to various combination and concentrations of BAP, TDZ and NAA and produced yellow to green friable and compact and dense calli. The maximum amount of callus per explants was produced on MS medium with 4.44 μM of BAP, 1.16 μM of TDZ and 1.135 μM of NAA combination and concentration gave the 84% response and produced green compact calli, 19.98 ± 0.03 shoots from the callus and the maximum mean number of rootlets per culture was also noted (15 ± 0.57) in this combination with MS medium of half strength. The *in vitro* developed entire plantlets were fruitfully acclimatized to thermocol cups and earthen pots.

Keywords: Callogenesis, Capparidaceae, *Cleome monophylla*, Growth Regulators, Shoot and Root Induction

1. Introduction

Plants, the golden gift of the nature are playing a crucial role in the life of human right from ancient period. Nature is forever a glorious sign to prove the prominent events of harmony. The main phenomena of the last three decades the tremendous increase in use of 'herbal products'. Every medicinal plant and the particular plant part used as crude drug material contains active principles or major chemical compounds with a specific profile that can be used for both chemical quality control as well as quality assurance. Medicinal plants are presently in demand and their acceptance is increasing progressively. Tissue culture technique is emerged as one of the most potential tools for the conserving medicinal plants. In this method, the mature plants are reproduced quickly as well as multiple of plants. It also permits the production of pathogen free materials by the plants¹. In conventional cultivation, several plants may not germinate at particular climatic conditions or take long duration of growth and multiplication. *Cleome monophylla* grows well in the loosely moistened soil and the growth dwindled during

summer months. Availability and occurrence of plants in summer months are gradually decreasing. These obstacles are overcome in tissue culture techniques. The advantages of micropropagation are sufficient supply of medicinally important plants, using limited space and time, plants are available throughout the year, production of secondary metabolites, conservation of endangered medicinal plant species and production of genetically engineered novel plants².

The over exploitation even result in ecological imbalance. Therefore, intensive studies on medicinal plant species pertaining to their conservation are disputatively needed. In Eratti hill, tribal society is using sporadically available *Cleome monophylla* for curing various illnesses. The sporadic status of *Cleome monophylla* needs to be conserved before it gets extinct. Therefore, unscrupulous practices and unscientific management are threatening the existence of *Cleome monophylla*. This results in the depletion of *Cleome monophylla*. Hence, in the present study the leaf explants were used for conserving *Cleome monophylla* through *in vitro* regeneration technique.

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2. Materials and Methods

2.1 Selection of Explants and Sterilization

The in vivo leaf explants was immersed in 10% (w/v) of (Bavistin)-methyl-3-benzimidazole carbonate and thoroughly washed with teepol in tap water. They were frequently surface sterilized with 0.12% (w/v) HgCl_2 solution for 4-8 min. and washed for 3-4 times in distilled water. The well surface sterilized explant was gently trimmed by sterile surgical blade (Lister No: 10) and carefully inoculated aseptically.

2.2 Culture Medium Composition

In³ full strength basal medium was employed in the present study.

2.3 Medium Preparation

For media preparation, analytical reagents of “Hi-media” grade chemicals, double distilled water and “Borosil” glass wares were used. The nutrient medium possesses all required nutrients. The prepared stock solutions were stored in sterilized well-stoppered bottles and kept in 4°C. The quantity of the stock solutions and growth regulators were pipette out into a 1 litre beaker. Required amount sucrose and other organic nutrients and complex additives (optional) were mixed. The final quantity was made up with sterilized distilled water and the pH was adjusted (5.8-5.9)

Agar 0.8% (w/v) (extra pure gelling point 32-35°C, Hi-media - Bombay) was added to the above said composition and melted in water bath and about 30 ml of the medium was transferred into 150 ml capacity bottles and were sterilized nearly 15 minutes at 121°C. Then the medium were allowed to solidify and stored for inoculation purpose.

2.4 Growth Regulators Preparation

The main growth regulators like auxins and cytokinins were used. All the prepared growth regulators and adjuvant were kept under 4°C.

2.4.1 Auxins Preparation

In the present experiment auxin is α -naphthalene acetic acid (NAA), was used. 10 mg of auxin was individually

dissolved for preparation of stock solution with 100 ml of sterilized distilled water. The needed volume of auxin was admixed to the media before sterilization and was used in various concentrations.

2.4.2 Cytokinins Preparation

By dissolving 10 mg of BAP (6-benzyl amino purine), TDZ (thidiazuron) and KIN (kinetin) in 1 ml of 0.1 N HCl (Hydrochloric acid) and the volume was made up to 100 ml with sterile distilled water. The various concentrations of BAP, TDZ and kinetin were used before autoclaving.

2.5 Culture Conditions

The culture bottles were maintained in the culture room at a temperature of $25 \pm 2^\circ\text{C}$ and relative humidity of 65-70%. The cultures were kept under white light at an intensity of 3000 Lux provided from white fluorescent lamps (Philips, India) with 14 hours photoperiodic duration.

2.6 In vitro Observations

The observations of the present study were carried out in the PG and Research Department of Botany, Vellalar College for Women, Erode and Government Arts College, Coimbatore India. The callus induction, shoot initiation and rooting were noted from the leaf explants.

2.7 Callus Induction

The enriched MS medium with different concentrations of BAP (2.22 to 13.32 $\mu\text{M/l}$) with stable concentrations of TDZ (1.16 $\mu\text{M/l}$) and NAA (1.135 $\mu\text{M/l}$) were allowed for callus induction. The six weeks old cultures were selected and calculated. The obtained Calli were regularly sub cultured at three weeks interval.

2.8 Proliferation of Shoot Buds

In order to produce differentiation, the calli were regularly sub cultured on basal medium supplemented with various concentrations (2.22 to 13.32 $\mu\text{M/l}$) of BAP (Benzylaminopurine) in combination with stable concentration of TDZ (1.16 $\mu\text{M/l}$) and NAA (1.135 $\mu\text{M/l}$).

2.9 Shoot Initiation from Roots

The shoots about 2 to 3 cm long were aseptically excised

and were transferred to half strength medium supplemented with various BAP concentrations (2.22 to 13.32 μM /l) and stable concentrations of TDZ (1.16 μM /l) and NAA (1.135 μM /l) in combination . The observation was noted after 4 weeks of transfer.

2.10 Acclimatization

For in vivo acclimatization, the culture bottles were opened and kept inside the culture room one day and transferred to outside and kept for 6 hours as such as. After that the in vitro cultured plants removed from the medium and rinsed with tap water for removal of adhered medium. Then the plantlets were transferred to thermocol cups with soil rite and finally transferred to garden soil, vermiculite, decomposed coir waste and mixture of decomposed coir waste and compost containing earthen pots for in vivo acclimatization.

2.11 Statistical Analysis

All the experiments were repeated three times 10 replicates were taken from each set of experiments for to

statistical analysis for computation of the Standard Error (SE) of the Mean. Data were recorded periodically for any noticeable change.

3. Results

3.1 Effect of BAP, TDZ and NAA on Callogenesis

The preliminary experiment carried out on MS medium alone revealed that the growth regulators are essential for induction of callus since there was no sign of growth on the explants. The MS medium supplemented with different concentrations (from 2.22 μM /l to 13.32 μM /l) of BAP and in combination with stable concentration (1.16 μM /l) of TDZ and (1.135 μM /l) NAA developed calli. Number of days taken for callus initiation from leaf explant ranged from 14 days to 19 days at various concentrations of BAP in combination with NAA and TDZ. Percent response of callus formation was maximum at 4.44 μM /l concentration of BAP with stable concentration of 1.16 μM /l TDZ and 1.135 μM /l NAA. This concentration produced 86% of

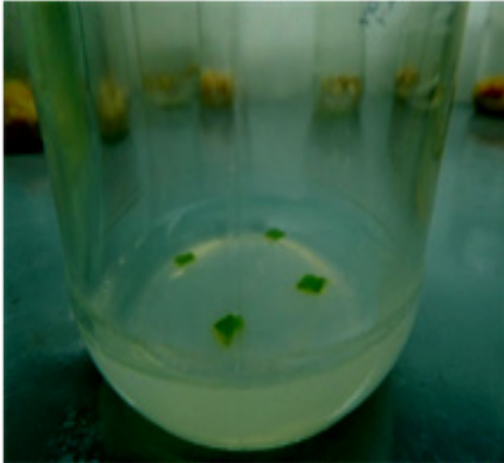
Table 1. Effect of MS medium with different concentrations and combination of BAP, TDZ and NAA on callogenesis and organogenesis from leaf explant of *Cleome monophylla* L

S. No.	BAP μM	TDZ μM	NAA μM	Days taken for Callus initiation	Callus formation (%)	Callus amount	Nature of the Callus	No. of Shoots initiation from callus	No. of Shoots from callus in subculture	Shoot length (cm)	No. of Roots from Shoots	Mean root length (cm)
1.	2.22	1.16	1.135	17	72.0 \pm 1.15	+++	GF	17 \pm 0.88	18.06 \pm 0.14	04.05 \pm 0.13	12 \pm 0.5	4.50 \pm 0.05
2.	4.44	1.16	1.135	15	86.0\pm0.57	+++	GC	25\pm0.57	19.98 \pm 0.03	04.41 \pm 0.10	15\pm0.57	5.0 \pm 0.15
3.	6.66	1.16	1.135	18	57.33 \pm 0.88	+++	YF	12 \pm 0.13	22.01 \pm 0.17	04.25 \pm 0.07	09 \pm 0.88	3.50 \pm 0.15
4.	8.88	1.16	1.135	14	48.0 \pm 1.00	++	YF	15 \pm 0.83	33.05 \pm 0.13	05.28 \pm 0.06	08 \pm 0.32	2.50 \pm 0.17
5.	11.10	1.16	1.135	17	42.66 \pm 0.88	++	GF	12.33 \pm 0.88	25.04 \pm 0.06	03.45 \pm 0.09	07 \pm 0.18	2.0 \pm 0.15
6.	13.32	1.16	1.135	19	39.0 \pm 0.57	++	GF	10 \pm 0.57	14.09 \pm 0.01	03.98 \pm 0.14	03 \pm 0.87	3.15 \pm 0.45

Values are mean \pm SE (n = 10)

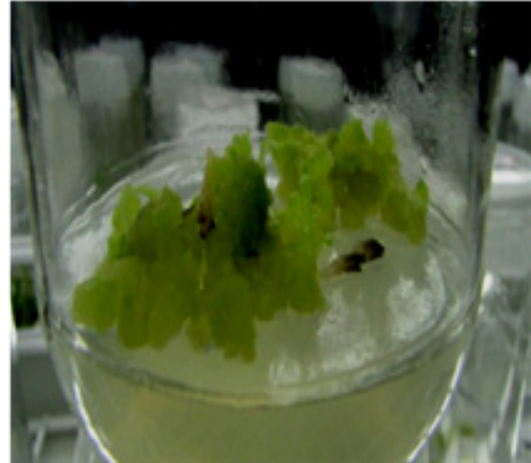
Abbreviations: YF - Yellow Friable and GF - Green Friable, GC - Green Compact.

+ Less callus, ++ Moderate callus, +++ Excellent callus.



(a)

Inoculation of Leaf Explants



(b)

Callus Induction



(c)

Shoot arises directly from Callus



(d)

Subculture of Callus



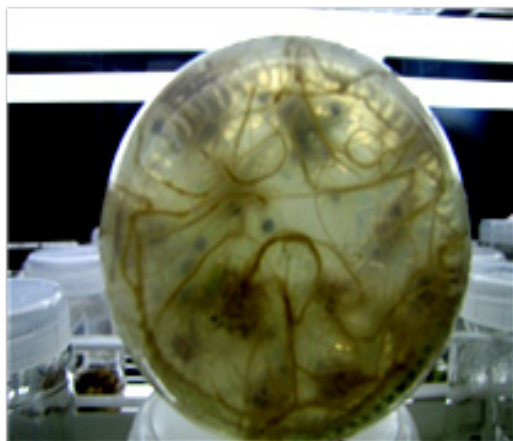
(e)

Shoot development from the sub cultured Callus



(f)

Multiple shoot formation



(g)



(h)

Root formation and elongation

Plate 1. In vitro regeneration of *Cleome monophylla* L

green, compact callus with less number of days (15 days), followed by 2.22 μM of BAP concentration (72%). The higher concentration of 13.32 μM of BAP developed lower percentage (39%) of callus. Callus amount was excellent in 2.22 μM , 4.44 μM and 6.66 μM concentration of BAP. Green, friable calli were produced at 2.22 μM , 11.0 μM and 13.32 μM concentration of BAP in combination with TDZ and NAA. 6.66 μM and 8.88 μM concentrations of BAP produced yellow, friable nature of callus (Table 1 and Plate 1 B, C and D).

3.2 Role of BAP, TDZ and NAA on Shoot Induction

The green and yellow, friable, dense calli were sub cultured in MS medium with different concentrations of BAP in combination with constant concentration of TDZ (1.16 μM) and NAA (1.135 μM). 4.44 μM concentration of BAP in combination with 1.16 μM concentration TDZ and 1.135 μM concentration of NAA initiated maximum number (25) of shoots directly from the callus as compared to the other concentrations of BAP in combination with stable concentration of TDZ and NAA. In BAP, TDZ and NAA combination, 8.88 μM concentration of BAP has produced maximum number of shoots (33) from callus in sub cultured condition with an moderate shoot length of 5.28 cm. Less number (14) of shoots produced in higher concentration of BAP (13.32 μM) (Plate- 1, E and F).

3.3 Effect of BAP, TDZ and NAA Root Induction

Root initiation was observed after 20 days of sub cultured shoot. 4.44 μM BAP concentration was found to be superior in inducing higher number (15) of roots as compared to the other concentrations of BAP in combination with TDZ (1.16 μM) and NAA (1.135 μM). Higher concentration (13.32 μM) of BAP has recorded a minimum number of roots (3). The average root length was recorded maximum at 4.44 μM BAP concentration. The remaining concentrations have recorded a moderate number of roots (Table – 1 and Plate – 1, G and H).

3.4 Acclimatization

After complete formation of shoots and roots, the plants were shifted to the thermocol cups for hardening in shaded condition for 20 days. Hardened plants were then transferred into earthen pots containing different planting substrates such as garden soil, vermiculite, decomposed coir waste and decomposed coir waste and compost. The regenerated plants were originally held in the shade for several days before planting. Those plantlets shifted to the garden soil alone recorded 44% of survival rate, the plantlets transferred to vermiculite mixture registered 60% survival rate. 76% of survival rate of plantlets was noted in decomposed coir waste substrates. 93% of

Table 2. Acclimatization of in vitro plantlets of *Cleome monophylla* L

S. No.	Planting substrates	No. of plants transferred	No. of plants survived	Survival rate (%)
1.	Garden soil	45	20	44.00
2.	Vermiculite	50	30	60.00
3.	Decomposed coir waste	55	42	76.00
4.	Decomposed coir waste and Compost	65	61	93.00

Values are mean \pm SE (n = 10)

plantlets were survived in the substrates containing decomposed coir waste and compost (Table 2).

4. Discussion

Herbal plants, the green gold are the most important resource of life saving drugs for the society. All over the world the safety and affordable remedies in traditional health system has attracted by people. Every day the demand for medicinal plants is growing due to the excess use of common as well as threatened herbal plants. On account of the increasing population their activities leads to ecosystem loss and are facing extinction. Hence, there is an urgent need to enhance alternate methods to propagate, cultivate and conserve the herbal plants and also to maintain the balance of ecosystem. To cope up with alarming situation, the development in tissue culture technology has come as a boon. Hence, in the present investigation the selected sporadic medicinal plant *Cleome monophylla* was subjected to in vitro regeneration strategy and a protocol has been developed for the conservation of this *Cleome monophylla* resource using leaf and nodal explants.

In the present study, provided a wide response to various concentrations and combination of growth regulators and produced green, compact, yellow and white, friable calli from leaf explants. The application of growth regulators to the basal medium is the most important aspect for the development of organogenesis in the tissue culture medium. This is in line with the report of^{4,5} who stressed that differentiation and development of shoots and roots from the explants depend upon the application of growth regulators.

4.1 Effect of BAP, TDZ and NAA on Callus Induction

Callus was successfully initiated in MS medium supplemented with different concentrations of BAP in combination with stable con of TDZ and NAA. The maximum number of callus per explant was produced on MS medium supplemented with 4.44 μ M BAP concentration, TDZ (1.16 μ M) and NAA (1.135 μ M) combination. This combination has produced 86% calli which are green and compact, callus amount was also maximum. Among the different concentrations tested in this observation, 4.44 μ M concentration of BAP provided the best response to the induction of calli. Previously it has been reported that callus induction was maximum in MS medium supplemented with BAP and NAA⁶. Callus was successfully initiated on BAP and NAA using leaf explant of *Cleome chelidonii*⁷. In the present investigation among the six concentrations of BAP, 4.44 μ M concentration of BAP in combination with TDZ and NAA was found to be superior in the induction of callus with less number of days and the calli were green and compact. The higher concentrations (11.10 μ M and 13.32 μ M) significantly reduced the callus induction.

4.2 Effect of BAP, TDZ and NAA on Shoot Induction

The calli resumed the shoot growth directly within three weeks at all concentrations of BAP. Among the various concentrations used, 4.44 μ M of BAP concentration in combination with TDZ and NAA has produced more number of shoots than other concentrations. This concentration was found to be superior in the initiation

of shoots directly from the callus. When the calli were sub cultured on MS medium with different concentrations of BAP with stable concentration of TDZ and NAA, the calli resumed efficient shoot development at 8.88 μM BAP with maximum shoot length. This result corroborates the observations of⁸ in *Turnera ulmifolia* and⁹ in *Alstroemeria*.

4.3 Effect of BAP, TDZ and NAA on Root Induction

The rooting results showed that 4.44 μM concentration of BAP in combination with stable concentration of TDZ and NAA has induced rhizogenesis efficiently. The higher concentrations of BAP, TDZ and NAA were slightly detrimental to root formations.

In the present study, the individual excised plantlets sub cultured in half strength MS medium with different concentrations of BAP, in combination with stable concentration of TDZ and NAA influenced adventitious roots on stem base of the plantlets. The MS medium without growth regulators did not showed rooting in this species. But¹⁰ reported the efficient rooting from in vitro propagated *Cleome gynandra* plants on MS medium in combination with IBA, NAA (2 naphthoxyacetic acid) and activated charcoal. Whereas¹¹ observed adventitious rootings from *Cleome spinosa* on MS medium without addition of plant growth regulators. In¹² revealed that the low level of NAA tested medium exhibited better rooting in shoot system of *Cleome viscosa*. This is in line with the present investigation.

5. Conclusion

The higher plants acting as a source of many phytoconstituents which may possess the therapeutical properties and so play a important role in human health. The pharmacognostical study of *Cleome monophylla* ensures the identification of novel bioactive compounds which may provide a path for the discovery of new drug. The in vitro regeneration study would be effective and fruitful for mass proliferation of *Cleome monophylla*.

6. References

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