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In vitro Propagation of *Cleome gynandra* L. *u*sing Nodal Explants

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Abstract: ***Mass propagation in plants of Cleome gynandra L. were regenerated from nodal explants on MS medium containing various concentration of cytokinins (BAP and KIN) ranging from 5 µM to 25 µM. When compared to KIN, BAP was found to act in response well in shoot multiplication and number of shoots. Large number of shoots was produced from all concentration of BAP and KIN. Maximum number of shoots and highest frequency of 100% shoot induction was observed on MS medium containing 10 µM KIN and BAP. The elongated shoots were then transferred to MS half strength medium augmented with NAA and IAA in various concentrations for root induction. The roots were initiated and well developed in 2µM of both the auxins.***

Keywords: *Cleome gynandra* L., BAP, KIN, IAA, NAA, Micropropagation

1. Introduction

The plant *Cleome gynandra* Linn (Fam: *Cleomaceae*) (Syn. *Gynandropsis gynandra*.L., *Gynandropsis pentaphylla* L.), is commonly known as „Hurhur‟and„Karaila‟ in India and „Cat‟s whiskers‟ in English. Velai keerai, Thaivelai in Tamil, it is an annual, erect, branched, 0.6-1.2 m in height, stems and branches striate, white spreading hairs. Leaves 3-5 foliolate, petioles 5-7.6 cm long, and seeds muricate dark [1]. *Cleome gynandra* is used as a medicinal plant and can be found in all over world. It grows as a weed in paddy fields and also in roadsides and in open grass lands [2]-[4]. In India it is not at all cultivated but grows unexpectedly all over the place. Different species of Cleome can be found in all states of India.

In the literature of pharmacopoeia of India and also in other ancient medical texts describe that the medicinal application of *Cleome gynandra* L is an important medicinal plant in Ayurveda. In Ayurvedic medicine these plant is a chief constituent in Narayana Churna [5]-[7]. Natural products are identified to take part in an important role in both drug discovery and chemical biology. Though some therapeutic benefits can be traced from the specific plant compounds, most of herbs contain several active phytochemical constituents; they are all therapeutic value either individually or in combination. *In vitro* clonal propagation is only the alternative way for the regeneration and expedites release of large number of *C. viscosa* plants under aseptic culture conditions. Earlier, there have been few reports on establishment of *in vitro* propagation through callus culture of *C. viscosa* [8-9], micropropagation and shoot organo-genesis from different explants of *Cleome spinosa* [10] – [13] and *Cleome gynanadra* [14], somatic embryogenesis [15] cell suspension and root culture with cryopreservation of *C. rosea* [16]. The present study was carried out to standardize a protocol for *in vitro* propagation of *C. gynandra* from nodal explants.

2. Materials and Methods

2.1 Sample collection

The plants of *Cleome gynandra* L. were collected from road sides of Thanthonimalai village, Karur District, Tamil Nadu, India and the name was confirmed with the help of Rapinat Herbarium, St. Joseph‟s College, Tiruchirappalli . The samples were washed thoroughly in running tap water to remove soil particles and adhered debris and finally washed with sterile distilled water.

2.2 Media Preparation

The Murashige and Skoog basal medium consisted of the mineral salts and organic nutrients of B5 vitamins with 3% sucrose and 0.8% agar [17]. After adding all the supplements the basal media pH was adjusted to 5.8 by adding 0.1N HCl or NaoH. The prepared media were poured in culture tubes or conical flasks and were closed with cotton plugs. The culture media were sterilized by autoclaving at 1.06 kg cm^{-3} and 121°C for 15-18 min.

2.3 Inoculation Procedure

The nodal segments were isolated from the collected plants of *C. gynandra* L. These explants were surface sterilized by rinsing in running tap water for 30 minutes. Then they were washed in an agitated solution of liquid detergent (Teepol) for 5 minutes and distilled water for 2-3 times. After thorough washing, the materials were taken in to the laminar flow chamber where they were disinfected with 70% alcohol for 45 sec followed by 0.1% mercuric chloride for 3 mins. Finally, the explants were thoroughly rinsed with sterile distilled water for 4-5 times to remove the surface traces of mercuric chloride. Finally, the nodal segments were placed on the medium.

2.4 Shoot Induction

For the shoot induction, young nodal region of the explants were transferred into the MS medium containing BAP (5 µM to 25 µM) or KIN (5 µM to 25 µM) concentration individually. Shoot initiation was first recorded in the nodal region. The optimum % of shoots was obtained from nodal segments.

2.5 Rooting

In vitro raised shoots were excised and transferred to half strength MS medium supplemented with various

concentrations of Indole butyric acid (NAA: 2-10 µM) or α Indole acetic acid (IAA:2-10 µM).

2.6 Culture Conditions and Maintenance

The cultures were maintained in a culture room at 25±2°C under 16 hours photoperiod with light intensity of 30-40 µM m^{-2} s^{-1} supplied by cool white fluorescent tubes. These growth conditions were referred to as standard culture conditions for *in vitro* studies. After 4 weeks of inoculation, the shoots were sub cultured on MS medium fortified with the same growth regulator concentrations and combinations or whichever is the best for further multiplication.

2.7 Statistical analysis

All above experiments were performed by a randomized completely block design and each experiment consisted of ten replicates and each experiment was repeated five times. The analysis of variance (ANOVA) appropriate for the design was carried out to detect the significance of differences among the treatment means were compared using Duncan"s Multiple Range Test (DMRT) at a 5% level of significance [18].

3. Result and Discussion

Shoot, Root induction and multiplication

The present study reported direct organogenesis of *Cleome gynandra* L. from nodal segment on MS medium supplemented with different concentrations of BAP or KIN. The nodal segments were initially grown on MS medium supplemented with BAP and KIN separately in different concentrations ranging from 5 - 25 µM [19]-[21]. The Maximum number of shoots was achieved in 30-45 days. Among these, the maximum number of shoots 13.2 ± 0.41 was developed on MS media fortified with 10 µM BAP, maximum shoot length was observed as 6.6± 1.66 cm with 90% shoot sprouting frequency on a medium supplemented with 10 µM BAP followed by 5 µM BAP with 7.6 ± 0.57 shoots per explant and mean shoot length of 4.86 ± 1.37 cm with 70% shoot induction frequency. The data in respect of shoot induction frequency, number of shoots and length of shoots on different concentrations of each hormone on node were presented in (Table.1, Fig.1a-c).

Table 1: Effect of different concentrations of cytokinins on shoot induction from the nodal explants of *C. gynandra* L

Concentration of cytokinins		Shoot Induction Frequency (%)	Number of Shoots	Shoot Length (cm)
BAP (µM)	KIN (µM)			
5	---	70.0	8.6 ± 0.57	4.86 ± 1.37
10	---	90.0	13.2 ± 0.41	6.60 ± 1.66
15	---	66.6	7.6 ± 0.40	4.86 ± 0.65
20	---	63.3	4.75 ± 0.47	4.08 ± 0.88
25	---	53.3	3.8 ± 0.41	4.02 ± 1.21
---	5	66.6	8.5 ± 0.28	5.14 ± 0.98
---	10	80.0	9.75 ± 0.47	5.66 ± 1.50
---	15	83.3	11.75 ± 0.75	6.62 ± 0.62
---	20	50.0	5.6 ± 0.57	4.88 ± 1.06
---	25	46.6	4.4 ± 0.44	2.82 ± 1.02

The basal medium containing different concentrations of KIN induced more or less similar number of shoots like BAP [22]. Maximum number of 11.75 ± 0.75 shoots per explants was proliferated on MS medium supplemented with 15 µM KIN and mean shoot length 6.62 ± 0.62 cm with 73.3% shoot induction frequency. Among these two cytokinins tested BAP was found to induce more number of shoots when compared to KIN. Similar to our findings, many researchers showed that BAP induced multiple shoot formation [23]-[24]. The shoot induction and proliferation depend on plant growth regulators and types of explants [25].

The shoots measuring about 3-5 cm in height were transferred to half-strength MS medium supplemented with various concentrations of NAA and IAA (2-10 µM) for rooting. IAA is clearly more effective in promoting root induction than NAA. Rooting frequency, the number of roots per shoot and root length were highly influenced by type and concentration of auxin. Significant ($P < 0.05$) difference was found between different concentrations of both the auxins and increasing auxin concentration which promoted roots (Fig. 1d) [26-27].

Of the two auxins tested for root induction IAA was more responsive than NAA. Here, the results showed consistency with other studies where the addition of IAA promotes the induction of roots in several systems including *Holarrhena* antidysenterica [28]; *Heliotropium kotschyi* [29-30]. However, NAA was more effective auxins for root induction in *Terminalia arjuna* [31].

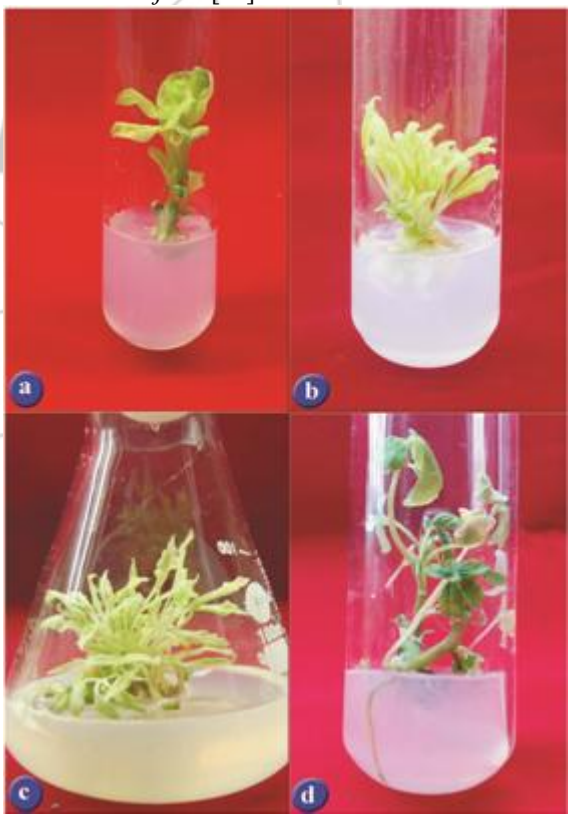

Figure 1: *In vitro* regeneration of *C. gynandra* L. a. Nodal explant growing on MS medium, b. & c. Shoot multiplication and shoot proliferation, d. Root initiation.

4. Conclusion

Micropropagation or Direct Organogenesis was carried out from the nodal explants of *Cleome gynandra* L. The nodal explants were grown on MS medium supplemented with different concentrations of BAP or KIN ranging from 5 – 25 µM. Both the cytokinins had more or less similar effect. However, BAP was found to have little higher effect for shoot multiplication when compared to KIN. Maximum number of 13.2 shoots per explant and mean shoot length of 6.6 cm with 90% shoot induction frequency were obtained from the nodal explants on MS medium containing 10 µM BAP. Maximum number of 11.75 shoots per explant and mean shoot length of 6.62 cm with 83.3% shoot induction frequency were obtained from the nodal explants on MS medium containing 15 µM KIN.

5. Acknowledgement

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