In vitro propagation of Cleome gynandra L. through direct organogenesis

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Abstract: An efficient protocol was developed for in vitro regeneration of Cleome gynandra L. as a herb belonging to the family Cleomaceae. In the present study, shoot tip explants of Cleome gynandra were cultured on MS medium supplemented with different concentrations of BAP and Kin (5-25 µM/l) alone or in combination with IAA (2 – 10 µM/l ) for multiple shoot bud induction. The highest frequency (100%) of shoot bud induction and maximum number of shoot buds (26.4±0.48) was noticed on 10µM BAP with mean shoot length of 8.2 cm. The shoot proliferation was further improved by combination of BAP+IAA. The maximum number of shoots (35.8± 1.42) was developed on MS media containing 10 µM/l BAP in combination with 2 µM/l IAA. The regenerative shoot buds were transferred to half strength MS medium fortified with different concentrations of IBA and NAA (2-10µM/l) for root induction. The maximum numbers of roots (17.2±1.95) were obtained from 4µM NAA with mean root length of 3.6 cm. In vitro regenerative plantlets were successfully acclimatized in red soil, farmyard manure and sand (2:1:1). Subsequently, the plants were successfully established in the field.

Keywords: Cleome gynandra, shoot tip explants, BAP, NAA, MS medium.

1. Introduction

Cleome gynandra L. is a member of Cleomaceae formerly capparaceae and it can be found in all over world. It grows as a weed in paddy fields and also in road sides and in open grass lands. In India it is never cultivated but grows spontaneously everywhere. Different species of Cleome can be found in all states of India. The medicinal application of this plant is also described in Ayurvedic pharmacopoeia of India and also in other ancient medical texts [1]. In Ayurvedic medicine it is a chief constituent in Narayana Churna. In Ayurveda it is used as an Antihelmentic, in ear diseases, pruritis and several other diseases like gastrointestinal disorders and gastrointestinal infections etc [2]-[4].

In several communities, boiled spider plant leaves are traditionally given to mothers before and after delivery of a child, and in other situations where blood has been lost, e.g. to warriors. Similarly, an infusion of the leaves is used to treat anemia. The leaves and seeds are used medicinally as rubefacient and vesicant, and to treat rheumatism, externally as well as internally. The whole plant is also used in the treatment of malaria, piles, rheumatism and also it shows antidiabetic and anticancer activity [5]-[7]. The methanol extract of Cleome gynandra possess very good antioxidant property [8]. The plant Cleome gynandra also possesses anti-inflammatory and lysosomal stability actions in adjuvant induced arthritic rats. An infusion of the roots is used as a medicine for chest pain, the leaves to treat diarrhoea. The seeds are used to feed birds. The seed contains edible polyunsaturated oil, which is extracted by simple pressing and does not need refining. The seed cake can be used as animal food [9].

During the last few years, many plants used in phytopharmaceutical preparations are being overexploited, and several species are disappearing at an alarming rate. In addition, medicinal plants can be associated with a broad variety of microbial contaminants and the main prerequisite for the development of high quality phytopharmaceutical products is a consistent source of high-quality plant material. Hence, advanced biotechnological methods for culturing plant cells and tissues should provide alternatives for propagating valuable and endangered medicinal plants and their metabolites [10]-[12]. In fact, these strategies contribute to the conservation and the sustainable use of biodiversity. Tissue culture technologies have been intensively used in afforestation and plantation programs, but also for plant improvement and in vitro production of metabolites. Considering the medicinal plants, the possibility of exploring bioactive compounds without commercial harvesting based on wild populations, represent the most efficient strategy to maintain the natural resources [13]-[16].

However, very few studies have been taken up on Cleome gynandra clonal propagation [17]. The present study was undertaken to develop a more efficient protocol for rapid in vitro multiplication of Cleome gynandra using shoot tip explants.

2. Materials and Methods

2.1. Plant material

Shoot tip segments from young and healthy plants of Cleome gynandra L. (Cleomaceae) were collected from in and around Thanthonimalai village, Karur district, Tamilnadu. They were washed with tap water for 30 minutes followed by treatment with 5 % teepol for 5 minutes. After repeated washes in double distilled water to eliminate the microbial contamination. They were then rinsed with 70% (v/v) ethanol for 30-45 seconds and subsequently surface sterilized with 0.1% (w/v) HgCl2 solution for 3-5 minutes under aseptic condition. After rinsing five times with sterile distilled water, the explants (approximately 2.0 cm in length) consisting of a lateral shoot tips were used for culture initiation.

2.2. Culture media and conditions

Basal medium used in this study was the MS medium containing 3% sucrose as a carbon source [18]. The pH of the
direct shoot multiplication of *C. gynandra*. The present study reports direct shoot multiplication of *C. gynandra*. For multiple shoot induction, the shoot tip explants were placed on MS medium supplemented with different concentrations of benzylaminopurine (BAP: 5-25 µM) or kinetin (Kin: 5-25 µM) alone or in combination with indole 3 acetic acid (IAA: 2-10 µM).

2.3. Shoot induction and multiplication

For multiple shoot induction, the shoot tip explants were placed on MS medium supplemented with various concentrations of indole butyric acid (IBA: 2-10 µM) or α naphthalene acetic acid (NAA: 2-10 µM). The root number and length were measured in each culture medium.

The complete plantlets were carefully removed from the medium and washed gently with sterilized doubled distilled water to remove any traces of medium on roots. Then they were transplanted to the paper cup containing a mixture of autoclaved red soil, farm yard manure and sand in the ratio of 2:1:1 mixture for two weeks before transplanted into soil under field conditions.

2.4. Rooting and hardening

*In vitro* raised shoots were excised from shoot cluster and transferred to half strength MS medium supplemented with optimal level of BAP(10 µM) and IAA(2-10 µM) showed emergence of multiple shoot buds after two weeks of inoculation. Presence of higher concentration of cytokinins and low concentrations of auxins positively influenced the induction of shoots. Among the various combination and concentration of hormone tested, the highest number of shoot was recorded on MS medium containing BAP (10 µM) + IAA(2 µM). The highest frequency of shoot regeneration (100 %) and maximum number of shoots (35.8±1.42) per explant were observed on MS medium fortied with 10 µM BAP + 2 µM IAA. The number of shoots induce was decreased when the combination of IAA is increased. Similar results were also reported in many plants [27]-[29]. In many plants, multiple shoots were obtained from the shoot tips or axillary buds by administering BAP or Kin [30] - [33].

2.5. Statistical analysis

All experiments were performed using 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 [19].

3. Results and Discussion

3.1. Shoot induction and multiplication

The present study reports direct shoot multiplication of *Cleome gynandra* L. from shoot tip on MS medium supplemented with different concentrations of BAP or Kin alone or in combination with IAA (Fig.1). The shoot tips were initially grown on MS medium supplemented with BAP and Kin separately in different concentrations ranging from 5 - 25 µM. The Maximum number of shoots was achieved in 30-45 days. Among these, the maximum number of shoots (26.4 ± 0.48) was developed on MS media fortified with 10 µM BAP, maximum shoot length was observed as 8.2± 1.38 cm with 100% shoot sprouting frequency on a medium supplemented with 10 µM BAP followed by 5 µM BAP with 22.7 shoots per explant and mean shoot length of 6.8 cm with 94% 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 [20]-[23]. The shoot induction and proliferation depend on plant growth regulators and types of explants [24]-[26].

Hence it is suggested that the optimum concentration of BAP promote multiple shoot induction. However the shoot implanted on MS medium supplemented with optimal level of BAP(10 µM) in combination with IAA (2-10 µM) showed emergence of multiple shoot buds after two weeks of inoculation. Presence of higher concentration of cytokinins and low concentrations of auxins positively influenced the induction of shoots. Among the various combination and concentration of hormone tested, the highest number of shoot was recorded on MS medium containing BAP (10 µM) + IAA(2 µM). The highest frequency of shoot regeneration (100 %) and maximum number of shoots (35.8±1.42) per explant were observed on MS medium fortied with 10 µM BAP + 2 µM IAA. The number of shoots induce was decreased when the combination of IAA is increased. Similar results were also reported in many plants [27]-[29]. In many plants, multiple shoots were obtained from the shoot tips or axillary buds by administering BAP or Kin [30] - [33].

<table>
<thead>
<tr>
<th>Concentration of hormone</th>
<th>Percentag e of Response</th>
<th>Number of Shoots</th>
<th>Shoot Length in cm</th>
</tr>
</thead>
</table>
| Kin 5µM --- --- 84 13.7±1.58b 5.4±1.04b | --- --- 90 17.5±1.66b 6.3±0.99b 10µM --- --- 25µM --- --- 96 21.3±1.14h 7.5±0.89d --- --- 82 12.9±2.25l 4.3±0.68l --- --- 68 6.6±0.96no 2.8±0.34o 15µM --- --- 90 18.6±1.51l 5.2±0.77jk 20µM --- --- 82 12.7±1.79m 4.3±0.50m 25µM --- --- 76 8.3±0.83b 3.2±0.41a --- 10µM --- 100 26.4±0.48d 8.2±1.38a --- --- 100 24.4±1.29a 8.5±0.87a --- 20µM --- 100 32.3±1.98b 8.0±0.71b --- 10µM --- 96 27.0±1.83c 6.2±0.30h 10µM --- 80 24.4±1.29a 7.8±1.08d 10µM --- 88 22.5±1.98bf 5.8±0.45hi --- --- 10µM --- 90 22.5±1.98bf 5.8±0.45hi 1369

--- --- 88 22.5±1.98bf 5.8±0.45hi
Results are repeated in five replicates. Each replicates have 10 test tubes. Values in the last two columns are Mean ± SE of Mean followed by the letters within the column indicating the level of significance at P<0.05 by Duncan’s Multiple Range Test (same letter within the column of the respective growth regulator indicates the absence of difference; different letters indicate the significant difference; and combination of letters indicate no significant difference).

**Table 2:** Effect of different concentrations of auxins on rooting of isolated shoots of *C. gynandra* L.

<table>
<thead>
<tr>
<th>Concentration of hormone</th>
<th>Percentage of Response</th>
<th>Number of Roots</th>
<th>Root Length in cm</th>
</tr>
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<tbody>
<tr>
<td>IBA</td>
<td>NAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2µM</td>
<td>---</td>
<td>84</td>
<td>7.8±0.64&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>4µM</td>
<td>---</td>
<td>96</td>
<td>8.2±0.80&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>6µM</td>
<td>---</td>
<td>100</td>
<td>12.9±0.43&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>8µM</td>
<td>---</td>
<td>78</td>
<td>5.2±0.73&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>10µM</td>
<td>---</td>
<td>64</td>
<td>3.5±0.65&lt;sup&gt;ce&lt;/sup&gt;</td>
</tr>
<tr>
<td>---</td>
<td>2µM</td>
<td>94</td>
<td>14.4±1.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>---</td>
<td>4µM</td>
<td>100</td>
<td>17.2±1.95&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>---</td>
<td>6µM</td>
<td>88</td>
<td>13.7±1.68&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>---</td>
<td>8µM</td>
<td>80</td>
<td>11.3±1.26&lt;sup&gt;bc&lt;/sup&gt;</td>
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<tr>
<td>---</td>
<td>10µM</td>
<td>76</td>
<td>10.5±1.69&lt;sup&gt;ef&lt;/sup&gt;</td>
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</table>

Results are repeated in five replicates. Each replicates have 10 test tubes. Values in the last two columns are Mean ± SE of Mean followed by the letters within the column indicating the level of significance at P<0.05 by Duncan’s Multiple Range Test (same letter within the column of the respective growth regulator indicates the absence of difference; different letters indicate the significant difference; and combination of letters indicate no significant difference).

Of the different concentrations of NAA, maximum number of 17.2 roots were induced on 4 µM NAA with mean root length of 3.6 cm with 100% root induction frequency, followed by 2 µM NAA with 14.4 roots, mean length of 3.2 cm with 94% root induction frequency (Fig.1d & e; Table 2).

The MS basal medium with 6 µM IBA showed 100% root induction frequency with mean number of 12.9 roots per shoot and the mean root length of 3.1 cm, followed by 4 µM IBA showing 96% root induction frequency with average number of 8.2 roots per shoot and mean length of 2.8 cm.

In the present study of the two auxins tested, NAA was found to induce more number of roots than IBA. The number of roots and root length decreased when the concentration of IBA and NAA was increased. However NAA was proved to be best for rooting. Similar to our findings, many researchers showed that NAA induced rooting in many plants [34]-[38].

**Figure 1:** *In vitro* propagation of *Cleome gynandra* L. a. Shoot tip explant growing on MS medium. b. Shoot multiplication. c. Shoot proliferation and elongation.

**3.2. Root induction and multiplication**

*In vitro* regenerated and elongated shoots (5 – 6 cm long) were excised and transferred on to the rooting medium containing half strength MS medium supplemented with different concentrations of IBA and NAA ranging from 2-10 µM. Roots were initiated and well established in all the concentrations of the two auxins studied.

**Figure 1:** *In vitro* propagation of *Cleome gynandra* L. d. Root initiation. e. Root multiplication.
3.3. Hardening and acclimatization

The rooted plantlets were carefully taken out from the culture tubes and washed thoroughly with distilled water to remove the culture medium. Then they were transplanted to the paper cup containing a mixture of autoclaved red soil, farm yard manure and sand in the ratio of 2:1:1 (Fig. 1f). The survival rate of these plants was 80% after acclimatization [39,40].

![Figure 1: *In vitro* propagation of *Cleome gynandra* L. f. Hardening and acclimatization](image)

**Acknowledgements**

The authors are grateful to Dr. P. Velayutham, Associate Professor and Dr. K. Murugesan, Head, P.G. and Research Department of Botany, Govt. Arts College, Karur for providing constant engorgement and support throughout the study.

References


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