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IN VITRO REGENERATION OF *COMMELINA DIFFUSA BURM*. F. USING NODAL EXPLANTS

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ABSTRACT

In Vitro Regeneration of plantlets has been achieved in an important medicinal plant of *Commelina Diffusa* (Commelinaceae) through nodal explants. The nodal explants were cultured on MS medium supplemented with different concentrations of BAP (5 µM -25 µM) and KIN (5 µM -25 µM) alone. Among the two cytokinins tested, 10 µM of BAP was found to induce more number of shoots (2.75 ± 0.47) than 10 µM KIN (3.2 ± 0.41). A maximum number of shoots was induced from all the various concentrations of BAP and KIN. The highest frequency of 90% shoot induction was observed on MS basal medium supplemented with 10µM BAP and 10 µM KIN alone. For root induction regenerated shoots were transferred to the half strength MS basal medium supplemented with different concentration of IBA and IAA. The maximum numbers of roots (15.6 ± 1.48) were obtained from 6 µM IAA with mean root length of 6.7cm. The plantlets were successfully transplanted to the paper cup for hardening and then it was transferred to the field with 85% survival rate.

KEYWORDS: *Commelina Diffusa Burm*. f., nodal explants, BAP, KIN, IAA, IBA, MS medium.

1. INTRODUCTION

Commelina Diffusa Burm. f. is an annual herb, commonly called climbing dayflower or spreading dayflower. This herb is widely distributed throughout Bangladesh and other South Asian countries. It is a sprawling, rhizomatous herb with jointed succulent, ascending stems growing to 75 cm tall. Leaves are alternate, mostly without petioles, parallel-veined.[1],[2] In China, traditionally this plant is highly useful to reduce swelling and inflammation. In

different parts of Asia and America it is commonly used in urinary tract infections, to remove a cough with sticky phlegm and in diarrhea, eye irritation, conjunctivitis and other eye problems like ophthalmia. The juice of stem is used in laryngitis, acute tonsillitis, pharyngitis (gargled juice or decoction), otitis media and nose bleeding.[3],[4],[5] This plant also exhibited good antimicrobial activity against wide range of gram +ve and –ve bacteria and fungi.[6],[7],[8] Diuretic compounds that stimulate the excretion of water are potentially useful in many disorders including most of those exhibiting edema such as congestive heart diseases, nephritis, toxemia of pregnancy, premenstrual tension, hypertension.[9]

Plant tissue culture has the potential to introduce genetic variability in genotypes through somaclonal variants, somatic hybrids or transgenic plants. However, a prerequisite to applied plant biotechnology is the development of a suitable and reproducible plant regeneration system at least cost.[10],[11] This plant may also serve as the alternative sources for the development of new diuretic agents due to their biological activities. However, very few studies have been taken up on *C.* diffusa. The present study was undertaken to develop a more efficient protocol for rapid *In Vitro* multiplication of C. diffusa using nodal explants.

2. MATERIALS AND METHODS

2.1. Chemicals

All the solvents and other reagents used in the present study are of analytical grade and purchased from Himedia laboratories, India.

2.2. Source of explants

The wild plants of *C. diffusa* Burm. f. were directly collected from roadsides of Thanthonimalai, Karur of Tamil Nadu, India. The nodal segments with auxiliary buds of the plants were selected for the source of explants in the present study.

2.3. Procedure for preparing culture media

The basal medium consisted of the mineral salts and organic nutrients of Murashige and Skoog (MS) salts with B_5 Vitamins were used for the study.[12] 30 g of sucrose (3%) and 8 g of agar (0.8%) were dissolved and made up to 1 litre. Finally, the pH of the media was adjusted to 5.8 using 0.1 N NaOH and 0.1 N HCl. The culture media were sterilized by autoclaving at 1.06 kg cm^{-1} and 121^{o}C for 15 min.

2.4. Explant preparation

The nodal explants were washed thoroughly under running tap water for 30 minutes followed by washed with surfactant (Teepol). They were rinsed with distilled water for 4-5 times. Then they were disinfected with 70% alcohol (v/v) for 45 seconds followed by 0.1% mercuric chloride (w/v) for 3 minutes. Finally, the explants were washed with sterile distilled water for 3-5 times to remove the traces of using chemicals.

2.5. Shoot induction

In the present study the protocol for shoot induction and regeneration in *C. diffusa* has been developed in culture medium. Young nodal region was used as explants for shoot induction on MS medium containing BAP or Kin separately in different concentrations ranging from 5-25 µM. Shoot initiation was first recorded in the nodal region and it was tabulated (Table.1).

2.6. Root induction

In Vitro raised shoots were excised from shoot cluster and transferred to half strength MS medium supplemented with various concentrations of indole butyric acid (IBA: 2-10 µM) or indole acetic acid (IAA: 2-10 µM). The root number and length were measured in each culture medium.

2.7. Culture conditions and maintenance

The growth room conditions were maintained for the culture at 25±2^{o}C under 16 hours photoperiod with photo flux of 30-40 µM m^{-2} s^{-1} (40 W cool-white fluorescent tubes, and 50–60% relative humidity). Each experiment was conducted at least thrice with 10 replicates per treatment.

2.8. Statistical analysis

The percentage of initiation, days for initiation, length and number of shoots and roots, and regeneration percentage during acclimatization of plantlets were recorded. The data were analyzed using analysis of variance (ANOVA), and the means were compared using the Duncan's multiple range test (DMRT) at 5%level of significance (Pb0.05).[13]

3. RESULTS

3.1. Shoot induction and multiplication

The nodal segments were initially grown on MS medium supplemented with BAP and Kin separately in different concentrations ranging from 5-25 µM. The Maximum numbers of

shoots were achieved in 30-45 days. In this study, the maximum number of shoots 3.7 ± 0.23 was developed on MS media contained with 10 µM BAP, maximum shoot length was observed as 8.7± 0.90 cm with 90% shoot sprouting. The data obtained in the present with respect of shoot induction frequency, number of shoots and length of shoots on different concentrations were presented in Table 1 and Fig. 1 a-c.

The basal medium containing different concentrations of Kin induced more or less similar number of shoots like BAP. A maximum number of shoots (2.75 ± 0.47 shoots per explants) was proliferated on MS medium supplemented with 10 µM Kin and mean shoot length 8.66 ± 1.50 cm with 80% shoot induction frequency. Among the two cytokinins tested, BAP was found to induce more number of shoots then Kin.

Table 1: Effect of Different Concentrations of Cytokinins on Shoot Induction from the Nodal Explants of *C. diffusa* Burm. f.

Concentration of Cytokinins		Shoot Induction Frequency (%)	Number of Shoots	Shoot Length (cm)
BAP	KIN			
5 µM	---	70	2.6 ± 0.57^{bc}	7.86 ± 1.37^{c}
10 µM	---	90	3.2 ± 0.41^{a}	9.6 ± 1.66^{a}
15 µM	---	66.6	2.0 ± 0.40^{e}	6.74 ± 0.65^{d}
20 µM	---	63.3	1.75 ± 0.47^{fg}	5.08 ± 0.88^{fg}
25 µM	---	53.3	1.8 ± 0.41^{f}	3.02 ± 1.21^{i}
---	**5 µM**	66.6	1.5 ± 0.28^{hi}	5.14 ± 0.98^{f}
---	**10 µM**	80	2.75 ± 0.47^{b}	8.66 ± 1.50^{b}
---	**15 µM**	70	2.25 ± 0.75^{d}	6.62 ± 0.62^{de}
---	**20 µM**	50	1.6 ± 0.57^{gh}	4.88 ± 1.06^{gh}
---	**25 µM**	46.6	1.4 ± 0.44^{ij}	2.82 ± 1.02^{ij}

Results are repeated in thrice. 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).

3.2. Root induction and multiplication

Rooting in regenerated this plants has been achieved on containing any growth regulators with half–strength MS medium.[14] The shoots measuring about 3-5 cm in height were transferred to rooting medium supplemented with various concentrations of IBA and IAA (2-10 µM) for rooting. The optimal medium for rooting contained 6 µM IAA, on which 86.6%

of the regenerated shoots developed roots with an average number of 15.6 roots per shoots within 30 days. The highest frequency of rooting (86.6%) and a maximum number of roots per shoot (15.6 roots) with root length (6.7 ± 1.15 cm) was obtained on MS containing 6 µM IAA. Of the two auxins, tested for root induction IAA was more responsive than IBA (Table.2; Fig.1 d–e).

Table 2: Effect of Different Concentrations of Auxins on Rooting of Isolated Shoots of *C. diffusa* Burm. f.

Concentration of Auxins		Root Induction Frequency (%)	Number of Roots	Root Length (cm)
IAA	IBA			
2 µM	---	73.3	9.4 ± 1.60^{ef}	5.6 ± 1.12^{ef}
4 µM	---	80	11.2 ± 1.85^{c}	6.02 ± 0.56^{bc}
6 µM	---	86.6	15.6 ± 1.48^{a}	6.7 ± 1.15^{a}
8 µM	---	76.6	9.6 ± 2.01^{de}	5.84 ± 1.42^{cd}
10 µM	---	60	7.2 ± 0.82^{gh}	4.13 ± 0.45^{h}
---	**2 µM**	76.6	10.2 ± 1.78^{cd}	5.76 ± 0.65^{de}
---	**4 µM**	83.3	13.8 ± 1.91^{b}	6.32 ± 0.49^{ab}
---	**6 µM**	70	7.8 ± 0.96^{g}	4.96 ± 0.77^{g}
---	**8 µM**	63.3	6.8 ± 1.29^{hi}	3.48 ± 0.74^{i}
---	**10 µM**	56.6	5.2 ± 0.65^{j}	3.26 ± 1.39^{ij}

Results are repeated in thrice. 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).

3.3. Hardening and Acclimatization

After 4 weeks, plantlets with a well-developed root system (**Fig. 1f**) were subjected to acclimatization experiment. Acclimatization was successful with the combination of red soil, farmyard manure and sand (2:1:1), showing 85% survival rate. In order to expose plantlets to *ex vitro* / environmental conditions, potting was subsequently moved from high humidity (80–85% RH) and low temperature (26–28ºC) to low humidity (50–55% RH) and high temperature (34–36ºC) zone under plant growth chamber **(Fig. 1g)**. The paper cups containing hardened plantlets survived in the field conditions. Acclimatized plants were very uniform and continued to grow; showing the development of new leaves and did not show any visible morphological abnormalities from the field grown plants.

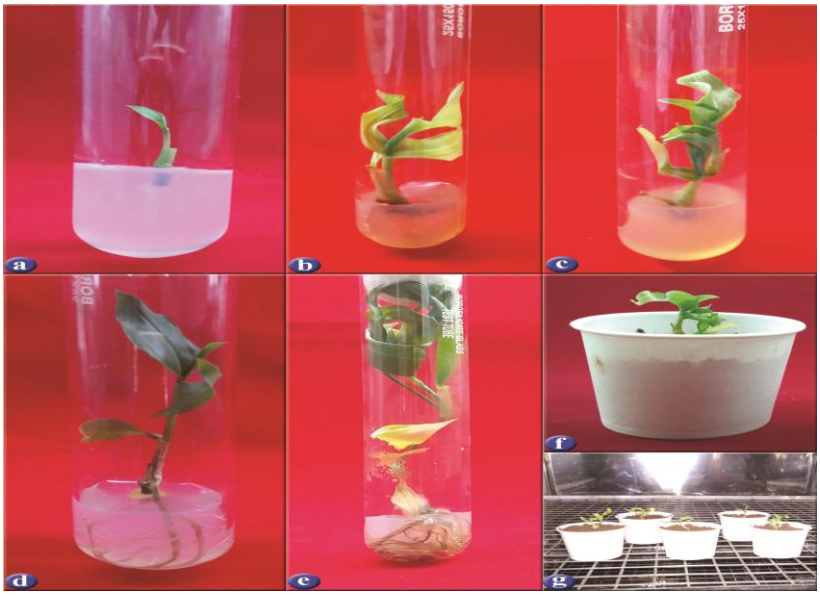

Fig. 1: *In Vitro* regeneration of shoots from the nodal explants of *C. diffusa* Burm. f. a. Shoot initiation; b & c. Shoot elongation and multiplication; d. root initiation; e. Root multiplication and f. & g. Hardening.

4. DISCUSSION

The results suggested that the cytokinin played an important role in the multiple shoot formation. The promotary effect of kinetin shows multiple shoot induction. Of the two cytokinins tested, BAP treated explants showed multiple shoot formation higher than the Kin. Shoot regeneration of this plant is similar to our findings, many researchers showed that BAP induced multiple shoot formation, *Heliotropium crassavicum*,[15] *Passiflora foetida*,[16] *Vitis vinifera*,[17] *Cleome gynandra*,[18] *Stevia rebudiana*.[19] The shoot induction and proliferation depend on plant growth regulators and types of explants, *Terminalia bellerica*,[20] *Commelina benghalensis*,[21] *Mentha viridis*,[22] *Tashnedari*.[23]

After 5 weeks the well developed shoots were transferred to MS medium supplemented with 6 µM of IAA and 4 µM of IBA in half strength of MS medium was found to be the most suitable for root induction. In rooting, the results showed consistency with other studies where the addition of IAA promotes the induction of roots in several systems including, *Cleome gynandra*,[24] *Bacopa Monneri*,[25] *Abrus precatorius*,[26] *Vitex negundo*.[27] However, IBA was more effective auxins for root induction in many plants, *Cleome chelidonii*,[28] *Blepharispermum subsessile*,[29] *Achyranthes aspera*,[30] *Costus sp*,[31] *Mentha piperata*,[32] *Rauvolfia tetraphylla*,[33] *Talinum triangulare*,[34] *Viola pilosa*.[35]

5. CONCLUSION

These results indicate that the best shoot induction (90%) with early response was obtained on MS medium supplemented with BAP (10 µM) from node explants. Plantlets obtained from the nodal region, rooted on half strength MS supplemented with 6µM IAA. So, this *In Vitro* regeneration studies is useful for production of a large number of plants.

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