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In vitro propagation of *Commelina ensifolia* R.Br.

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Abstract

Commelina ensifolia R.Br. is a rare edible herb belonging to family Commelinaceae. In the present study, *in vitro* regeneration of *C. ensifolia*. from nodal explants has been established. Nodal explants were cultured on MS medium supplemented with different combinations of BAP, NAA and IBA. The nodal explant of C. ensifolia is responsible for root induction, mean number of roots and root length and mean number of elongated shoots and shoot length. In the observed results, *C. ensifolia* 90% showed root regeneration responses in IBA 3.0 mg/L^{-1} and NAA 1.0 mg/L^{-1}. A well developed micro shoots and rooting media for complete regeneration of *Commelina ensifolia* has been developed in the present study.

Abbreviations: *Commelina ensifolia*, micropropagation; BAP: 6-benzylaminopurine; NAA:1-naphthaleneacetic acid IBA: indole-3-butyric acid; MS: Murashige and Skoog Medium.

Introduction

Loss of biodiversity of tropical forests is mainly due to degradation and destruction of habitat by anthropogenic activities, which recognized as a global problem. The ever increasing demand of medicinal plants leads to indiscriminate collection of plants from the wild create a constant pressure on existing resources, which forms leading to continuous depletion of some of the species in the forests and at the same time forest land is also losing its natural flora at an alarming rate of 1.5 mha every year (Shreekar Pant and Virbala Sharma Pant, 2011). The pharmacological significance along with quiescent nature of seeds and lengthy cycle are collectively responsible for its endangered status. Therefore, conservation of plants through tissue culture is a vital need and has been developed nowadays (Eti Sharma *et al*., 2012). *In vitro* culture techniques is the best method for multiplication and germplasms conservation of rare, endangered, aromatic and medicinal plants (Mallon *et al*., 2010; Kaur and Bhutani, 2011).

Commelinaceae family is consists of 650 species in 40 genera (Robert, 1998). *C. ensifolia* R.Br. is an annual or perennial herb. The whole parts are used as edible. Therefore, there is an urgent need to conserve the *C. ensifolia* R.Br. So, the present work was carried out to conserve this plant through *invitro* propagation from its nodal regions.

Materials and Methods

Explants

Nodal regions of *C. ensifolia* R.Br. (Commelinaceae) were used as explants for invitro culture. The explants of nodal region was thoroughly washed in mercuric chloride (w/v) for 2mins followed by rinsing with sterile distilled water and followed by explants were shaken for 10minutes in Tween 20 and washed several times with sterile water. MS medium (1962) with different concentration of NAA, IBA and BAP were used as growth regulators (Table-1 and 2). Twenty grams of sucrose and 8.0g of agar were used per litre of the medium. The pH of the medium was adjusted to 5.8 before adding agar. The media were autoclaved at $1.1kg/cm^2$ pressure and 121°C for 20 mm. The inoculated cultures were incubated at 26 ± 1°C under approximately about 2000 lux by cool white fluorescent tubes for 16/8 light/ dark cycle. The percentage of nodal explants was responsible for rooting, number and length of roots / shoots after one month of culture. The rooted plantlets were transferred to plastic cup filled with humus soils and covered with polythene bags to ensure high humidity. They were grown under confined

conditions before their transfer into the soil in the greenhouse.

Statistical analysis

Data were statistically analysised using the program package SPSS 11.5.

Results and Discussion

The nodal region of *C. ensifolia* used for root induction, mean number of roots and root length and mean number of elongated shoots and shoot length represented in the table-1 and 2 and plate 1a-i. The results of *C. ensifolia* showed that 90% of root regeneration in IBA 3.0 mg/L^{-1} and NAA 1.0 mg/L^{-1} (Plate-1h). These results are in accordance with those of Mustafa Anand *et al*., (1997) on *Kaempteria rotunda*, Manickam *et al*., (2000) on *Withania somnifera* (Indian ginsens) and Segio *et al*. (2000) on *Anthemis robilis*. The best shooting medium was observed in MS medium containing 0.25 mg/L^{-1}BAP (Plate 1g). The similar results were observed in several species such as *Castilleja tenuiflora* Benth, *Cannabis aativa* L., *Solanum nigrum, Stevia rebaudiana* (Bert.), (Guadalupe Salcedo-Morales *et al*., 2009; Ren Wang *et al*.,2009; Sundari *et al*., 2010; Bochra Laribi *et al*., 2012). The present study helps in developing a protocol for conserving this type of rare and endemic species.

Table-1: Effect of different concentration of BAP and NNA for rooting induction from nodal explants of *Commelina ensifolia*

Plant growth regulators	% responses	Mean no of roots	Mean no roots length
IBA			
0.5	30	2.7±1.5	17.0±1.5
1.0	30	2.7±0.5	22.0±3.0
1.5	40	2.7±0.5	15.0±1.5
2.0	60	4.0±0.5	12.0±1.5
2.5	70	3.3±0.5	8.0±2.0
3.0	90	3.3±0.5	5.0±1.5
3.5	70	2.7±0.5	6.0±1.5
4.0	80	2.7±0.5	7.0±1.5
4.5	70	2.7±0.5	10.0±1.5
5.0	80	2.3±0.5	4.6±1.5
NAA			
0.1	50	2.7±1.5	17.0±1.5
0.25	70	1.7±0.5	21.0±3.0
0.5	80	6.7±0.5	12.0±1.5
1.0	90	2.0±0.5	15.0±1.5
1.25	80	3.3±0.5	8.0±2.0
1.5	60	3.3±0.5	5.0±1.5
2.0	70	2.7±0.5	6.0±1.5
2.5	70	2.7±0.5	6.0±1.5
3.0	80	2.7±0.5	8.0±1.5
3.5	60	1.3±0.5	3.0±1.5

All data were expressed as Mean ±SD (10 Nos.)

Table-2: Effect of different concentration of BAP for shoot elongation from nodal explants of *Commelina ensifolia*

Plant growth regulators BAP	% responses	Mean no of roots	Mean no roots length
0.1	30	2.7±1.5	21.0±2.0
1.0	30	2.7±0.5	25.0±2.0
0.25	80	2.7±0.5	19.0±2.0
0.5	60	4.0±0.5	24.0±2.0
1.0	70	3.3±0.5	33.0±2.0
1.25	50	3.3±0.5	21.0±2.0
1.5	70	2.7±0.5	14.0±2.0
1.25	60	2.7±0.5	26.0±2.0
1.5	70	2.7±0.5	11.0±2.0
2.0	60	2.3±0.5	7.0±2.0

All data were expressed as Mean ±SD (10 Nos.)

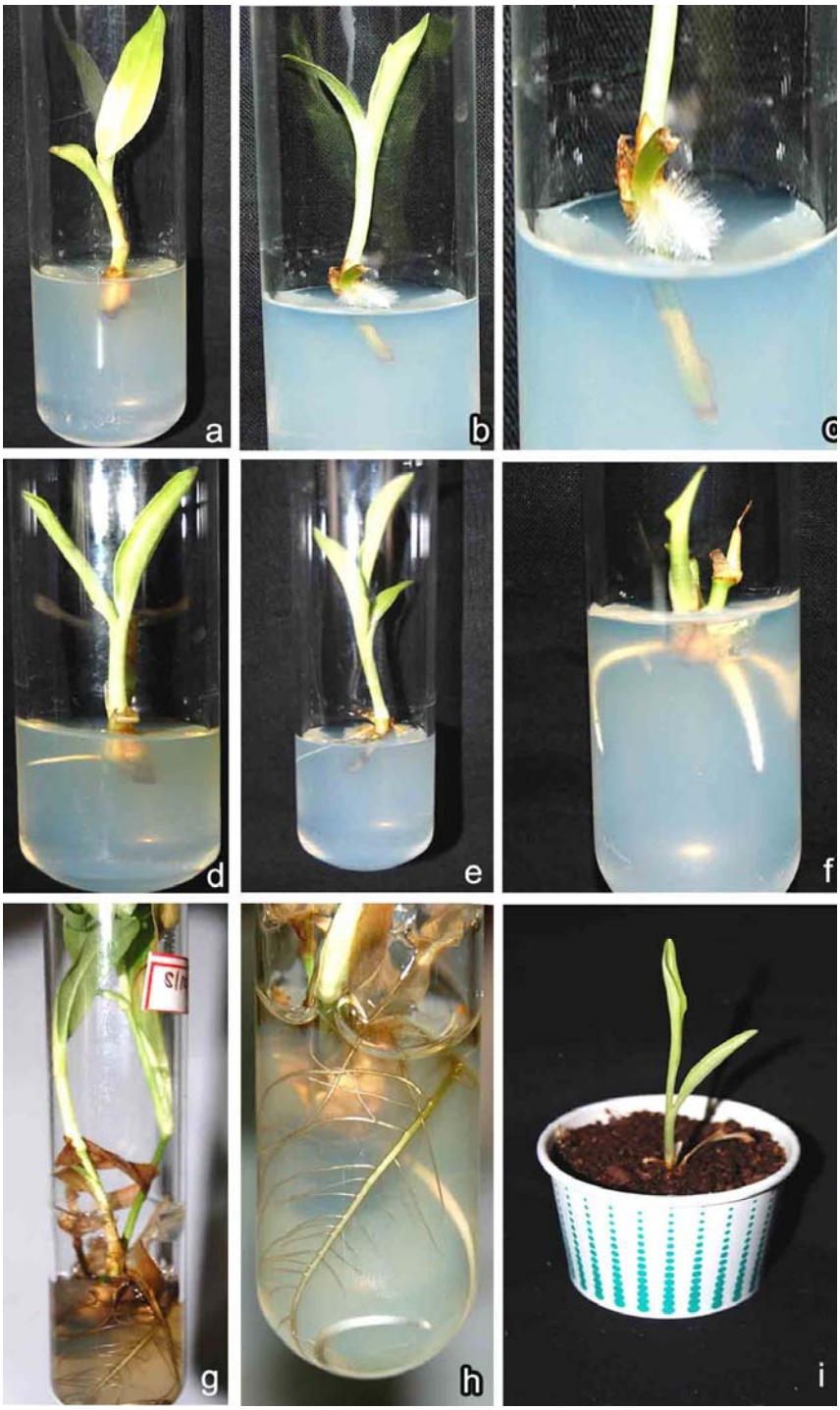

Plate - 1: *In vitro* multiple shoots and root induction of *Commelina ensifolia*

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