Available online at www.jobpcr.com

ISSN-2394:3076
CODEN(USA) : JBPCBK

Journal of Biological Pharmaceutical And Chemical Research , 2015,2(2):1-5

(http://www.jobpcr.com/arhcive.php)

Induction of callus and shoot proliferation of a medicinally important herb. (*Cleome chelidonii* L.f.)

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ABSTRACT
Green globular callus was induced from leaf and nodal explants of cleome chelidonii L.f. On MS medium fortified with 2.0mg/ml BAP+1.0 IAA mg/ml maximum number of shoot buds were proliferated from the same explants on MS medium supplemented with 4.0mg/mlBAP+2.0mg/ml IAA. These plantlets were allowed for further growth on the same medium then transferred to full strength MS medium supplemented with 2mg/ml IBA for rooting. The rooted plantlets were transferred to peet and vermicompost pots for acclimatization

Keywords : Cleome *chelidonii* L.f, BAP-Benzyl amino purine, node, shoot buds, acclimatization.

INTRODUCTION

Cleomaceae is a small family of flowering plants in the order Brassicales, comprising more than 300 species belonging to 9 genera, of which *Cleome* is the largest genus with about 180-200 species of medicinal, ethno medicinal ecological importance. The species ***Cleom chelidonii* L.f.** grows as perennial with penta or hexa foliate leaves, pink flowers and 2-3 inch pods. A new variety of *Cleome chelidonii* (Reddy C.S and V.S Raju 2001) was identified in Pakal lake of Warangal District Telangana state. It is a Endamic herb commonly called as adavi avalu and seeds used as condiment and leaves known to have antipyretic, antirheumatic, antioxidant, anti-inflammatory, antinociceptive and anticancerous properties (Parimalakrishna *et al.*,2007). Production of two volatile Glucosinolate hydrolasis compounds such as glucocapparin, glucocleomin (Songsak *et al.*, 2004). Rutin a bioflavonoid can be isolated from whole plant. In view of its medicinal importance the species is being over exploited hence there is an urgent need for its conservation before they get extinct. There is an urgent investigation is need to propagate large amount of callus for extraction of useful compounds cultures. Here we have developed a rapid and simple protocol for the production of callus and plantlets

MATERIALS AND METHOD

Cleome chelidonii plants collected from Pakal lake of Warangal district Telangana state.This species was indentified with help of flora of Andra pradesh .These plants grown in the college Reaserch field The leaf and nodal explants were thoroughly washed under running tap water for 10

minutes and surface sterilized with 1% $Hgcl_2$, 2-4 minutes, rinsed 3-4 times with sterilized distilled water. The sterilized leaves and nodes were cut into small pieces and inoculated on MS medium supplemented with 2.0mg/ml BAP+1.0 IAA mg/ml for callus induction ,MS medium with 4.0mg/mlBAP+2.0mg/ml IAA for regeneration and 2mg/ml IBA for rooting with 30 gm/l sucrose and 6 gm/l agar. P^H was adjusted to 5.7and autoclaved for sterilization at 121^0c, the cultures were incubated under fluorescent light of 16 hrs photoperiod. The cultures were responded after 10 days results recorded with different intervals of time.

RESULTS AND DISCUSSION

Plant growth regulators (PGR) showed a significant impact percentage of callus was observed initially on explants in MS medium supplemented with different combinations. The highest percentages of callus formation (90%) were obtained from explants culured on MS medium containing 2.0 mg/l BAP and 1.0 mg/ml IAA. After 10 days the explants were form callus and callus induction was 50 % (Table-1). Interesting and unique aspect was the formation of yellow, globular, green compact, white callus which initiated when the explants were enlarged and swelled, however, they remained in green globular callus was observed. Even though no shots were produced, callus initiation and growth of callus first. Shoot and embryo like structure formation from cultured tissue of Sorghum were reported by Thomas et al., (1977).

After the establishment phase, different concentration of plant regulators enabled plant propagation via nodal explants were placed horizontally on a surface of a solidified culture medium in a test tube . The nodal explants haves shoot proliferation when cultures on MS media supplemented with lower or higher concentration of BAP .On the medium both leaf

And nodal explants was failed to regenerate shoots. The medium supplemented with 2.0 mg/l BAP and 1.0 mg/ml IAA (Table-1) at same medium, the nodal explants was induced shoot proliferation. Increased no of shoots (13 shoots) with enhanced level of BAP 4.0 mg/ml
Regenerative shoots were singled out and cultured on MS medium containing IBA. The shoots were produced roots, the number of roots was observed at 2.0 mg/ml IBA. Acclimatization of *in vitro* regenerated plants has been established in *Cleome chelidonii* for the first time. The new leaves were formed after 10 days transferred .The planlets were transferred into green house for their maintenance. The potting mixture containing peet+vermicompost (1:1) showed better results 70% of survival

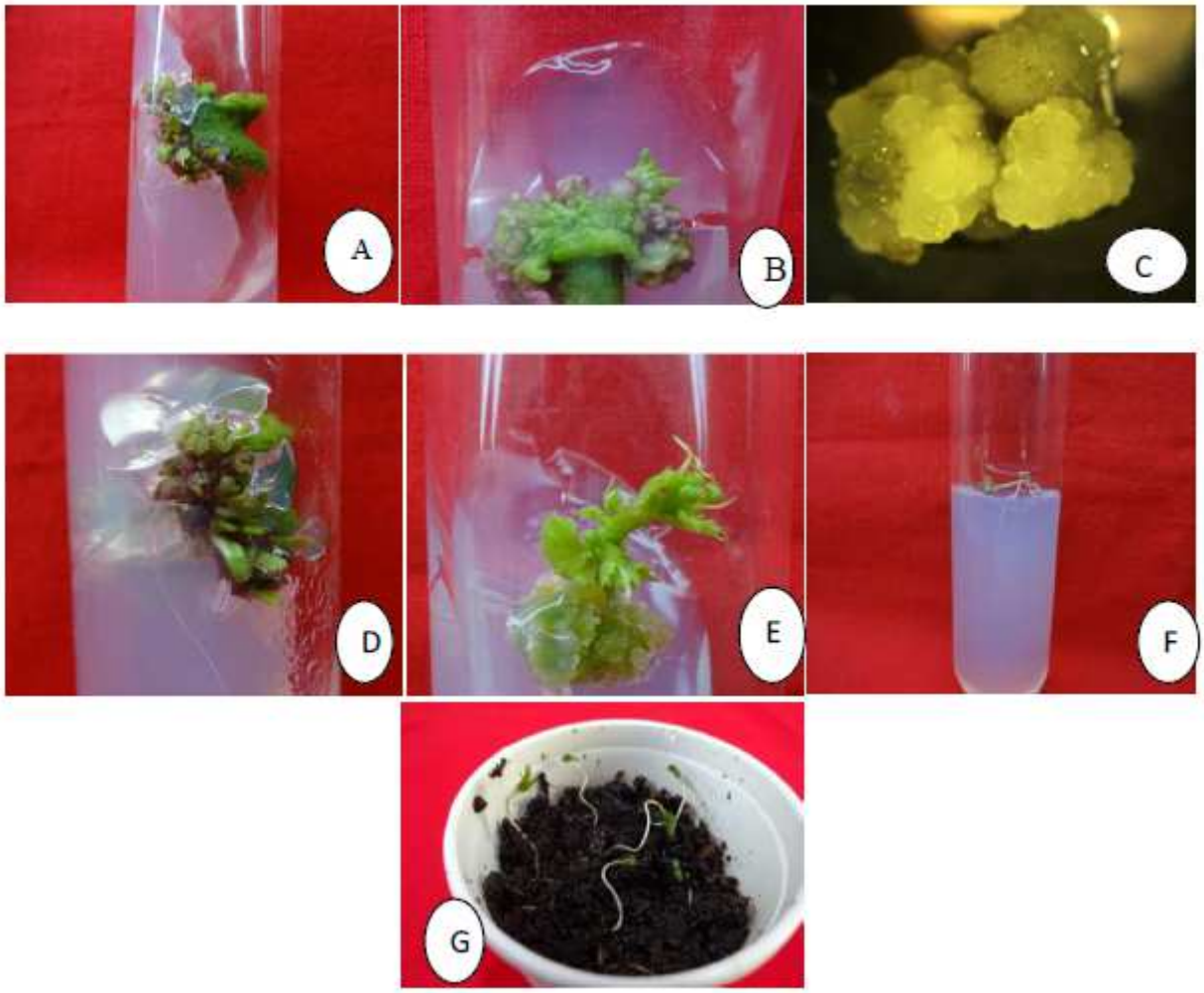

Fig-1.A. Green compact callus: **B**. green globular callus: **C** .Yellow friable callus D. Shoot proliferation: **E**. Number of shoots: **F.** *In vitro* rooting: **G**. acclimatization:

Table-1

Induction of callus from leaf explants (*C.chelidonii*) on MS medium supplemented with different concentrations of 2.0 mg/ml BAP+1.0mg/ml IAA.

2.0mg/mlBAP+1.0mg/ml IAA	% of response	Morphogenetic response
0.5+0.1	8	Green compact callus
1.0+1.0	10	Yellow friable callus
1.5+1.0	20	Green callus
2.0+1.0	50	**Green globular callus**
2.5+1.0	30	White friable callus
3.0+1.0	20	Brown callus
3.5+1.0	30	White callus
4.0+1.0	20	Green callus

*Data was collected after 3 weeks of cultures.

Table-2

Shoot proliferation of (*C.chelidoonii*) on MS medium supplemented with different concentrations of 4.0 mg/ml BAP+2.0 mg/ml IAA.

S.No	MS+BAP+IAA	% of response	Mean. No. of shoots	Mean. No of shoot length
1	0.5+0.5	8	1.5±0.11	2.2±0.06
2	0.5+1.5	15	2.1±0.14	4.1±0.05
3	0.5+2.0	25	3.2±0.11	2.6±0.05
4	0.5+2.5	40	4.2±0.12	1.5±0.06
5	0.5+3.0	30	5.5±0.23	3.2±0.04
6	1.0+0.5	35	2.3±0.17	3.6±0.04
7	1.0+1.5	50	2.2±0.16	3.7±0.06
8	1.0+2.0	40	4.0±0.21	2.1±0.07
9	1.0+2.5	40	5.1±0.12	3.0±0.08
10	1.0+3.0	60	6.3±0.14	2.5±0.04
11	2.0+0.5	30	7.8±0.16	2.2±0.02
12	2.0+1.0	50	8.4±0.21	1.8±0.03
13	2.0+1.5	60	9.0±0.24	2.2±0.01
14	2.0+2.0	40	8.6±0.21	2.3±0.04
15	2.0+2.5	20	6.2±0.21	1.6±0.06
16	2.0+3.0	30	6.0±0.35	2.0±0.05
17	3.0+0.5	20	6.4±0.24	2.4±0.01
18	3.0+1.5	10	5.6±023	1.2±0.03
19	3.0+2.0	40	4.5±0.36	3.2±0.04
20	3.0+2.5	50	5.1±0.35	2.5±±0.03
21	3.0+3.0	40	4.6±0.2.1	1.4±0.02
22	4.0+0.5	50	3.2±0.35	3.0±0.03
23	4.0+1.5	70	9.0±0.32	2.8±0.05
24	**4.0+2.0**	**80**	**13.10±0.35**	**3.2±0.06**
25	4.0+2.5	40	8.0±0.33	2.3±0.03
26	4.0+3.0	40	7.0±0.40	2.8±0.02

*Data was collected after 3 weeks of cultures.

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