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RESEARCH ARTICLE

INDUCTION OF CALLUS AND SHOOT PROLIFERATION OF A MEDICINALLY IMPORTANT HERB. CLEOME RUTIDOSPERMA DC

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ABSTRACT

Green globular callus was induced from leaf and node of ***Cleome rutidosperma* DC** 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 peat + vermicompost pots for 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. *Cleome rutidosperma* DC (Family: Capparidaceae) is a low-growing herb, up to 70 cm tall with trifoliate roots and small violet-blue flowers, which turn pink as they age, found in waste grounds and grassy places. The plant is native to West Africa and has become naturalized in various parts of tropical America as well as South East Asia (Widespread, 1972; Waterhouse, 1998). Traditionally, the roots, leaves and seeds of the plants of Cleome genus are used as stimulant, antiscorbutic, anthelmintic, rubifacient, vesicant and carminative (Kiritikar and Basu, 1991). In Malaysia, planting of C. rutidosperma around field edges may be considered as part of an insect control programmed (Burkill, 2004). The antiplasmodial activity of the chloroform-methanol (1:1) extract of the leaves was reported by Bidla *et al.* (2004), simmilarly diuretic, laxative, analgesic, antiinflammatory, antipyretic, antimicrobial, antioxidant and free radical scavenging activities of the aerial parts of C. rutidosperma have been reported earlier (Bose *et al.*, 2008). The roots are also reported to have hypoglycaemic effect (Mondal *et al.*, 2009) and anthelmintic activity (Mondal *et al.*, 2009). As per the folklore information the tribal people apply the juice of roots over severe open wounds and claim for its promising effectiveness towards healing of wounds, so in the present study was undertaken to provide on wound healing activity of the roots using all possible models and provide a scientific support to its use in the folklore medicines for treating wound 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

MATERIAL AND METHODS

***Cleome rutidosperma* DC.** Plants collected from Khammam district Telangana state. This species was indentified with help of flora of Andra pradesh .These plants grown in the college Research 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 5.7 and 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.

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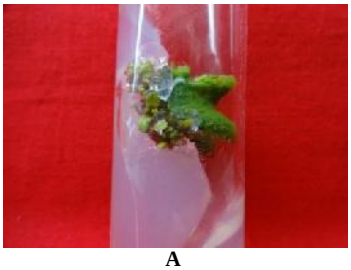
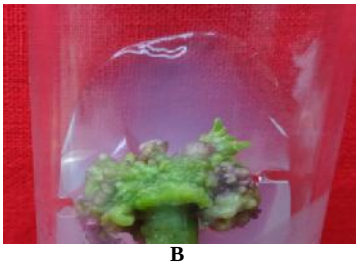
RESULTS AND DISCUSSION

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 cultured 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, how ever, they remained in green globular callus was observed. Even though no shots were produced, callus initiation and growth of callus first.



A



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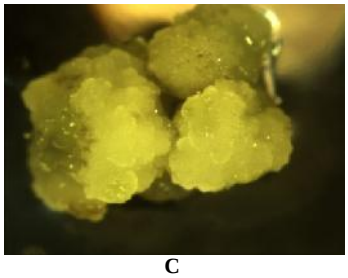

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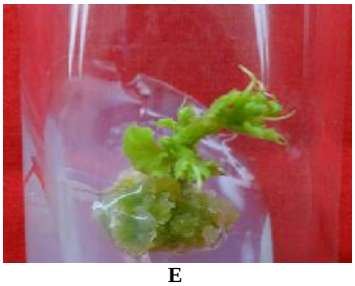

E

F

G

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.rutidosperma*) 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.rutidosperma*) 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.

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 rutidosperma* for the first time. The new leaves were formed after 10 days (transferred) .The plantlets were transferred into green house for their maintenance. The potting mixture containing peat+vermicompost (1:1) showed better results 70% of survival

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