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Research Article

IN-VITRO SHOOT PROLIFERATION OF *CALOTROPIS PROCERA*

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ABSTRACT
Calotropis procera, a plant of family Asclepiadaceae is of immense importance as multipurpose plant with medicinal as well as traditional uses. Latex of this plant has been used to cure disease like leprosy, syphilis, inflammation, cutaneous infections, and malarial fever and in many more. *In vitro* culture of this pharmacologically plant is required otherwise it leads the segregated loss of desirable traits. A comparative study of this plant has come out through *in vitro* culture of nodal segment of the mature plant. The explants were inoculated in MS medium fortified with different plant growth regulators alone or in combinations. Maximum shoot proliferation was observed i.e. 4 shoots per explants on 5+1 mg/L of BAP+NAA within 30 days of inoculation. Maximum shoot length was 1.41±0.0141 cm on 2+1 mg/L of BAP+NAA within 20 days after inoculation.

Keywords: *Asclepiadaceae, Calotropis procera, Growth Regulators, MS Medium, Nodal Segment*

INTRODUCTION
Calotropis procera belongs to family *Asclepiadaceae*. Plants are native to the North Africa, Western Asia, South Africa and Indochina. It is a shrub or small tree upto 2.5 m high (Kumar and Basu, 1994). A number of secondary metabolites are present in latex of this plant having significant medical role. These are used against cold and cough and as an analgesic. Flowers are used in aborifacient, asthama and piles (Sharma and Sharma, 2000). In religious view the *Calotropis procera* have been used for the worship of the Sun from Vedic times. Hindus obtain Swetark Ganpati from the root of this shrub that sometimes takes the shape of the lorg Ganesha. In the present study attempts were made for *in vitro* propagation of *Calotropis procera* using nodal explant. *In vitro* propagation of plants by tissue culture to produce genetically similar copies of a plant is referred as micropropagation or clonal propagation. Sexually propagated plants show a high level of heterogeneity since their seed progenies are not true to type whereas asexual reproduction gives rise to genetically identical copies of parent plant. Thus, it keeps remain of the parental characters of the plant resulting from micropropagation.
Each single cell has the ability to regenerate the whole plant (Haberlandt, 1902). The totipotency of the cell can be understood through the process of differentiation, in which a single cell can give rise to the organ, this process is called as organogenesis. Root formation is known as rhizogenesis and shoot formation is known as caulogenesis (White, 1963). Each somatic cell has potential for the whole development of this plant that is derived from the zygotic cell through mitosis. When a single cell is cultured in suitable medium and conditions, it will regenerate the whole plant.

MATERIALS AND METHODS
The explants (axillary branch) of *Calotropis procera* were obtained from the garden, Department of Botany, School of Life Sciences, Khandari Campus, Agra. On the basis of their physical performance and appearance (elites) healthy plants were selected for *in-vitro* establishment. The explants (1-2 cm.) were washed thoroughly under running tap water for 10 min. and then treated with few drops of tween-20 (Polyoxyethylene sorbitan monolaurate) for one min. with constant shaking by hand. The shaking was followed by three successive washings again with distilled water. The surface sterilization was carried out with 0.1% $HgCl_2$ for one min. followed by gentle shaking. After surface sterilization the segmented parts were thoroughly washed for several times with sterile (autoclaved) distilled water and explants were transferred in 25x 150 mm culture tubes with 15 ml MS media (Murashige and Skoog, 1962) supplemented with hormone.

Various hormones were used in different concentrations for obtaining multiple shoots. The pH was adjusted to 5.8 prior to autoclaving. The cultures were incubated at 25±5 °Ċ with 16 h light and 8 hours dark period having of light intensity of 1000 Lux.

Explants after disinfection were inoculated on MS medium supplemented with BAP and NAA in the flame with the help of sterilized forceps and scalpel. After the inoculation in culture tubes/flasks the mouth of tube or flask quickly tightened with plastic caps or cotton plugs.

For shoot proliferation, MS medium was supplemented with different concentration of various plant growth regulators (BAP 0 to 2mg/l, NAA 0-4.0mg/l). Data was recorded as inoculation per culture tube/ flask and was measured every day after the inoculation.

Multiplied sprouted node (explant) were transferred to MS media supplemented with different concentration of BAP and NAA for regeneration of multiple shoots. Sub culture was carried out at an interval of 2-3 weeks regularly.

RESULTS AND DISCUSSION

Shoot induction of the nodal segment was inoculated in three types of media these were water agar media, MS media and MS+PGR media. In each medium the rate of axillary bud sprouting was different (Table 1). In water agar medium the axillary bud sprouted within 12 days of inoculation while in MS medium the bud was found different sprouted in 10 days of inoculation. The highest rate of bud sprouting (3.3±1.86) among these three types of media was observed in MS+PGRs (BAP+NAA) (Table 1). It was observed within one week of inoculation (Figure 1B, C).

Earliest bud sprouting was observed in MS medium augmented with BAP+NAA at 5+1 mg/L concentration (Table 2). Zaki *et al*., (2011) also documented in his study on *Morus nigra* in which the best shoot induction was observed in 5 mg/L of BAP. In terms of shoot induction, obtained the MS medium and MS+PGR are considered better than the water agar medium (Figure 1 C, D)

Shoot multiplication and elongated multiple shoots were obtained in all three medium. In water agar medium the average number of shoots 2±1 per nodal explants were denoted while in MS medium the multiplied shoots per explants were 2.0±0.81 (Table 1). MS medium with different plant growth hormones showed the best result when the inoculation of BAP+NAA was applied (3.3±1.86). In terms of shoot multiplication also similar result has been achieved (Figure 1G, H).

Table 1: Effect of Different Plant Medium on Shoot Multiplication in the Nodal Segment of *Calotropis Procera*

Medium	Inoculated Explant	Established Explant	Average No. of Shoots Per Explant	Average Length of Shoots Per Explants
Water Agar Medium	15	6	2.0±1.00	1.04±0.036
MS Medium	20	8	2.0±0.81	1.22±0.219
BAP	5	2	3.0±1.41	2.00±1.00
KIN	5	3	3.0±2.0	1.10±0.00
2,4-D	10	4	1.0±0.0	0.85±0.70
IBA	5	2	3.0±1.41	1.32±0.02
BAP+NAA	15	6	3.3±1.86	1.29±0.134
BAP+GA_3	10	4	2.0±0.81	1.10±0.043

The maximum number of shoots 4±2.82 as well as highest percentage of explant response was observed in 5mg/L of BAP+1mg/L of NAA (Table 2). Similarly, in *Asclepias curassavica* the highest shoot multiplication was observed on BAP+NAA (2.0+0.5 mg/L) by Reddy *et al.,* (2012). These multiple shoots in this medium had grown within 4-5 weeks of inoculation. The minimum number if shoots were observed at 2, 4-D at 2.5 and 3 mg/L conc (Table 2 Fig 1G, H). Kanungo and Sahoo (2011) observed the average number of shoot was 4.16±0.05 in *Withania somnifera* in RT medium supplemented with NAA

(0.5 mg/L). Prema *et al*., (2013) reported 5 shoots per explant in *Cryptolepis grandiflora*. All explants and plantlets were transferred frequently after 20 days of inoculation into the medium.

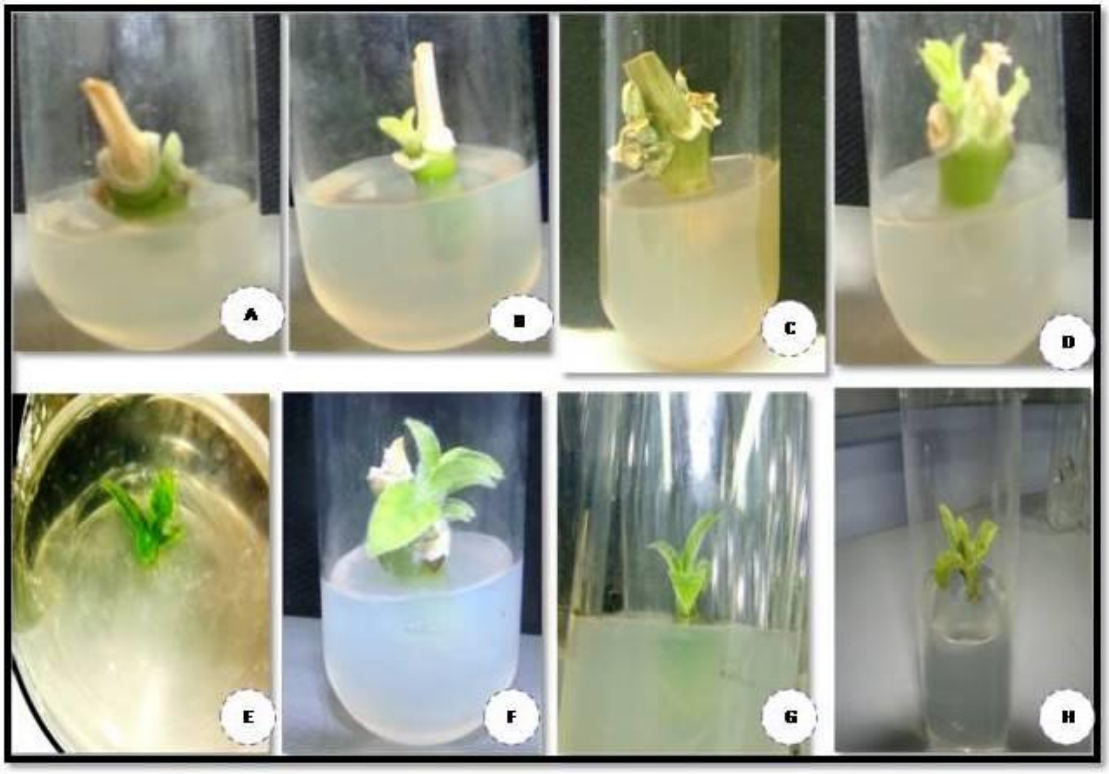

Fig. 1 Complete *in vitro* Shoot Multiplication of *Calotropis procera*

A. Inoculation of Explant B. Plant response after 10 days of inoculation C. Growth of explant after 15 days of inoculation D. Growth of explant after 20 days of culture E. Subculturing of plantlet in multiplication medium F. Subculting of second plantlet in to multiplication medium G. Multiplied shoot after 12 days H. Multiplied shoot after 20 days.

A significant increment in the length of the inoculated explants as well as in multiple shoots was also observed. The highest shoot length 2.00±1.00 was in BAP (3.0mg/L) alone (Table 1, 2) was observed. The minimum height of shoot 0.85±0.70 was observed in 2,4-D (Table 1).

Table 2: Effect of Different PGRs (Alone or in Combinations) at Different Concentration in MS Medium on Shoot Multiplication in the Nodal Segment of *Calotropis Procera*

Medium with Various PGRS	Conc.(mg/l)	Average No. of Shoots per Explants	Status of Shoot Induction	Average Length of Shoots in cm
BAP	3	3±1.41	++	2.0±1.00
KIN	3	3±2.00	++	1.1±0.00
2,4-D	2.5	1±0.00	+	0.8±0.00
2,4-D	3	1±0.00	+	0.9±0.00
IBA	3	3±1.41	++	1.32±0.02
BAP+NAA	5+1	4±2.82	+++	1.22±0.150
BAP+NAA	4+1	3±2.82	++	1.33±0.042
BAP+NAA	2+1	3±0.00	++	1.41±0.0141
BAP+GA_3	.5+2	2±1.41	+	1.10±0.028
BAP+GA_3	1+2	2±0.00	+	1.10±0.070

Conclusion

Maximum destruction of *C. procera* have resulted in the loss of several populations and plant have reached in the common and rare plant category as per the recorded of Red Data Book. Hence, this attempt of *in vitro* cultivation of this species would be able to regenerate and replant in the specific areas where threat have been reported. Further being rich in the alkaloids content through this study elite selection will be made for proper isolation and characterization.

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