Research Article

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# MICRO PROPAGATION AND ORGANIC STUDIES IN *ASTERACANTHA LONGIFOLIA* (L) NEES AND VARIATION IN PHENOL CONTENT IN CALLUS

**Santajit Bag, Harishankar Lal* and Prashant Kumar Mishra**
Department of Botany, Vinoba Bhave University, Hazaribag-825301, Jharkhand, India

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**Abstract:** Multiple shoots were induced from nodal segments and shoot tips of *Asteracantha longifolia* (Acanthaceae) and Ms Medium containing NAA (0.05mg/l) +Kinetin (0.25mg/l) was found to give the best shoot proliferation rate. Callus was induced from the leaf explants on the same medium supplemented with NAA (1.0 mg/l) + BAP (0.75mg/l). The *in vitro* proliferation shoots were rooted and later transferred to the soil.

**Keywords:** *Asteracantha longifolia* Medicinal plant, Acanthaceae, Plant Tissue Culture

## INTRODUCTION

Uniformity in the qualitative and quantitative traits among the cloned plants and their relative stability in the subsequent generation is an essential perquisite in plant improvement programmes. The perquisite for successful application of tissue culture technique is the systematic evaluation of different factors affecting plant cell growth and different *In vitro* conditions. In this regard it may be mentioned that advances in *In vitro* manipulation of plant have been achieved mainly on the environmental condition of cell culture. Beside the physiological state of the culture condition, plant genotypes are also important in development of plasticity and introduction of new genotypes sometimes face the problems of acclimatization plant is attacked by large number of disease, which cause tremendous loss in yield. Inter specific crosses do not accomplish by selective procedures. The limited successes in sexual hybridization in some cases affect manipulation of desirable one. Therefore biotechnological methods can be applied in selection and multiplication as well as plantlets regeneration from embryogenic cultures of plants. The test plants, when used as tincture of whole plants, (one part in three part of the alcohol) is effective in urinary ailments particularly dysuaria. Ashes are useful in dropsy and gravel, seeds, leaves and roots are used as diuretic, and for treatment of jaundice, rheumatism, and urinogenital[6]. Progress in plant cell tissue and organ culture as an unconventional tools has opened several new possibilities for the induction of genetic diversity and selection of genetic variants[9,3,2,10,1]. One of the practical applications of culture totipotency was *In vitro* multiplication of true types of plant from small tissue fragment or isolated cell of desire genotype. [4]has shown that small /inoculate grow at a transfer rate than larger inoculate. *Asteracantha longifolia* Nees was placed in a priority list of most important Indian medicinal plants. Evaluated on the basis of their medicinal importance, commercial value and potential for further research and development. In the present investigation attempts have been made to study the mode of regeneration from kulkhera tissue with different concentration of growth regulators in selective medium. Shoot bud plantlets regeneration *In vitro* may occur by the phenomenon of organogenesis i.e through the formation of shoot bud and root from nodal segment and callus or embryogenesis.

## MATERIAL AND METHODS

Nodal and shoot tip segments were collected from selected areas of elite donor plants, and washed thoroughly under running tap water. For sterilization, explants were treated with 0.5% (v/v) teepol for 10 minutes, washed three to four times with sterile distilled water and then immersed in 0.1% $Hgcl_2$ for 5 minutes and thoroughly washed in sterile distilled water at least three times.[8] (MS) medium[8] with 3% sucrose (w/v) adjusted to pH 7-7.8 with 8% (w/v) autoclaved at 1.05kg cm2 for 10 minutes. It was used as culture medium. For micro propagation the nodal segment were inoculated on MS medium fortified with various concentrations and combinations of NAA (0.25-0.75mg/l) + Kinetin (0.25-2mg/l). For organogenesis, the leaf discs along with midrib were inculcated on MS medium fortified with various concentrations and combinations of NAA and BAP (0.5-2mg/l) either individually or in combination. *In vitro* raised shoots were transferred to leaf strength MS media supplemented with NAA (0.5-1.5mg/l) or IBA (0.5-1.5mg/l) for rooting. All cultures were maintained at 25+_20◦C under at 16 hr photoperiod providing light intensity of 2000-3000 lux.

***Corresponding Author:**
**Harishankar Lal,**
Institute Of Forest Productivity,
Lalgutwa, Ranchi-835303,
Jharkhand, India.

## RESULTS AND DISCUSSION

Multiplications of multiple shoot formation were taken from the nodal segment for establishment of micro propagation. The medium was fortified with different types of hormones (NAA and Kinetin). Interestingly maximum percentage of establishment of explants was dependent much upon the concentration of auxin. The percentage of establishment of micro shoot formation gradually decreased due to increase in cytokinin. Constant amount of auxin (0.5mg/lo + Kinetin (0.25mg/l) was most suitable concentrations for the micro shoot initiation as it showed significantly highest regeneration of micro shoots (Table.1 fig a-b). Callus ignition was started from leaf explants on Ms basal medium when supplemented with NAA (1.0mg/l) + BAP (0.75mg/l). On MS medium (NAA 1.0mg/l) + BAP (1.0mg/l) resulted significantly highest callus induction than other combination. Through MS medium the combination NAA (1.0mg/l) +BAP (3.0mg/l) has shown significantly highest regeneration of plantlet 70 (56-79) a% (Table-2, fig. c-c) from callus. It was also found that further increase in BAP concentration up to 4.0mg/l reduce such regeneration of plantlets (Table.3). It is mentionable that the absolute growth factor requirements for both callus initiation and plantlet development are genotype dependence[7]. The percentage of phenol increased the fresh callus with advancement of days till 45 as studied. But this percentage of phenol reduces day by day when the callus is treated with charcoal under the same study period. (Table-4, fig.g). *In vitro* shoots were subsequently rooted (fig-f) in the medium supplemented with NAA or IBA (0-1.0mg/l). In the present investigation we have regenerated plantlets of *Asteracantha longifolia* through shoot tip and nodal culture to examine the performance of these plantlets in the field, prior to exploring commercialization of this protocol[5].

**Table.1:** Effect of growth regulators on direct regeneration of shoots from nodal segment and stem apices

| Auxin NAA(mg/l) | Cytokinin Kinetin (mg/l) | Direct regeneration of shoot % in MS basal medium |
|---|---|---|
| - | - | NR |
| 0.25 | - | NR |
| 0.5 | - | 10(18.38)$^{d}$ |
| 0.5 | - | 10(18.42)$^{d}$ |
| 0.75 | - | NR |
| - | 0.25 | NR |
| - | 0.5 | NR |
| 0.5 | 2.0 | 55(47.87)$^{b}$ |
| 0.5 | 0.25 | 70(54.98)$^{a}$ |
| 0.5 | 0.5 | 25(30.00)$^{c}$ |
| 0.5 | 0.75 | 10(18.43)$^{d}$ |
| SE (mean) | = 1.02 | |
| CD at (0.01) | =4.428 | |
| CV (%) | =5.66 | |
| Symbol | = without hormone | |
| NR | = No response | |

Superscripts (a-d) denote Duncan's test (DMRT) results at 5% level of significance where similar alphabets indicate hormongenous means.

**Table.2:** Effect of growth regulators on callus induction from leaf explants and nodal segments

| Auxin NAA (mg/l) | Cytokinin BAP (mg/l) | Induction and proliferation of callus (%) in MS basal medium |
|---|---|---|
| 0.5 | 0.25 | NR |
| 0.5 | 0.5 | NR |
| 1.0 | 0.75 | 42(40.39)$^{b}$ |
| 1.0 | 1.0 | 80(63.44)$^{a}$ |
| 1.0 | 1.5 | 40(39.23)$^{b}$ |
| 1.0 | 2.0 | 30(33.21)$^{c}$ |
| SE (mean) | = 0.60 | |
| CD at (0.01) | =2.867 | |
| CV (%) | =2.375 | |
| Symbol: | | |
| NR | =No response | |

Superscripts (a-c) denote Duncan's test (DMRT) results at 5% level of significance, where similar alphabets indicate homogenous means.

**Table.3:** Plantlet regeneration from callus on different media

| Auxin NAA (mg/l) | Cytokinin BAP (mg/l) | Regeneration of plantlet % from callus in MS Basal media |
|---|---|---|
| 0.5 | 0.75 | NR |
| 0.5 | 1.0 | NR |
| 0.5 | 1.5 | NR |
| 0.75 | 1.0 | NR |
| 1.0 | 2.0 | 15(22.74)$^{d}$ |
| 1.0 | 2.5 | 40939.23)$^{b}$ |
| 1.0 | 3.0 | 70(56.79)$^{a}$ |
| 1.0 | 3.5 | 32(34.45)$^{c}$ |
| 1.0 | 4.0 | 10(18.42)$^{e}$ |
| SE (mean) | =0.612 | |
| CD at (0.0100 | =2.744 | |
| CV (%) | =3.091 | |
| Symbol NR | = No response | |

Superscript (a-e) denotes Duncan's test (DMRT) results at 55 level of significance, where similar alphabets indicate homogenous means.

**Table.4:** Estimation of phenol content from callus.

| Days | Phenol from fresh callus | Phenol from treatement of callus with charcoal on MS basal medium |
|---|---|---|
| 15 | NR | 0.007408 |
| 20 | NR | 0.005728 |
| 25 | 0.003496 | 0.00428 |
| 30 | 0.00608 | 0.00296 |
| 35 | 0.00676 | 0.00208 |
| 40 | 0.00864 | 0.00204 |
| 45 | 0.00867 | 0.00200 |
| Mean (n=5) | | |
| Symbol | | |
| NR=No response | | |

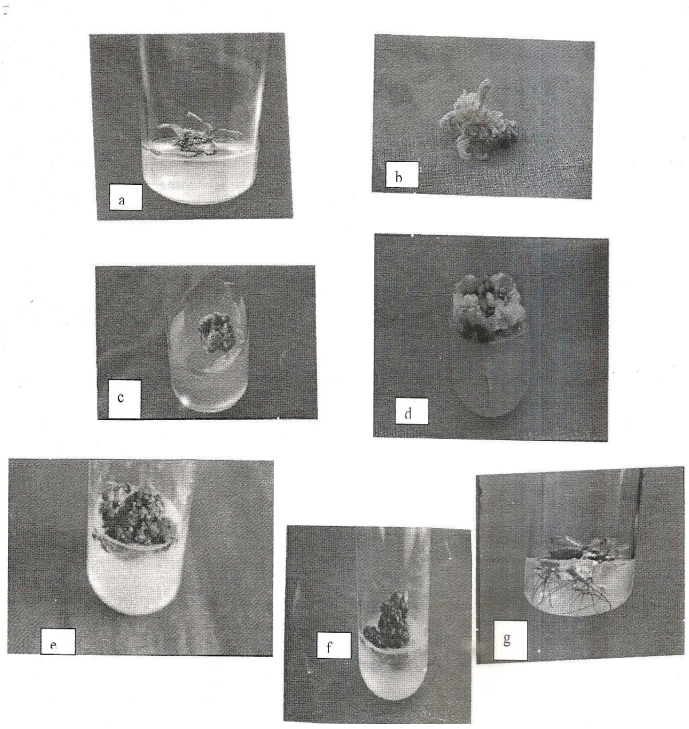


**Fig: (a-b)** Induction and multiple shoot formation from nodal explants
**(c)** Induction of callus from the cut surface of the young leaf explants
**(d)** Proliferation of callus
**(e)** Regeneration of shoot buds to plantlets
**(g)** Induction of root
**(f)** Secretion of phenolic compound on callus

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