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# Callus induction in a magic herbal medicinal plant *achyranthes aspera* L. on leaf explant at different hormonal combinations

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**Abstract :** To promote *in vitro* callus induction, in leaf segments of *Achyranthes aspera* L. were inoculated on MS medium with different concentrations of 2,4 –D alone and different combinations with NAA ,BAP ,IAA ,IBA. The explants were maintained at (25°C ± 1°C) and 16 h light cycle. The best calluses develop with IBA (1.0 ppm) in combination with BAP (1.0 ppm). On this combination of hormones light green and soft compact callus was observed.The responses of callus induction with other hormonal combinations were negative. The present paper depicts the callus induction in *Achyranthes aspera* L. in *in vitro* conditions that provide a novel broad spectrum among researchers as a protocol for further research and investigation in tissue culture of magic herbal plant *Achyranthes aspera* L.

**Key words: Achyranthes aspera , 2, 4 - D, BAP in vitro, Callus, Pharmaceutical.**

## INTRODUCTION

Advances in plant biotechnology research in the last decade has opened a new vistas in the propagation of plants with improved resistance to diseases, pests, herbicides, and stress etc. Plants have been used in traditional medicine for several thousand years.The use of traditional medicine in most developing countries is a normative basis for the maintenance of good health as quoted by (Lucy Hoareau and Edger Dasila,1999).

Medicinal plants have been used as an exemplary source for centuries as an alternative remedy for treating human diseases because they contain numerous active constituents of therapeutic value (Nostro *et. al.,*2000).

In India, herbal medicines have been the basis of treatment and cure for various diseases and physiological, conditional in traditional methods practiced such as Aurveda,Unani and Sidha (Perumalsamy *et. al*.,1999).

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*Achyranthes aspera* L. (chirchira or latjira) is an abundant indigenous herb found throughout the country. This plant belongs to the family Amaranthaceae, all parts (root, stem , leaf and seed) of the plants are commonly used by traditional healer for the treatment of number of diseases like malaria, dysentery, asthma, diabetes, bronchitis, piles, dyspepsia and cardiac disorder.

The secondary metabolites of the plants like saponin, oleanic acid, achyranthine, ecdysterone, alkaloids, flavonoids, steroids and terpenoids are good sources of medicines, that cure above mentioned diseases magically.The secondary metabolites of the plants are the major sources of pharmaceutical, food additives and fragrances(Danhankar *et.al*.,2000).

The dried aerial parts are taken orally in case of diabetes ,the powder made from the dried plant is given orally against whooping cough .Roots of achyranthes are used as medicine for diarrhoea and the leaves are used against hypoglycemic activity and asthma (Chakrabarti and Vasudeva,2006). Cancer chemopreventive activity of *Achyranthes aspera* L.metholic extracts of leaves reported

by Chakarbarti and Adelhaid Branter,2002.

In last few decades, due to rapid decrease in natural vegetations there is limited supply of medicinal plants, which has prompted new methods development for their supply. *In vitro* propagation of plant is the best method for production of plant base medicines. Present paper describes callus development in leaf explants of *Achyranthes aspera* L. with different combinations of NAA, BAP, and IAA for micropropagation.

In India tissue culture of *A. aspera* was not been previously studied. However, Kayani 2008 Pakistan did work on this plant for callogenic studies. presence of wide range of chemical compounds in *achyranthes aspera L.* indicates that it could serve as "lead" for the development of novel agents having good efficacy in various pathological disorders in the coming years.

## MATERIALS AND METHODS

*Achyranthes aspera* L. (Amaranthaceae) plant has been collected from field near Argora. The fresh new and soft leaves were collected and washed with bavestin for 10-15 min followed by washing with running tap water and rinsed with distilled water. The leaves were sterilized by 0.1% $HgCl_2$ solution for 2-3 min and washed 3-4 times with sterilized distilled water in laminar air flow cabinet. The explants were dried using sterile filter paper and inoculated into culture jars and flasks with MS medium (Murashige and Skoog, 1962) containing 3% sucrose solidified with 0.8% agar. The aqueous solutions of phytohormones Indole-3-butyric acid (IBA), 6- benzyl amino purine (BAP), 2,4-Dichlorophenoxy acetic acid(2,4-D) and naphthaline-1- acetic acid (NAA) were added in required concentrations. The PH of the medium was adjusted to 5.8 followed by autoclaving at 121°C and 1.06 $kg/m^2$ for 15 min in autoclave. The cultures were incubated at 25°C ±1°C with 16 h light and 8 h dark cycle.Each experiment was repeated three times.

## RESULTS

*Achyranthes aspera* leaf explant in MS medium supplemented with different concentrations of 2,4-D(0.5 ppm.1.0 ppm,1.5 ppm,2.0 ppm) gave scanty callus. Other auxins like IAA and NAA alone in different concentrations given no callus. However, MS medium supplemented with 2,4-D(1.0 ppm) + BAP(1.0 ppm) and IBA(1.0 ppm) + BAP(1 ppm) showed callus induction. Initially hypertrophy of leaf was seen after seven days and callus formation started from 15 days onwards. The callus colour was either light green or yellowish brown depending on nature of phytohormones. The callus was compact and non-embryonic. On 5$^{th}$ week the callus growth was maximum, afterwards browning of callus started and showed necrosis at later stage.

When nodal segment was taken as explant, no callusing was seen rather shoots developed directly. The callusing responses with respect to various concentrations of hormones with leaf explants are presented in table 1.

**Table 1:Callus induction in leaf explant of *Achyranthes aspera* L.**

| Hormones | Conc.(ppm) | No. of culture vessels | Culture vessel with callus | % callusing | Morphological character |
|---|---|---|---|---|---|
| 2,4-D | 0.5 | 5 | 1 | 20 | Only swelling occurs in leaf explants |
| | 1.0 | 5 | 1 | 20 | Scanty callus |
| | 2.0 | 5 | 0 | 0 | Callus not develop |
| 2,4-D/BAP | 0.5/0.5 | 5 | 2 | 40 | Medium callus |
| | 1.0/1.0 | 5 | 3 | 60 | Light green friable |
| | 2.0/2.0 | 5 | 3 | 60 | Light green friable |
| IBA/BAP | 0.5/0.5 | 5 | 4 | 80 | Light green and yellowish brown soft non-embryonic |
| | 1.0/1.0 | 5 | 5 | 100 | Light green and yellowish soft compact non-embryonic |
| | 2.0/2.0 | 5 | 4 | 80 | Light green and yellowish |
| NAA/BAP | 0.5/0.5 | 5 | 0 | 0 | Callus not develop |
| | 1.0/1.0 | 5 | 0 | 0 | Callus not develop |
| | 2.0/2.0 | 5 | 0 | 0 | Callus not develop |

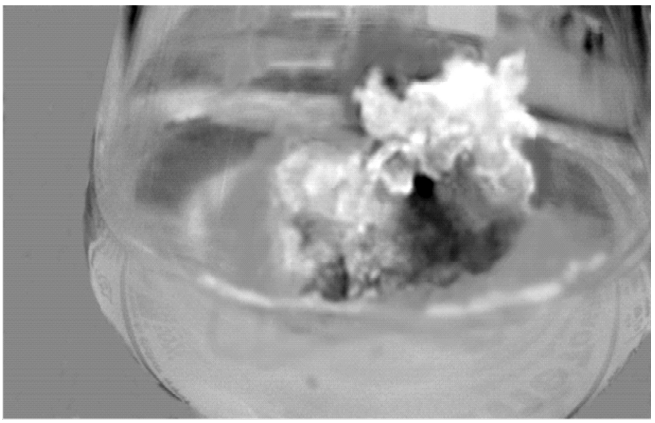

**Fig 1: 28 days old callus induction from leaf explant of A. aspera in a MS + IBA(1 ppm) + BAP(1 ppm)**

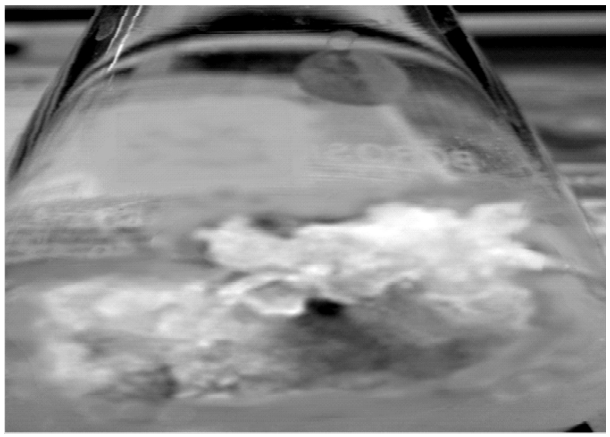

**Fig 2 : 35 days old callus intionduc from leaf explant of A.aspera in a MS+IBA(1 ppm) + (BAP(1 ppm)**

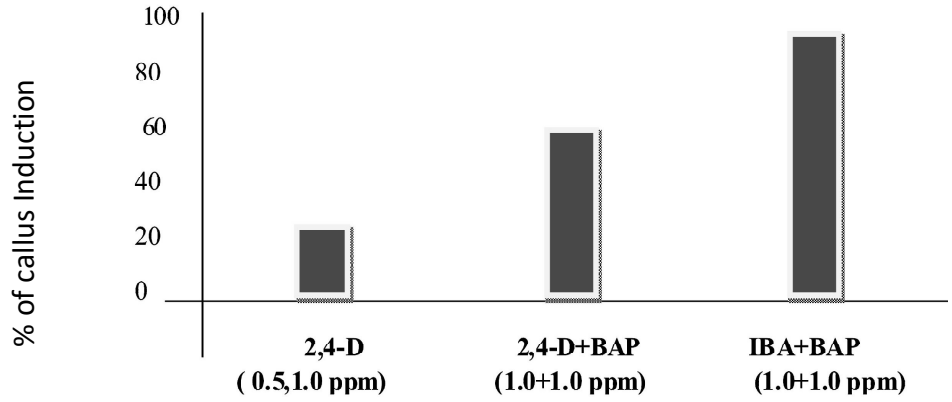


**Fig.3: Histogram showing percentage of callus response with respect to phytohormones on leaf explant.**

## DISCUSSION

In order to carry out any advance studies, callus induction and regeneration are preconditions. *Achyranthes aspera* L. is known to be widely used in different types of diseases. However, reports on tissue culture studies in *A.aspera* is less. In the present investigation callus induction was observed from leaf explant of *A.aspera* by different types of hormones supplemented in the MS medium. The percentage of callus induction with different concentrations of 2,4-D alone was very less, i.e. 20% but callus response with 2,4-D + BAP was recorted to be 60%, and the nature of callus was found to be friable. The callus response with combination of IBA and BAP was 100% and the nature of callus was seen to be compact. The callus growth was maximum in 4 - 5 week old culture. These findings are in agreement with the results of Kayani *et. al.*(2008) and Wesely *et.al.*(2010). The timing of surface sterilization was found to be very important in the present investigation. $HgCl_2$ treatment more than 3 min has lethal effect.The same result was also reported by Kayani et. al.(2008) and Wesely et. al.(2010).

The effect of 2,4 -D on callus induction have been already been reported by large number of scientists(Zenk ,1978, Wakhlu and Sharma, 1998 and De-Silva *et.al.*,2003). The synergistic effect of two or more hormones have already been reported by other scientists (Mischenko and Fedoreyev,1999, Kayani *et. al.*,2008).

In the present investigation best result for callus

induction was seen on MS supplemented with IBA(1ppm) + BAP(1ppm).

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