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Impact of growth regulators on callus production of *Asystasia gangetica* (L) T. Anderson

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ABSTRACT

In vitro callogenesis was achieved from the leaf explants of Asystasia gangetica (L) T. Anderson, an important medicinal plant belonging to the family Acanthaceae. The leaf explants were inoculated on MS medium provided with 3% sucrose and 0.8% agar, supplemented with various concentrations and combinations of 2,4-D, NAA, IAA, IBA, BAP and KIN. The inoculated explants were maintained in culture room at $25^0C \pm 2^0C$ and 16 hr light period. Healthy, profuse, green and fast growing callus but with low regenerative capacity was observed on MS medium provided with 15µM NAA in combination with 3µM BAP and 3µM KIN. 2,4-D showed sticky , fragile, white and non-regenerative callus but with poor callus induction percentage. The other two auxins, IAA and IBA showed better callus induction in combination with cytokinins and the callus so formed were pale green to brown. The synergistic effect of auxins with cytokinins in callus induction was found to be better. This is the first report of in vitro callogenesis in Asystasia gangetica.

Keywords*: Asystasia gangetica*, callogenesis, auxins, cytokinins, MS Medium.

INTRODUCTION

Asystasia gangetica (L) T.Anderson (Acanthaceae), commonly known as Chinese Violet or Creeping Foxglove is distributed in Tropical and Subtropical Old World regions (Mabberley, 1987). It is a rapidly growing perennial herb reaching 600 mm in height or up to 1 m if supported. The stems root easily at nodes. The leaves are simple or opposite. The fruit is an explosive capsule which starts out green in colour but dries to brown after ripening. It is occasionally planted as an ornamental herb and used as a leafy vegetable in time of food scarcity. *Asystasia* is known to have high nutritional value as it contains rich amount of proteins, aminoacids, minerals like calcium, phosphorus, sodium, manganese, copper, zinc, iron and magnesium and fibers (Yeoh and Wong, 1993; Odhav *et al*., 2007; Tilloos *et al*.,2012).

Asystasia is mainly used in mild hypoglycaemia (Kirtikar and Basu, 1998; Suvarchala Reddy *et al*., 2010). It has also been claimed to have anti-asthmatic, antihelminthic, antidiabetic, anticancer and anti-oxidant properties (Kirtikar and Basu, 1998; GuhaBakshi *et al*.,1998 Akah *et al*., 2003; Yang *et al*., 2006; Sudhakar *et al*., 2006 and Gopal *et al*.,2013 ; Kavitha Sama *et al*.,2013). High medicinal value of *A. gangetica* is due to the presence of various biologically active substances such as carbohydrates, proteins, alkaloids, tannins, steroidal aglycones, saponins, flavonoids, iridoids, megastigmanes and terpenoids (Morokola, 2002; Kanchanapoom and Ruchirawat, 2007; Hamid *et al*.,2011 and Mary Kensa, 2011).

As the plant *Asystasia* found to contain several important secondary metabolites, the present investigation aims to produce callus from leaf explants. This is the first tissue cultural report in *A.gangetica.*

MATERIALS AND METHODS

The whole plants of *A. gangetica* were collected from the locality of Thanthondrimalai, Karur district, Tamilnadu, India. Healthy and young leaves were selected as explants source. The leaves were initially washed with Teepol followed by rinsing with running tap water for 30 minutes. Then the explants were treated with 70% ethanol for 45 seconds and washed with sterile distilled water for 4-5 times. Later, the explants were sterilized with 0.1% $HgCl_2$ solution for 2 to 3 minutes and finally washed 3-4 times with sterilized distilled water, inoculated into culture tubes inside the Laminar Flow Chamber.

Culture media

The basal MS medium (Murashige and Skoog, 1962) with 3% sucrose solidified with 0.8% agar was used. Several concentration and combination of growth regulators were tested including 2, 4-D, NAA, IAA, IBA, BAP and KIN. The pH of the medium was adjusted to 5.8 before autoclaving at 121ºC for 20 minutes. The cultures were maintained at 25ºC±2ºC with 16 hr photoperiod. After 30 to 45 days, the cultures were visually observed for the formation of callus texture, coloration, etc. Well grown callus induced from cultures were selected to sub- culture on the same medium after every 4-5 weeks.

Statistical Analysis

Experiments were carried out with 10 replicates and were repeated five times. Mere visual observations of the cultures were made every week and the percentage of cultures showing callus were recorded.

RESULTS AND DISCUSSION

Callus production from leaf explants of *A.gangetica* were observed at different concentrations and combinations of auxins and cytokinins.

1)Effect of 2, 4-D on Callus Induction (Table 1. Fig.1-a,b)

2,4-D at very low and high concentrations showed low percentage of callus induction. At 1 µM 2,4-D only 38% of callus response was recorded and at 25µM 2,4-D 48% of callusing was recorded. But 15 µM 2,4-D produced 60% of callusing which proved moderate concentration of 2,4-D is effective in callus induction in *A. gangetica*. Moreover, 15 µM 2,4-D when combined with 3µM BAP and 3µM KIN produced 80% of callusing proved the synergistic effect of auxins with cytokinins in induction of callus. Likewise, 10 µM 2,4-D showed only 64% of callusing, whereas, 10µM 2,4-D with 2 µM BAP and 2µM KIN produced 74% of callusing.

But surprisingly, 20 µM and 25 µM 2,4-D produced only 62% and 48% of callus induction respectively when tested alone and also in combination with BAP and KIN. So it was cleared that the synergistic effect of auxins with cytokinins in callus induction was effective only up to certain level of concentration and after reaching certain level of concentration there was no effect. The callus induced from 2,4-D was found to be white, sticky and light green, fragile and non-regenerative. The white callus turns browning gradually. It might be due to high phenol content and due to oxidation of phenolic compounds. In contrast to this results obtained, 2,4-D was found to be effective in callus induction of many plant species (Sumathi *et al.*, 2003; Kayan i*et al.*, 2008; Kaladhar *et al.*, 2012).

2) Effect of NAA on Callus Induction (Table 1, Fig.2)

NAA supplemented medium showed flourished growth of callus. Of various concentrations of NAA tested, 15 µM NAA showed good callusing (94%) followed by 10 µM NAA which produced 90% of callusing. The synergistic effect of auxins with cytokinins in callus induction was also found to be greater in NAA. 15 µM NAA in combination with 3 µM BAP and 3 µM KIN produced 100% callus induction.

Likewise, 5 µM NAA produced only 68% of callus induction, whereas, the same concentration of NAA (5 µM) with cytokinins (1 µM BAP and 1 µM KIN) produced 84% of callusing indicating the synergistic effect of auxins with cytokinins in callus induction. This finding is in harmony with those results obtained by Rao *et al.*, (2006) in *Gossypium* species, Ghada Abd El-MoneimHegazi (2011) in *Delonix elata*, L and Mousavi *et al*., (2012) in *Eustoma grandiflorium.*

The callus observed in NAA supplemented medium was fragile turned into compact, green and regenerative in nature with few adventitious buds in regenerative medium. But the regenerative potential was found to be very low.

3) Effect of IAA on callus Induction (Table 2, Fig.1-c,d)

At low concentration (1µM IAA) produced only 38% of callus and at higher concentration (25 µM) produced 66% of callusing. But 10 µM and 15 µM concentrations of NAA showed 72% and 84% of callusing respectively.

Moreover the callus induction capacity was very much pronounced when IAA was combined with cytokinins. Basal medium with 10µM and 15µM IAA when combined with cytokinins produced 84% and 96% of callusing respectively showing the synergistic effect of auxins with cytokinins. The callus formed due to IAA fortified medium was found to be white fragile and pale green and non-regenerative in nature. In *Phyllanthus stipulus*, the plant growth regulators 2,4-D, IAA, IBA, BAP and 2ip were equally effective in callus induction (Elizabete Catapan *et al*., 2001).

4) Effect of IBA on callus Induction (Table 2, Fig.1- e,f)

IBA showed moderate callus induction. IBA at 1 µM, 5 µM, 15 µM, 20 µM and 25 µM produced 36, 52, 82,80 and 62 percentage of callusing respectively. But the same concentrations of IBA when combined with cytokinins produced 40, 66, 96, 88 and 76 percentages of callusing respectively. The callus observed on IBA supplemented medium was compact, brown in colour and non-regenerative in nature. The calli were turned brown in colour after few days and are necrosesed. David Paul Raj *et al*., (2010) reported that in *Callistemon citrinus* L., IAA and IBA in combination with KIN showed significant callus induction.

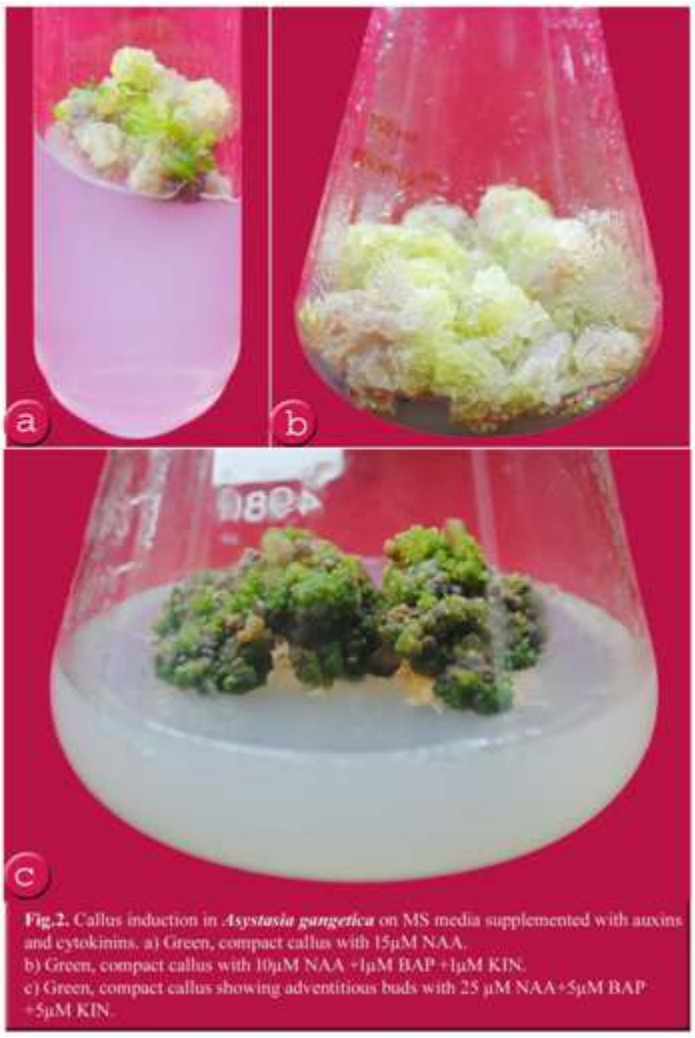

Fig.2. Callus induction in ***Asystasia gangetica*** on MS media supplemented with auxins and cytokinins. a) Green, compact callus with 15µM NAA.
b) Green, compact callus with 10µM NAA +1µM BAP +1µM KIN.
c) Green, compact callus showing adventitious buds with 25 µM NAA+5µM BAP +5µM KIN.

Table-1: Effect of 2, 4-D and NAA alone and in combination with cytokinins on callus induction of *Asystasia gangetica* (L) T. Anderson

Name of Growth Regulators	Concentration of Growth Hormone (µM)	Percentage of Callus Induction (%)	Nature of Callus
2,4-D		38±3.34	White , Sticky
2,4-D	1	46±4.55	White , Sticky
2,4-D	2	44±3.57	White , Sticky
2,4-D	3	52±3.34	White , Sticky
2,4-D	4	42±3.34	White , Sticky
2,4-D	5	64±2.18	White , Sticky
2,4-D	10	60±3.99	White , Sticky
2,4-D	15	62±3.34	White , Sticky
2,4-D	20	48±3.45	White , Sticky
2,4-D+BAP+KIN	25	50±2.83	Light Green, Fragile
2,4-D+BAP+KIN	5 +1+1	74±4.55	Light Green, Fragile
2,4-D+BAP+KIN	10+2+2	80±3.99	Light Green, Fragile
2,4-D+BAP+KIN	15 +3+3	62±3.34	Light Green, Fragile
2,4-D+BAP+KIN	20 +4+4	48±3.45	Light Green, Fragile
NAA	25 +5+5	42±5.21	Green, Compact
NAA	1	44±4.55	Green, Compact
NAA	2	48±5.21	Green, Compact
NAA	3	62±3.34	Green, Compact
NAA	4	68±3.34	Green, Compact
NAA	5	90±2.83	Green, Compact
NAA	10	94±2.18	Green, Compact
NAA	15	84±2.18	Green, Compact
NAA	20	80±2.83	Green, Compact
NAA+BAP+KIN	25	72±3.34	Green, Compact
NAA+BAP+KIN	5 +0.5+0.5	96±2.18	Green, Compact
NAA+BAP+KIN	10 +1+1	94±2.18	Green, Compact
NAA+BAP+KIN	15 +1.5+1.5	82±3.34	Green, Compact
NAA+BAP+KIN	20 +2+2	76±2.18	Green, Compact
NAA+BAP+KIN	25 +2.5+2.5	84±2.18	Green, Compact
NAA+BAP+KIN	5 +1+1	96±2.18	Green, Compact
NAA+BAP+KIN	10 +2+2	100±0	Green, Compact
NAA+BAP+KIN	15 +3+3	88±3.34	Green, Compact
NAA+BAP+KIN	20 +4+4	86±2.18	Green, Compact
	25 +5+5		

Values are Mean±SE Experiments were repeated 5 times with 10 replicates

Table-2: Effect of IAA and IBA alone and in combination with cytokinins on callus induction of *Asystasia gangetica* (L) T. Anderson

Name of Growth Regulators	Concentration of Growth Hormone (µM)	Percentage of Callus Induction (%)	Nature of Callus
IAA	1	38±3.34	White , Fragile
IAA	2	40±2.83	White , Fragile
IAA	3	42±5.21	White , Fragile
IAA	4	50±2.83	White , Fragile
IAA	5	54±4.56	White , Fragile
IAA	10	72±3.34	White , Fragile
IAA	15	84±2.18	White , Fragile
IAA	20	76±2.18	White , Fragile
IAA	25	66±2.18	White , Fragile
IAA+BAP+KIN	5 +0.5+0.5	48±3.34	Pale Green, Fragile
IAA+BAP+KIN	10 +1+1	44±4.55	Pale Green, Fragile
IAA+BAP+KIN	15 +1.5+1.5	52±5.21	Pale Green, Fragile
IAA+BAP+KIN	20 +2+2	60±2.83	Pale Green, Fragile
IAA+BAP+KIN	25 +2.5+2.5	62±3.34	Pale Green, Fragile
IAA+BAP+KIN	5 +1+1	66±2.18	Pale Green, Fragile
IAA+BAP+KIN	10 +2+2	84±2.18	Pale Green, Fragile
IAA+BAP+KIN	15 +3+3	96±2.18	Pale Green, Fragile
IAA+BAP+KIN	20 +4+4	90±2.83	Pale Green, Fragile
IAA+BAP+KIN	25 +5+5	78±3.34	Pale Green, Fragile
IBA	1	36±3.57	Brown, Fragile
IBA	2	38±3.34	Brown, Fragile
IBA	3	44±4.55	Brown, Fragile
IBA	4	50±2.83	Brown, Fragile
IBA	5	52±1.78	Brown, Fragile
IBA	10	86±2.18	Brown, Fragile
IBA	15	82±3.34	Brown, Fragile
IBA	20	80±3.99	Brown, Fragile
IBA	25	62±3.34	Brown, Fragile
IBA+BAP+KIN	1 +0.5+0.5	40±4.89	Brown, compact
IBA+BAP+KIN	2 +1+1	44±4.55	Brown, compact
IBA+BAP+KIN	3 +1.5+1.5	54±4.55	Brown, compact
IBA+BAP+KIN	4 +2+2	62±3.34	Brown, compact
IBA+BAP+KIN	5 +2.5+2.5	66±2.18	Brown, compact
IBA+BAP+KIN	5 +1+1	70±2.83	Brown, compact
IBA+BAP+KIN	10 +2+2	90±2.83	Brown, compact
IBA+BAP+KIN	15 +3+3	96±2.18	Brown, compact
IBA+BAP+KIN	20 +4+4	88±1.78	Brown, compact
IBA+BAP+KIN	25 +5+5	76±2.18	Brown, compact

Values are Mean±SE Experiments were repeated 5 times with 10 replicates

CONCLUSION

Because of lack of previous report on *in vitro* callus production of *Asystasia gangetica* (L) T. Anderson, it was decided to test different media for callus induction. Of various auxins tested for callus induction of *A. gangetica*, MS medium fortified with 15 µM NAA + 3 µM BAP + 3 µM KIN showed best results with 100% callus induction. Superiority of NAA for callus induction has also been reported in different plant species, viz., in Flax (Burbulis *et al*., 2007), *Momordica dioica* (Nabi *et al*., 2002), *Rauwolfia sserpentiana* (Tomar and Tiwari, 2006),*Erigeron breviscapus* (Lei *et al.*, 2007) and *Eustoma grandiflorium* (Mousavi *et al*., 2012). But the rate of regeneration observed in this study was very low. IAA and IBA showed moderate callus induction capacity and 2,4-D poor callusing. Callus growth and development are strongly related to genetic background and physical state of the culture medium and cultural conditions (Cassells and Curry, 2001).

Effective callus induction due to the synergistic effect of auxins with cytokinins was also proved with all type of growth hormones tested. Moreover, it was also found that the synergistic effect was effective only up to certain level of concentration of hormones and after reaching certain level of concentration there was no effect. A number of plant tissues in *in vitro* synthesize growth regulators in sub-optimal amounts and therefore, require addition of external phytohormones (Reinert and White, 1956; Gamberg *et al.*, 1968). It might be concluded that the external phytohormones should also be effective at sub-optimal concentrations for callus induction or shoot regeneration. All those calluses obtained from different media were necrosesed after 45 days. This might be due to high phenol content of the plant (Rabha Abdelwahd, 2008) or due to high concentrations of auxin and low concentration of cytokinin (Bornman, 1983; Bonga, 1988).

The present investigation first reports the production of callus from *Asystasia gangetica* (L) T. Anderson.

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