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# Micropropagation Studies of Senna Alata (L.) Roxb. an Anti Allergenic Plant

KEYWORDS: Senna alata, Anti allergenic plant, Micropropagation, Rooting and Acclimatization

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**ABSTRACT** *Senna alata (L.) Roxb., an important medicinal plant, belongs to the family Ceasalpiniaceae. It is used in several ways for different ailments like skin infections against fungi constipation and intestinal worms etc. The present investigation outlines the in vitro propagation of S. alata, to develop a standard protocol to initiate multiple shoot induction which provides a good source of pharmacologically active plant constituents like chrysophanic acid, sennosides and anthraquinones which were used in an industrial level for preparation of drugs. An in vitro propagation has been investigated by MS medium containing 6-Benzyl adenine (8.8µM and 6.6µM), Kinetin (6.9µM and 9.2µM), Gibberellic acid (1.4µM) and 3% sucrose which induced maximum number of shoots and beneficial shoot lengths by nodal and hypocotyl explants. The rooting was initiated at half strength MS medium consist indolebuteric acid (7.3µM), which promoted well rooted plantlets and showed highest percentage (81%) survival in the field conditions without any morphological variation.*

## Introduction

*Senna alata* L. is a large shrub with very thick finely downy branches. It is named for its flower buds which grow in a column and looks like fat yellow candles each complete with a flames. It is found commonly in Somalia, Saudi Arabia, some parts of Pakistan and Kutch area of Gujarat. It is largely cultivated in Madurai, Ramanathapuram, Salem and Tiruneleveli, districts of Tamilnadu for its medicinal values. It occurs in diverse habitats in the tropics and containing some chemical substances like, chrysophanic acid, rhein, emodin, sistosterol and sennoside A, B, C and b-physcione. It is used in several ways for different ailments like skin infections against fungi, constipation and intestinal worms etc. The leaves are pounded and rubbed on the skin to cure eczema, ringworm and fungal infections (Pieme et al. 2006; Palanichamy and Nagarajan, 1990). The ethanolic extract of *S. alata* leaves showed efficient results against antifungal (Sule et al. 2010), bacterial activity (Ibrahim and Osman, 1995; Khan et al. 2001; Ogunjobi and Abiala, 2013), analgesic activity (Villasenor et al. 2002) and used in treatment of pityriasis vesicular, anti inflammatory activity. Stem bark could be potential source of active antimicrobial agents, and a detailed assessment of its *in vivo* potencies and toxicological profile is therefore advocated. The present investigation report delivered the micropropagation of *S. alata* through nodal and hypocotyl explants and subsequent establishment of plantlets in the field.

## Material and Methods

Matured pods were collected in the months of December 2010 from Mulugu Division of Warangal District, Andhra Pradesh, India. Seeds were extracted manually and air dried at normal temperature. Complete dry seeds were considered for testing of germination. The morphological traits of pod (pod length, width and number of seeds per pod) and seed (seed length, width and thickness) were measured. The experiment was laid out in complete randomized designed with five replications each, 100 seeds were taken for replication under both *in vitro* and *in vivo* conditions. Seeds were germinated in natural soil and *in vitro* on MS basal medium. But in both the cases showed effective seed germination of around 85-86% was seen, when explants taken from such plant lets.

Hormones obtained from Himedia laboratories and analytical grade chemicals were used for preparing the stock solutions and subsequent media preparation. The basal medium consisted of MS salts (Murashige and Skoog 1962) supplemented with $B_5$ vitamins (0.5mg/l nicotinic acid, 0.5mg/l pyridoxine HCl, 1mg/l Thiamine HCl and 100mg/l Myoinsosital (Gamborg et al. 1968). 30g/l sucrose and 0.8% agar for gel rite. The pH of the medium was adjusted to 5.8 prior to autoclaving, at molten medium was dispensed in to test tube (Borosil) at 20ml per tube and plugged with non absorbent cotton. Culture tubes containing the media were autoclaved at 121°c for 20min. Surface sterilized explants were inoculated vertically and horizontally on to the culture medium one or two explants per each test tube. All the cultures were maintained at 25 ± 2°c under 16h photoperiod at photosynthetic flux of 35-50 µmol $m^{25^1}$ provided by 100/daylight florescent lamps.

Hypocotyl and nodal explants were cultured on MS medium supplemented with 3% sucrose with 0.8% agar and supplemented with different combinations and concentrations of plant growth regulators including 2.2-13.3µM BA, 2.4-11.5µM Kinetin and addition of 0.5µM Gibberellic Acid. Elongated shoots were excised from each culture passage and transferred to half strength MS medium containing 3% sucrose and 0.8% agar. The medium was further supplemented with different concentrations of Indole Acetic Acid (IAA), IBA and NAA individually.

Plantlets with well developed roots were removed from the culture medium and washed the roots gently for removal of agar traces, and then plantlets were transferred to plastic cups containing autoclaved garden soil, farm yard manure and sand (1:1:1). Each was irrigated with one-eighth strength MS basal salt solution devoid of sucrose and inositol every $4^{th}$ day for 2 weeks. The potted plantlets were covered with porous poly ethylene sheets for maintain high humidity and maintained inside the culture room conditions. The relative humidity was reduced gradually after 40 days the plantlets were transferred to a Botanical garden and kept under shading in a net house for further growth and development. The morphological characteristics, growth characteristics and floral features were examined. Total plants developed *in vitro* (81%) were transferred on to the field. The developed protocol will be useful for large scale production at industrial level.

## Statistical analysis

Ten to 15 explants were used per treatment in each replication. Ten replications were considered and repeated thrice.

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The observations were recorded on the frequency (number of cultures responding for axillary shoot proliferation and root development) and the number of shoots per explant, shoots length, roots per shoot, and root length respectively. The analysis of variance (ANOVA) appropriate for the design was carried out to detect the signature of differences among the treatment means. The treatment means were compared using Duncan's (Duncan 1955) multiple range tests at a 5% probability level according to Gomez and Gomez (1976).

**Results and Discussion**

In the present investigation, the hypocotyl and nodal explants were excised from in *vitro* germinated seedlings after 28 days of germination. Excised explants cultured on MS medium used for culturing of shoot induction contained different levels of BA, Kn, TDZ and combination with NAA for shoot regeneration. The MS medium contained different concentrations of Cytokinins BA (2.2-13.3µM), Kn (2.4-11.6µM), TDZ (2.2-9.0µM) and combination with NAA (2.6, 5.3 &b10.7µM). Carbon source also one of the major component in high frequency of shoot regeneration. MS medium also contain 3% sucrose 0.8% agar. This media was satisfactory for shoot proliferation for both nodal and hypocotyl explants. Sucrose is better source of carbon than other carbohydrates like glucose fructose, its showed better shoot regeneration (Romano et al. 1995).

MS medium supplemented with 8.8µM BA, 83% of nodal explants produced shoot induction after 7 weeks of culture, average of 4.2 shoots per each explant (Fig.1 a,b,c). Bhaskaran and Jayabalan,(2005) reported the similar results in *Eclipta alba* with 8.8µM BA. 85% shoot proliferation was initiated from the hypocotyl explants cultured on MS medium containing 6.6µM BA (Table 1). The hormonal combination (BA+NAA) at level of 6.6µM+5.3µM showed 90% of highest frequency of regeneration and average number of 4.1 shoots (Fig. 1 d, e). However, combinations of Kn+NAA used were failed to produce the shoot multiplication in nodal explant (Table 2).

Increasing the Cytokinin concentration, the shoot regeneration intensity was decreased in the all levels of Cytokinin in both explants. Higher concentration of cytokinin BA at 13.3µM and Kn at 11.6µM, showed reduced number of shoots production. Hu and Wang (1983) reported that higher concentration of Cytokinins reduces the shoot proliferation strength. Cytokinin BA from range of 2.22 -13.3µM was reduced the number of shoots as well as shoot lengths. The Kn (2.3-6.9µM), NAA (2.6-10.7µM) along with BA (8.8µM) and TDZ alone (2.2-9.0µM) were analysed for shoot induction from nodal and hypocotyl explants. BA was shown sufficient number of shoots from individually at 8.8µM in nodal explants. The appearance of smaller shoots in lengths occurred in BA alone and in combination of NAA for various concentrations. BA was tried along with $GA_3$ (0.2-5.7µM) for shoot elongation. At optimum concentration of BA (8.8µM) and lower concentration of $GA_3$ (1.4µM) was shown maximum 90% response for shoot enhancement and maximum shoot lengths (4.77) were noticed (Table 2 & Fig. 1 f). Hypocotyl explants were cultured on MS medium supplemented with different growth regulators like NAA (2.6-10.7µM) along with BA (6.6 & 8.8µM) and Kn (9.2µM) and compared with individual. The BA when combined with NAA was given optimum response with hypocotyl explant. Individual Cytokinins Kn (9.2µM) and TDZ (2.2µM) and BA (6.6 µM), and also auxin NAA (5.3µM) shown high frequency shoot proliferation with hypocotyl on MS medium (Table 2). Similar reports were observed *Cassia tora* with BA considering nodal explants by Afaque et al. (2011).

Excised shoots were rooted within four weeks on half strength MS medium with supplementation of different types of auxins IAA, IBA and NAA at various levels (Aravind et al. 2011; Neha Goel et al. 2009; Parveen et al. 2010). While the shoots implanted on MS medium containing half strength salts, 3% sucrose and fortified with IBA (7.3µM) was found effective than IAA and NAA. In regeneration at this hormone level, profuse roots with 90% efficiency (Fig. 1 g, h) and average number of roots (4.00) and root lengths (3.82) were recorded (Table 3). However, IAA and NAA formed less frequency of roots in half strength MS medium. Light brownish callus development was seen with all types of auxins along with rooting. IBA was showed successful root induction on half strength MS medium comparing with IAA and NAA. Similar reports were observed in *Bacopa monneri* (Vijaya Kumar et al. 2011; Vibhav tiwari et al. 2001). IBA was most favorable for root induction in several medicinal plants (Rajender et al. 2012; Gbadamosi and Shaibu 2013). Maximum frequency (90%), number of roots (around 4.00) and mean root length (3.82) were recorded on 40$^{th}$ day on MS with IBA (7.3µM). It was observed that *in vitro* hardened plantlets transferred on to the field was 81% (Fig. 1 i). The high survival rate of *in vitro* plants in the present study indicates that this procedure may be easily adopted for large scale multiplication and cultivation which facilitate to meet the medicinal demand in the market. *In vitro* propagated plantlets resemble the general growth and morphological characteristics of mother plants (Fig. 1 j).

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**Table 1. Effect of different concentrations (BA, Kn and TDZ) on shoot multiplication from nodal and hypocotyl explants of *Senna alata*.**

| Growth regulators / Conc.(µM) | | | Node | | | Hypocotyl | | |
|---|---|---|---|---|---|---|---|---|
| No. of Shoots | | | | No. of Shoots | Shoot length | | No. of Shoots | Shoot length |
| BA | KN | TDZ | % of response | Mean ± S.E. | Mean ± S.E. | % of response | Mean ± S.E. | Mean ± S.E. |
| 2.22 | - | - | 43 | 1.50±0.29$^{ab}$ | 2.15±0.33$^{b}$ | 30 | 1.66±0.33$^{a}$ | 2.00±0.05$^{a}$ |
| 4.4 | - | - | 60 | 1.20±0.20$^{a}$ | 1.42±0.24$^{a}$ | 60 | 2.16±0.30$^{ab}$ | 2.13±0.19$^{a}$ |
| 6.6 | - | - | 77 | 2.14±0.14$^{b}$ | 2.22±0.22$^{b}$ | 85 | 3.25±0.25$^{b}$ | 2.92±0.18$^{ab}$ |
| 8.8 | - | - | 83 | 4.20±0.73$^{c}$ | 4.11±0.20$^{c}$ | 60 | 2.83±0.40$^{b}$ | 1.95±0.27$^{a}$ |
| 11.1 | - | - | 67 | 1.50±0.22$^{ab}$ | 1.95±0.06$^{ab}$ | 45 | 2.50±0.28$^{ab}$ | 1.95±0.15$^{a}$ |
| 13.3 | - | - | 57 | 1.60±0.24$^{ab}$ | 2.04±0.04$^{ab}$ | - | - | - |

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|---|---|---|---|---|---|---|---|---|
| - | 2.3 | - | 30 | 1.33±0.33[a] | 1.93±0.49[a] | 40 | 1.50±0.28[ab] | 2.02±0.33[a] |
| - | 4.6 | - | 43 | 1.50±0.28[ab] | 2.17±0.21[a] | 45 | 1.75±0.47[ab] | 2.20±0.32[a] |
| - | 6.9 | - | 70 | 2.57±0.42[b] | 2.11±0.27[a] | 55 | 2.00±0.31[a] | 1.94±0.30[a] |
| - | 9.2 | - | 50 | 1.40±0.24[ab] | 2.36±0.31[a] | 78 | 2.71±0.28[b] | 2.32±0.20[a] |
| - | 11.6 | - | 33 | 1.33±0.33a | 1.96±0.07[a] | 55 | 1.60±0.24[a] | 1.82±0.16[a] |
| - | 13.9 | - | 47 | 1.60±0.24[ab] | 2.16±0.47[a] | - | - | - |
| - | - | 2.2 | 43 | 1.25±0.25[a] | 2.62±0.26[a] | 20 | 1.00±0.36 | 1.40±0.50 |
| - | - | 4.5 | 53 | 1.60±0.24[a] | 1.80±0.35[a] | 10 | 1.00±0.57 | 2.00±0.29 |
| - | - | 6.8 | 60 | 1.66±0.33[a] | 2.20±0.34[a] | - | 0.00±0.00 | 0.00±0.00 |
| - | - | 9 | 53 | 1.64±0.81[a] | 1.68±0.15[a] | - | 0.00±0.00 | 0.00±0.00 |

**Data were recorded after 40d of culture**
Treatment means followed by different alphabets within PGR and response variable combinations are significantly different from each other (p<0.05); comparison by Duncan's Multiple Range Test (DMRT).

**Table 2. Effect of different concentrations and combinations (BA, Kn and NAA) on shoot multiplication from nodal and hypocotyl explants of *Senna alata*.**

| Growth regulators / Conc.(µM) | | | | Node | | | Hypocotyl | | |
|---|---|---|---|---|---|---|---|---|---|
| BA + NAA | BA + KN | KN + NAA | BA + $GA_3$ | % of response | No. of Shoots | Shoot length | % of response | No. of Shoots | Shoot length |
| | | | | | Mean ± S.E. | Mean ± S.E. | | Mean ± S.E. | Mean ± S.E. |
| 6.6+2.6 | - | - | - | - | - | - | 50 | 2.40±0.24[a] | 1.88±0.08[a] |
| 6.6+5.3 | - | - | - | - | - | - | 90 | 4.11±0.20[bc] | 3.51±0.18[b] |
| 8.8+2.6 | - | - | - | 40 | 2.25±0.47[a] | 1.87±0.50[a] | 65 | 2.50±0.34[a] | 2.50±0.37[a] |
| 8.8+5.3 | - | - | - | 77 | 4.00±0.30[b] | 3.07±0.48[a] | 65 | 3.16±0.47[ab] | 2.40±0.45[a] |
| 8.8+8.0 | - | - | - | 53 | 1.80±0.37[a] | 1.98±0.34[a] | - | - | - |
| 8.8+10.7 | - | - | - | 50 | 2.00±0.31[a] | 1.76±0.18[a] | - | - | - |
| - | 8.8+2.3 | - | - | 57 | 3.83±0.30[b] | 1.96±0.20[ab] | - | - | - |
| - | 8.8+4.6 | - | - | 53 | 2.00±0.31[a] | 1.66±0.14[a] | - | - | - |
| - | 8.8+6.9 | - | - | 67 | 3.87±0.23[b] | 2.93±0.43[b] | - | - | - |
| - | - | 9.2+2.6 | - | - | - | - | 50 | 2.00±0.31[a] | 2.00±0.04[a] |
| - | - | 9.2+5.3 | - | - | - | - | 70 | 2.42±0.36[a] | 2.87±0.18[b] |
| - | - | 9.2+10.7 | - | - | - | - | 50 | 1.80±0.37[a] | 2.06±0.31[a] |
| - | - | - | 8.8+0.2 | 77 | -- | 3.02±0.35 [b] | - | - | - |
| - | - | - | 8.8+1.4 | 90 | - | 4.77±0.14 [c] | - | - | - |
| - | - | - | 8.8+2.8 | 60 | - | 2.18±0.29 [ab] | - | - | - |
| - | - | - | 8.8+5.7 | 30 | - | 1.70±0.20 [a] | - | - | |

**Data were recorded after 40 d of culture**
Treatment means followed by different alphabets within PGR and response variable combinations are significantly different from each other (p<0.05); comparison by Duncan's Multiple Range Test (DMRT).

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**Table 3. Effect of different concentrations of Auxins (IAA, IBA and NAA) on rooting from *in vitro* developed shoot buds of *Senna alata* cultured on Half strength MS medium**

| Growth regulators / Conc.(µM) | | | % of response | No. of roots Mean ± S.E | root length Mean ± S.E. |
|---|---|---|---|---|---|
| IAA | IBA | NAA | | | |
| 0.5 | - | - | 0 | 0.00±0.00 | 0.00±0.00 |
| 2.8 | - | - | 0 | 0.00±0.00 | 0.00±0.00 |
| 5.7 | - | - | 43 | 1.50±0.29[a] | 1.40±0.32[ab] |
| 8.5 | - | - | 57 | 1.40±0.24[a] | 1.72±0.22[b] |
| 11.4 | - | - | 47 | 1.00±0.00[a] | 0.82±0.05[a] |
| - | 0.4 | - | 0 | 0.00±0.00 | 0.00±0.00 |
| - | 2.4 | - | 30 | 1.50±0.29[a] | 1.57±0.36[a] |
| - | 4.9 | - | 57 | 2.40±0.51[a] | 1.44±0.32[a] |
| - | 7.3 | - | 90 | 4.00±0.24[b] | 3.82±0.16[b] |
| - | 9.8 | - | 70 | 1.85±0.34[a] | 1.87±0.32[a] |
| - | - | 0.5 | 0 | 0.00±0.00 | 0.00±0.00 |
| - | - | 2.6 | 0 | 0.00±0.00 | 0.00±0.00 |
| - | - | 5.3 | 37 | 2.00±0.41[a] | 2.7±0.23[a] |
| - | - | 8.0 | 40 | 2.00±0.41[a] | 2.77±0.36[a] |
| - | - | 10.7 | 60 | 2.83±0.48[a] | 2.26±0.27[a] |

**Data were recorded after 40d of culture,**
Treatment means followed by different alphabets within PGR and response variable combinations are significantly different from each other ($p<0.05$); comparison by Duncan's Multiple Range Test (DMRT).

**Fig .1 Micropropagation studies of *Senna alata* through node and hypocotyl explants. a,b,c: Shoot initiation from nodal explant on BA(8.8µM); d,e:Shoot initiation from hypocotyl explant on BA+NAA(6.6+5.3µM); f:Shoot elongation on BA+$GA_3$ (8.8+1.4µM); g,h: Root iduction on half MS medium with IBA(7.3µM); i,j:Transplanted plantlets**

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