Research Paper

Influence of the phytohormones on the in-vitro regeneration in Senna alata (Linn)

Accepted 15th February, 2013

ABSTRACT

Plants growth regulators, especially auxins and cytokinins are critical for in-vitro plant regeneration. The effect of plant growth regulators on the in-vitro propagation of Senna alata (Linn) was investigated. Mature seeds were collected from the medicinal garden of National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan (Lat. 7°22' N and long. 3°50' E). Seeds of the species were inoculated into Murashige and Skoog (MS) media only. Proliferated shoots were excised; one shoot-tip cutting per test-tube was inoculated into MS supplemented with benzylaminopurine (BAP) + naphthalene acetic acid (NAA) at concentrations of 0.05+0.01, 0.075+0.01, 0.10+0.01, 0.125+0.01 and 0.15+0.01 mg/l; kinetine (KIN) + NAA (0.05+0.01, 0.075+0.01, 0.10+0.01, 0.125+0.01 and 0.15+0.01 mg/l) and a control (MS only). Within 14 weeks of culture, elongation of shoots was observed. There were significant differences (P≤0.05) in all seedling parameters assessed; the highest number of shoots/plantlet at concentration 4.00 ± 0.00 was obtained in MS media that had 0.05 mg/l BAP and 0.01 mg/l NAA, 0.075 mg/l BAP and 0.01 mg/l NAA. The highest values of 4.05 ± 0.46 cm and 3.50 ± 0.22 for shoot length and number of nodes per sprouted shoot respectively were obtained in MS media that had 0.05 mg/l BAP and 0.01 mg/l NAA; the highest number of leaves of 13.90 ± 0.41 was obtained in MS media that had 0.05 mg/l KIN and 0.01 mg/l NAA. MS media that had 0.15 mg/l KIN and 0.01 mg/l NAA gave the highest values of 4.80 ± 0.51 and 2.96 ± 0.05 cm for number and length of roots respectively. Control treatment gave the lowest values for all parameters assessed. Eighty percent of transplanted plantlets survived and grew successfully on the field. Therefore, it is recommended that Senna alata be propagated in MS media supplemented with low concentration of BAP (0.05 mg/l) and relatively high concentration of KIN (0.15 mg/l); and 0.01 mg/l of NAA for optimum growth.

Key words: In vitro, regeneration, plant growth regulators, Senna alata.

INTRODUCTION

Medicinal plants produce many organic chemicals that are vitally important to the pharmaceutical industry. Also, about two-third of the world' population use these plants for their primary health care (Eloff, 1998).

Senna alata have various end-uses; apart from the primary use as a laxative or purgative, abscesses and wounds, and ringworm (Monkheang et al., 2011); other ailments treated with the species include stomach pain during pregnancy, dysentery, haemorrhoids, blood urine (schistosomiasis, gonorrhoea), convulsion, jaundice, headache, hernia and paralysis (Kulka, 2006). Oguntuyi et al., (2005) reported that the leaf decoction is used as an expectorant in bronchitis and dyspnoea, astringent, a mouth wash and a wash in cases of eczema.

Although S. alata has become naturalized and is often considered a weed in most countries of tropical Africa...
(Arbonnier, 2004), it is a native to South America and is naturally and widely cultivated in Americas. It is a shrub that grows to about 2–5 m in height. It produces pretty yellow flowers in a column that resemble yellow candle sticks thus, the common name candle stick or candle bush (Burkil, 1995).

Micro-propagation which includes tissue culture procedures used to propagate plants has proven to be a potential technology for large scale production and conservation of medicinal plant species (Martin, 2003; Hassan and Roy, 2005; Hasan et al., 2008). Fett-Neto et al. (2000) opined that multiplication of S. alata by micropropagation may aid in the production and selection of elite genotypes. For a plant which almost all parts are used for one medicinal purpose or the other, it is necessary to enlarge its population via a rapid process which in-vitro regeneration offers. This study was conducted to investigate the response of this plant species under in-vitro regeneration using different plant growth regulators (PGR) at varied concentration, in order to develop an efficient in-vitro regeneration protocol for the species.

MATERIALS AND METHODS

Seed source

Mature seeds were collected from the medicinal garden of National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan (Latitude 7°22' N and longitude 3°50' E).

Seed pretreatment

The seeds were washed thoroughly under running tap water for 5 mins and then treated with conc. H₂SO₄ for 15 min after which the seeds were rinsed thoroughly with distilled water to remove all traces of acid. The seeds were again soaked in warm water (55°C) for 19 h to scarify properly. The scarified seeds were soaked in 3% concentration of sodium hypochlorite (NaOCl) and later rinsed thoroughly (six times) in sterile distilled water to remove all traces of chemical. 70% Ethanol was used to soak the seeds again for 5 min before they were properly rinsed several times using autoclaved distilled water to remove all traces of ethanol. Murashige and Skoog (MS) (1962) media was prepared with pH adjusted to 5.7 and autoclaved at 121°C for 15 min. The seeds were then inoculated into 25 x 150 mm culture tubes each containing 10 ml MS media.

Growth and development

The culture tubes were incubated in the culture room of Tissue Culture and Biotechnology laboratory of NACGRAB at 28° ± 2°C with photo period of 16/8 h at 3000 – 4000 Lux. Light was provided by cool white fluorescent tubes.

After 4 weeks of culture, proliferated shoots were subculture into a fresh medium, supplemented with varying concentration of BAP, KIN or NAA. Ten shoot-tip cuttings were used for each treatment which include BAP + NAA (0.05 + 0.01, 0.075 + 0.01, 0.10 + 0.01, 0.125 + 0.01, 0.15 + 0.01 mg/l); KIN + NAA (0.05 + 0.01, 0.075 + 0.01, 0.10 + 0.01, 0.125 + 0.01, 0.15 + 0.01 mg/l) and a control (MS only).

Data collection was done weekly for 14 weeks. The following growth parameters: shoot length, number of shoots, root length, number of roots, number of nodes, and number of leaves were recorded. The data were subjected to analysis of variance (ANOVA) using SAS software package and the means were separated using Duncan Multiple Range Test at 5% level of significance.

Hardening and acclimatization

Plantlets with well developed root system were removed from the culture medium, washed properly under running tap water to remove any adherent gel and transferred to plastic pot containing stone dust/river sand, coconut fiber and top soil in the ratio of 1: 7: 2. Plants were covered with transparent polythene bags to maintain adequate moisture (Plate C). After 3 weeks, the plastics cover was gradually removed and the plantlets were maintained in the green house in earthen pots containing normal garden soil until they were transplanted to the nursery.

RESULTS

Metrical traits of Senna alata (Linn) seedlings as influenced by PGR

Number of shoots

The effect of PGR was significant (P≤0.05) on the number of shoots of in-vitro propagated S. alata (Table 1). The highest number of shoots 4.00 ± 0.00 was obtained in MS media that had 0.05 mg/l BAP and 0.01 mg/l NAA or 0.075 mg/l BAP and 0.01 mg/l NAA, this was followed with a value of 2.80 ± 0.13 obtained in MS media that had 0.125 mg/l BAP and 0.01 mg/l NAA. The lowest mean value of 1.00 ± 0.26 was obtained under the control (Table 2).

Shoot length

Shoot length of Senna alata was significantly (P≤0.05) affected by PGR (Table 1). The highest value of shoot length (4.05 ± 0.46 cm) was obtained in MS media containing 0.05 mg/l BAP and 0.01 mg/l NAA (Plate A), this was followed with a value of 3.14 ± 0.07 cm obtained in MS media that had 0.125 mg/l KIN and 0.01 mg/l NAA. The lowest mean
Table 1. Analysis of variance for seedlings parameters in *Senna alata* grown under different concentrations of plant growth regulators.

<table>
<thead>
<tr>
<th>Seedlings parameter</th>
<th>Sources</th>
<th>D.F</th>
<th>Sum of Square</th>
<th>Mean Square</th>
<th>F - Value</th>
<th>P &lt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Shoots</td>
<td>Mode</td>
<td>10</td>
<td>75.05</td>
<td>7.51</td>
<td>36.60</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>99</td>
<td>20.30</td>
<td>0.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>109</td>
<td>95.35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of Nodes</td>
<td>Mode</td>
<td>10</td>
<td>55.29</td>
<td>5.53</td>
<td>9.29</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>99</td>
<td>58.90</td>
<td>0.59</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>109</td>
<td>114.19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of Leaves</td>
<td>Mode</td>
<td>10</td>
<td>1321.56</td>
<td>132.16</td>
<td>132.56</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>99</td>
<td>98.70</td>
<td>0.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>109</td>
<td>1420.26</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of Roots</td>
<td>Mode</td>
<td>10</td>
<td>473.65</td>
<td>47.37</td>
<td>34.66</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>99</td>
<td>135.30</td>
<td>1.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>109</td>
<td>608.95</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root Length(cm)</td>
<td>Mode</td>
<td>10</td>
<td>164.96</td>
<td>16.49</td>
<td>115.12</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>99</td>
<td>14.19</td>
<td>0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>109</td>
<td>179.15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoot Length(cm)</td>
<td>Mode</td>
<td>10</td>
<td>97.35</td>
<td>9.73</td>
<td>26.20</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>99</td>
<td>36.78</td>
<td>0.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>109</td>
<td>134.13</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

value of 0.11 ± 0.03 cm was obtained under the control (Table 2).

**Number of nodes**

The effect of different PGR on the mean number of nodes was significant (P≤0.05) (Table 1). The highest number of nodes 3.50 ± 0.22 was obtained in MS media that had 0.05 mg/l BAP and 0.01 mg/l NAA, this was followed with a value of 3.10 ± 0.23 obtained in MS media that had 0.075 mg/l BAP and 0.01 mg/l NAA. The lowest mean value of number of nodes 1.10 ± 0.28 was obtained in MS media under the control (Table 2).

**Number of leaves**

The effect of plant growth regulators was significant (P≤0.05) on the production of leaves in the species (Table 1). The highest number of leaves of 13.90 ± 0.41 was obtained in MS media that had 0.05 mg/l KIN and 0.01 mg/l NAA, this was followed with a value of 13.80 ± 0.39 obtained in MS media that had 0.125 mg/l KIN and 0.01 mg/l NAA. The lowest mean value of 1.20 ± 0.29 was obtained under the control (Table 2).

**Number of roots**

The effect of different plant growth regulators on the number of roots was significant (P≤0.05) (Table 1). The highest number of roots 4.80 ± 0.51 was obtained in MS media that had 0.15 mg/l KIN and 0.01 mg/l NAA (Plate B), this was followed with a value of 4.40 ± 0.92 obtained in MS media that had 0.075 mg/l KIN and 0.01 mg/l NAA. The lowest mean value of 3.40 ± 0.27 was obtained in MS media that had 0.125 mg/l KIN and 0.01 mg/l NAA.

There was no root response at all in MS media that had 0.10 mg/l KIN and 0.01 mg/l NAA, 0.075 mg/l BAP and 0.01 mg/l NAA, 0.10 mg/l BAP and 0.01 mg/l NAA, 0.125 mg/l BAP and 0.01 mg/l NAA, 0.15 mg/l BAP and 0.01 mg/l NAA and those under the control (Table 2).

**Root length**

The effect of different plant growth regulators on root length in *S. alata* was significant (P≤0.05) (Table 1). The highest root length value of 2.96 ± 0.05 cm was obtained in MS media that had 0.15 mg/l KIN and 0.01 mg/l NAA (Plate B), this was followed with a value of 2.86 ± 0.18 cm obtained in MS media that had 0.075 mg/l KIN and 0.01 mg/l NAA. The lowest mean value of 1.71 ± 0.28 cm was
obtained in MS media that had 0.05 mg/l KIN and 0.01 mg/l NAA. MS media that had 0.10 mg/l KIN and 0.01 mg/l NAA, 0.075 mg/l BAP and 0.01 mg/l NAA, 0.10 mg/l BAP and 0.01 mg/l NAA, 0.125 mg/l BAP and 0.01 mg/l NAA, 0.15 mg/l BAP and 0.01 mg/l NAA and those under the control showed no response (Table 2).

DISCUSSION

Whole plant could be raised from single cell, tissue or organ due to the potency of cells. Most plant cells, given a correct stimuli regenerates an entire plant from single cells or tissues. Plant growth regulators are the critical media components in determining the developmental pathway of plant cell (Slater et al., 2003; Faheem et al., 2011). The result of the present study revealed that the synergism between cytokinin – auxin combination influenced growth parameters such as multiple shoots, nodes, leaves, and roots proliferation significantly.

Slater et al. (2003) reported that a high cytokinin to auxin ratio generally favours shoot formation; however, in this study a higher BAP to NAA combination gave a lower shoot growth and number of nodes response. Generally, combination of BAP and NAA gave a better shoot performance than combination of KIN and NAA, and this is supported by the findings of Rahman et al. (2006) who reported the superiority of BAP over other cytokinins in shoot proliferation. Agrawal and Sardar (2003) reported that cotyledonary node (CN) explants of senna supplemented with 1.0 µM BA induced 2.4 shoot per explants; while the number of shoots increased to a maximum of 17.6 shoots per CN explants when pretreated with 1.0 µM TDZ for 4weeks on MS basal medium (Siddique and Anis, 2007a,b). NAA supplemented media favoured the regeneration of Cassia; Parveen and Shahzad (2011) reported that NAA provided better shoot response than IAA in combination with BA in the in-vitro regeneration of C. angustifolia- a closely related species with S. alata.

In this study, combination of KIN and NAA stimulated root development better than combinations of BAP and NAA, this agreed with the findings of Kalimuthu et al. (2007) who found that KIN enhanced root growth better compared to shoots in the in-vitro propagation of orchid. Furthermore, Kaviani et al. (2011) demonstrated that the addition of NAA and NAA+KIN in culture media was effective for increasing the number of root and root length in Matthiola incana L. On the contrary, the combination of BAP and NAA actually inhibited root induction in S. alata.

Rooting is an important phase in the successful micro propagation of plant species. Without effective rooting system, plant acclimatization will be difficult and the rate of plant propagation may be severely affected (Goncalves et al., 1998). In addition, media composition seems to be the key factor affecting morphogenesis in cells grown in-vitro (Elkonin and Pakhomova, 2000). In this study, combination of KIN and NAA effectively promoted the regeneration of roots than combination of BAP and NAA; Hasan et al. (2008) reported a better rooting result for IBA supplemented media than NAA ones. Rooting in Cassia auriculata was induced from the cut end of the hypocotyls in MS medium supplemented with IBA 1.0 mg/l or NAA 1.0 mg/l (Negi et al., 2011). The explants cultured on pure MS medium without the addition of growth regulators showed a rather poor regeneration response; Faheem et al. (2011) obtained similar result for Catharanthus roseus. On the
contrary, Fett-Neto (2000) reported that shoots of *S. alata* were rooted on root induction medium with or without auxin (IBA) supplementation; while percentage rooting decreased with increasing concentration of IBA.

The results of this research suggested that the regeneration of shoot tips into plantlets could be influenced by the presence of phytohormones and the media composition. Plantlets derived from the tissue culture experiment were established in the green house and grown to maturity. However, the choice of medium composition influenced the growth of specific embryogenic stages and their regeneration into plants. Nevertheless, the overall indication of the results suggest that there may still be room for additional adjustment to the BAP and NAA concentration in *S. alata* cultures.

**Conclusion and Recommendation**

From these results, shoot parameter of *S. alata* performed better in MS media supplemented with low concentration of BAP (0.05 mg/l) while root initiation and growth was aided by relatively higher (0.15 mg/l) concentration of KIN. Thus, these media composition supplemented with 0.01 mg/l NAA could be used for mass propagation of *S. alata* seedlings for conservation.

**REFERENCES**