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A Note on *In Vitro* Regeneration of Balfour aralia (*Polyscias balfouriana marginata* L.) Shoots Using Shoot Tip Explants

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Abstract: The objective of this study was to investigate the effect of the cytokinins benzylaminopurine (BAP) and Kinetin on *in vitro* shoot regeneration on shoot tips of *Polyscias balfouriana marginata.* Shoot tips were cultured on Murashige and Skooge (1962) medium (MS) supplemented with various concentrations of BAP and Kinetin at 0.0, 0.75, 1.5, 3, 6 mg/L for both. Results showed that *Polyscias balfouriana marginata* responded positively to growth regulators application and there was significant difference among concentration treatments. The highest percentage of responded explants was given by BAP and Kinetin - free medium and the one containing the lowest concentration of both (0.75 mg/L). The concentration 3 mg/L BAP showed significantly the highest number of shoots/explant. There was no significant difference between kinetin concentratios 0.75 and 1.5 mg/L, yet they resulted in significantlys higher number of shoots/explant compared to the control.The concentration 0.75 mg/L of both BAP and kinetin recorded the highest shoot length while the shortest shoot resulted from 6 mg/L of both cytokinins and the control of kinetin.

Key words: Aralia**,** *in vitro* regeneration, Cytokinins.

Polyscias balfouriana "Marginata" is a common foliage plant in the world' floriculture industry, especially in countries with tropical climate. It is grown mostly for its beautiful and attractive leaves. (Gunadasa and Dissanayake,2012). It is extensively used in landscaping purposes and also

popular as a potted ornamental plant. Traditionally, this plant is vegetatively propagated by grafting and stem cuttings. These methods are slow and commercially not practical to produce enough plants for commercial purposes. Hence, regeneration through *in vitro* culture provides viable alternative methods for mass production of healthy plants with uniform characteristics (Ilyas *et al*. 2012). The importance of cytokinins in *in vitro* shoot regeneration was recorded widely in research work as they stimulate adventitious but formation (Mohamed and Özzambak 2014 and Mohamed *et al*. 2015). The objective of this research was to study the effect of different concentrations of the growth regulators BAP and Kinetin on *in vitro* shoot regeneration on shoot tips of *Polyscias balfouriana marginata*.

Shoot tips 1- 2 cm long of *Polyscias balfouriana marginata* used as explants for *in vitro* cultures initiation were taken from potted plants growing in the nursery of the Department of Horticulture, Faculty of Agriculture, University of Khartoum. The shoot tips were washed under running tap water for 60 minutes to remove all surface dust and dirt and then washed using autoclaved distilled water. They were then transferred to the laminar air flow cabinet for surface disinfestation. The explants were immersed in 15 % bleach (Clorox) to which two drops/100 ml of Tween 20 were added with shaking for 15 minutes. Then the explants were rinsed five times with autoclaved distilled water and inoculated into the medium. The primary nutrient medium used in this study was MS (Murashige and Skoog, 1962) inorganic elements + Thiamine-HC1 0.4 mg/L + Sucrose 30 g/L + Adenine sulphate 20 mg/L + Citric acid 20 g/L + Ascorbic acid 20 g/L + NAA 0.1 mg/L + Agar 7 g/L. The pH of all media was adjusted to 5.5. Twenty five ml of medium were distributed in (GA7) culture containers. The culture vessels were sterilized by autoclaving at 121^{o} C and pressure of 1.05 kg/ cm^{2} for 20 minutes. Cultures were incubated at 25 $\pm$ 1^{o}C at photon flux density 13.5 µmol m^{-2} s^{-1} and 16/8 h light/dark cycle using fluorescent lamps during September 2013-March 2014. Different concentrations (0, 0.75, 1.5, 3, 6 mg/L) of the cytokinins BAP (experiment 1) and Kinetin (experiment 2) representing treatments were tested. Each treatment was replicated 5 times. Each replication composed of 4 explants per culture vessel. The parameters measured were percentage of

responded explants, number of shoots/explant and shoot length. The parameters were measured seven weeks after culture. The experimental design for each experiment was a completely randomized design with 5 replications. All data were subjected to analysis of variance using SAS statistical software V11, 2002 (SAS 2002) and mean separation was done using Duncan's Multiple Range Test at 5% level of probability.

Tables 1 and 2 illustrate the influence of BAP and kinetin concentrations on shoot regeneration on shoot tips in experiment 1 and experiment 2 respectively. Among different concentrations used, the highest percentage of explants responded was obtained on the concentrations 0.0 mg/L (control) and 0.75 mg/L of both BAP and kinetin. This result is in agreement with that of Mohamed *et al*. (2015) who cultured nodal explants of *Chrysanthemum morifolium* on MS basal medium supplemented with the cytokinins BAP and kinetin both at 0.0, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0 mg/L for shoot regeneration and found best response towards shoot proliferation (60%) at 0.1 mg/L concentration of both cytokinins. Results recorded at concentrations 0.0 mg/Land 0.75 mg/L of both BAP and kinetin might be due to the fact that the cytokinin concentration found naturally in the tissues of this plant was sufficient for shoot initiation. In experiment 1 (Table 1) the number of shoots/explant was significantly highest with the concentration 3 mg/L BAP. In experiment 2 (Table 2) there was no significant difference between kinetin concentrations 0.75 and 1.5 mg/L, yet they resulted in higher number of shoots/explants compared to the control. Lower number of shoots/explant obtained from the higher concentrations 3 and 6 mg/L kinetin were not significantly different from the control. The concentration 0.75 mg/L of both BAP and kinetin in experiments 1 and 2 (Tables 1 and 2) recorded the highest shoot length while the shortest shoot resulted from 6 mg/L of both cytokinins and the control of kinetin. Working on chrysanthemum, Mohamed *et al*. (2015) reported the highest length of shoot on cytokinin - free medium. In *Munronia pinnata* BAP at 2 mg/L produced the maximum number of shoots with highest average length while increasing BAP concentration to 5 mg/L negatively modified the frequency of shoot induction and growth (Gunathilake *et al*. 2008*)*. Such an inhibitory effect of higher concentrations

of cytokinins was also reported by many other workers (Ahmad *et al*. 2008; Ilyas *et al.*2012). In conclusion BAP at 3 mg/l seems to be a good choice for shoot regeneration on shoot tips of *Polyscias balfouriana marginata.*

Table 1. Influence of BAP concentration on shoot regeneration on shoot tips of *Polyscias balfouriana marginata* cultured for 7 weeks on MS basal medium.

BAP concentration (mg/L)	Explants responded (%)	Number of shoots/ explant	Shoot length (cm)
0.0	80 a	1.8^{d}	1.64^{b}
0.75	80 a	2.89^{c}	2.38^{a}
1.5	65 ab	4.24^{b}	2.06^{ab}
3.0	70 ab	5.88^{a}	2.12^{ab}
6.0	25 b	1.6^{d}	1.28^{b}

*Means within column followed by the same letter (s) are not significantly Different at P=0.05 according to Duncan's Multiple Range test.

Table 2. Influence of kinetin concentration on shoot regeneration on shoot tips of *Polyscias balfouriana marginata* cultured for 7 weeks on MS basal medium.

Kinetin concentration (mg/L)	Explants responded (%)	Number of shoots⁄explant	Shoot length(cm)
0.0	90 a	0.5^{b}	0.24^{c}
0.75	55 ab	1.57^{a}	1.41^{a}
1.5	40 ab	1.67^{a}	1.13^{ab}
3.0	45 ab	1.1^{ab}	1.04^{ab}
6.0	25 b	1.1^{ab}	0.65^{bc}

*Means within column followed by the same letter (s) are not significantly different P=0.05 according to Duncan's Multiple Range test.

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إعادة تكوين الأفرع خارج الجسم الحي في نبات البنكس باستخدام القمم النامية

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المستخلص: الهدف من هذه الدراسة كان بحث تأثيرتراكيزمختلفة للسايتوكاينينات بنزايل أمينو بيورين (BAP) والكاينتين (Kinetin)على نشأة الأفرع خارج الجسم الحي على القمم النامية لنبات البنكس. زرعت القمم النامية في وسط MS (Murashige and Skoog, 1962) المحتوي على تراكيز مختلفة (0.0،0.75 ، 1.5 ،3 ، 6 ملجرام/ لتر) من BAP و kinetin. أظهرت النتائج استجابة نبات البنكس ايجابيا لإضافة منظمات النمو و أن هنالك فروق معنوية بين معاملات التركيز. أعلى نسبة للقمم النامية التي استجابت سجلت في الوسط الخالي من منظمات النمو BAP و Kinetin و في ذلك الذي يحتوي على أقل تركيز منهما (0.75 ملجرام/ لتر). التركيز3 ملجرام/لترBAP أعطى معنويا أعلى عدد من الأفرع بالقمة النامية. لم يكن هنالك فرق معنوي بين تركيزي الكاينتين 0.75 و 1.5 ملجرام/لتر لكن نتج عنهما و بدرجة معنوية أعلى عدد من السيقان بالقمة النامية مقارنة بالشاهد. التركيز0.75 ملجرام/لتر لكل من ال BAP و الكاينتين سجل أعلى طول للفرع بينما أقصر فرع نتج من التركيز 6 ملجرام/لتر لكل من ال BAP و الكاينتين وشاهد الكاينتين.