

COMPARISON BETWEEN USES OF GROWTH REGULATORS AND NATURAL ADJUVANT ON *in vitro* MICROPROPAGATION OF *Ixora coccinea*

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Abstract: *Ixora coccinea* is a beautiful ornamental plant so far it is an important medicinal plant that traditionally propagated by stem cutting. Many attempts have been made to propagate this plant through tissue culture using chemical growth regulators which is more expensive and may be harmful to the environment in different ways. This study was conducted to compare the effect of growth regulators with natural adjuvant on morphogenesis of *Ixora coccinea* shoot tip cultured on Murashige and Skoog (1962) (MS) media. Shoot tip explants were cultured on MS media supplemented with 2.0mg/l Benzyl aminopurine (BAP) combined with different concentrations of Indole-3-butyric acid (IBA) (0.0,0.1,0.25, and0.5mg/l). Other three experiments contained different concentrations of peptone (0.0,1.0, 2.0 and 3.0 g/l), banana powder (0.0,10.0, 20.0 and 30.0 mg/l) and coconut water (0.0, 5.0 ,10.0 and 15.0 ml/l) were also evaluated for shoot morphogenesis using shoot tips explant. Addition of 2.0 mg/l BAP and 0.5 mg/l IBA to MS media enhanced significantly morphogenesis rate of shoot and gave the highest value of all measured parameters; number of leaves (17.1), number of shoots (2.5), length of shoots (3.4cm), length of explants (1.9cm) and number of nodes (7.0). The best response of natural adjuvant on morphogenesis was occurred when added 2.0g/l peptone and this appeared just on number of leaf with an average1.3.10 mg/l banana powder showed significantly higher result compared to other treatments, number of leaves (2.0), number of shoots (1.6), length of shoots (0.3cm) and(0.4cm) length of explant. Coconut water affected slightly *Ixora coccinea* shoot initiation rate where initiation of leaves and shoot (1.6 and 1.3 respectively) appeared only at 5.0ml/l and no significance different in a number of leaf between 5.0 and 10.0 ml/l. Different treatments did not enhance root initiation.

Keywords: *Ixora coccinea*, *in vitro*, growth regulators BAP; IBA; peptone; banana powder; coconut water

General over view

Ixora coccinea one of the Rubiaceae member (Stewart, 1982), and it is cultivated throughout the tropics and sub- tropical regions as an ornamental and medicinal plant. It is a shrub or

small tree which is grown as beautiful garden plants for their cluster of flowers of various hues and evergreen foliage (Malathy & Pai,1998). Traditionally *Ixora* is propagated from stem cuttings of mature shrubs which have been replaced recently by tissue culture technique. Many studies have been reported on micropropagation of this plant using plant growth regulators, however, only one report by Khan *et al.* (2004) studied the effect of some adjuvant (banana powder, malt extract and peptone) supplemented to MS media on *Ixora* shoot multiplication. There are many substances that can be added to culture media to improve the multiplication rate, some of them chemical substance like growth regulators (BAP, IBA,2,4 D *etc.*) and others from natural sources like peptone, banana powder, and coconut water. Peptone was used as carbon and nitrogen sources for plant culture to promote the growth of explant at an efficient concentration (Chen and Chang, 2002). Banana powder is an excellent source of potassium and sodium, also it is a rich source of vitamins like A, B₆, C and D (Kumar *et al.*, 2012), banana homogenate or powder usually used to promote growth in plant tissue cultures. The extensive use of coconut water promoting component in tissue culture medium formulation started more than half a century ago when Overbeek *et al.* (1941) introduced coconut water as a new component of the nutrient medium for cultures. Now a day coconut water is widely used in the plant tissue culture industry and has been used to stimulate shoot proliferation in many species of plants (Arditti, 2008).

The aim of this study was to compare the uses of 2.0 mg/l BAP combined with different concentrations of IBA (0.0,0.25, and 0.5 mg/l) with the uses of a natural source of adjuvant (peptone, banana powder, and coconut water) in different concentration on shoot morphogenesis of *Ixora coccinea*.

Materials and Methods

Explant of *Ixora coccinea* (pink) was obtained from a mature plant grown as an ornamental hedge. New branches about 8 cm length were taken from the mother plant to obtain shoot tips and washed under running tap water to remove dust and reduce contamination. The explant was sterilized by immersing in a 20% sodium hypochlorite solution (v/v) and 2-3 drops of Tween 20 with continuous shaking for 15 mins. For culture establishment MS media was used and incubation room was adjusted to 25°C ± 2 temperature under a light intensity of 1000 lux using white fluorescent lamp.

The sterile shoot tip explants with 1cm length were cultured on MS media supplemented with 2.0mg/l BAP combined with different concentrations of IBA (0.0,0.1,0.25, and 0.5 mg/l). Other three different experiments were conducted to test the effect of different level of

peptone (0.0,1.0, 2.0 and 3.0 g/l), banana powder (0.0,10.0, 20.0 and 30.0 mg/l) and coconut water (0.0,5.0 ,10.0 and 15.0 ml/l) on morphogenesis rate of *Ixora coccinea* shoot tip explants. Number of leaves, number of shoots, length of shoots and number of nodes were measured after 8 weeks as growth parameters. Each experiment was arranged in a completely randomized design with four treatments replicated five times. All growth parameters were measured weekly until the 8th week. Data were recorded and analyzed using a Statistic Analysis System software program (SAS) using analysis of variance (ANOVA). Treatments means of data comparison was done using Duncan Multiple Range Test (DMRT) at a 5% probability.

Result and Discussion

The effect of different concentrations of IBA (0.0,0.1, 0.25, 0.5 mg/l) combined with 2.0 mg/l BAP on *Ixora coccinea* direct shoot regeneration was highly significance (table 1). 2.0 mg/l BAP combined with 0.5 mg/l IBA gave the highest result of all measured parameters, 0.5mg/l and 0.1 mg/l with 2.0mg/l BAP gave a comparable number of leave, shoot and node which was significantly higher than two other treatments. Shoot tip explant was grown normally and look healthy Plate (1). The result emphasis the previous one stated that a high concentration of cytokinin and low concentration of auxins promote vegetative growth (George *et al.*,2008). This result also in agreement with Lakshmanan *et.al.* (1997) who found that 2.5 mg/l BAP with 0.25 mg/l IAA gave the best result on shoot multiplication of *Ixora coccinea*.

Table (1): The combination effect of 2.0 mg/l BAP and different concentrations of IBA on morphogenesis of *Ixora coccinea* shoot tip explant after 8 weeks of culture on MS media

IBA (mg/l)	No. of leaves (Mean ±SE)	No. of shoots (Mean ±SE)	Length of shoots(cm) (Mean ±SE)	Length of explants(cm) (Mean ±SE)	No. of nodes (Mean ±SE)
0.0	5.7±0.47 ^c	0.0 ^b	0.0 ^c	0.0 ^c	0.0 ^c
0.1	16.2 ±1.20 ^a	2.3±0.37 ^a	1.3±0.40 ^b	0.5 ±0.16 ^b	7.0±0.62 ^a
0.25	10.0 ±1.30 ^b	1.4±0.35 ^a	1.1±0.37 ^b	0.6±0.20 ^b	4.0±0.61 ^b
0.5	17.1±1.70 ^a	2.5±0.12 ^a	3.4±0.60 ^a	1.9±0.39 ^a	7.0±0.77 ^a

Means with same letters in the same column are not significantly different at 5% using Duncan multiple range test. SE=Standard Error

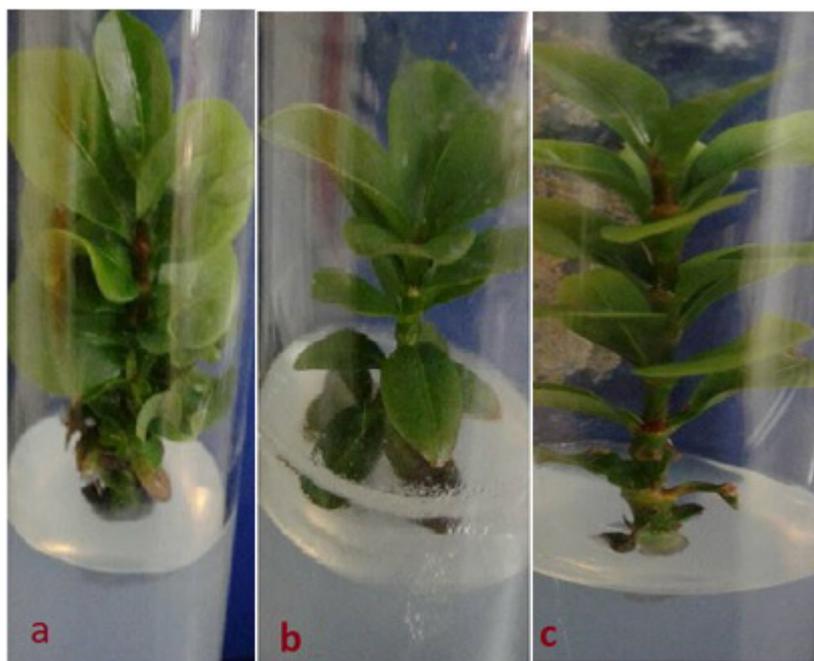


Plate (1): Plantlet derived from shoot tip explant cultured on MS media supplemented with (a) 2.0mg/l BAP + 0.10 mg/l IBA, (b) 2.0mg/l BAP + 0.25 mg/l IBA (c) 2.0mg/l BAP combine + 0.5 mg/l IBA

Initiation of the shoot did not take place on MS medium without peptone supplementation, however, the addition of peptone (2.0 g/l) slightly induced initiation of leaves after 8 weeks from culture with significant different from other treatments. On 3.0g/l, explants looked browned and necrotized. Table (2) and Plate (2). In contrast, Khan, *et.al* (2004) found that MS supplemented with 5.0 g/l peptone affected positively *Ixora coccinea* morphogenesis not just on a number of leave but also in a number of shoots and shoot length of the explant. Also 2.0 g/l peptone in MS culture media enhanced shoot formation as well as root development of avocado woody plant (Nhut; *et.al* 2008).

Table (2): Effect of different concentrations of peptone supplemented to MS media on shoot initiation of *Ixora coccinea* explant after 8 weeks from culture

Peptone g/l	Vegetative Parameters			
	No. of leaves (Mean \pm SE)	No. of shoots (Mean \pm SE)	Length of shoots (cm) (Mean \pm SE)	Length of explants (cm) (Mean \pm SE)
0.0	0.0 ^b	0.0 ^a	0.0 ^a	0.0 ^a
1.0	0.3 \pm 0.19 ^b	0.0 ^a	0.0 ^a	0.0 ^a
2.0	1.3 \pm 0.38 ^a	0.0 ^a	0.0 ^a	0.0 ^a
3.0	0.0 ^b	0.0 ^a	0.0 ^a	0.0 ^a

Means with same letters in the same column are not significantly different at 5% using Duncan multiple range test. SE=Standard Error

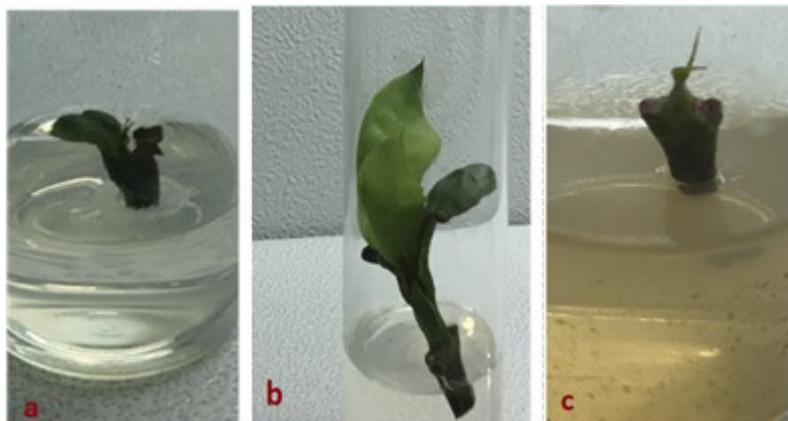


Plate (2): Leave initiation from shoot tip explant cultured on MS media supplemented with (a) 1.0mg/l peptone (b) 2.0 mg/l peptone (c) 3.0mg/l peptone

Addition of banana powder to MS media promoted shoot initiation of *Ixora* derived from shoot tip explant (Table3). 10.0 g/l banana powder enhanced shoot initiate of *Ixora* shoot tip explants significantly and gave a higher number of leaves (2.0), number of shoots (1.6), length of shoots (0.3 cm) and length of explants (0.4 cm). There was no significant different between 10 and 20mg/l of banana powder whereas 30.0mg/l gave a similar result to control without any effect on all parameters Plat(3). This result in the same line with Zeng;*et al.* (2005) who reported 10.0g/l of banana powder improved the multiplication of *Bletilla striata* and *Dendrobium candidum*. Chlorophyll content of *Solanum laciniatum* enhanced significantly when added 3.0–8.0 g/l banana powder to the culture media Handayani (1991).

Table (3): Effect of different concentrations of banana powder on shoot initiation of *Ixora coccinea* shoot tip explant after 8 weeks from culture

Banana Powder mg/l	No. of leaves (Mean ±SE)	No. of shoots (Mean ±SE)	Length of shoots (cm) (Mean ±SE)	Length of explants (cm) (Mean ±SE)
0.0	0.0 ^b	0.0 ^b	0.0 ^b	0.0 ^b
10.0	2.0±0.00 ^a	1.6±0.30 ^a	0.3±0.07 ^a	0.4±0.04 ^a
20.0	1.6±0.30 ^a	1.3±0.50 ^a	0.18±0.08 ^b	0.1±0.08 ^a
30.0	0.0 ^b	0.0 ^b	0.0 ^b	0.0 ^b

Means with same letters in the same column are not significantly different at 5% using Duncan multiple range test. SE=Standard Error

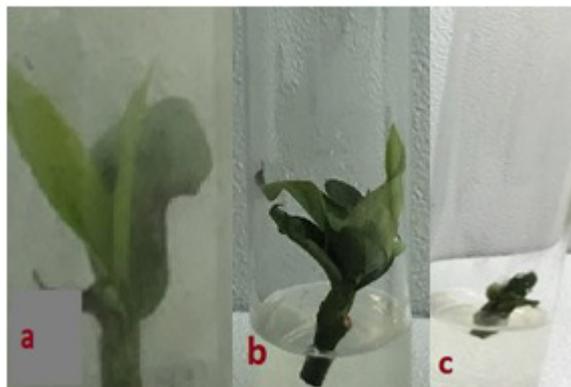


Plate (3): Shoot morphogenesis from shoot tip explant cultured on MS media supplemented with (a) 10mg/l banana powder (b) 20 mg/l banana powder (c) 30 mg/l banana powder

Coconut water affected slightly *Ixora coccinea* shoot initiation rate (Table 4), where initiation of leaves and shoot took place only at 5.0 (1.6 and 1.3 respectively) and no significance different in a number of leave between 5.0 and 10.0 ml/l Plat (4). That is mean addition of low concentration of coconut water to media may promote the multiplication rate more than a high concentration which on line with Matsui; *et al.* (2008). Kende and Zeevaart, (1997) stated that a coconut water concentrations range between (5- 20% (v/v)) could be used in tissue culture media depend on plant species to promote shoot multiplication and if it exceeded it may inhabit the growth of plant.

Table (4): Effect of coconut water on shoot morphogenesis of *Ixora coccinea* shoot tips after 8 weeks from culture

Coconut water ml /l	Vegetative Parameters			
	No. of leaves (Mean \pm SE)	No. of shoots (Mean \pm SE)	Length of shoots(cm) (Mean \pm SE)	Length of explants(cm) (Mean \pm SE)
0.0	0.0 ^b	0.0 ^b	0.0 ^a	0.0 ^a
5.0	1.6 \pm 0.30 ^a	1.3 \pm 0.38 ^a	0.0 ^a	0.0 ^a
10.0	2.0 \pm 0.00 ^a	0.0 ^b	0.0 ^a	0.0 ^a
15.0	0.0 ^b	0.0 ^b	0.0 ^a	0.0 ^a

Means with same letters in the same column are not significantly different at 5% using Duncan multiple range test. SE=Standard Error

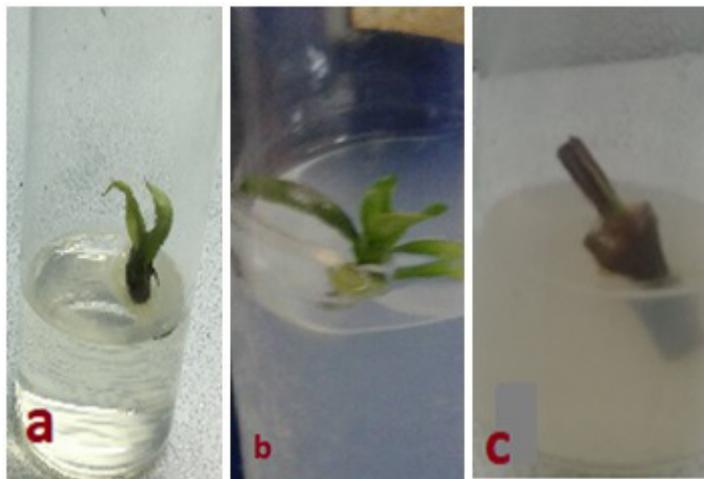


Plate (4): Shoot initiation from shoot tip explant cultured on MS media supplemented with (a) 5.0ml/l coconut water (b) 10.0ml/l coconut water (c) 15.0 ml/l coconut water.

Conclusion

To obtain well and high rate of shoot morphogenesis from shoot tip explant of *Ixora coccinea* to be used as ornamental plant better to use growth regulators (2.0mg/l BAP combine with 0.5 mg/l IBA) in MS media more than adjuvants from natural sources. But natural adjuvant could be used when looking after medicinal value to reduce the harmful from chemical growth regulators that may affect on the medicinal content in the plant.

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