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AN ECONOMICAL AND EFFICIENT METHOD FOR MASS PROPAGATION OF *IXORA COCCINEA*

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Abstract

A simple and efficient micropropagation protocol was developed for *Ixora coccinea* cv. Super King, a beautiful ornamental plant using shoot tip and nodal segments as explants. The explants were cultured in Woody Plant Medium (WPM) supplemented with 0.5 mg/L BAP incubated for 10 weeks initially and subcultured onto fresh medium after every 4 weeks. The proliferating clusters were cultured in WPM basal medium fortified with Peptone (40 mg/l) and 3% sucrose for elongation of shoots, formation of root initials and further proliferation of axillary shoots. Separation and culturing of elongated shoots in fresh medium for 2 to 3 weeks yielded 7 to 8 cm long plantlets which were acclimatized in polytunnels. The most suitable medium for rooting was half-WPM enriched with IBA at a concentration of 0.05 mg/L. An increase in axillary shoot number of up to 10 per shoot were observed. This exponential rise in plants was very encouraging for the development of an efficient commercial micropropagation system.

Introduction

In the recent years, propagation of the numerous ornamental plants by tissue culture has become an accepted commercial practice (Frankenberger *et al*., 1981). Current production techniques for the *In vitro* propagation of plants have allowed for strong and continued growth within the micropropagation industry. *Ixora coccinea* of the family Rubiaceae, is cultivated throughout the tropics as ornamental plants. *Ixora* belongs to a genus of shrubs and small trees distributed in the tropical and sub- tropical regions and more than 30 species occur in the Indo-Pak subcontinent (Stewart, 1982). *Ixora* are beautiful garden plants grown for their cluster of flowers of various hues and evergreen foliage, they are also known to possess anti- carcinogens in them and prove to be an effective remedy against tuberculosis (Malathy & Pai, 1998). The common name “Flame of Woods” was given to this tropical plants because of its brilliant red flowers, which remains open for a long time, contrast with the glossy, dark green leaves. They grow well in acidic soil and can be raised by seeds and cuttings (Holttum & Enoch, 1991). Traditionally *Ixora* is propagated from stem cuttings of mature shrubs and is raised in polybags. This method is not economical since the collection of stem cuttings leads to arrest of growth and development of the mother plant (Ayyappan, 1990). Moreover, the market demand for propagules is hardly met with such cuttings. Mass propagation for commercial cultivation requires a simple, economical, rapidly multiplying and highly reproducible protocol employing tissue culture technology. Thus a more economical and rapid means of propagation of *Ixora* is a strong need of time in order to meet the ever-rising market demand of this beautiful plant (George & Sherrington, 1993). In the present study such a protocol for large- scale micropropagation of *Ixora coccinea*, cv. Super king is reported.

Materials and Method

Shoot tips and nodal buds excised from green house grown vines were used as explants. The terminal portion of the stem was cut into 3cm pieces. The leaves were removed by gentle cutting, leaving the base intact, and the remaining stems were thoroughly washed under running tap water for 15-20 minutes. The explants were then soaked in a jar containing distilled water with 2-3 drops of Tween 20. The jar was placed on a gyratory shaker for 15 minutes. The explants were given a quick dip in 70% ethanol for 5 seconds followed by placing in 20% NaOCl with 3 drops of Tween 20 where the explants were placed with continuous but very gentle stirring on a gyratory shaker for 20 minutes. This was followed by rinsing the explants in sterile distilled water 3 times for 5 minutes each in order to remove traces of NaOCl. The stems were cut into transverse sections containing one nodal bud. They were cut into approximately 2cm long segments and used as explants for shoot initiation under *In vitro* conditions.

Preparation of medium

Woody Plant Medium (WPM) was used as the basal medium. Sucrose (3%) was added as the sole carbon source. The pH of the medium was adjusted to give a final value in the range of 5.7—5.8. Phytagel (Sigma) was added at a concentration of 0.25% w/v to solidify the medium. The medium was heated in a microwave to melt and allow Phytagel to thoroughly dissolve for even distribution, and dispensed into jars, capped and labelled. The medium was then autoclaved at 15 psi for 15 minutes. The medium was allowed to cool and set prior to its use for culturing. In the first experiment, three different concentrations of BAP @ 0.005, 0.05, and 0.5 mg/l were used in medium M1, M2 and M3 respectively. Each medium combination had six replicates. In each replicate, five shoots having at least one node each were placed in an upright position. The explants were then incubated in growth rooms having an average temperature of 22 ± 2°C with light provided by cool white florescent lamps for 16h photoperiod. Data regarding increase in shoot length and increase in number of nodes per shoot was recorded at regular interval of 5 days. After a period of 10 weeks, the shoots were subcultured onto fresh medium. This was done by removing all the leaves on the shoot, and cutting segments of one node each. These segments were then placed into fresh medium and subjected to the same physical conditions as mentioned earlier.

In the second experiment, the effect of adjuvant like banana powder, malt extract and peptone were evaluated at concentrations of 20, 30 and 40 mg/l respectively to determine their effect on shoot proliferation. The basal medium used was WPM as explained earlier. The interactions and individual effects of data was recorded at regular interval of one week by observing the increment in the number of nodes and shoot length. The plants were subcultured at regular intervals of 5 weeks in the same way as described.

In the final experiment, the basal medium employed were full and half WPM. Each basal medium were supplemented with IBA at concentrations of 0.1 mg/1 and 0.5 mg/1. All four types of media were prepared as described above. The media used were solidified with Phytagel at 0.25%. Shoots of height more than 4 cm were introduced into each medium-containing jar in a vertical position. Callus formed, if any were gently removed using a sharp blade. However, the leaves remained intact.

Results and Discussion

Shoot proliferation was investigated in order to determine the optimum medium for maximum shoot growth. After a period of 4 weeks, the maximum length of shoots accompanied by maximum number of nodes per shoot was observed in medium containing BAP at a concentration of 0.05 mg/L (M2).

In the second series of experiments where three different types of adjuvants were evaluated, a profound effect on the increase in axiliary shooting of the plants was observed. Media containing only Peptone at a concentration of 40-mg/l showed a significant increase in the shoot length and the number of nodes per shoot (Fig. 1). Results obtained showed a negative tendency towards shooting when two or more adjuvants were added into the media. Likewise, addition of only banana powder or only malt extracts also showed an improvement in shoot growth as compared to interaction of the adjuvants (Table 1). It was observed that trimming the leaves prior to subculture, enhances the axillary shoot proliferation, most probably because of suppression of apical dominance. A two fold increase in multiplication was observed in the second subculture, followed by three to four fold increase in the subsequent subcultures.

Based on these observations, the explants cultured for 12 weeks on the same medium i.e. BAP (0.05mg/L) with Peptone (40mg/L) had attained the stage of vigorous proliferation, consisting of 6 to 8 shoots (Fig. 2). Small clumps, consisting of 3 to 4 shoots were further subcultured on the same medium, where it was observed that the dwarf shoots recovered to normal growth with more number of axillary shoots arising from the base. At the same time, 2 to 3 vigorously growing shoots from each clump elongated with expanded leaves by 4 weeks (Fig. 3). Such shoots had healthy root initials. The elongated shoots (4 cm to 5 cm long) were excised and cultured separately in the medium containing half- WPM with IBA (0.1 mg/ L), in order to encourage the formation of long shoots, broad leaves and basal roots. The basal tufts of roots were observed in more than 95% of the transferred shoots (Fig. 4). The elongated shoots attained a height of 8 to 9 cm in about two weeks and were ready for transplantation.

Regenerated shoots with sufficient roots were removed from the jars. Roots were washed thoroughly in running tap water to remove traces of agar and handled very gently. The plants were transferred to the mixture of equal ratio of sand and compost in disposable plastic pots. Pots were placed in green house where the relative humidity was about 75% and were watered once a day. A survival rate of 90% was achieved during the hardening process in greenhouse (Fig. 5).

The results showed that *Ixora* can be propagated *In vitro* using shoot tips as explants. Multiple shoot regeneration in *Ixora coccinea* has been reported by Lakshman *et al*., (1997), however, they had reported a severe problem of basal callus and vitrification of shoots, which had to be rectified by the addition of TIBA in the medium. In the present study, no such problem was observed, most probably by the addition of adjuvants like Peptone in the medium, which had reduced shoot vitrification considerably.

In the present study, the axillary proliferation was initiated only when the shoot tip was injured by giving a longitudinal bisection. This might be due to the strong apical dominance exerted by axillary buds, also found by Lakhsman *et al*., (1997).

Table 1. Effect of adjuvants on the increase in shoot length (cm) and number of nodes in *Ixora coccinea*.

Media	Adjuvants			Mean no. of nodes	Mean shoot length (cm)
	Banana	Peptone	Malt		
	mg/L	mg/L	mg/L		
1	50	0	0	1.99	1.87
2	100	0	0	1.55	1.35
3	200	0	0	1.49	1.3
4	0	50	0	1.07	1.99
5	0	100	0	1.45	1.95
6	0	200	0	1.28	1.06
7	0	0	50	1.91	1.75
8	0	0	100	1.4	1.25
9	0	0	200	1.39	1.19
10	50	50	0	1. 22	1
11	100	100	0	1.71	1.57
12	200	200	0	1.77	1.61
13	50	0	50	1.34	1.16
14	100	0	100	1.67	1.5
15	200	0	200	1.46	1.24
16	0	50	50	1.39	1.17
17	0	100	100	1.66	1.43
18	0	200	200	1.44	1.11
19	50	50	50	1.58	1.4
20	100	100	100	1.51	1.28
21	200	200	200	1.34	1.1

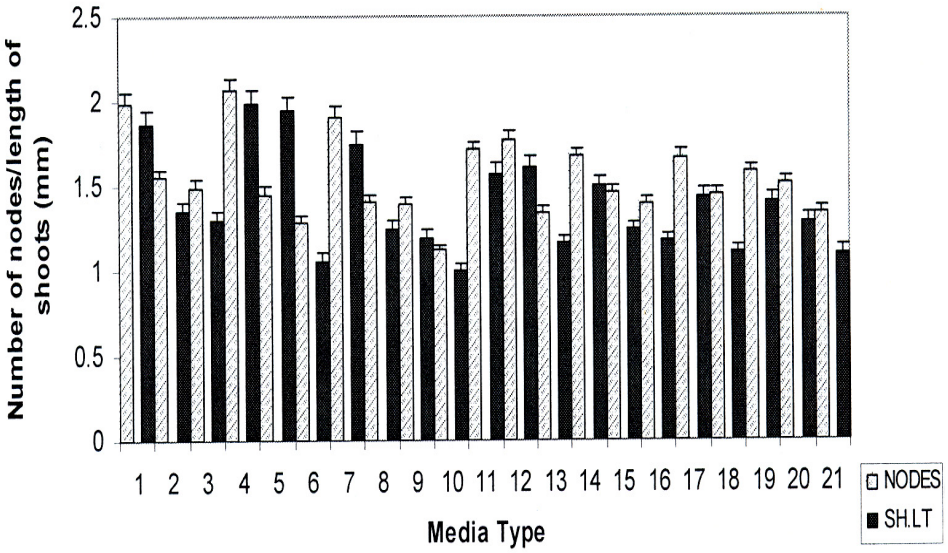

Fig. 1. Response of different media on the increase in length (mm)/number of nodes in *Ixora coccinea*.

Fig. 2. Two weeks after cultures were subjected to subculture.

Fig. 3. Shoot elongation was observed 4 weeks after plants were subjected media containing BAP@ 0.05mg/L.

Fig. 4. Profouse rooting was observed on media containing IBA @1mg/L.

Fig. 5. Plants thriving well in green houses.

It has been shown in this study that after obtaining substantial proliferation of 8 to 10 shoots per explant per subculture, transfer of multiplying clusters to a basal medium with lower salt concentration is essential for conditioning the cultures as observed by Misra *et al*., (1999). A similar observation has been made in *Ixora singaporensis* by Malathy (1998) in which roots were induced by transferring the shoots previously cultured in MS basal to B5, a lower salt medium.

A micropropagation protocol for *Ixora coccinea* has been successfully developed in which explants are initiated and cultured for 3 to 4 months at a 4 week subculture cycle by which proliferating shoots are obtained. From these proliferating shoots, supply of both rooted plantlets and proliferating clusters of shoots are available continuously, which are an essential requirement for mass propagation (Rajaseger, 1997, 1999). By the end of five subcultures, more than 500 shoots were obtained from a single explant, which were in active proliferation besides simultaneously generating rooted plantlets in an increasing order of 50, 100, 200 and 400 for each subculture cycle. Thus, from a single explant, it is possible to regenerate as many as 100,000 plants in about 20 subcultures, making this a commercially viable protocol.

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