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Effect of Growth Regulators In *in vitro* Propagation of *Ixora* spp.: An Short Review

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ABSTRACT

Various factors that affect culture establishment, shoot growth proliferation and rooting of *Ixora* were discussed in this article. Stem segment with node from *Ixora coccinea* was best suited as explant. Micropropogation of *Ixora* involves culturing shoot tips on Murashige and Skoog (MS) medium supplemented with different concentration of cytokinin. The use of cytokinin alone had a low effect on shoot morphogenesis of *Ixora*. The results showed that a lot of calli be induced in MS medium with 2,4-D (2.0 mg/l), increased shoots and a little callus be induced in medium MS with BA (1.0 mg/l) and NAA (0.2 mg/l) . It also indicated that axillary buds of *Ixora* treated with NAA and IBA, using any substrate as support, allowed reaching an optimal morphology development ensuring the survival and rooting of the plants in 100%. The use of NAA encourages the initiation of the root and the number and length of roots after 25 days from culture. The basal application of auxin to the cuttings of treated shoots improves rooting. By sequential reculturing and subculturing 15-20 usable shoots (more than 1cm length) could be produced from the 2-node shoot segments after 12-15 weeks of cultures. Microcuttings taken from *In vitro* proliferated shoots were rooted on half-strength MS medium having of NAA, IBA, and IAA (0.1-0.5 mg/l). The 90% of the plantlets can be established under the conditions when transferred them on a specially made plastic tray containing coco-peat as a potting mix.

Keywords: BAP; Explant; IAA; Ixora sp.; Micropropagation; NAA; Sub-culturing

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INTRODUCTION

Ixora coccinea belongs to Rubiaceae family, is an ornamental plant in many parts of the world. It is one of the important horticultural crop and it gains its commercial importance as a cut flower. The native of *Ixora* is Malay, Peninsula and its adjacent area (Amin et al., 2002). It is distributed in the tropical and sub-tropical regions. It comprises 160 species, and 30 are found in India (Thakur and Kumar, 2014). The common name of *Ixora* includes "Flame of woods" because of its bright colours (Khan et al., 2004). The other terms of *Ixora* are Jugle-geranium and Flame of forest, and it is named as *Ixora* in Sudan. Many cultivars and hybrids come to market in the last 20 years for commercial cultivation (Onsa et al., 2018). It even grows in sunny weather. *Ixora chinensis* is a widespread tropical woody ornamental plant for its abundant and bright flowers. They are known to possess anti-carcinogens and prove to be an effective remedy against TB (tuberculosis) (Malathy and Pai, 1998) and contain antibacterial property. The flowers of *Ixora* spp. are used to treating whooping cough, and the decoction of the bark is used to treat anaemia (Thakur and Kumar, 2014). It has a large, round cluster of tubular flowers with flat, flared petals, which are brilliant red that remains open for a long time, contrast with the glossy, leathery dark green leaves. They also produce yellow, orange, pink flowers. They grow well in acidic soil and can be raised by seeds and cuttings (Holttum and Enoch, 1991). *Ixora* plant care is negligible, which makes it useful as a part of the low maintenance landscape. The conventional method of propagation is stem cutting. Since it is a time consuming, labour intensive method and low multiplication rate (Amin et al*.,* 2002). Only a few *in vitro* studies were available for regeneration of *Ixora* plants through plant tissue culture (Lakshmanan et al., 1997, Mishra and Datta, 1999, Songjun et al., 1999, Noreen et al., 2001, Amin et al., 2002, Khan et al. 2004, George et al., 2008, Thakur and Kumar, 2014, Onsa et al., 2018, Li et al., 2019).

Effect of explant

Shoot apex (Lakshmanan et al., 1997), leaf (Li et al., 2019), and floral bud (Rania Ahmed et al., 2018) all in their early stage of development were taken as explant. Stem cuttings (decapitated shoot, three nodes) were the most suitable explants for multiple-shoot proliferation, and when cultured on a woody plant medium (WPM) containing BA (2.5 μM) produced axillary shoots which repeatedly branched, yielding an average of 27 shoots per explant after six weeks in culture. A regeneration system of *I. chinensis,* the using young leaves as explants on the MS medium supplemented with different Plant Growth Regulators (PGRs). The optimal conditions for shoot regeneration include BAP)(4.0 mg/l), Thidiazuron (TDZ) (1.0 mg/l)and α-NAA (1.0 mg/l)with a shoot regeneration rate of 23.7% was obtained. The subculture of shoots on MS medium fortified with BAP (1.0 mg/l) and NAA (0.5 mg/l) achieved a survival ratio of 80.8% and the rooting ratio of 30.5%. For callus initiation, *I. coccinea* sterile leaves or flower petals explant showed little to respond to different concentrations of 2,4-D, TDZ and NAA.

Effect of hormones

The result of adding different types of cytokinins with MS medium appeared only on the initiation of leaves on the shoot tip of *I. coccinea* after eight weeks from culture. The best result obtained on Zeatin (1.0 mg/l) (5.9 leaves) followed by BAP (2.0 mg/l), (5.7 leaves) treatments without significant difference between them, but both have a considerable difference from other treatments. This result was confirmed by Lakshmanan et al. (1997) reported that BAP (2.0 and 2.5 mg/l)in culture media affected shoot tips explant of *I. coccinea* that continued to grow as a single shoot and did not branch even after three months. A similar observation was also achieved by Khan et al. (2004) in *I. chinensis* .Direct shoot regeneration was highly significant in *I. coccinea* using different PGRs IBA (0.5 mg/l) combined with BAP (2.0 mg/l). BAP (2.0 mg/l) combined with IBA (0.5 mg/l) showed the highest number of all measured parameters. The shoot regeneration is optimal in the BAP (4.0 mg/l) TDZ (1.0mg/l), and NAA (1.0 mg/l) with a shoot regeneration rate 23.7% (Li et al., 2019). These results emphasize the previous finding that the high concentration of cytokinin and low concentration of auxins promote regeneration of shoots (George et al., 2008). Add BAP (2.5 mg/l)with IAA (0.25 mg/l)observed best result on shoot multiplication of *I. coccinea*, and also in the same line with Amin et al. (2002) who found that 100% of *I. fulgens* explant proliferation on MS media contains BAP (0.5mg/l)and NAA (0.1mg/l)while in same concentration of BAP but with IBA instead of NAA showed 60% of shoot tip explant growth commonly and healthy this also in agreement with Lakshmanan et al. (1997).

Different types and concentrations of auxins showed a significant effect on rooting of plantlets. Rooting 100% occurred on NAA (10.0 mg/l) followed by 80% on NNA (5.0 mg/l) and IBA (5.0 mg/l). Othertreatments, including all concentrations of IAA, did not initiate roots. Lakshmanan et al. (1997) who noticed that NAA (10.0 mg/l) and IBA showed that 100% and 60% root initiation, respectively. Amin et al. (2002) observed that IBA (0.2 mg/l) was effectively more than auxin and 100% rooting happened in IBA and NAA of *I. fulgens* plantlets.

Callus initiation of *I. coccinea* leaves or petal explants showed little respond in different concentrations 2,4-D, TDZ, and NAA (0.0, 2.5, 5.0, 7.5 and 10.0 mg/l). There was no response in above treatments in petals, and it remains normal and healthy for more than three months. Songjun et al. (1999) noticed that more shoots and little callus initiation are best in IBA (1mg/l) and NAA (0.2mg/l). This result disagreed with Noreen et al., (2001) who found 80% callus formation with maximum fresh weight (1.16g) in *I. chinensis* leaf explant cultured in MS medium fortified with 2,4-D (3.0mg/l), after eight days from the culture.

NAA alone was found suitable for proliferation of roots from internode, cultures proliferation of axillary shoots were established on MS medium with different concentrations of either BA alone or in combinations of BA with NAA or IBA and with shoot explants from the field-grown mature plant of *I. fulgens*. Nodal segments found to be the best explants for axillary shoot formation on agar gelled MS medium with BA (0.5 mg/L) and NAA (0.1 mg/L). iiN this medium, axillary buds showed sprouting within two weeks of incubation. By sequential reculturing and subculturing 15-20 usable shoots (>1 cm length) could be produced from node segments after 12-15 weeks of culture. Micro-cuttings taken from *In vitro* proliferated shoots were rooted on ½ strength MS medium having NAA, IBA or IAA (0.1-0.5 mg/l). It was found that 90% of the plantlets could be established under *Ex-vitro* conditions. Calli were produced from shoot apex, leaf, internode, and node explants when cultured on KIN,NAA or 2,4-D. MS Medium with KIN (0.5-2.0 mg/l) and NAA (1.0-2.0 mg/l) induced average growth in the cultured floral bud. The best proliferation of roots was achieved when internodal explants were cultured on NAA (1.5 mg/l). MS medium with Kin (0.5-1.0 mg/l) and NAA (1.0-2.0 mg/L) induced average growth in the cultured shoot apex.

Avoid the Browning of medium

Plant parts of the *Ixora* have the property of exudation of phenolics and are the reason for the browning of medium in the micropropagation. It can be avoided by repeated subculturing (Thakur and Kumar, 2014). When compared with other parts, leaves and floral buds exhibit fewer phenolics. Phenolics are exhausted during repeated subculturing.

Table 1. Effect of PGRs in *In vitro* regeneration of *Ixora* spp.

Sl. No	Species	Explant	Medium and its composition (shooting and rooting)	Reference
1.	*I. coccinea*	Stem cuttings (decapitated shoot)	MS+BAP+ IAA (2.5+0.25 mg/l)	Lakshmanan et al. (1997)
2.	*I. coccinea*	Stem segment with node	MS+BA+NAA (0.5+0.5 mg/l)	Songjun et al. (1999)
3.	*I. chinensis*	Young leaves	MS/WPM+GA_3 (2.5 µm)	Noreen et al. (2001)
4.	*I. fulgens*	Nodal segments	MS+BA+ NAA (0.5+0.1 mg/l)	Amin et al. (2002)
5.	*I. coccinea*	Shoot tips and Nodal segments	WPM+BAP (0.5mg/l) WPM+IBA (0.05 mg/l)	Khan et al. (2004)
6.	*I. parviflora*	Shoot apex, leaf, inter node and nodes	MS+KIN+NAA (0.5-1+1-2mg/l)	Thakur and Kumar (2014)
7.	*I. coccinea*	Shoot tip	MS+BAP (2 mg/l)+ IBA(0.5mg/l)	Onsa et al. (2018)
8.	*I. chinensis*	Young leaves	MS+BA+NAA (1.0mg/l)+0	Li et al. (2019)

CONCLUSION

The major problems observed in *Ixora* species micropropagation are browning in medium and high degree of contamination from the experiments. Contamination can be reduced by proper surface sterilization of the explant, and the surface sterilization is different for different *Ixora* species. Browning can be eliminated by repeated

subculturing as described above. The higher concentration of cytokinin and a lower concentration of auxin promote i*n vitro* plant regeneration. Through plant tissue culture techniques without disturbing wild, ornamental plant produce with in short duration, conserved endangered medicinal plants.

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DISCLOSURE STATEMENT

No potential conflict of interest was reported by authors.

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