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Callogenesis and Embryogenesis from Leaf Disks of *Ixora chinensis*

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ABSTRACT

In the present study callus culture were initiated from leaf (explants) of *Ixora* taken from expanding young leaves. Murashige and Skoog (MS) salt mixture containing various concentrations of 2, 4-Dichlorophenoxy acetic acid (2, 4-D) used for callus initiation. Callus initiation was significantly higher in MS medium having 2, 4-D @ 3 mg/L. Earliest callus induction was also found on 2, 4-D (3 mg/L) on the 8th day. Maximum callus fresh weight 1.16 g was also on media containing 2, 4-D (3 mg/L). Direct root hairs formation occurred from calluses induced on 2, 4-D @ 1 and 2 mg/L. Two media (Woody Plant Medium, WPM and MS) were tested for embryogenesis. No embryogenesis occurred but callus multiplication was observed. The best combination which showed excellent multiplication was both WPM and MS media supplemented with Gibberellic acid (2.5 uM).

Key Words: Callogenesis; Embryogenesis; Dichloro phenoxy acetic acid; Gibberellic acid

INTRODUCTION

Ornamental plants decorate and beautify the environment but also add to reduction of pollution. Flowers are also being used as therapy for the serious patients to sooth and relax them. Each plant has its own qualities and uses in the modification of out door environment. The use of shrubs for the beautification of surrounding is main objective. The genus *Ixora* is one of the most important genera of shrubs. Many members of this genus are cultivated through out the tropics as ornamental plants. It belongs to the family Rubiaceae that consist of 400 species. All the species have evergreen glossy green leaves, which attract eyes of observers. *Ixora chinensis* (prince of orange) is often used as a bedding plant in the tropics, but its flower fade badly. The flowers (blooming period is summer) are pale pink but turn reddish with age (Randhawa & Nukhopadhay, 1994). *Ixora* is propagated through stem cuttings in spring as well as through seeds in autumn. It is difficult to propagate through vegetative method of propagation because the cuttings are difficult to root. Similarly, sexual propagation is time consuming and labor oriented method (Lakshmanan, 1997). A little work is done on *in vitro* propagation of *Ixora*. The intention of initiation of this research venture was the mass propagation of *Ixora chinensis* by inducing somatic embryogenesis through *in vitro* culture of leaf disks, so that ever increasing demand of *Ixora* could be met properly.

MATERIALS AND METHODS

Leaf disks 1 cm^2 of *Ixora* were used as explant. Leaves were sterilized with 0.1% mercuric chloride ($HgCl_2$) with 1-2 drops of Tween 20 for one minute, a quick dip in 70% ethyl alcohol (C_2H_5OH) followed by 2-3 washings with autoclaved distilled water. Murashige and Skoog (1962, MS) medium supplemented with four levels of 2, 4-D was used for callus initiation. For embryogenesis, two media were tried. In phase A, the calluses were cultured in Woody Plant Medium (WPM) having different growth regulators. While in phase B, the calluses were inoculated in MS media having same growth regulators as in WPM. Leaf cultures for callus initiation were placed in growth room at 27^0C under dark condition. The cultures for embryogenesis were kept under both dark and light conditions.

RESULTS AND DISCUSSION

Statistical analysis of callus initiation indicated significant combination of MS with 2, 4-D (3 ml/L), which presented 80% callus formation (Table I). The next best combination turned out to be MS with 2, 4-D (2 mg/L), which yielded 73% callus formation. The lowest callus initiation (5%) was observed at control. Callus initiation enhanced significantly with increase in 2, 4-D concentration (Table I). These results are in agreement with Hamidah *et al.* (1997) who obtained best callus from Antherium on 2, 4-D (3mg/L). Maximum fresh weight (1.16 g) of callus was recorded on 2, 4-D (3 mg/L) and second best fresh weight (0.35 g) of callus on 2, 4-D (2 mg/L) (Table I). The treatment 2, 4-D (3 mg/L) also showed the highest fresh weight of callus (Table I). These results are not in line with the findings of Javaid *et al.* (1996) who worked on geranium and reported that it is not necessary that treatments which show the highest percentage of callus initiation, also show the highest mean of callus fresh weight. The difference in results may be due to difference of explant and cultural conditions.

Data recorded on time (week) to initiate callus indicated that earliest callus induction was found on 2, 4-D (3 mg/L) on 8th day, followed by on 2, 4-D (2 mg/L) on 15th day, and on 1mg/L after 15 days. The current results are contrary to the findings of Khosh-Khui and Sink (1982)

who reported best callus induction in stem explants of rose after 3rd week on MS media having 2 mg/L 2, 4-D. Though the basic salts and growth regulators employed are the same but their concentrations vary and may be the possible factor of difference.

Table I. Effect of different 2, 4-D concentrations on callus initiation, callus fresh weight and days to initiate callus from leaf disks of *Ixora*

Treatment 2, 4-D (mg/L)	Callus Initiation (%)	Callus fresh weight (g)	Days to Initiate callus
0	5.00 b	0.09 b	20
1	65.00 a	0.28 b	15
2	73.33 a	0. 35 b	15
3	80.00 a	1. 16 a	08

Means carrying same letter do not differ significantly from each other (with in each column) at 0.05 probability level

Callus growth and morphology (on visual basis). Table II revealed that maximum growth was recorded on 3 mg/L 2, 4-D. The color of callus was off-white and yellow with spongy or friable structure. The lowest callus growth with off- white friable callus was observed on control. Although callus growth on 2 4-D (1 mg/L) was lower than 2, 4-D (2 mg/L) but produced good quality callus with green color. Both of the treatments also showed unusual initiation of root hairs on calluses. These results are in confirmation with those of Amin and Razzaque (1993), who obtained creamy white callus. However, these findings are contrary to those of Javaid *et al.* (1996) who reported that treatment [LS + NAA (2 mg/L)] showed best callus initiation of hard continuous devisable callus growth of white color. The difference may be due to difference in media or variation in the explant used.

No embryogenesis occurred on both WPM and MS media (Table III) when used in combination with different growth regulators, but callus multiplication was observed. In phase A (WPM) and in phase B (MS medium), the best combination which showed excellent callus multiplication under both cultural conditions were both WPM and MS media supplemented with Gibberellic acid (GA_3, 2.5 uM). WPM having BAP (8.8 uM) + IBA (2.5 uM) also showed excellent callus multiplication rate while WPM supplemented with BA (0.5 uM), NAA (0.1 uM) and GA_3 (2.5 uM) showed no callus multiplication.

When callus cultured in MS media having IAA (22.8 uM) and kinetin (0.046 uM), callus remained unchanged i.e. no embryogenesis and multiplication. It was also noted that treatments, which showed maximum callus multiplication resulted in off white color and friable callus. In contrast, the treatment showed the minimum callus multiplication produced by green and hard callus. In phase A and B, callus multiplication occurred instead of embryogenesis. Therefore, there was a deviation from the findings of Buneo *et al.* (1992) who obtained cork oak embryoid on MS + BA (0. 44 uM) + NAA (0.05 uM). The results are also in contradiction with those of Jose and Vieitz (1993) who reported embryogenesis on WPM + BA (8.8 uM) + IBA (2.5 uM) under dark condition. Most of the calluses used for embryogenesis were friable and non-devisable and hence showed no embryogenesis.

Table III. Effect of WPM media and different growth regulators on embryogenesis in *Ixora* (Phase A)

Treatment	Culture conditions	
	Light	Dark
WPM	++++	+
WPM+BA (0.5 uM) +NAA (0.1 uM)	+	–
WPM+ GA_3 (2.5 uM)	++++	++++
WPM + BA (0.5 uM)+NAA (0.1 uM) + GA_3 (2.5 uM)	–	–
WPM + BAP (8.8 uM) + IBA (2.5 uM)	++++	++++

Table III. Effect of MS media and growth regulators on embryogenesis in *Ixora* (Phase B)

Treatment	Culture conditions	
	Light	Dark
MS	+	–
MS + BA (0.5 uM) + NAA (0.1 uM)	+	–
MS + GA3 (2.5uM)	++++	++++
MS + BA (0.5 uM) + NAA (0.1 uM) + GA_3 (2.5 uM)	+	+
MS + IAA (22.8 uM) + Kinetin (0.046 uM)	–	–

++++= Excellent; += Poor; – = Nil

CONCLUSION

It was concluded that MS media having 3 mg/L 2, 4-D was best for callus initiation, maximum growth and earliest callus induction. The procedure of embryogenesis should be improved by testing different growth regulators alone or in combination and by changing the cultural conditions.

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Table II. Effect of 2, 4-D on Callus Growth and Morphology

2, 4-D (mg/L)	Callus growth	Color	Structure	Other Observation
0	+	Off-white	Friable	No other changes were observed
1	++	Green	Hard	Some cultures developed root hairs in up word direction
2	+++	Green	Hard	Some cultures formed root hairs in down ward, mostly in up word direction.
3	++++	Light yellow	Spongy & Friable	No other changes

++++= Excellent; +++= Good; ++= Fair; += Poor

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