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IN VITRO PLANT REGENERATION FORM SHOOT TIP EXPLANTS OF *HIBISCUS SYRIACUS* (L.)

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ABSTRACT
The effect of Tributyltin chloride on freshwater bivalve, *Lamellidens marginalis* was assessed. The freshwater bivalves were exposed to 3.5, 2.5, 1.8 and 1.0 ppm experimental concentration (50% lethal concentration) of Tributyltin chloride up to 96 hours. After 24, 48, 72 and 96 h of exposure the lipid level were estimated in gonad, gill and digestive gland. The results showed a decrease in lipid in both the tissues with an increase in concentrations and time of exposure to the Tributyltin chloride.

INTRODUCTION

Hibiscus syriacus (L.) is a widely cultivated ornamental plant in the genus *Hibiscus*. It is commonly known as Rose of Sharon. The flowers are hermaphrodite (have both male and female organs). *Hibiscus syriacus* (L.) is a national flower of South Korea. It is a shrub with great economic as well as medicinal value. It is used in the production of fibre, medicinal products and beverage additives in some Asian countries (Shimizu *et al.,* 1986; Hotto *et al.,* 1989). *Hibiscus syriacus*, is highly resistant to pests and disease (Zhoa *et al.,* 1991) and is frost tolerant .This species is suitable for genetic improvement through somatic hybridization technique. A preparation of the flower is used in the treatment of itch and other skin disease. Root is used in the treatment of dysentery and abdominal pain. The present investigation describes the development of milky white and green callus and development of shoots and roots from shoot tip culture on Murashige and Skoog media supplemented with various phytohormones ad growth adjutants (Murashige and Skoog, 1962).

MATERIALS AND METHODS

Young green shoot tip of *Hibiscus syriacus* (L.) were collected from Birsa Agriculture University, Kanke. The explants (shoot tips) were washed with running tap water for 15 – 20 minutes and was followed by wash with 1–2 drops of savelon for 2 minutes mixed with 250 mL of sterile water. The explant was further surface sterilized with 70% ethanol for 30 seconds and washed with 3–4 times with autoclaved distilled water again to remove in effects of sterilizing agents. The explants were further sterilized with 0.1% $HgCl_2$ for one minute and again washed with autoclaved distilled water. Shoot tip were inoculated in test tube containing Murashige and Skoog media (1962) with different concentration of phytohormones. Combination of auxin and cytokinin was also used for plant regeneration. The pH of the media was adjusted to 5.8 by adding 0.1% NaOH or 0.1% $HgCl_2$ before gelling with Agar–Agar (0.8% W/V). The media was autoclaved at 121ºC for 15 minutes. All inoculation was done in a laminar Air flow cabinet. All inoculation tools like forceps, needles, scissors, blade, and scalpel were thoroughly autoclaved. Culture area glass wares and all tools used were properly sterilized with UV light, Inoculation tools were flamed to sterilize them. Callus obtained from shoot tip cultures were subculture for further multiplication. Fully grown shoot were then transferred to MS media containing IAA, NAA and BAP.

RESULTS AND DISCUSSION

Shoot tip of *Hibiscus syriacus* L. was cultured on Murashige and Skoog medium (1962) supplemented with different concentration of Phytohormones. After 10–15 days callus was produced. When shoot tip was cultured on Murashige and Skoog medium supplemented with 2,4-D 2.5 mgL^{-1} only white callus and no organogenesis was observed .When shoot tip derived callus was cultured in combination of auxin (2, 4-D) and cytokinin (BAP) supplemented with Murashige and Skoog medium (1962) green embryogenic callus was observed. Roots and shoots were observed when callus was sub cultured on the MS medium supplemented with combination of auxin and cytokinin. BAP was found to be the most effective cytokinin for shoot differentiation in combination with IAA. When these callus were cultured on media containing higher concentration of BAP 5mgL^{-1} with lower concentration of IAA 2.5 mgL^{-1} shoot formation were observed. Similar types of responses were reported by various workers in different plant

Table1: Effect of different concentration of growth regulators on callus induction and morphogenic response of *Hibiscus syriacus* L. grown on MS Media

Growth regulator(mgL^{-1})	Nature of callus	Response organogenesis
2,4-D		
0.5	— —	
1.5	+ + +	
2.5	+ +	
5	+ +	
NAA		
0.5	— —	
1.5	— —	RT
2.5	+	RT
5	+ +	RT
BAP		
0.5	— —	
1.5	+	
2.5	+ +	SH
5	+ + +	SH
BAP + IAA		
2 + 1	— — — —	SH
2.5 + 2		SH
BAP + NAA		
1 + 0.5		SH + RT
2 + 1		SH + RT
2.5 + 1.5		— —

— — — = No response; + = Good; + + = Very Good; + + + = Excellent; SH = Shooting; RT = Rooting

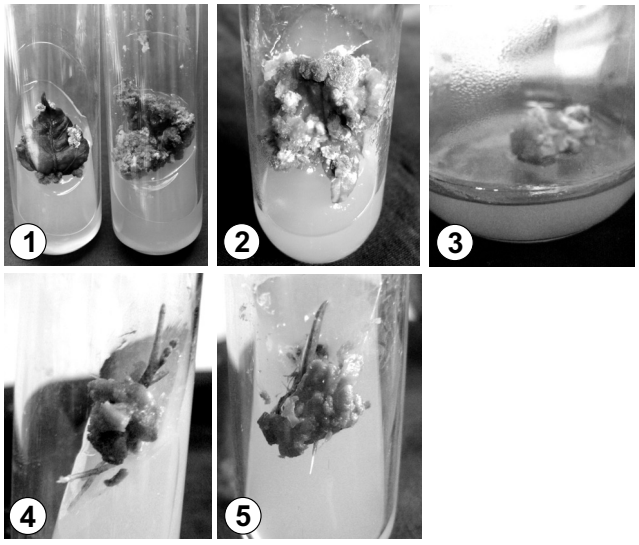

Figure 1 to 5: Showing different stages of callus formation and shoots and roots development in *Hibiscus syriacus* (L.)

species (Wang, 1986; Jorge *et al.,* 1998; Mishra *et al.,* 2004; Vidya *et al.,* 2007). In vitro rooting of regenerated shoots was efficiently obtained after a period of 6 weeks on MS medium containing higher concentration of auxin and lower concentration of cytokinin. When callus was cultured on NAA (0.5 to 2.5 mgL^{-1}) and BAP (0.5 to 2.5 mgL^{-1}) profused growth of root was observed. When BAP was used with NAA or KN was used with NAA large numbers of roots were observed which are in conformity with the findings reported for few species of other medicinal plants (Hatrilazarous *et al.,* 2003; Antar *et al.,* 2007) and In conclusion, this study clearly demonstrated that in vitro propogation of *Hibiscus syriacus* can be obtained by proper manipulation of explants age and concentration of different plant growth regulators. Disease free plants of *Hibiscus syriacus* L. could be produced in lesser time in large scale by this method. The present studies also highlight the season independent availability of material during micropropogation of *Hibiscus syricus* L.

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