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MICROPROPOGATION OF *HIBISCUS ROSA SINENSIS* (L.)

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ABSTRACT
Hibiscus rosa sinensis L. commonly known as China rose is an ornamental plant belonging to family *Malvaceae*. This is a sacret plant with great economic importance as medicinal plant. Differentiation of shoots were initiated on Murashige and Skoog (1962) medium supplemented with various combination of phytohormones such as IAA, IBA, NAA, KN, BAP and 2,4-D.

INTRODUCTION

Plants are major source of our food and they provide medicine for cure of many of our diseases (Arvind *et al.,* 2007). Due to its medicinal importance there has been uncontrolled exploitation of these valuable resources (Namedeo, 2007). The techniques of tissue and organ culture is used for rapid multiplication of plants for genetic improvement of crops, for obtaining disease free clones and for preserving valuable germplasm. *Hibiscus rosa sinemsis* (L.) (Malvaceae) commonly known as China Rose, lipstick flower, shoe flower, etc is an ornamental as well as medicinal plants. It is an evergreen prennial shrubs and native of China. The flowers are large, generally red in colour in original varieties, but lack any scent. It is a sweet astringent cooling herbs that checks bleeding and it also soothes (Bown, 1995). The flowers are aphrodisiac, demulcent, emollient and refrigerant (Chopra *et al.,* 1996). They are used internally in the treatment of excessive and painful monsuration, feverish, illness cough and to promote hair growth. The leaves and flowers are beaten into paste and polished onto concerous swelling and mumps. A paste made from root is used in the treatment of veneral disease.

Commercially *Hibiscus rosa sinensis* L. is important and its flowers are also used for religious purposes. *Hibiscus rosa sinensis* is a shrub and is widely grown for its religious importance and medicinal value. Long item usefullness of tissue culture for woody species has been described by Marziah, 1995. Thus it is important to determine conditions formation of tissue culture for this economically important plant. The benefit of a successful tissue culture protocol, would allow the desired commercially valuable species such as *Hibiscus rosa sinensis* L. to be propogated aseptically.

The present study described the most suitable concentration of various phytohormones along with Murashige and Skoog (1962) medium for callus induction. The technique of plant tissue culture and organ culture has been used for multiplication of *Hibiscus rosa sinensis*.

MATERIALS AND METHODS

Green young leaves explants of 4 - 8 months old *Hibiscus rosa sinensis* L. plants were collected from B.A.U. Botanical Garden. The explants were washed with running tap water for 15 - 20 minutes to wash surface contaminations. This was followed by wash with 2 drops of savelone mixed with 250 mL of sterile water. They were further surface sterilized with 70% ethanol for 30 seconds and washed 3 to 4 times with autoclaved distilled water again to remove its effects to sterilizing agents. They were sterilized with 0.1% $HgCl_2$ for one minute and again washed with autoclaved distilled water. Leaves were inoculated in test tube containing MS basal media supplemented with various concentration of phytohormones. Callus obtained from above culture was also used as culture material.

Semisolid (Murashige and Skoog, 1962) media containing various concentration of phytohormones were used for callus formation and shoot regeneration. Combination of auxin (IAA, IBA, NAA, 2, 4-D) was used for shoot regeneration. Coconut milk (cw -= 20% v/v) was used in MS basal medium for regeneration system. The pH of media was adjusted to 5.8 by adding 0.1% NaOH or 0.11 $HgCl_2$ before gelling with Agar - Agar (0.8% v/w) and autoclaved for 15 to 20 minutes at 121°C. Surface sterilized leaves were inoculated into the culture medium in culture room under cool fluorescent light (1500 - 20000 Lux) with a 16 hr/8hr light dark cycle. Each treatment consisted of 24 explants and all experiments were carried out under sterile condition. All inoculation tools like forceps, needles, scissors, blade etc. were thoroughly autoclaved. Culture area glass wares and all the tools used were properly

sterilized with UV light and floor surface were swabble with 95% ethyl alcohol inoculation tools were flamed time to time.

RESULTS AND DISCUSSION

Explants of *Hibiscus rosa sinensis* L. cultured in Murashige and Skoog medium (1962) supplemented with different growth regulators produced callus and shoot differentiation after 15 - 28 days of culture. Callus showed a different response according to the growth regulator used. Callus formation was observed from leaf on medium containing 2, 4-D (0.5mg/L to 5mg/L). Concentration above 5mg were found to be quite inhibitory. There was only callusing and no organogenesis was seen in media containing phytohormones 2,4-D. IBA proved to be more effective than IAA in inducing callus from leaf culture. Shoot formation was observed from leaf explant on medium containing BAP + IAA.

BAP was found to be the more effective cytokinin for shoot differentiation in combination of auxin. Most suitable medium for shoot formation from leaf tip explant was BAP (2mg/L) + IAA (1mg/L). The effect of different auxins and cytokinins for inducing callus and morphogenic response from explants is shown in Table 1 and Fig. 1a to 1d.

The synergistic effect of higher concentration of cytokinin with lower concentration of auxin induced better shoot formation was reported by various workers in different plant species (Mishra *et al.,* 2004 and Vidya *et al.,* 2005). Similar result was obtained in *Hibiscus rosa sinensis* L. It was found that in higher cytokinin concentration shoots developed with short internodes and apical leaves which were also reported in few other medicinal plants (Rudra and Juwarkar, 2002).

Table 1: The effect of *Hibiscus rosa sinensis* L. cultured on Murashige and Skoog Medium (1962) with different concentration of Phytohormones

Growth regulator (mg^{L-1})	Nature of Callus	Response organogenesis
2,4-D		
0.5	— —	
1.5	+ +	
2.5	+ + +	
5	+ +	
IAA		
0.5	— —	
1.5	+	
2.5	+ +	
5	+ +	
IBA		
0.5	— —	
1.5	+	
2.5	+ +	
5	— —	
BAP + 2,4-D		
2 + 1	— — — —	SH
3 + 2.5		SH
BAP + IAA		
2 + 1		SH
3 + 2.5		SH
KN + 2,4-D		
2 + 1		SH
3 + 2.5		SH
BAD + 2,4-D		
2 + 1		SH
3 + 2.5		SH

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

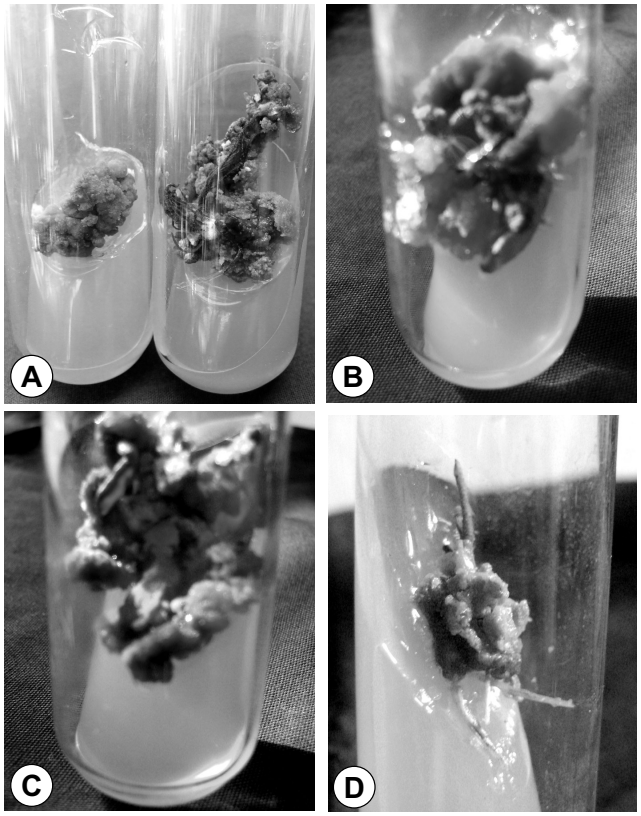

Figure 1a to 1d: Various stages of callus formation and root and shoot development in *Hibiscus rosa sinensis* (L.)

Callus formation was influenced by type and age of explant and growth regulators used. Importance of tissue culture technique for efficient and reliable vegetative propogation has been described by De Fossard (1976) and Hussey (1978). Rapid asexual multiplication can be obtained by axillary shoot formation production of adventitious shoots or by simatic embryogenesis (Murashige, 1978).

Disease free plant for *Hibiscus rosa sinensis* L. could be obtained in large scale on a short period of time bond. The present protocol also highlights the age of explants and different concentration of phytohormones on shoot regeneration and micropropogation of *Hibiscus rosa sinensis* L.

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