Micropropagation of *Hoya Kerrii* (Valentine Hoya) Through Callus Induction for Long Term Conservation and Dissemination

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Abstract: ***Micropropagation is applied to multiply those species which are difficult to produce conventionally. The purpose of this study was to access in vitro propagation of Hoya kerrii, an important ornamental plant to explore its potential for micro-propagation. Microprogation of Hoya kerrii was initiated using leaf, petiole, root and inter-nodal segments of the selected plant as explants on MS medium containing 2,4-D at 1, 2, 3, 4 and 5 mg/L for callus induction. Leaf segments initiated callus earlier than inter-node, petiole and root. A significant amount of callus was produced in MS medium with 5.0 mg/L 2, 4-D and MS medium with 1.0 mg/L 2, 4-D gave the poorest callus.***

Keywords: Hoya kerrii, Micropropagation, 2, 4-D, Callus**.**

1. Introduction

Hoya kerrii is a species of Hoya native from the south-east of Asia, Australia and Polynesia. As their thick leaves are heart-shaped, the plant is sometimes called "Lucky-heart" or valentine hoya. The unique characteristics of *Hoya kerrii* is its heart shape leaf which gives it a high demand as an ornamental plant. Usually Valentine hoys can be propagated through either leaf or stem cutting which is a slow process. *In vitro* culture has been widely used for the propagation of agricultural and horticultural crops and for the conservation of crop genetic resources [1].

Mass propagation of these plants can be obtained from a small explants shoot tip or leaf explants within few months. It is rapid and cost effective and clonal propagation of plants can be produced throughout the year [2]. Tissue culture studies on Hoya species are very limited. Actually there are very few studies on *Hoya kerrii* Micropropagation. In this study I tried to micropropagate *Hoya kerrii* through callus induction.

2. Materials and methodology

2.1 Explants Sterilization

The mother plants were collected from BRAC nursery and planted in pots for regular procurement of explants for experiment. For experiment, young leaf, middle aged leaf, flowers, roots of *Hoya kerrii* were procured and were kept under running tap water for about 30 minutes and then kept in a solution of liquid detergent (Teepol) for 2-3 minutes to remove surface dirt. They were then rinsed with sterile double distilled water at least thrice to remove the traces of detergent. The explants were then surface sterilized with 20% Chlorox (commercial bleach containing 5.2% (w/v) NaOCl solution for 5-6 minutes. Surface sterilized explants were again treated with 0.1% mercuric chloride solution for 4-5 minutes under aseptic condition then washed five to six times with sterilized distilled water. The explants were then inoculated aseptically into MS [3] medium with different concentrations and combination of growth regulators (Table 1).

2.2 Media preparation and culture condition

The MS basal media was supplemented with various growth regulators 2,4-D .After mixing all salts, organic components and 3% sugar the pH of the medium was adjusted to 5.8±0.02 .Then 6.9 g agar was mixed to make the media semi-solid. Test tubes containing media were then autoclaved at 121°C at 15 psi for 20 min.

All the cultures were grown in an air conditioned culture room at a temperature of 26±2ºc. The source of illumination was 40 w white florescent tubes light with intensity varied from 2000-3000 lux. The photoperiod was maintained as 16 h light and 8 h darkness and 55±5% humidity was maintained. Visual observation of culture was made every week and data were recorded after 4 weeks of inoculation. Various concentrations of 2, 4-D (1-5 mg/l) were used for callus initiation through leaf, inter-node, root and petiole explants.

3. Results and Discussion

In this study, the medium was supplemented with various concentrations of growth hormone for the induction of callus from various explants like leaf, root, inter-node and petiole. Growth hormone 2, 4-D alone could initiate callusing from explants but the growth of callus was slow. The results of callus production and multiplication are presented in Tables 1 and 2. Leaf segments produced profuse callus than petiole, root and inter-nodal segments. Roots failed to produce any callus. A significant amount of callus was found in the MS medium supplemented with 5.0 mg/L 2, 4-D.

Table 1: Callus formation of *Hoya kerrii* on MS medium supplemented with different concentrations of 2, 4-D

2,4-D	*Explants*	*Test tube*								
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
1 mg/L	Leaf	F	F	F	F	F	F	F	F	F
	Inter-node	F	F	F	F	F	F	F	F	F
	Petiole	F	F	F	F	F	F	F	F	F
	Root	F	F	F	F	F	F	F	F	F
2 mg/L	Leaf	+	+	F	+	F	+	+	+	F
	Inter-node	F	F	F	F	F	F	F	F	F
	Petiole	F	F	F	F	F	F	F	F	F
	Root	F	F	F	F	F	F	F	F	F
3 mg/L	Leaf	+	+ +	+ +	++	+	+	++	+	+
	Inter-node	+	+	+	F	+	F	+	+	F
	Petiole	+	F	+	+	F	F	F	F	F
	Root	F	F	F	F	F	F	F	F	F
4 mg/L	Leaf	++	++	+ ++	+ +	++	++	+++	++	++
	Inter-node	+	+	+	F	+	+	+	+	+
	Petiole	++	+	+	+	+	F	+	+	+
	Root	F	F	F	F	F	F	+	F	F
5 mg/L	Leaf	+++	+++	+ ++	+++	+ ++	+++	+++	+++	+++
	Inter-node	++	++	++	++	++	++	++	++	++
	Petiole	+	++	+	++	+	+	+	+	+
	Root	F	F	+	F	F	F	F	F	F

F, failed; +, poor callus; ++, medium callus; +++, profuse callus

Table 2: Influence of different concentrations of 2, 4-D on callus formation

Hormone	*Days of callus initiation*	*Color*	*Amount of callus formed*
1 mg/L	29	No callus formed	Failed
2 mg/L	29	Blakish	Poor
3 mg/L	28	Brown to blakish	Poor
4 mg/L	28	Brown	Medium
5 mg/L	28	Brown to light green	Profuse

Here the study of Hoya micropropagation has been demonstrated which can be an important alternative to conventional propagation and breeding procedures for this ornamentally important plant species for its ex situ conservation.

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Author Profile

Romana Siddique received the B.Sc. and M.S. degrees in Genetic Engineering and Biotechnology from University of Dhaka in 2007 and 2009, respectively. She did her Bachelor and Master’s thesis at Plant Biotechnology Laboratory of University of Dhaka on salt tolerant rice. Later she worked in BRAC Biotechnology Lab, a commercial research lab working on Plant Biotechnology. She is now with BRAC University as a faculty member.