Micropropagation of *Adenium Obesum* (Dessert Rose) *In Vitro*

Rasad F.M.*, Hasbullah N.A, Daud N.F, Azis N.A, Amin M.A.M, and Lassim M.M

Abstract**—***Adenium obesum* is an exotic plant that is also known as dessert rose, mock azalea and pink begonia. It belongs to the family *Apocynaceae*. *Adenium obesum* is an attractive succulent shrub with a stout swollen stem, grayish bark and striking pink flowers. This study highlights micropropagation of *Adenium obesum* through tissue culture techniques. *Adenium obesum* seeds were first rinsed using 50%, 20%, 10% sodium hypochlorite followed by distilled water. This series of seed sterilization was done sequentially. Subsequently seeds were rinsed with 70% alcohol and finally with distilled water prior to culture. Seeds were cultured on Murashige and Skoog, 1962 (MS) basal medium containing 8% agar technical and 30% sucrose. *In vitro* seed germination was observed within two weeks. Complete plantlets were obtained after 6 weeks of culture. Slight callus growth was observed at the base. Plantlets will then subcultured onto fresh MS basal medium for root growth and elongation. Explants such as leaf and stem will later be transferred onto MS medium supplemented with various concentration of plant growth regulator such as Benzylaminopurine (BAP) and Napthalene Acetic Acid (NAA) to see further organogenesis response. The study showed that *in vitro* propagation of *Adenium obesum* to develop new plantlets was successfully obtained.

Keywords**—***Adenium obesum*, micropropagation, in vitro, callus, plantlets, plant growth regulators.

I. Introduction

WITH the garden and nursery industry, it is very important to ascertain the consumer returning year after year for the new discoveries, most unusual and most exciting species. *Adenium obesum* also known as dessert rose, belongs to the family *Apocynaceae*. It is native to the East Africa and has been a very popular ornamental plant for decades. Adenium flower resemble frangipani (*Plumeria sp.*). Therefore, it's not suprising that many of these ornamental plants fans called it kemboja cina or pokok cina. *Adenium obesum* is a beautiful succulent shrub with a stout swollen stem, grayish bark and striking pink flowers. *Adenium obesum* can be proapagated by seeds, cuttings or transplants.

Fatimah Bt Mat Rasad, Sultan Idris Education University, Malaysia
Email id:- caeq_pex@yahoo.com
Nor Azlina Bt Hasbullah, Sultan Idris Education University, Malaysia
Email id:- azlina.h@fptv.upsi.edu.my
Noor Fara'ain Bt Daud, Sultan Idris Education University, Malaysia
Email id:- fare_inmush258@yahoo.com
Nurul Aufa Bt Azis, Sultan Idris Education University, Malaysia
nurulauffa@yahoo.com
Muhamad Azizi Bin Mohamed Amin, Sultan Idris Education University, Malaysia
Mohammad Bin Mohd Lassim, Sultan Idris Education University, Malaysia

Cutting is inefficient propagation method for pot plant, since the planting material has a very low multiplication rate and it requires a large area of stock plants [1] [2].

In vitro propagation technique has come up as a credible and cost-effective alternative tool for rapid production or ornamental in recent years. In India, the market of ornamentals has steadily increased over the last few years [3] and Adenium has a good opportunity to occupy a significant share of this nascent market. Kanchanapoom et al. [1] reported that *Adenium obesum* could be micropropagated by utilizing multiple shoots derived from seedling shoot tips. Not more information has been reported on the in vitro propagation of potted *Adenium obesum* but the commercial application of *in vitro* techniques in Apocynaceae has been well demonstrated and the regeneration of plants has been reported [4], [5], [6], [7], [8], [9].The present work describes *in vitro* regeneration and callus induction from leaf explants.

II. Material and Method

The seeds of *Adenium obesum* (Dessert rose) were obtained from Ho Chi Minh City in Vietnam. *Adenium obesum* seeds were first rinsed using 50%, 20% and 10% sodium hypochlorite for 3 minutes respectively followed by distilled water. This is series of seed sterilization was done sequentially. Subsequently seed were rinsed with 70% alcohol for 1 minutes and finally with sterilized distilled water prior to culture. Rinsing of seeds with 70% alcohol was done in the laminar flow (under aseptic conditions). Seeds were cultured on Murashige and Skoog (MS)(1962) basal medium containing 8% agar technical and 30% sucrose for germination.

Culture were incubated in dark photoperiod at 26°C .Seeds were germinated after 14 days of culture. Prior to germinating, cultures were transferred to cool-white-fluorescent light room and incubated at 25+1°C with 16 hours light and 8 hours dark photoperiod.

Leaf and stem explants excised from in vitro plantlets containing were then placed on MS medium containing 30 g/L sucrose supplemented with various concentrations of BAP (0.5 - 2.0 mg/L) and NAA (0.5 – 2. 0 mg/L) (Tables 1). Media was solidified with 8 gram agar and autoclaved at 121°C at psi 20 minutes. Ph of media was adjusted to 5.8 prior to autoclaving. All cultures at 25 + 1°C under white fluorescent light of 40-60 µ mol m-2 s-1 intensity for 16 hrs light /8 hrs dark photoperiod were incubated. Total number of explants in each treatment was 30. Subculturing to a new fresh medium was done after 4 weeks of cultures.

III. Statistical Analysis

All data obtained were statistically analyzed using Microsoft Excel. The data gathered from the experiments were analyzed according to mean percentages and analysis variance (ANOVA) at 5% level of significance. Each treatment was replicates 3 times with 2 explants for each replicates. The experiment was conducted twice.

IV. Result And Discussion

TABLE I: PERCENTAGE OF CALLUS FORMATION FROM LEAVES AND STEMS ON MS MEDIUM SUPPLEMENTED WITH NAA AND BAP CULTURE AT 25 ± 1 °C

No.	Hormone concentration (mg/L) NAA + BAP	Explants	% Callus
{1}	0.5mg/L + 0.5mg/L	Leaves Stems	73.33 ± 13.58 99.04 ± 0.95
{2}	0.5mg/L + 1.0mg/L	Leaves Stems	68.57 ± 6.61 95.45 ± 2.52
{3}	0.5mg/L + 1.5mg/L	Leaves Stems	85.18 ± 3.75 100 ± 0
{4}	0.5mg/L + 2.0mg/L	Leaves Stems	NR NR
{5}	1.0mg/L + 0.5mg/L	Leaves Stems	100 ± 0 100 ± 0
{6}	1.5mg/L + 1.0mg/L	Leaves Stems	100 ± 0 100 ± 0
{7}	Control	Leaves Stems	NR NR

Callus formation was observed from leaf and stem explants after 4 weeks of inoculation in MS medium with different combination and concentrations of Napthalene Acetic Acid (NAA) and Benzylaminopurine (BAP) (Table 1). The highest callus induction percentage (100%) was observed in the MS medium supplemented with two different concentrations, 1.0mg/L NAA + 0.5mg/L BAP {5} and 1.5mg/L NAA + 1.0mg/L BAP {6} both for leaves and stems. However, callus for concentration {5} very small with light green color (Fig. 1a), while the callus with concentration {6} was biggest and healthy green color (Fig. 1b). [10] explained that the callus formation is depending upon the several factors including the culture, environment, nature of explants and hormonal and non hormonal regulators which may act synergistically in determining the proper induction, proliferation of callus and regeneration into plants.

In addition, callus induction percentage (100%) also obtained in culture media consisting of 0.5mg/L NAA + 1.5mg/L BAP {3} for stems and 85.18 ± 6.61 for leaves. The color of callus was turning brown at the base (Fig. 1c). The control culture media and the concentrations of 0.5mg/L NAA + 2.0mg/L {4} did not response in this study (Fig. 1d).

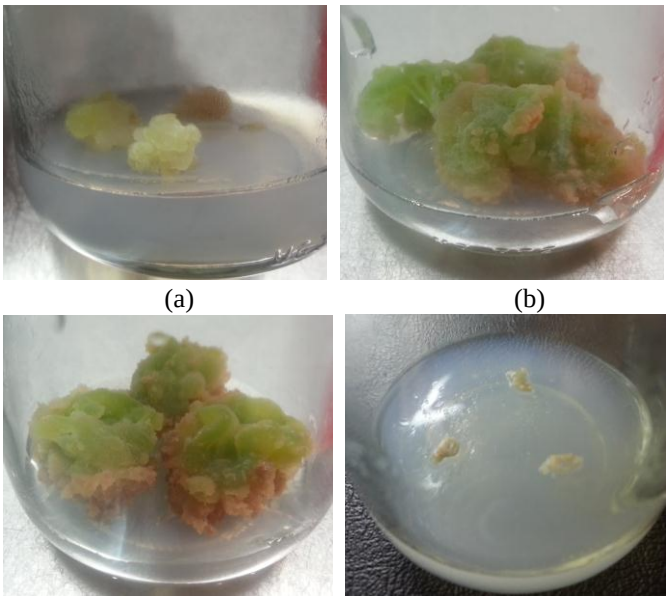

Fig.1. Micropropagation of *Adenium obesum*. (a)Callus induction for concentration {5}; (b) Callus induction for concentration {6}; (c) Brown base callus for concentration {3}; (d) Non response for control and concentration {4}

The callus induction also high for both combination of concentration 0.5mg/L NAA + 0.5mg/L BAP (stem: 99.04 ± 13.58; leaves: 73.33 ± 13.58) and 0.5mg/L NAA + 1.0mg/L BAP (stem: 95.45 ± 2.52; leaves: 68.57 ± 6.61) but in the end, after 4th weeks the callus become brown and dead. The best result for this study is on concentration {6}, 1.5mg/L NAA + 1.0mg/L BAP because of the biggest callus and healthy green color. In this study, we have developed a callus induction protocol for *Adenium obesum* (Dessert rose). This protocol will promote the application of tissue culture technology to facilitate the genetic transformation of this species.

V. Conclusion

In this study, micropropagation and callus induction of *Adenium obesum* was successfully obtained. Stem explants has been identified as the move regenerative explants for induction of callus. Studies of *Adenium obesum* clonal propagation and callus induction could also be efficiency adapted for other crops in future research.

APPENDIX

A

B

Figure 2: (A) In Vitro Seedling.
(B) 8th week old of *Adenium obesum*

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