

Callogenesis and Plant Regeneration of *Jasminum sambac* L. (Jasmine)

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Abstract— In the present study, regeneration of *J. sambac* var. Maid of Orleans through direct and indirect organogenesis has been investigated. In direct organogenesis, shoot development from young stems (each contained one nodal part) cultured in MS media supplemented with a combination of BAP (3.0 mg L⁻¹) and NAA (1 mg L⁻¹) showed 20% shoot regeneration.

For indirect organogenesis, among different explants only young stems inoculated in MS media supplemented with 2, 4-D (0.5 mg L⁻¹) yielded callus. In order to identify embryogenic cells in callus, double staining test was carried out, and embryogenic callus was detected in all formed callus, in MS media supplemented with different concentrations of 2, 4-D. Further studies on cell suspension culture by using liquid MS media supplemented with various concentrations of 2, 4-D (0.5 mg⁻¹) has also been done. Different colors of callus were obtained in the present study including green, yellowish and whitish. However, the entire callus did not show any development of organogenesis.

Keywords— Callogenesis, Regeneration, *Jasminum Sambac*, Jasmine.

I. INTRODUCTION

Plant tissue culture offers an alternative method for the conservation of rare, valuable and endangered plant genotypes. This technique can successfully preserve the plant species, which do not produce seeds or some of seeds that cannot be stored for an extended period. However, *in vitro* methods can be applied for preservation of vegetative tissues, to keep the genetic background [1].

Jasminum sambac L. (family: Oleaceae) is a genus containing approximately 600 species of small trees and shrubs.

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Based on old Chinese books, the origin of *J. sambac* was from Eastern south and Southeast Asia. The ten species of this genus, including *J. sambac* were cultivated into Arabia and Persia (Iran) in gardens under the common name "sambac" in the 18th century and was introduced to Europe as ornamentals [2]. According to its high medicinal value, *J. sambac* is one of the most cultivated species in many countries in Asia including Thailand. There are plenty of secondary metabolic activities such as anti-bacterial, anti-cancer, anti-tumor, anti-diabetic, anti-acne, anti-oxidant and anti-stress from this plant that had been reported [3]. The phytoconstituents of this plant contain iridoidal glycosides, linalyl 6-O-malonyl-β-D-glucopyranoside, β-primeveroside, 2-phenylethyl β-primeveroside, β-rutinoside, dotriacontanoic acid, dotriacontanol, oleanolic acid, daucosterol, and hesperidin [4]. Furthermore, flower of *J. sambac* displayed the efficacy to suppress puerperal lactation [5] and the essential oil was determined to possess antibacterial activity [6].

There are numerous cultivars of *J. sambac* on the basis form of flower bud, petal shape and number of whorls [3]. The variety, Maid of Orleans is the national flower of Philippine (Sampaguita) and cultivar of *J. sambac* that possesses flowers with a single layer of five or more oval shaped petals [7]. The goal of the present study was to develop an efficient regeneration protocol for direct and indirect organogenesis of *J. sambac* var. 'Maid of Orleans' and to examine callogenesis using various hormones and explants.

II. MATERIALS AND METHODS

A. Plant Materials

The mother plants (8- month- old) were bought from a nursery (Ipoh-Malaysia). The fresh and young leaves, stems and petioles were surface sterilized by using calcium hypochlorite containing, ethanol and distilled water [8].

Hormone preparation: Based on preliminary *in vitro* hormones optimization and effect of auxin/cytokinin ratio to plant regeneration [9], for callus initiation, high concentration of auxins and low concentration of cytokinins were used. A combination of NAA (α-Naphthalene acetic acid) and BAP (6-Benzylaminopurine) as high cytokinin to auxin (3 mg L⁻¹ BAP, 1 mg L⁻¹ NAA) and high auxin to cytokinin (3 mg L⁻¹ NAA, 1 mg L⁻¹ BAP) for direct

organogenesis was applied. To achieve indirect organogenesis 2, 4-D (2, 4 Dichlorophenoxyacetic acid) from 0 to 2 mg L⁻¹ with 0.5 increment was used.

B. Media preparation

MS basal media [10] included 30 g sucrose, 5 g L⁻¹ gelrite supplemented with two different combinations of NAA/BAP and five different concentrations of 2, 4-D was used as inoculation medium. All media were adjusted to pH 5.8 with 1 N, KOH and autoclaved at 121 °C for 20 min and was dispensed into 60 mm specimen containers under aseptic condition. The sterilized explants were cut and were cultured onto Murashige and Skoog (MS) medium supplemented with various concentrations of hormones with 16 hours light and 8 hours dark conditions in a sterile container.

The 2-month callus formed *in vitro* was tested by double staining method adopted from [11] to obtain embryogenic cells. The callus (20 g) established *in vitro* (MS supplemented with 0.5 mg L⁻¹ of 2, 4-D) was weighed out under laminar flow cabinet and was cultured on liquid MS media supplemented with different concentrations of 2, 4-D (0 to 2 mg L⁻¹ with 0.5 increment). The cultures were maintained on a horizontal shaker (120 rpm) in a growth chamber at 24°C. All experimental units were monitored every day to record the contamination of explants. The regular subcultures were carried out at the intervals of two weeks into the same media and concentration of the hormone.

C. Statistical analysis

Callus induction experiment was arranged as RCBD (Randomized Completely Block Design) with 10 replications. Mean comparison was performed using Duncan multiple range tests. The data were subjected to normality test using one sample Kolmogorove-Smirnove. Before ANOVA, the percentage data were arcsine transformation to normalize the distribution. All data analysis was done using SPSS.

III. RESULTS

The different types of explants showed various responses to media and hormones. After two weeks, the highest amount (89%) of greenish friable calli was obtained from stem explants cultured on MS medium supplemented with a combination of 3 mg L⁻¹ BAP and 1 mg L⁻¹ NAA. The compact green-yellowish calli formed from petiole (64%) and leaf (58%) explants on the same concentration of hormones, respectively (Fig. 1).

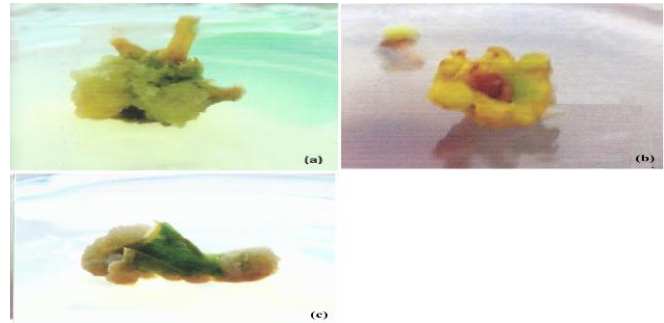


Figure 1: Callus formation from stem (a), petiole (b) and leaf (c) explants of *J.sambac* on MS media supplemented with high ratio of BAP and NAA

Shoot was formed from stem explants cultured on MS media with addition of 3 mg L⁻¹ BAP and 1 mg L⁻¹ NAA as a combination of high ratio cytokinin to auxin (Fig. 2).



Figure 2: Shoot formation from stem explant of *J. sambac* on MS media supplemented with high ratio of BAP to NAA.

As shown in Fig. 3, calli were formed (94%) and 28% on liquid media supplemented with 0.5 and 2 mg L⁻¹ of 2, 4-D, respectively.

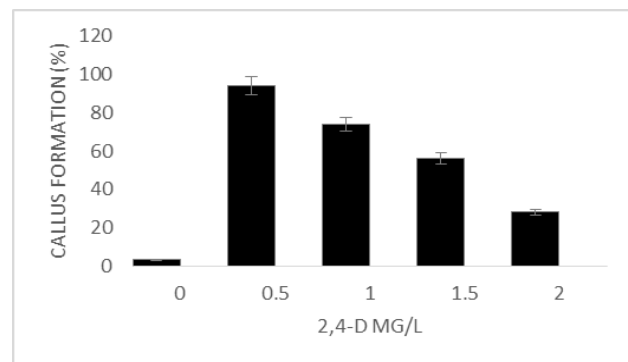


Figure 3: Callus formation of *J. sambac* on MS liquid media and various concentrations of 2, 4-D.

According to double staining test, the embryogenic cells observed in callus formed in MS medium supplemented with auxin.

IV. DISCUSSION

Effects of explants and different ratios of auxin/cytokinin to callus formation was reported by [9, 12], in the present study, stem explant inoculated on MS media with addition of a high ratio of BAP to NAA gave the better response to callus formation compared to other explants and PGR's ratio. However, [13, 14] reported the same results of shoot and root formation when different explants cultured on MS media containing different 2,4-D/Zeatin and IBA/BAP. The double staining test and callus formation in liquid media supported our previous results of embryogenic cells from callogenesis of *G. jasminoides* by 2,4-D [15] and [7].

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