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Callus induction on *Jasminum sambac* L. by 2,4-dichlorophenoxy acetic acid hormone

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Abstract

Jasminum sambac (L.) Aiton (Family: Oleaceae), an ornamental plant extensively used in perfumery and religious purposes, is a herb which shows shrub-like appearance after two years. It is locally known as '*Motia'* and produces white flowers with a very pleasant fragrance. In recent years, plant tissue culture techniques has become a powerful tool for the propagation of many plant species. In this research weight and diameter of callus were analyzed to determination of appropriate medium culture. This study were performed on spring of 2014 in complete randomized design with three replicated. The MS medium culture was contain 2,4-D hormone. ANOVA statistical analysis showed significant difference at 1% level. Highest callus diameter belong to medium contain 0.3 mg/l 2,4-D , also maximum callus weight belong to 0.3 mg/l 2,4-D. Therefore, hormones amount which used in this research can induced callus in sesame.

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Introduction

Jasminum sambac (L.) Aiton (Family: Oleaceae), an ornamental plant extensively used in perfumery and religious purposes, is a herb which shows shrub-like appearance after two years. It is locally known as '*Motia*' and produces white flowers with a very pleasant fragrance. The plant attains a height of 1-2 feet in later stages. *J. sambac* is vegetatively propagated by ground layering and stem cuttings. As seeds are not formed, the vegetative propagation is the only reproductive method. Normally vegetative propagation is achieved through ground layering but it is not convenient for transportation purpose of germplasm and success rate is also very low. Hormonal treatment of stem cuttings shows induction of high frequency multiplication. A dip and grow3 solution is helpful in vegetative propagation of garden plants (*Hartman et al, 1998*). It has been reported that softwood tissues of stems tend to form adventitious roots in plants (Berry, 1984). In the structural development of plants, environment is responsible to some extent but hormones play important role in transforming cells towards formation of adventitious roots (Barnes, 1989). Phytohormones play important role in stress responses and adaptation10 and the exogenous application of auxins (*Khan et al, 2004*), gibberellins (*Afzal et al, 2005*) and cytokinins (*Gul et al, 2000*) produce some benefits by accumulating at damaged site in plants and induce formation of callus (*Akira et al, 2011*). It also plays role in alleviating the adverse effects of environment as well as improve growth and development. Plant is propagated using medium-mature stems (8 to 10 inches long) by planting in perforated plastic bags filled with sandyloam soil and watered daily. In its cultivation, water is critical especially during the establishment period where rooting and rapid plant growth occurs. The soil should be saturated with moisture to the root zone for good growth. The flowering of jasmine is not correlated with the amount of rainfall although the water status in the soil prior to induction may influence the intensity of flowering. The harvesting of flowers is done from 2nd year after planting and the commercial yields commence from third year onwards (*Sanchez et al, 2010*). Tissue culture, an important area of biotechnology can be use to improve the productivity of planting material through enhanced availability of identified planting stock with desired traits. Micro propagation is one of the important contribution of Plant Tissue Culture to commercial plant propagation and has vast significance. Tissue culture has helped to develop new strain of food crops, cereals, vegetables flowers, oil seeds and plantation crops such as spices, coffee, tea and rubber. Therefore the present preliminary study was we have chosen callus mediated shoot organogenesis as an alternative method to achieve a higher rate of leaf multiplication for crop improvement, during January until June , 2014, Sari Agriculture Sciences and Natural Resources University (SANRU), Sari, Iran.

Material and methods

Plant material and disinfection

The leafs were washed with running tap water for 60 minutes, followed by soaking in 3 % of teepol soap solution for 15 minutes then reported rinsing in distilled water. The leafs were disinfected with 70 % ethanol treatment for 45 seconds and washed with sterile distilled water for three times, followed by 0.1 % (w/v) aqueous mercuric chloride for 3 minutes and washed again three times with sterile distilled water. The disinfected seeds were germinated in 25 × 150 mm test tubes containing non-absorbent cotton. Initially the culture were maintained in dark condition for 48 h at 25 ± 2°C and then under 16 h light and 8 h dark photoperiod condition with the light intensity of 3000 lux. Shoot tip and nodal segments were excised from seven-day-old aseptic seedlings and used as explants.

Culture Condition

MS basal medium (Murashige and Skoog, 1962) supplemented with 3 % (w/v) sucrose and 0.8 % (w/v) agar was used for subsequent experiments. The pH of the medium (supplemented with respective growth regulators) was adjusted to 5.8 with 1 N NaOH or 1 N HCL before galled with 0.8 % (w/v) agar (Himedia Ltd., Mumbia, india). The medium was

dispensed into culture tubes (borosli, India) and autoclaved at 105 kPa and 121°C for 15 minutes. The shoot tipe and nodal explants were inoculated on the culture medium in test tubes 150 × 25 mm, containing 10 ml medium and plugged tightly with non-absorbent cotton. All the cultures were incubated at 25 ± 2°C under 16/8 (light/dark) hours photoperiod of 30 µmol m^{-2} s^{1} of cool white fluorescent tubes (Philips, India). All subcultures were done at 20 or more day's intervals at appropriate stages.

Organogenic Callus Induction

leafs segments from in vitro grown 12 days old seedlings were used as explants and placed on callus initiation medium which contained MS salts (Murashige and Skoog, 1962), B_5 vitamins supplemented with diverse concentration of 2,4-D (0.1 - 0.2 - 0.3 - 1 - 2 - 3 mg/l) alone or in combination 2.4-D for callus induction.

Results and discussion

Callus culture induction

The leafs were cut into small segments and used as explants. They were cultured on callus induction medium (MS) consisting of different auxins. Among the one auxin investigated 2,4-D was more effective than the other auxins with the highest percentage (68 %) of callus initiation (Table 1). The auxins in different concentration produced different types of callus. However, used 2,4-D at mid-level concentrations gave best percentage of organic callus induction (68 %, Table1) in the present study. Result of ANOVA statistical analysis according by Complete Random Design (CRD) with three replicates showed that the leaf in MS medium with 2,4-D (0.3 mg/l) has produced high quality callus (Table 2). Auxins are the most likely candidates in the regulation of developmental switches (Nomura and Komamine, 1985 and Feher *et. al.,* 2003). The influence of exogenously applied auxins particularly 2,4-D on the induction of leaf are well documented (Dudits *et. al.*, 1991 and Yeung, 1995). It is suggested that 2,4-D above certain concentration has a dual effect in the culture medium as an auxin directly (Michalczuk *et. al.,* 1992a and 1992b) and as a stress inducing agent (Feher *et. al.,* 2001, 2002 and 2003). However at higher concentrations of auxins the number and frequency of callus induction decreased (Table 1). The results were obtained has many differences with Sharma *et al* (2014) in amount of using 2,4-D hormone. Their result in compare with our research have significant in diameter and weight of callus. Therefore, we can introduce these hormone concentrations' for JASMINUM callus induction.

Table 1. Data on effects of 2,4-D on callus induction and callus growth of leaf explants.

Plant Growth Regulators (mg/l) 2,4 D	Percentage of organogenic callus induction	Type and nature of callus
0.1	32	Light Brown
0.2	36	Light Brown
0.3	68	Light Brown
1	56	Golden
2	0	------
3	0	------

Table 2. Sambac ANOVA statistical analysis.

Source	df	Diameter	Weight
2,4-D	5	57.757**	0.499**
Error	12	1.177	0.027

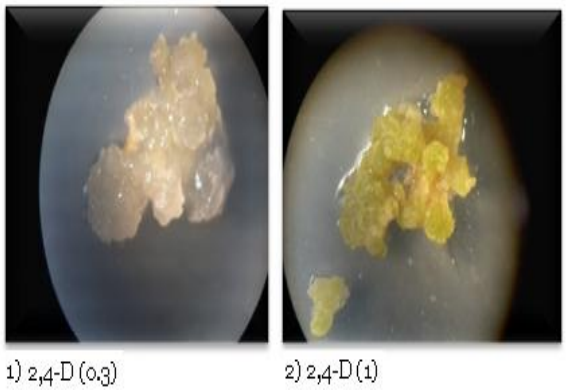

Fig. 1. Callus induction of sambac by 2,4-D hormones.

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