Regeneration of adventitious shoots from induced callus of Lantana Camara L. plant by in vitro culture technique

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ABSTRACT

The results showed that the MS medium supplemented with a concentration of 1.4 mg L-1 of NAA and 0.1 mg L-1 BA resulted in the highest significant value in the percentage of response to callus induction of 96.67% compared with the other combinations of growth regulators. The results showed that a combination of 9.5 mg L-1 BA and 0.2 mg L-1 NAA which was added to the MS medium recorded the highest percentage of response to indirect adventitious shoot formation from callus of Lantana Camara, several shoots per 100 mg callus and shoot length reached 96.67%, 10 shoots per 100 mg callus and 3.00 cm, respectively, after 12 weeks of culturing. The adventitious shoots of indirect organogenesis of Lantana Camara were rooted when cultured on the MS medium supplemented with a concentration of 1.0 mg L-1 of NAA and 0.1 mg L-1 BA after 8 weeks of planting.

Keywords: Agar, benzyl adenine, growth regulator, indirect organogenesis, rooting

INTRODUCTION

The big sage (Lantana camara L.) is an evergreen ornamental shrub that is cultivated for the beauty of its flowers and ability to cut and form (Francis, 2004). The native habitat of big sage is a subtropical and subtropical region of the Americas and in the tropics of Asia and Africa where sources indicate that the natural presence of Lantana in Mexico, the Caribbean. The species was widely spread around the world during the 19th and early 20th centuries (Gentle and Duggin, 1997). This plant belongs to the Verbenaceae family, which includes 100 genera and about 2000 species and the Lantana genus, it contains about 150 species that fall within the group of ornamental plants. Their flowers are small in size and are clustered in small-sized bouquets, as well as small-sized fruits, with smooth stone seeds that are easy to germinate. The height of the big sage plant is about 2 meters (Liogier, 1995). L. camara plant propagates by seeds but the producing plants are few because of the hardness of the seed coat (testa) and the plants are genetically different. The traditional vegetative propagation methods are not favoured for the difficulty of rooting using stem cuttings. Therefore, many researchers resorted to the micropropagation method of the big-sage plant because of its advantages, such as the production of plants in large numbers and the plants are genetically identical to the mother plant and free from diseases and micro-organisms causing them. The plant is characterized by quadrangular stems covered with sharp spines (Henderson, 2001). Waoo et al. (2013) found that culturing the shoot tips or nodal segments of the big-sage plant as explants on the Murashige and Skoog (1962) (MS) medium, which is supplemented with different benzyl adenine (BA) at concentrations of 0.1 to 0.7 mg L-1. In another study carried by Veraplakorn (2016) on callus induction from leaves of L. camara L. by in vitro culture technique, the result showed that callus induction and formation on leaves when cultured on the MS medium

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supplemented with combinations 0.0 and 9.0 mg L-1 BA with 3.7 or 7.4 mg L-1 NAA. The current study was conducted to determine the optimal combination of plant growth regulators that are added to the MS medium for the induction of callus and the indirect organogenesis by culturing the shoot tips of the big-sage (L. camara L.) plant using the plant tissue culture technique.

MATERIALS AND METHOD

The study was conducted in the plant tissue culture laboratory at the Faculty of Agriculture, University of Basra, Iraq. The shoot tips were taken as explants of seedlings of big-sage (L. camara L. cv. Local) plant. Then were washed with tap water to get rid of the dust and impurities suspended. Then they were surfaces sterilized with 1% sodium hypochlorite solution with a few 2-3 drops of polysorbate 20 (Tween 20) under the Laminar air flow cabinet for 20 min. The sterilized shoots were cultured MS medium (Murashage and Skoog, 1962), obtained from American Coisson Labs Company. This medium of callus induction consisted of 4.33 g L-1 of MS salts, 170 mg L-1 of sodium hydrogen orthophosphate, 40 mg L-1 of adenine sulfate and 1.0 mg L-1 for each of glycine, thiamine-HCl, pyridoxine and nicotinic acid. Naphthalene acetic acid was supplemented with different concentrations (0.8, 1.0, 1.2 and 1.4 mg L-1) and benzyl adenine at a concentration of 0.1 mg L-1 to the MS medium prepared for the callus induction experiment. For the indirect shoot regeneration experiment, 7.5, 8.5 and 9.5 mg L-1 of benzyl adenine and 0.2 mg L-1 of naphthalene acetic acid were added to the MS medium. While for rooting combination of 1.0 mg L-1 naphthalene acetic acid and 0.1 mg L-1 benzyl adenine was added to the MS medium. The pH of the medium was adjusted on 5.8 by sodium hydroxide and hydrochloric acid solutions at a concentration of 1.0 N for each. Then medium was solidifying using agar at a concentration of 6.0 g L-1.

MS medium was poured at a size of 20 ml per culture tube and then the culture tubes were then sterilized in the autoclave instrument at a pressure of 1.0 kg cm-2 and 121 °C for 20 minutes. The response percentage of the shoot tips to callus induction, callus to indirect adventitious shoot formation, shoot formed per 100 mg callus and length of the shoot (cm) were calculated. Each experimental treatment repeated 10 times.

Experimental design and statistical analysis

Simple experiments were designed according to Complete Randomized Design (CRD). The experimental data were statistically analyzed according to the analysis of variance. Compare the mean of the treatments according to the Revised-LSD test depending on Al-Rawi and Khalaf Allah (2000).

RESULTS AND DISCUSSION

Callus induction experiment showed that the callus tissue was at the base of the shoot tips of L. camara that were cultured on MS medium with different combinations of naphthalene acetic acid (0.8, 1.0, 1.2 and 1.4 mg L-1) with benzyl adenine (0.1 mg L-1) after eight weeks of culture (Figure 1, A and B). Results from Figure 1 showed that MS medium with a concentration of 1.4 mg L-1 of naphthalene acetic acid and 0.1 mg L-1 benzyl adenine recorded the maximum callus induction percentage (96.67%) compared to other treatments. While the shoot tips cultured on the MS medium with 0.8 mg L-1 NAA and 0.1 mg L-1 BA recorded the lowest callus induction percentage (67%) (Figure 2). The results of this study were agreed with previous studies conducted on the same plant (Saxena et al., 2013; Veraplakorn, 2016). They indicated the possibility of stimulating callus from culturing the shoot tip and leaf explants at a concentration of 0.8 mg L-1 NAA and 0.1 mg L-1 BA. The cause of the stimulation of the explants on callus induction was may be due to the importance of the role of auxins and cytokinins in cell division. Since auxins activate enzymes responsible for the formation of the cellular wall (Taiz and Zeiger, 2010; George et al., 2008).

Table 1 indicates that the combination of 9.5 mg L-1 BA and 0.2 mg L-1 NAA recorded maximum adventitious shoot formation from callus of L. camara, with ten shots per 100 mg callus, and shoot length reached 3.00 cm with the percentage of callus induction reached 96.67%, after 12 weeks periods (Figure 1, C, D, E and F). This is because the high concentration of cytokinin with a low concentration of auxin leads to the re-differentiation of callus cells, which has led to the formation of adventitious shoots. The results of the study were agreed with a previous study conducted on the Citrus grandis plant (Ibrahim, 2012). While the combination of 7.5 mg L-1 BA and 0.2

mg L-1 NAA resulted in the lowest percentage of shoot formation (66.7%) and produced 3.0 shoots per 100 mg callus (Table 1). The cause of indirect organogenesis is the hormonal balance between endogenous and exogenous cytokinins and auxins, which has led to the growth and differentiation of callus cells that have led to the adventitious shoot formation (Firoozabady and Moy, 2004). As well as cytokinins and auxins activate the cellular division of meristematic tissues, especially during the stage of callus formation and differentiation and their role in the growth and elongation of plant organs (Dello'loio, 2007; Ibrahim and Draaj, 2015). The adventitious shoots of indirect organogenesis of L. camara were rooted when cultured on the MS medium supplemented with a concentration of 1.0 mg L-1 of NAA and 0.1 mg L-1 BA after 8 weeks growth periods (Figure 1, G). The adventitious shoots cultured on MS medium produced a maximum rooting percentage (100%). A number of roots that formed at the shoot base were 11 roots shoot-1. This is because this combination of growth regulators, auxin and cytokinin that have been added is optimal for stimulating the division and differentiation of cells in the shoot base that led to production of adventitious roots (Al-Ramdan, 2012).

CONCLUSION

The combination of 1.4 mg L-1 BA and 0.1 mg L-1 NAA is optimal concentrations for callus induction from the shoot tip explants of the Lantana camara plant. While using 9.5 mg L-1 BA and 0.2 mg L-1 NAA, resulted in maximum adventitious shoot formation from the callus. On the other hand, MS medium supplemented with combination 1.0 mg L-1 BA and 0.1 mg L-1 NAA, resulted in maximum root formation.

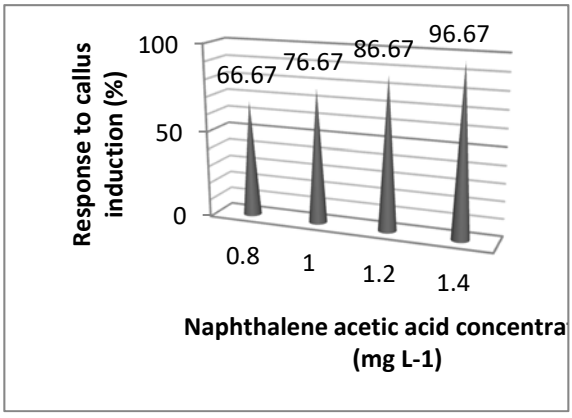

Figure 2: Effect of different concentrations of naphthalene acetic acid on the percentage of response to callus induction of Lantana camara L. cv. Local. (R-LSD p≥0.05=9.66).

Table 1: Effect of different concentrations of benzyl adenine with 0.2 mg L-1 naphthalene acetic acid on adventitious shoot formation from callus of Lantana camara L. cv. Local.

Benzyl adenine (mg L-1)	Response to shoot formation (%)	Number of shoots per 100 mg callus
7.5	66.67	3.00
8.5	90.00	9.00
9.5	96.67	10.00
R-LSD p≥0.05	1.85	2.22

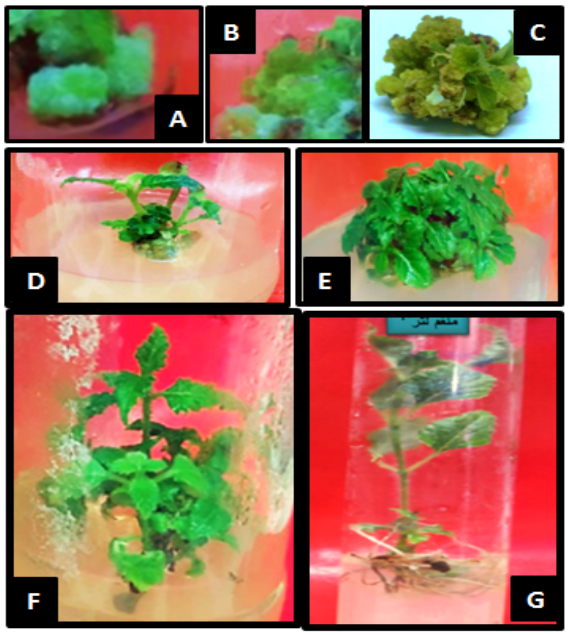

Figure 1: Adventitious shoot formation of big-sage (Lantana camara L. cv. Local).

A, B- Callus induction from shoot tip explants after 8 weeks of culturing.

C, D, E, F- Indirect organogenesis after 12 weeks of culturing.

G- Shoot rooting after 8 weeks of culturing

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توليد الأفرع العرضية من الكالس المستحث من نبات المينا الشجيري *Lantana camara L.* بتقنية الزراعة خارج الجسم الحي

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ملخص

أوضحت النتائج أن الوسط الغذائي *MS* المجهز بتركيز 1.4 ملغم لتر-1 نفثالين حامض الخليك (*NAA*) و0.1 ملغم لتر-1 بنزيل أدنين (*BA*) نتج عنه فرق عالي المعنوية في النسبة المئوية للاستجابة إلى استحثاث الكالس بلغ 96.67% مقارنة مع التوليفات الأخرى من منظمات النمو. وأشارت النتائج إلى أن التوليفة المكونة من 9.5 ملغم لتر-1 *BA* و0.2 ملغم لتر-1 *NAA* التي اضيفت إلى الوسط الغذائي *MS* أدت إلى تسجيل أعلى نسبة مئوية للاستجابة إلى تكوين الأفرع الخضرية غير المباشر من كالس المينا الشجيري وأعلى عدداً للأفرع الخضرية لكل 100 ملغم كالس، وأعلى طول للفرع الخضري الذي بلغ 96.67% و10 أفرع خضرية لكل 100 ملغم كالس و 3.0 سم بالتتابع بعد 12 أسبوعا من الزراعة، إن الأفرع العرضية لتوليد الأعضاء غير المباشر لنبات المينا الشجيري جذرت عندما تم زراعتها على الوسط الغذائي *MS* المجهز بتركيز 1.0 ملغم لتر-1 *NAA* و 0.1 ملغم لتر-1 *BA* بعد ثمانية أسابيع من الزراعة.

الكلمات الدالة: آجار، بنزيل أدنين، منظم نمو، توليد أعضاء غير مباشر، تجذير.

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