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Callus induction and indirect and direct organogenesis from culturing the axillary buds of the tulip (*Tulipa gesnerina* L.) bulbs by *in vitro* culture technique

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Abstract: The study was conducted with the aim of callus induction and indirect and direct shoot formation from culturing the axillary buds of the tulip cv. Arma on the MS medium equipped with 1.0 mg L^{-1} NAA and 0.5, 1.0, 1.5, 2.0 or 2.5 mg L^{-1} BA. The results showed that the callus tissue did not grow when the axillary buds were cultured on the MS medium which supplied with 1.0 mg L^{-1} NAA and 0.5, 1.0 or 1.5 mg L^{-1} BA after eight weeks from culture. However, the axillary buds cultured on the MS medium which supplied with 1.0 mg L^{-1} NAA and 2.0 or 2.5 mg L^{-1} BA which led to callus induction after eight weeks from culture. The treatment of 1.0 mg L^{-1} NAA and 2.5 mg L^{-1} BA was a significant increase in the percentage of callus induction and indirect shoot formation and the number of shoots. The results showed that the callus cultured on the MS medium that supplied with 1.0 mg L^{-1} NAA and 2.0 or 2.5 mg L^{-1} BA led to the formation of the adventitious shoots formation eight weeks after culturing. While, the axillary buds cultured on the MS medium which supplied with 1.0 mg L^{-1} NAA and 0.5, 1.0, or 1.5 mg L^{-1} BA led to the direct adventitious shoot formation eight weeks after culturing. The MS medium that supplied with 1.0 mg L^{-1} NAA and 1.5 mg L^{-1} BA was a significant increase in the percentage of response to direct adventitious shoot formation and the number of shoots.

Keywords: Adventitious shoot, benzyl adenine, naphthalene acetic acid.

INTRODUCTION

Tulips belong to the Liliaceae family of about 150 species (Jaap *et al.*, 2007). Tulips are ranked among the top ten best types of flowers sold worldwide. The Netherlands is one of the most producing countries for tulip bulbs, which account for about 87% of global production (Buschman, 2005). The tulip plant is propagated vegetative in the traditional method by the bulbs which are not optimum because the number of bulbs formed is very little, meaning that the production of a new cultivar for this plant needs about 25 years (Le Nard and De Hertogh, 1993). The tulip plant is usually characterized by the length of the juvenile stage that takes about 4-5 years (Rees, 1992; Custers *et al.*, 1997). Moreover, the number of tulip bulbs made from the bulb is very limited when propagating by traditional methods, ranging from 2-3 new bulbs (Podwyszynska, 2005). Tulip bulbs are also exposed to infection with many fungal and viral diseases when propagating in the traditional method, as there are about 22 species of viruses that infect them (Mowat, 1995). All of these reasons led to the use of the plant tissue culture technique in the proliferation of tulip to obtain true to type and free-viruses plants from the mother plant and obtain large numbers of bulbs up to 1000 bulbs within a short period (20 months) instead of 16 years (Podwyszynska, 2001; Podwyszy´nska and Marasek, 2003; Sochacki and Podwyszy´nska, 2006). Maślanka and Bach (2014) indicated that the best medium of callus induction for tulip plant is the MS medium supplemented with 0.11 mg L^{-1} benzyl adenine and 3% sucrose as the response rate to callus induction was 96%. Kabir *et al.*, (2014) obtained the highest percentage of response to the callus induction from culturing the corm segments of gladiolus (*Gladiolus dalenii*) on the MS medium supplied with 7.5 mg L^{-1} naphthalene acetic acid amounted to 90% after 90 days of culture. This study also found that the culture of induced callus in the MS medium supplied with 0.5 mg L^{-1} benzyl adenine and 0.5 mg L^{-1} kinetin led to high response to the indirect organogenesis of 70%. The average number of shoots that formed from induced callus and shoot length were 20±2.40 shoots and 4.50 ± 0.45 cm, respectively, after 60 days of culture. Kizil *et al.*, (2016) indicated to the callus induction from the leaf sheath of the hyacinthus (*Hyacinthus orientalis* L.) plant when cultured on the MS medium supplied with concentrations of 0.5, 1.0 and 1.5 mg L^{-1} benzyl adenine and 0.1 mg L^{-1} naphthalene acetic acid. The MS medium supplied with 1.5 mg L^{-1} benzyl adenine and 0.1 mg L^{-1} naphthalene acetic acid led to the highest percentage response to callus induction of 97.98%. The current study aims to propagate the tulip (*Tulipa gesinerina* L.) plant cv. Arma by indirect and direct organogenesis using the plant tissue culture technique to obtain true to type plants and free from infection by fungal and viral diseases.

MATERIALS AND METHODS

The study was conducted at the Plant Tissue Culture Laboratory at the College of Agriculture, Basra University, and Basrah, Iraq. The bulbs of the Arma plant tulip were kept in the refrigerator at 5 ° C until they were used as explants in tissue culture. Bulbs were washed with tap water and liquid soap several times to remove dust and impurities. Next, the surface sterilization of the bulb was carried out using mercury chloride at a 0.1% concentration for 15 minutes. The bulbs were then washed with sterile distilled water 3 to 4 times in a laminar airflow cabinet. Tulip axillary buds were taken from bulbs after anatomy and removal of leafy scales of them (Plate 1, A). These explants were cultured directly on the MS medium (Murashige and Skoog, 1962) obtained from ZAS (Zist) Arman Sabz at 4.33 g L^{-1} MS salts, 30 g L^{-1} sucrose, 40 mg L^{-1} adenine sulfate, 100 mg L^{-1} myo-inositol, 170 mg L^{-1} $NaH_2PO_4.2H_2O$, 7 g L^{-1}agar-agar, 1 mg L^{-1}vitamins supplemented with a constant concentration of NAA at 1.0 mg L^{-1} and BA at different concentrations 0.5, 1.0, 1.5, 2.0 and 2.5 mg L^{-1}. Each treatment in the experiment was repeated ten times. The cultures were incubated at a temperature of 25 ± 2 °C and a light density of 1000 lux. The measurements were included:

1. The percentage of response to callus induction.
2. The percentage of response to indirect and direct organogenesis.
3. The number of shoots per explant.
4. The number of leaves per shoot.
5. The length of shoot (cm).

Experimental design and statistical analysis

The experiment of the study was designed using a complete randomized design. Data were analyzed statistically using analysis of variance. The revised least significant difference test was used to compare the means of treatments at a 5% probability level (Snedecor and Cochran, 1986; Al-Rawi & Khalaf Allah, 2000).

RESULTS AND DISCUSSION

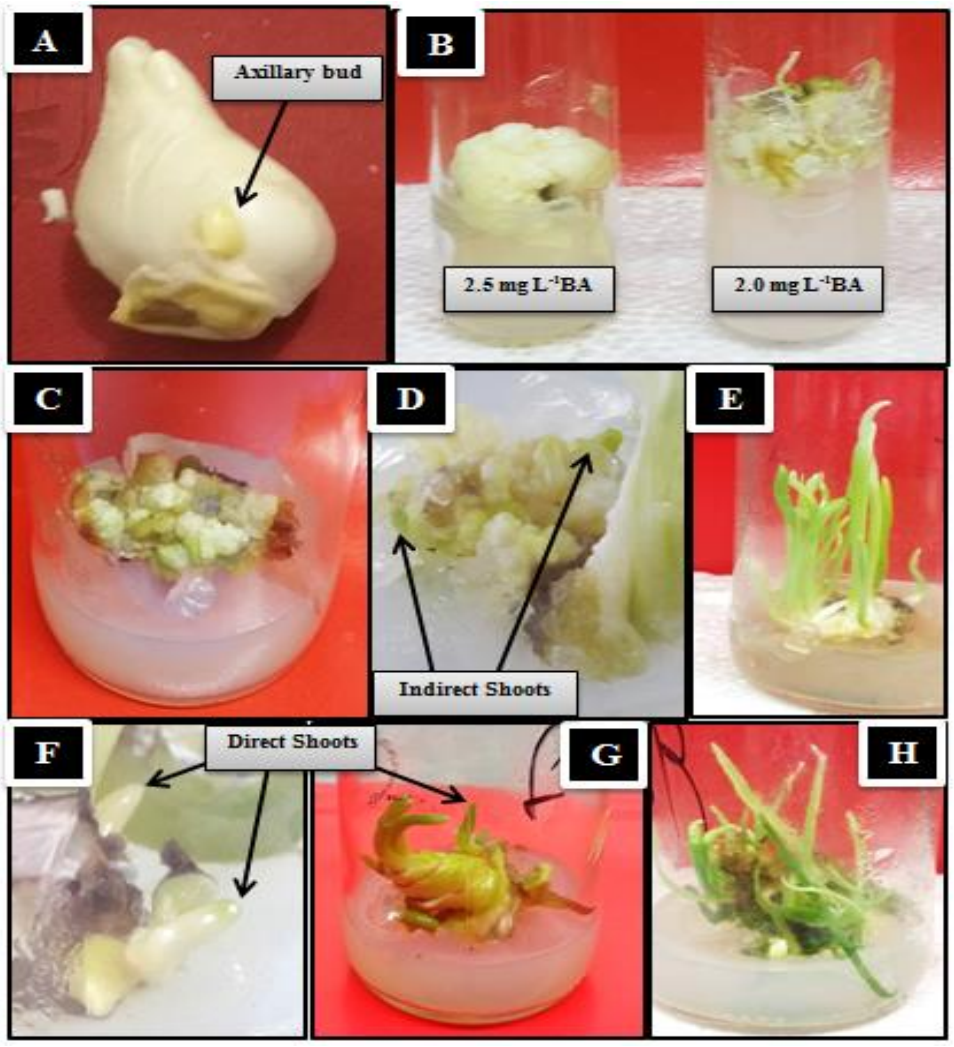

Plate 1: Callus induction and indirect and direct shoot formation of tulip (*Tulipa gesnerina* L.) plant by *in vitro* culture technique. A- Bulb of Arma cultivar. B- Callus induction on MS medium + 1.0 mg L^{-1} NAA and 2.0 or 2.5 mg L^{-1} BA after eight weeks from culture. C, D, E- Indirect shoot formation on MS medium + 1.0 mg L^{-1} NAA + 2.5 mg L^{-1} BA after eight weeks from culture. F, G, H- Direct shoot formation on MS medium + 1.0 mg L^{-1} NAA + 1.5 mg L^{-1} BA after eight weeks from culture.

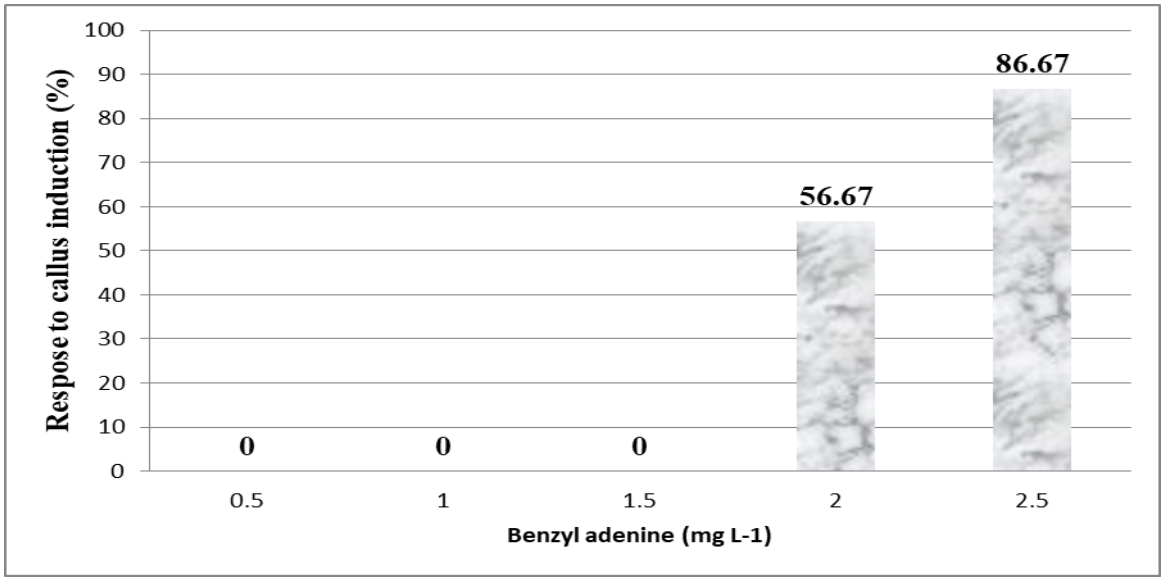

Figure 1: Effect of different concentrations of benzyl adenine on callus induction of tulip plant after eight weeks from culture (R-LSD p≥0.05= Significance).

The results in Figure 1 show that the callus tissue did not grow when the axillary buds were cultured on the MS medium which supplied with 1.0 mg L^{-1} naphthalene acetic acid and 0.5, 1.0 or 1.5 mg L^{-1} benzyl adenine after eight weeks from culture. However, the axillary buds cultured on the MS medium which supplied with 1.0 mg L^{-1} naphthalene acetic acid and 2.0 or 2.5 mg L^{-1} benzyl adenine which led to callus induction after eight weeks from culture (Plate 1, B). The optical cytokinin and auxin combination play an important role *in vitro* organogenesis (Zhao, 2008). The treatment of 2.5 mg L^{-1} benzyl adenine was significant superior in the percentage of response to callus induction compared to the treatment of 2.0 mg L^{-1} benzyl adenine which recorded 86.67% and 56.67%, respectively. The induced callus on the MS medium which supplied with 1.0 mg L^{-1} naphthalene acetic acid and 2.5 mg L^{-1} benzyl adenine was white, granular and friable. While the growing callus at 1.0 mg L^{-1} naphthalene acetic acid and 2.0 mg L^{-1} benzyl adenine was compact, non-granular, and greenish-white in color (Plate 1, B). The callus texture used to determine the quality of callus induced by explant. Friable callus grows separately into small pieces easily, and contains a lot of water (Sitorus *et al.,* 2011). But the compact callus has a texture that is not easily cut off and looks solid (Amid *et al.,* 2011). Callus tissue varies from friable to compact depending on the type of explants used, the components of the medium, the plant growth regulators, and the environmental conditions of the culture. In general, friable callus tissue is best than callus with a solid texture (Manuhara, 2014).

Table 1: Effect of different concentrations of benzyl adenine on indirect shoot formation of tulip plant cv. Arma after eight weeks from culture

Treatment	Response to indirect shoots (%)	Number of shoots per explant	Number of leaves per shoot	Shoot length (cm)
0.5	-	-	-	-
1.0	-	-	-	-
1.5	-	-	-	-
2.0	13.33	2.33	2.90	3.33
2.5	80.00	8.00	2.07	1.37
R-LSD p≥0.05	**	**	**	**

**: Significance.

The results in Table 1 show that the callus cultured on the MS medium that supplied with 1.0 mg L^{-1} naphthalene acetic acid and 2.0 or 2.5 mg L^{-1} benzyl adenine led to the indirect adventitious shoots formation eight weeks after culturing. Results from the same table indicate that 2.5 mg L^{-1} benzyl adenine treatment was significant superior compared to 2.0 mg L^{-1} benzyl adenine in the percentage of response to adventitious shoot formation and number of shoots which were 80.00% and 13.33%, 8.00 and 2.33 shoots per explant, respectively (Plate 1 C, D and E). The benzyl adenine and naphthalene acetic acid combination in an adequate balance was able to control and promote callus and indirect shoots formation (Arellano-Perusquia and Lopez-Peralta, 2013). Whereas, treatment 2.0 mg L^{-1} benzyl adenine was significant superior in number of leaves and shoot length compared to 2.5 mg L^{-1} benzyl adenine which were recorded 2.90 and 2.07 leaves per shoot, 3.33 and 1.37 cm, respectively. The reason for the increase in the length of the shoots and the number of leaves per shoot with the decrease in the concentration of cytokinin in the MS medium is due to the decrease in the number of formed shoots and the number of leaves in them, which caused less competition for medium, which led to increased division and elongation of cells and growth in the formed shoot tissues.

Table 2: Effect of different concentrations of benzyl adenine on direct shoot formation of tulip plant cv. Arma after eight weeks from culture

Treatment	Response to indirect shoots (%)	Number of shoots per explant	Number of leaves per shoot	Shoot length (cm)
0.5	13.33	1.67	2.50	3.20
1.0	33.33	2.67	3.07	3.40
1.5	76.67	6.67	2.07	2.20
2.0	-	-	-	-
2.5	-	-	-	-
R-LSD p≥0.05	6.75	1.67	N.S*	0.29

N.S: Non significance.

The results in Table 2 show that the axillary buds cultured on the MS medium which supplied with 1.0 mg L^{-1} naphthalene acetic acid and 0.5, 1.0 or 1.5 mg L^{-1} benzyl adenine led to the direct adventitious shoot formation. Direct bud regeneration is formed through a dedifferentiation process in which a single parenchyma cell located either in the epidermis or just below the surface of the explant develops into a shoot system with no callus production (Hartmann *et al.*, 2011). The MS medium that supplied with 1.0 mg L^{-1} naphthalene acetic acid and 1.5 mg L^{-1} benzyl adenine was significant increase in the percentage of response to direct adventitious shoot formation and number of shoots compared to 0.5 or 1.0 mg L^{-1} benzyl adenine which recorded 76.67% and 6.67 shoots per explant, respectively (Plate 1, F, G and H). The main reason for the superiority of this treatment is due to its stimulating to the enzymes and RNA synthesis that are responsible for proteins synthesis and releasing energy inside the cells and occurring the cellular division and growth that lead to the adventitious shoot formation (Taiz and Zeiger, 2010). Whereas, the MS medium which equipped with 1.0 mg L^{-1} naphthalene acetic acid and 0.5 mg L^{-1} benzyl adenine recorded the lowest percentage response to the direct adventitious shoot formation and the number of shoots that reached 13.33% and 1.67 shoots per explant, respectively. Table 2 also shows that there were no significant differences between the treatments in the number of leaves in the adventitious shoots formed from direct organogenesis. Whereas, the two treatments at 1.0 mg L^{-1} naphthalene acetic acid and 0.5 or 1.0 mg L^{-1} benzyl adenine which were significant superior in the direct adventitious shoot length, compared to treatment at 1.5 mg L^{-1} benzyl adenine which recorded 3.20, 3.40 and 2.20 cm, respectively.

CONCLUSION

The MS medium which supplied with 1.0 mg L^{-1} NAA + 2.5 mg L^{-1} BA was the best to callus induction and indirect shoot formation, and 1.0 mg L^{-1} NAA + 1.5 mg L^{-1} BA for direct shoot formation after eight weeks from culture.

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