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Micropropagation of dahlia plants *Dahlia variabilis* Wild (Desf.). Effect of explant and plant growth regulators on shoot regeneration and growth

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Abstract. The present study was conducted to determine the effect of explant, BA (6-benzyl amino purine) and NAA (α- naphthalene acetic acid) in the shoots regeneration and growth. The results showed the superiority of the shoot tip on hypocotyl in response to shoot formation reached 86.67% and 43.33% respectively. While the cotyledon, nodal segment and root segment did not respond for it. Also the results indicate that MS medium (Murashige & Skoog medium) supplemented with BA and NAA at 2.0 + 2.0 mg L^{-1} concentration gave the highest significantly response to shoot formation reaching 86.67%. While the MS medium supplemented with 2.5 + 2.5 mg L^{-1} BA and NAA combination was less responsive to form shoots, reaching 46.67%. But the two combinations of BA and NAA (1.5 + 1.5 and 3.0 + 3.0 mg L^{-1}) did not give any response for shoot regeneration. The 2.0 + 2.0 mg L^{-1} combination was significantly superior on 2.5 + 2.5 mg L^{-1} in number of formation shoots reaching 5.0 and 2.0 shoots/shoot tip respectively. The regenerated shoots cultured on half strength of MS supplemented with 0.6 mg L^{-1} IBA (Indole-3- butyric acid) proved to be significantly superior beside other concentrations of IBA in the response of root formation which reached 96.67%, when compared with the concentration of 1.0 mg L^{-1} of IBA which gave the lowest response for root formation (56.67%). As well as the 0.6 mg L^{-1} IBA concentration gave the highest rates in number of main and secondary roots and root length compared with other concentrations of IBA. The plants produced by micropropagation have been acclimatized at high success rate of 100%.
Key Words: Micropropagation, hypocotyl, shoot tip, benzyl adenine, regeneration.

Introduction. Dahlia plant belongs to the composite family (De Hertegh 1989). Original home of the dahlia plant is the center of America, and then spread to Mexico, then from there to England and other countries of the world. The Dahlia flowers are cut flowers; it is large sized and multi-colored, being one of the most beautiful cut flowers. Dahlia is a dicotyledonous plant and a leaf type is composite consisting of 3-7 leaflets (Dole & Wilkins 1999). Dahlia plants reproduce sexually by seed and vegetative tuberous roots. It can be more susceptible to fungal, bacterial and viral infections if is propagate by traditional vegetative methods. The plant tissue culture technique is used to overcome this inconvenient (George et al 2008).

Fatima et al (2007) produced the largest number of dahlia plants by indirect organogenesis technique when used the shoot tip, hypocotyls and cotyledon leaf as explants. These explants was cultured on MS medium (Murashige & Skoog medium) supplemented with a combination of benzyl adenine (BA) and naphthalene acetic acid (NAA) at 3.0 mg L^{-1} concentration each respectively. Salman et al (2010) reported similar results when they cultured the shoot tips on MS medium supplemented with 0.41 mg L^{-1} for each of BA and NAA respectively. In other flowering crops like carnation and gerbera results are reported from certain explant sources like shoot meristems (Poupet et al 2006), floral buds (Posada et al 1999), and hypocotyl (Sharma et al 2001) on different media. The study was conducted in order to produce large number of dahlia plants free of pathogens and matching the genetic traits of the mother plant by plant tissue culture technique.

Material and Method

The study was conducted in the laboratory of plant tissue culture, College of Agriculture, University of Basra, Iraq. Dahlia hybrid seeds were used, produced by the Dutch company "Aviflora", sterilized with a solution of sodium hypochlorite at 1.05% concentration for a period of 15 minutes. These seeds were then washed three times in sterile distilled water. Then the sterilized seeds was cultured on MS medium (Murashige & Skoog 1962) containing 2.0 mg L^{-1} BA, 0.3 mg L^{-1} NAA, 30 gm L^{-1} sucrose, 2 gm L^{-1} polyvinylpyrrolidone (pvp). The pH of the media was adjusted to 5.7 with 0.1 N NaOH or HCl after adding 5 gm L^{-1} agar, and before autoclaving at 1.04 Kg cm^{-2} for 20 minutes. All media were dispensed in 25 x 150 mm test tube containing 25 mL medium. Cultures were incubated under 1000 Lux light intensity provided by white fluorescent lamps for 16 hrs photoperiod at 27 ± 1°C. These seeds germinated after two weeks of culturing and then were used after cutting as explants.

First experiment: Effect of explants type on shoot regeneration. The explants (cotyledon, hypocotyl, shoot tip, nodal and root segment) cultured on MS media supplemented with different concentrations of BA and NAA at 2.0 mg L^{-1} for each of them. The sucrose and agar were added to medium and then preparation and sterilization as in the previous paragraph using adenine sulfate 40 mg L^{-1}. The notes and studied traits were recorded after eight weeks from culture. The recorded characteristics included: percentage of response to shoot formation, number of shoots formation, length of shoot (cm), number of leaves/shoot and leaf area (cm^2).

Second experiment: Effect of concentration of BA and NAA on shoot regeneration. The shoot tip was cultured on MS medium supplemented with a combination of different concentrations of BA and NAA (1.0 + 1.0, 1.5 + 1.5, 2.0 + 2.0, 2.5 + 2.5 and 3.0 + 3.0 mg L^{-1}, respectively). The organic materials were added to medium and preparation of media was performed, sterilization and incubating was conducted as in the preceding paragraph. After eight weeks from culture the same traits were recorded like in the previous experiment.

Third experiment: Effect of different concentrations of IBA on rooting of shoots. The shoots regenerations from previous experiment were cultured on half strength of MS medium supplemented with different concentrations of IBA (Indole-3- butyric acid) for rooting. The organic materials were added to medium and preparation of media was performed, sterilization and incubating conducted as in the preceding paragraph except for sucrose, which was added at 45 gm L^{-1} concentration. The data was recorded after eight weeks which included: percentage of response of root formation, number of main and secondary roots and length root (cm).

Dahlia plant acclimatization. The dahlia plants produced from shoot regeneration were acclimatized by grown in plastic pots 10 cm in diameter containing peat moss and soft sand at a ratio of 1:2, placed in a growth room under controlled conditions (temperature 27 ± 2°C, 16/8 hrs photoperiod and light intensity 1500 Lux). The studied characteristics in acclimatization included: percentage of survived plants, plant height, number of shoots, number of leaves and leaf area recorded after six weeks from acclimatization.

Experimental design and statistical analysis. Completely randomized design was used with five replicates. The data were subjected to the analysis of variance and mean values were compared using revised-LSD as described by Snedicor & Cochran (1980).

Results and Discussion

The effect of explant on shoot regeneration. Results from Table 1 shows that the explants (hypocotyl and shoot tip) given positive response to the formation of shoots while other explants did not give any response. The shoot tip was significantly superior on a hypocotyl as it gave the highest response to shoots formation reached 86.67% and 43.33% respectively (Figure 1, A & B). The cause of the increase of shoots formed by shoot tip culture was that BA stimulates the auxiliary buds on the growth and elongation compared with hypocotyl. Similar results were

obtained by other researchers in their studies on shoot tip of dahlia plant via *in vitro* culture (Salman et al 2010).

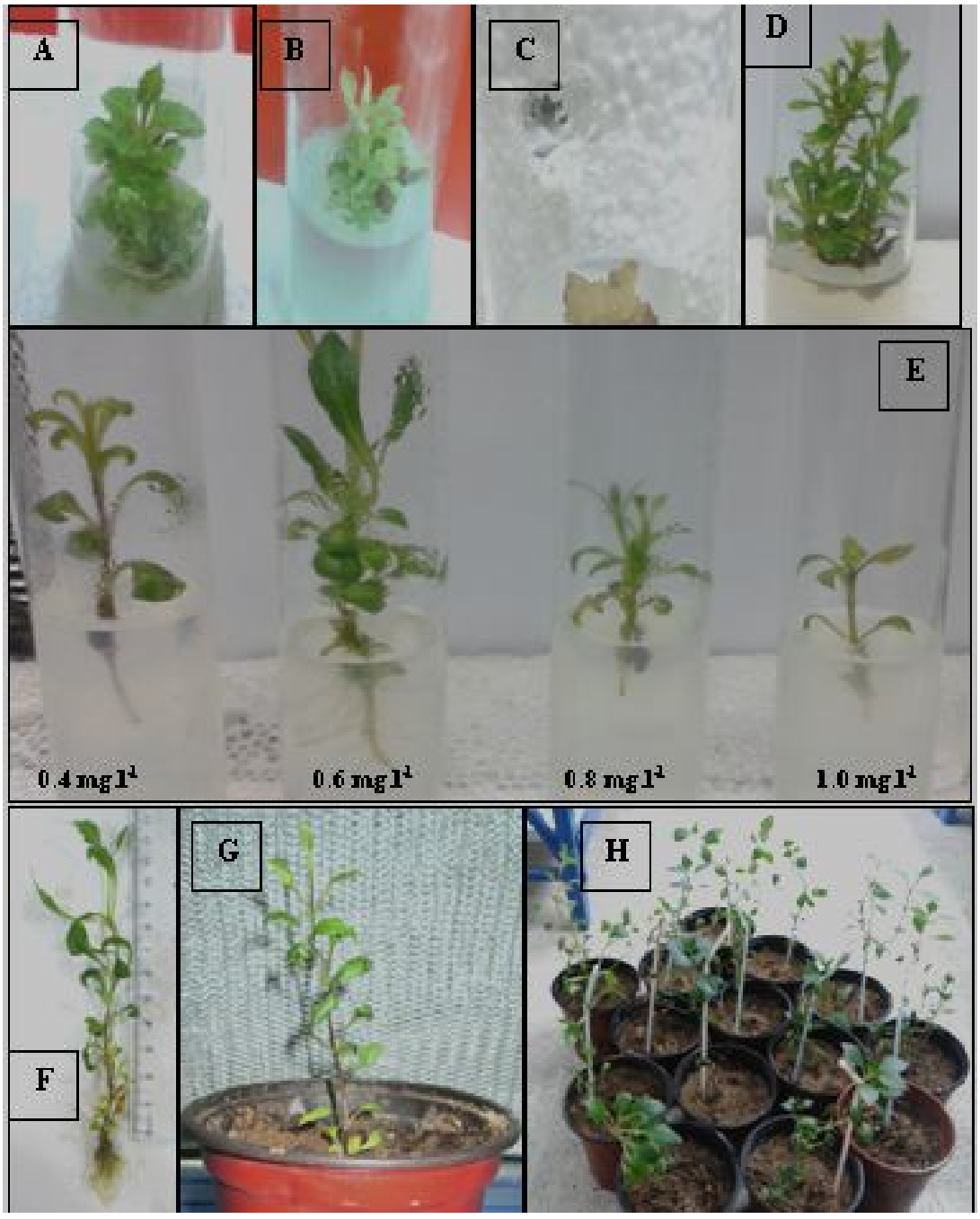

Figure 1. Micropropagation of dahlia hybrid plants (*Dahlia variabilis*). A– Shoot regeneration from shoot tip cultured on MS medium supplemented with 2.0 mg L^{-1} BA + 2.0 mg.L^{-1} NAA. B– Direct shoot regeneration from hypocotyl cultured on MS medium supplemented with 2.0 mg L^{-1} BA + 2.0 mg L^{-1} NAA. C– Callus initiation from nodal segment cultured on MS medium supplemented with 2.0 mg L^{-1} BA + 2.0 mg L^{-1} NAA. D– Shoot regeneration from shoot tip cultured on MS medium supplemented with 2.0 mg L^{-1} BA + 2.0 mg L^{-1} NAA. E– Rooting shoot cultured on half strength of MS supplemented with different concentrations of IBA (0.4, 0.6, 0.8 and 1.0 mg L^{-1}) after eight weeks. F- Plantlet of dahlia before acclimatization. G- Plantlet of dahlia after acclimatization. H- Acclimatized plants after six weeks from the planting.

Table 1

Effect of explant on shoot regeneration and some traits of vegetative growth

Type of explants	*% response of shoot formation*	*No. of shoots/ explant*	*Shoot length (cm)*	*No. of leaves/ shoot*	*Leaf area (cm^2)*	*Notes*
Cotyledon	0	0	0	0	0	Green and overgrown cotyledon
Hypocotyl	43.33	3.67	4.33	8.00	0.73	Low number of directly regenerated shoots
Shoot tip	86.67	5.00	3.33	5.33	0.40	Highest number of regenerated shoots
Nodal segment	0	0	0	0	0	Callus induction
Root segment	0	0	0	0	0	White and overgrown root
Significance	+	-	-	-	+	-

(+) - significant difference; (-) - no significant difference.

The same table shows no significant differences between hypocotyl and shoot tip in number of shoots/explant, shoot length and number of leaves/shoot except leaf area. Hypocotyl was significantly superior on shoot tip in leaf area which it reached 0.73 and 0.40 cm^2 respectively. The decrease in the leaf area resulting from shoot tip is due to the increased number of shoots formation, which had a negative impact in this decrease (Table 1). Also, the results showed that nodal segment gave white callus grew on the explant surface (Figure 1C). This result is not consistent with another study on dahlia plant as they had noticed the lateral shoot formation (Salman et al 2010). But, similar results were obtained by other researchers in their studies on callus induction when they cultured the explants on MS medium containing 2.0 + 2.0 mg L^{-1} concentrations of BA and NAA (Fatima et al 2007).

The effect of BA and NAA combinations on shoot formation. The results in Table 2 indicate that MS medium supplemented with BA and NAA at 2.0 mg L^{-1} concentration for each of them gave the highest significantly response to shoot tip to form shoots reached 86.67% (Figure 1D).

Table 2

Effect of BA and NAA concentrations on shoot regeneration and some traits of vegetative growth

BA + NAA (mg L^{-1})	*% response of shoot formation*	*No. of shoots/ explant*	*Shoot length (cm)*	*No. of leaves/ shoot*	*Leaf area (cm^2)*	*Notes*
1.5 + 1.5	0	0	0	0	0	Did not grow and green colored
2.0 + 2.0	86.67	5.00	3.33	5.33	0.40	Highest number of regenerated shoots
2.5 + 2.5	46.67	2.00	2.50	3.33	0.30	Low number of shoots regenerated
3.0 + 3.0	0	0	0	0	0	Did not grown and brown colored
Significance	+	+	-	-	-	-

(+) - significant difference; (-) -nNo significant difference.

The MS medium supplemented with 2.5 + 2.5 mg L^{-1} BA and NAA respectively was the less responsive to form shoots reaching 46.67%. But the two combinations of BA and

NAA (1.5 + 1.5 and 3.0 + 3.0 mg L^{-1}) did not give any response for shoot formation (Table 2). The reason may be the 2.0 + 2.0 mg L^{-1} BA and NAA which is the perfect combination for shoot forming.

The 2.0 + 2.0 mg L^{-1} combination was significantly superior to 2.5 + 2.5 mg L^{-1} in number of shoots formation which reached 5.0 and 2.0 shoots/shoot tip respectively, while the two combinations 2.0 + 2.0 and 2.5 + 2.5 mg L^{-1} were not significantly different in other traits (shoot length, number of leaves/shoot and leaf area) (Table 2). The present result isn't consistent with other studies, because they used low concentrations of BA and IAA (0.41 + 0.53 mg L^{-1}) that gave highest response for shoot formation (Salman et al 2010).

The effect of IBA on root formation. The Table 3 shows that shoot cultured on half strength of MS supplemented with 0.6 mg L^{-1} IBA gave significantly superior results against other concentrations of IBA in the response of root formation which reached 96.67% (Figure 1E). When compared with the concentration of 1.0 mg L^{-1} of IBA which gave the lowest response for root formation results reached 56.67%. Also, the 0.6 mg L^{-1} IBA concentration gave the highest rates in number of main and secondary roots and root length compared with other concentrations of IBA (Table 3). The 0.6 mg L^{-1} IBA gave the highest response to root formation because of the role of type and optimum concentration of auxin in stimulating the root initiation. Similar results were obtained by other researchers in their studies on dahlia plant via *in vitro* culture (Salman et al 2010). The reason for the low response to root formation at 1.0 mg L^{-1} IBA (high concentration) resulting from the interaction between the exogenous and endogenous auxins that led to inhibition of the formation and growth of roots.

Table 3

Effect of different concentrations of IBA on root initiation and some traits of root growth

IBA (mg L^{-1})	*% response of root formation*	*No. of main roots/shoot*	*No. of secondary roots/shoot*	*Root length (cm)*
0.4	80.00	1.33	2.00	1.57
0.6	96.67	3.00	11.33	1.63
0.8	63.33	2.67	5.67	1.50
1.0	56.67	2.33	4.33	0.53
RLSD (0.05)	12.63	n.s.	2.17	0.68

n.s. - no significant difference.

Plant acclimatization. The plants produced by micropropagation have been acclimatized at high success rate of 100%. Similar results were obtained by other researchers in their studies on dahlia plant (Salman et al 2010) and strawberry plant (Ibrahim et al 2013) acclimatization. Table 4 shows a significant increase in plant height and leaf area after six weeks from acclimatization while these were none significantly increased in the number of shoots and leaves/plant (Figure 1, F, G & H).

Table 4

Some growth traits of dahlia plants after six weeks from acclimatization

Weeks after acclimatization	*Plant height (cm)*	*No. of shoots/plant*	*No. of leaves/plant*	*Leaf area (cm^2)*
0	16.17	2.00	19.33	0.85
6	24.67	4.00	34.00	2.95
Significance	+	-	-	+

(+) - significant difference; (-) - no significant difference.

Conclusions. The results of this study revealed that best method for micropropagation of dahlia plants was the shoot multiplication using shoot tips as explants and MS medium

supplemented with BA and NAA at 2.0 + 2.0 mg L^{-1} concentration for mass propagation, resulting healthy and true plants.

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