AAB BIOFLUX

Advances in Agriculture & Botanics-
International Journal of the Bioflux Society

Micro propagation of dahlia plants (*Dahlia variabilis*). Direct and indirect organogenesis techniques

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Abstract. The present study was conducted for dahlia plants propagation by direct and indirect organogenesis methods. The results showed that a significant superiority of the combination of 2.0 + 2.0 mg L^{-1} (NAA + BA) on other combinations added to the MS medium components in the averages number of direct shoots/shoot tip and shoot length reached 10.00 shoots and 6.67 cm, respectively. While, the shoot tip cultured on MS medium supplemented with a combination 1.5 + 1.5 mg L^{-1} (NAA + BA) did not respond to adventitious shoot formation after eight weeks from culture. Also the results showed that the shoot tips and nodal segments developing on MS medium supplemented with 3.0 +3.0 mg L^{-1} (NAA + BA) were superior significantly on the hypocotyl in the percentage of the induction of callus reached 75.00%, 60.00% and 23.33%, respectively after eight weeks from culture. The shoot tip gave the largest amount of the white callus fragile. The results showed indirect shoots regeneration from the developing callus of shoot tip and hypocotyl cultured on the MS medium supplemented with 3.0 + 3.0 mg L^{-1} (NAA + BA) after eight weeks from culture. The two combinations of 2.0 + 2.0 and 2.5 + 2.5 mg L^{-1} (NAA + BA) were superior significantly on the other combination (3.0 + 3.0 mg L^{-1}) in the rates of the number and length of regenerated shoots from the developing callus of shoot tip recorded 6.33, 5.67 and 5.00 shoots, and 6.67, 5.67 and 5.00 cm, respectively after eight weeks from culture. As well as the direct and indirect shoots were rooted when they cultured on half strength of MS medium supplemented with 0.6 mg L^{-1} IBA concentration. The dahlia plants produced by micro propagation have been acclimatized at high success percent of 100%.
Key Words: Callus, dahlia, nodal segment, organogenesis, regeneration, shoot tip.

Introduction. Dahlia [*Dahlia variabilis* (Wild.) Desf.] belongs to the worldwide highly appreciated perennial ornamental plants. The original home of this plant is Mexico and then spread cultivated to all countries of the world at the beginning of the seventeenth century (Dole & Wilkins 1999). To the genus of *Dahlia* belongs 42 species (Rowlands 1999). This plant can be easily infected with fungal, bacterial and viral diseases (Bose & Yadav 1989). The *in vitro* culture technique is the best method which can successfully eliminate these infections and healthy dahlia plants can be obtained (Sediva et al 2006). Fatima et al (2007) found the maximum response to cotyledon leaf and hypocotyl of Dahlia explants on callus induction and indirect shoot organogenesis. In contrast, there are reports on response of other plant explants to direct shoot regeneration instead of callus formation, when these are cultured on MS media (Murashige and Skoog medium) containing naphthalene acetic acid (NAA) and Benzyl adenine (BA) combination, as Snapdragon (Cui et al 2004), Perilla (Hou & Jia 2005) and Dioscorea (Chen et al 2007) reported. The aim of this current study is mass propagation to good Dahlia cultivars using the seedling segments as explants and so as to obtaining healthy and true to type plants.

Material and Method

The study was conducted in the laboratory of plant tissue culture at the College of Agriculture, University of Basra, Iraq. Dahlia hybrid seeds produced by the Dutch company "Aviflora" was used, sterilized with a solution of sodium hypochlorite at 1.05% concentration for a period of 15 minutes. The seeds were then washed three times in

sterile distilled water. The sterilized seeds was cultured on MS medium (Murashige & Skoog 1962) containing 2.0 mg L^{-1} BA, 0.3 mg L^{-1} NAA, 30,000 mg L^{-1} sucrose, 1 mg L^{-1} vitamins and glycine, 2,000 mg L^{-1} polyvinyl pyrrolidone (pvp). The pH of the media was adjusted to 5.7 with 0.1 N NaOH or HCl after adding 5,000 mg 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 was used after cutting as explants.

The effect of NAA + BA combination in direct organogenesis. The internodes segment of shoot base (1.0 cm length) was cultured on MS media supplemented with different concentrations of NAA and BA (1.5 + 1.5, 2.0 + 2.0, 2.5 + 2.5 and 3.0 + 3.0) mg L^{-1}. The sucrose and agar was added to medium, and medium preparation and sterilization was performed as in the previous paragraph with adenine sulfate at 40 mg L^{-1} concentration addition. The notes and studied traits were recorded after eight weeks from culture. The followed traits included: number of direct shoots, shoot length (cm), number of leaves/shoot and leaf area (cm^2).

The effect of source of explants in the callus induction. The explants (cotyledon, hypocotyl, shoot tip, nodal and root segments) cultured on MS medium was supplemented with a combination of NAA and BA (3.0 + 3.0 mg L^{-1}). The organic materials added to medium, preparation of media, sterilization and incubating was conducted as in the preceding paragraph. The percentage of response to callus induction was recorded after eight weeks from culture.

The effect of the combination of NAA + BA in callus induction. The shoot tips cultured on MS medium supplemented with different concentrations of NAA and BA (1.5 + 1.5, 2.0 + 2.0, 2.5 + 2.5 and 3.0 + 3.0) mg L^{-1}. The organic materials added to medium and preparation of media, sterilization and incubating was conducted as in the preceding paragraph. The same traits has been recorded as in the previous experiment after eight weeks from culture.

Effect of the developing callus from explants in indirect shoots regeneration. The developing callus (100 mg weight) from explants cultured on MS medium was supplemented with a combination of NAA and BA (3.0 + 3.0 mg L^{-1}). The traits included: The number of indirect shoots/100 mg callus, shoot length (cm), number of leaves/shoot and leaf area (cm^2) after eight weeks from culture.

The effect of combination of NAA + BA in indirect shoots regeneration. The developing callus (100 mg weight) from shoot tips cultured on MS medium was supplemented with different concentrations of NAA and BA (1.5 + 1.5, 2.0 + 2.0, 2.5 + 2.5 and 3.0 + 3.0) mg L^{-1}. The same trait has been recorded as in the previous experiment after eight weeks from culture.

Rooting shoots and acclimatization of dahlia plants. The regenerated direct and indirect shoots were rooting on half strength of MS medium supplemented with IBA at 0.6 mg L^{-1} concentration. The dahlia plants produced from rooted shoots were acclimatized by grown in plastic pots 10 cm in diameter containing peat moss and soft sand 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).

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 Snedecor & Cochran (1980).

Results and Discussion

The effect of the combination of NAA + BA in direct organogenesis. Results shows in Table 1, adventitious shoots formation by direct organogenesis from culturing of shoot bases on MS medium supplemented with 2.0 + 2.0, 2.5 + 2.5 and 3.0 + 3.0 mg L^{-1} (NAA + BA) except 1.5 + 1.5 mg L^{-1} combination, which did not respond to adventitious shoot formation after eight weeks from culture (Figure 1 A and B). The balance between auxins and cytokinins plays an important role in shoot formation. It caused increasing the synthesis of RNA and proteins and enzymes inside the cell, which stimulates cell division and the adventitious shoot regeneration (Taiz & Zeiger 2006). These results are in agreement with results of other searchers on other plants as Snapdragon (Cui et al 2004) and Dioscorea (Chen et al 2007).

The results also showed the significant superiority of the combination of 2.0 + 2.0 mg L^{-1} (NAA + BA) on other combinations added to the MS medium components in the averages number of shoots proliferating and length (10.00 shoots and 6.67 cm, respectively). Similar results are reported on other plants by Pal & Dhar (1985), Hitmi et al (1998), Hedayat et al (2009), Mendi et al (2009). There was no significant difference between the two combinations 2.5 + 2.5 and 3.0 + 3.0 mg L^{-1} (NAA + BA) in number and length of shoots (7.00 and 5.67 shoots, and 4.00 and 3.33 cm, respectively). The results also showed no significant difference between the combinations of NAA + BA in the number of leaves/shoot and leaf area (Table 1).

Table 1

The effect of the combination of NAA + BA in direct organogenesis

NAA + BA (mg L^{-1})	*No. of shoots/explant*	*Shoot length (cm)*	*No. of leaves/shoot*	*Leaf area (cm^2)*
1.5 + 1.5	-	-	-	-
2.0 + 2.0	10.00	6.67	8.00	0.43
2.5 + 2.5	7.00	4.00	7.33	0.37
3.0 + 3.0	5.67	3.33	6.67	0.33
R-LSD (0.05)	2.65	2.01	ns	ns

ns - non significant difference.

The effect of source of explant in the callus induction. The results in the Table 2 showed that the shoot tips and nodal segments developing on MS medium supplemented with 3.0 + 3.0 mg L^{-1} (NAA + BA) were superior significantly on the hypocotyl in the percentage of callus induction reached 75.00%, 60.00% and 23.33%, respectively after eight weeks from culture (Figure 1 C, D, E & F). Fatima et al (2007) reported similar results on hypocotyl.

Table 2

The effect of source of explants in the callus induction

Source of explant	*% Response of callus formation*	*Notes*
Cotyledon	-	0
Hypocotyl	23.33	+
Shoot tip	75.00	+++
Nodal segment	60.00	++
Root segment	-	0
R-LSD (0.05)	30.29	-

0 - no formation of callus, + - small amount of callus, ++ - middle amount of callus, +++ - large amount of callus.

The cotyledons and root segments developing on the same as the previous MS medium components did not respond for callus initiation. The results of this study did not agree with another study on the callus initiation from cotyledons (Fatima et al 2007), when

they indicated the growth of callus from cotyledons cultured on MS medium supplemented with NAA and BA. The results also indicated (Table 2) that the shoot tip gave the largest amount of the white callus fragile, while the callus grew less quantity from nodal segments.

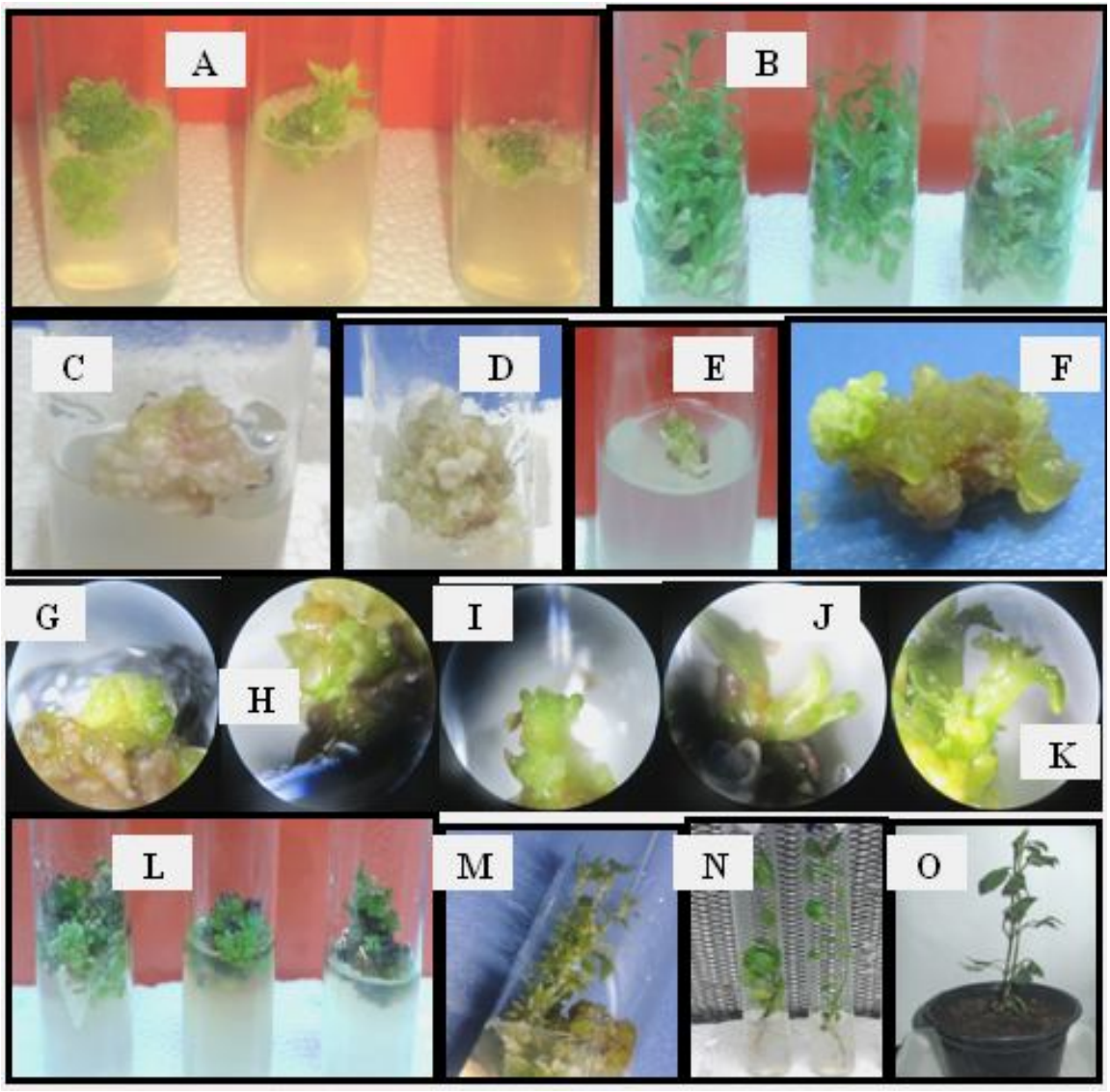

Figure 1. Micro propagation of dahlia hybrid plants [*Dahlia variabilis* (Wild.) Desf.] via direct and indirect organogenesis techniques. A & B – Direct adventitious shoot regeneration from shoot tip cultured on MS medium supplemented with different combinations of NAA and BA (2.0 + 2.0, 2.5 + 2.5, 3.0 + 3.0 mg L^{-1}, respectively (Left to right). C, D, E, F – Callus formation, growth and development from shoot tips cultured on MS medium supplemented with 3.0 mg L^{-1} NAA and 3.0 mg L^{-1} BA concentrations. G, H, I, J, K – Indirect shoots regeneration and growth stages from developing callus of shoot tip cultured on MS medium supplemented with 2.0 mg L^{-1} NAA and 2.0 mg L^{-1} BA concentrations. L – Indirect shoots regeneration from callus cultured on MS medium supplemented with different combinations of NAA and BA (2.0 + 2.0, 2.5 + 2.5 and 3.0+3.0 mg L^{-1}, respectively (Left to right) after six weeks. M - Indirect shoots regeneration from callus cultured on MS medium supplemented with 2.0 mg L^{-1} NAA and 2.0 mg L^{-1} BA concentrations after eight weeks. N - Rooting shoot cultured on half strength of MS supplemented with 0.6 mg L^{-1} concentration of IBA after eight weeks. O - Plant of dahlia after six weeks from the acclimatization.

The increase in the amount of the developing callus on the shoot tip is due to the continuing division of the cells in the meristematic region (Firoozabady & Gutterson 2003). The amount of callus formed on hypocotyl was very few (Table 2). The difference in the growth of callus is due to an increase in the concentration of BA in MS medium, which has become the supra-optimal cause to cell division.

The effect of NAA + BA combination in callus induction. The results in the Table 3 indicate that the shoot tips cultured on MS medium supplemented with 3.0 + 3.0 mg L^{-1} (NAA + BA) gave the highest response rate of callus initiation, reached 75.00% (Figure 1 C & D). Several researchers have also reported similar response of explants on the same components of MS medium to callus formation in dahlia (Fatima et al 2007) and asparagus (Pontaroli & Camadro 2005), while the combination of 2.0 + 2.0 mg L^{-1} (NAA + BA) gave a lowest rate in response to the callus induction, reached 13.33%, after eight weeks from culture. The results showed the significant superiority of the combination of 3.0 + 3.0 mg L^{-1} (NAA + BA) on the other in the percentage of response to shoot tip for callus initiation, while the shoot tips did not respond to callus induction when cultured on MS medium supplemented with 1.5 + 1.5 mg L^{-1} (NAA + BA). That shoot tips cultured on MS medium supplemented with 3.0 + 3.0 mg L^{-1} (NAA + BA) gave a large amount of white callus fragile compared with the amount of callus produced from the combination of 2.5 + 2.5 mg L^{-1} (NAA + BA), while a weak growth of callus on shoot tips cultured on MS medium supplemented with 2.0 +2.0 mg L^{-1} of NAA + BA was observed (Table 3). The callus induction is due to the balance between auxins and cytokinins which plays an important role in increasing the synthesis of RNA and proteins and enzymes inside the cell, which stimulates cell division and the formation of callus (Taiz & Zeiger 2006).

Table 3

The effect of the combination of NAA + BA in callus induction

NAA + BA (mg L^{-1})	*% Response of callus formation*	*Notes*
1.5 + 1.5	-	0
2.0 + 2.0	13.33	+
2.5 + 2.5	46.67	++
3.0 + 3.0	75.00	+++
R-LSD (0.05)	28.15	-

0 - no formation of callus, + - small amount of callus, ++ - middle amount of callus, +++ - large amount of callus.

Effect of the developing callus from explants in indirect organogenesis. The results in the Table 4 show shoots regeneration from the developing callus on shoot tip and hypocotyl cultured on the MS medium supplemented with 3.0 + 3.0 mg L^{-1} (NAA + BA) after eight weeks from culture. Fatima et al (2007) reported similar response of explant tissues of dahlia plant on MS medium containing NAA and BA and obtained indirect shoot regeneration. Also these results are in accordance with results of other studies on indirect organogenesis from callus (Salman & Al-Dabagh 2000; Al-Taha et al 2012; Ibrahim et al 2013). The callus produced from cotyledons, nodal and root segments did not respond to shoots regeneration when grown on the same MS medium components. The results also showed that developing callus from shoot tip achieved significant superiority in the number of shoots (5.00), compared with the shoot produced from the developing callus of hypocotyl (2.67 shoots). The regenerated shoots from callus produced from hypocotyl were superior significantly on the shoots produced from the developing callus of shoot tip in leaf area recorded 0.63 and 0.43 cm^2, respectively. The results showed no significant differences between the shoots proliferating from callus producing shoot tip and hypocotyl in the means of shoot length and number of leaves/shoot (Table 4). The shoot initiation from the callus tissue was recorded in many plants as the source of these shoots are plant tissue cultured in medium that lose differentiation (de-differentiation) and return to meristematic cells consisting of new cells (Figure 1 C & D) and then re-differentiation again by the medium components and

environmental conditions (Figure 1 E, F, G, H, I, J, K). This process causes the promeristmoids which develop into adventitious buds having the same morphogenesis of the auxiliary bud of leaf (Thorpe 1978).

Table 4

Effect of the developing callus from explants in indirect organogenesis

Developing callus from:	*No. of shoots/explant*	*Shoot length (cm)*	*No. of leaves/shoot*	*Leaf area (cm^2)*
Cotyledon	0	0	0	0
Hypocotyl	2.67	2.67	5.33	0.63
Shoot tip	5.00	5.00	8.00	0.43
Nodal segment	0	0	0	0
Root segment	0	0	0	0
Significance	+	-	-	+

+ = significant difference, - = non significant difference.

The effect of combination of NAA + BA in indirect organogenesis. Results from Table 5 show that the two combinations of 2 + 2 and 2.5 + 2.5 mg L^{-1} (NAA + BA) were superior significantly against 3 + 3 mg L^{-1} combination in the number and length of shoots from the developing callus of shoot tip recorded 6.33, 5.67 and 5.00 shoots, and 6.67, 5.67 and 5.00 cm, respectively after eight weeks from culture (Figure 1 L & M). These results are in accordance with the findings of Fatima et al (2007). The reason for the long shoots at the lower concentrations is due to reduce the role of endogenous auxin in stimulating cell elongation. Also those results showed no significant differences between the combinations (NAA + BA) in the number of leaves/shoot and leaf area of the regenerated shoots from callus (Table 5). The callus of shoot tip cultured on MS medium supplemented with 1.5 + 1.5 mg L^{-1} (NAA + BA) did not respond to the adventitious shoots regeneration. The balance between auxin and cytokinin lead to re-differentiation of the cultured explant and generate callus which result in adventitious shoots (Firoozabady & Moy 2004).

The direct and indirect shoots were rooting on half strength of MS medium supplemented with 0.6 mg L^{-1} concentration of IBA after eight weeks from culture (Figure 1 N). Dahlia plants produced from tissue culture acclimatized at high success at a percent of 100% (Figure 1).

Table 5

The effect of combination of NAA + BA in indirect organogenesis

NAA + BA (mg L^{-1})	*No. of shoots/explant*	*Shoot length (cm)*	*No. of leaves/shoot*	*Leaf area (cm^2)*
1.5 + 1.5	0	0	0	0
2.0 + 2.0	6.33	6.67	10.67	0.27
2.5 + 2.5	5.67	5.67	8.67	0.40
3.0 + 3.0	5.00	5.00	8.00	0.43
R-LSD (0.05)	1.03	1.28	ns	ns

ns - non significant difference.

Conclusions. The present studies results concluded that the most suitable explants that can be used in the propagation of dahlia plant via *in vitro* are shoot tips. Also the most affective combinations of NAA + BA added to MS medium components prepared for direct and indirect organogenesis is 2.0 + 2.0 and 3.0 + 3.0 mg L^{-1} respectively.

References

Al-Taha H. A., Ibrahim M. A., Saleh A. M., 2012 [Regeneration of plantlets form callus induced from shoot tips of pineapple (*Ananas comosus* (L.) Merr. Cv. DelMonte) by tissue culture technique]. Kufa J Agric Sci 4(1):333-343. [In Arabic].

Bose T. K., Yadav L. P., 1989 Floriculture in India. Allied Publishers Ltd., New Delhi, 14:41-48.

Chen F.-Q., Fu Y., Wang D.-L., Gao X., Wang L., 2007 The effect of plant growth regulators and sucrose on the micropropagation and microtuberization of *Dioscorea nipponica* Makino. J Plant Growth Regul 26:38-45.

Cui M.-L., Takayanagi K., Handa T., 2004 High frequency of shoot regeneration from hypocotyls and stem segments of *Antirrhinum majus* (Snapdragon). Plant Cell Tissue Organ Cult 78:51-53.

Dole J. M., Wilkins H. F., 1999 Floriculture principles and species. Prentice Hall, New Jersey, pp. 287-291.

Fatima B., Usman M., Ashraf T., Waseem R., Ali M. A., 2007 *In vitro* regeneration from cotyledon and hypocotyl explants of dahlia cultivars. Pak J Agri Sci 44(2):312-316.

Firoozabady E., Gutterson N., 2003 Cost effective *in vitro* propagation methods for pineapple. Plant Cell Rep 21:844-850.

Firoozabady E., Moy Y., 2004 Regeneration of pineapple plants via somatic embryogenesis and organogenesis. In Vitro Cell Dev Biol Plant 40:67-74.

Hedayat M., Abdi G., Khosh-Khui M., 2009 Regeneration via direct organogenesis from leaf and petiole segments of pyrethrum (*Tanacetum cinerariifolium* (Trevir.) Schultz-Bip). Am Eurasian J Agric Environ Sci 6(1):81-87.

Hitmi A., Barthomeuf C., Sallanon H., 1998 Rapid mass propagation of *Chrysanthemum cinerariaefolium* Vis. by callus culture and ability to synthesis pyrethrins. Pant Cell Rep 19:156-160.

Hou S. W., Jia J. F., 2005 *In vitro* regeneration of *Perilla frutescens* from hypocotyl and cotyledon explants. Biol Plant 49:129-132.

Ibrahim M. A., AL-Taha H. A., Seheem A. A., 2013 Effect of cytokinin type and concentration, and source of explant on shoot multiplication of pineapple plant (*Ananas comosus* '' Queen'') *in vitro*. Acta Agric Slov 101(1):15-20.

Mendi Y. Y., Curuk P., Kocaman E., Unek C., Eldogan S., Gencel G., Cetiner S., 2009 Regeneration of begonia plantlets by direct organogenesis. Afr J Biotechnol 8(9):1860-1863.

Murashige T., Skoog F., 1962 A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant 15:437-497.

Pal A., Dhar K., 1985 Callus and organ development of pyrethrum (*Chrysanthemum cinerariaefolium* Vis.) and analysis of their cytological status. Pyrethrum Post 16:3-11.

Pontaroli A. C., Camadro E. L., 2005 Plant regeneration after long-term callus culture in clones of *Asparagus officinalis* L. Biocell 29(3):313-317.

Rowlands G., 1999 The gardener's guide to growing dahlias. Timber Press, Portland, Oregon.

Salman M. A., Al-Dabagh F. M., 2000 [Vegetative propagation of Loquat trees (*Eriobotrya japonica* Lindle.) using tissue culture technique]. Iraqi Agric J 5(3):141-150. [In Arabic].

Sediva J., Novak P., Kanka J., Laxa J., 2006 Micro propagation, detection and elimination of DMV in the Czech collection of dahlia. Acta Hortic 725:495-498.

Snedecor G. W., Crochran R. W., 1980 Statistical methods. Iowa State University Press, Ames, Iowa.

Taiz L., Zeiger E., 2006 Plant physiology. 4th Edition, Sinauer Associates, Inc. Publishers, Sunderland, MA, 764 pp.

Thorpe T. A., 1978 Physiological and biochemical aspects of organogenesis *in vitro*. In: Frontiers of plant tissue culture. Thorpe T. A. (ed), pp. 49-58, Univ Calgary, Alberta, Canaela.

Received: 28 January 2015. Accepted: 01 April 2015. Published online: 09 April 2015.
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How to cite this article:
Ibrahim M. A., Daraj I. A., 2015 Micropropagation of dahlia plants *Dahlia variabilis*. Direct and indirect organogenesis techniques. AAB Bioflux 7(1):28-35.