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EFFECT OF SOME TREATMENTS ON SEEDS GERMINATION, SHOOTS MULTIPLICATION AND ROOTING OF DAHLIA PLANTS VIA *IN VITRO* CULTURE

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Abstract : The results showed that germination of dahlia seed when cultured on MS medium supplemented with 2.0+0.3 mg. L^{-1} combination of BA and NAA, respectively as the percentage of germination 100%. The seeds germinated in the darkness when cultured on MS medium supplied with combination of BA and NAA at 2.0+0.3 mg. L^{-1}, respectively, which represented 100%. But these seeds did not germinate when exposed to light. The shoot tip and hypocotyl showed the highest response to the formation of shoots amounted to 86.67% and 43.33%, respectively. While cotyledon leaf, nodal and root segments did not give any response to shoot formation. The MS medium supplemented with 2.0+2.0 mg. L^{-1} of BA and NAA was significantly superiority that the 2.5+2.5 mg. L^{-1} combination of BA and NAA in the percentage of response of shoot tips for shoots multiplication, which amounted to 86.67% and 46.67%, respectively. The results showed that shoots gave the highest significant response to rooting when cultured on a half strength of MS medium supplemented with 1.5 mg. L^{-1} NAA concentration compared with 0.5 and 1.0 mg. L^{-1} NAA, which reached 81.67%, 63.33% and 53.33%, respectively. Treatment 45 g. L^{-1} sucrose gave highest rate of number of secondary roots and root length when compared to treatment with 30 g. L^{-1} sucrose, which amounted to 11.33 and 6.00 roots/ shoot and 1.63 and 1.00 cm, respectively.

Key words : Cotyledon leaf, germination, Hypocotyl, *in vitro*, rooting, shoot multiplication.

Introduction

Dahlia plant belongs to Asteraceae family (De Hertegh, 1989). The genus name of this plant derived from the Swedish scientist Andreas Dahl. This genus belongs to 42 species (Rowlands, 1999). The original home of the dahlia plant is Middle America. Then its cultivation spread to Mexico and from there to England and to all over the world (Tawajin, 1987). This plant reproduces sexual method in seed for new varieties or vegetative method to get the plants similar to mother plant such as the use tuberous roots or cuttings (Twajin, 1987). It cans also propagation by plant tissue culture technique because of their many advantages when compared to other traditional methods vegetative propagation. Also, the producing plants from this tissue culture technique will be similar to the mother plant. Kongthong (1996) could dahlia plant propagation *in vitro* using shoot tips and axillary buds cultured on MS medium supplemented with different combinations of BA and NAA. Qassab Bashi (1998) has succeeded in dahlia plant propagation *in vitro* by culturing shoot tip, nodal segment, leaf and petiole on MS medium supplemented with different concentrations of kinetin, BA or 2ip (2-isopentenyl adenine). He got

the best results when used shoot tip cultures. Fatima *et al*. (2007) used cotyledon and hypocotyl of dahlia plant for callus induction and indirect organogenesis when cultured explants on MS medium supplemented with BA with NAA or IAA. Salman *et al*. (2010) received the highest percentage of response dahlia shoot tip and nodal segment to shoot multiplication when cultured on MS medium supplied with 2.0 mg. L^{-1} BA. The aim of the study was to determine the effect of certain treatments in the germination of seeds, shoot multiplication and rooting of dahlia plant cultivated *in vitro*.

Materials and Methods

The study was conducted in the laboratory of plant tissue culture in the College of Agriculture, University of Basrah in Iraq. The dahlia hybrid seeds used in the current study are produced by Dutch Company Aviflora. Seeds were sterilized with a solution of sodium hypochlorite at 1.05% concentration for a period of 15 minutes. Then seeds were washed three times in sterile distilled water.

1.Effect of different combinations of BA and NAA on seed germination:

The sterilized seeds were cultured on MS medium (Murashige & Skoog 1962) containing different combinations of BA and NAA (0.0+0.0, 1.0+0.0, 1.0+0.3 and 2.0+0.3 mg. L^{-1}) respectively, 30 g. L^{-1} sucrose, 2 g. 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 in Darkness.

2.Effect of light on seed germination:

The sterilized seeds were cultured on the same components of the previous medium except the combination between BA and NAA that added (2.0+0.3 mg. L^{-1}). Then cultures were incubated in darkness or 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.

3.Effect of source of explant on shoot multiplication:

The cotyledon leaf, hypocotyl, shoot tip, nodal and root segments taken from the seedling which produced from seed germination. These explants cultured on the same components of MS medium added 2.0+2.0 mg. L^{-1} of BA+NAA. The percentage of the response to shoot multiplication estimated after eight weeks from culture.

4.Effect of different combinations of BA and NAA on shoot multiplication:

The shoot tips cultured on MS medium supplemented with different combinations of BA and NAA (1.5+1.5, 2.0+2.0, 2.5+2.5 and 3.0+3.0 mg. L^{-1}). The percentage of the response to shoot multiplication estimated after eight weeks from culture.

5.Effect of different concentrations of NAA on shoot rooting:

The producing shoots by multiplication were cultured on half strength of MS medium supplemented with different concentrations of NAA (0.5, 1.0, 1.5 and 2.0 mg. L^{-1}). The measurements were taken after eight weeks of culture, which included:The percentage of response for shoot rooting , Root length, Number of main roots/plantlet and Number of secondary roots/plantlet , Effect of sucrose on shoot rooting:

The producing shoots by multiplication were cultured on half strength of MS medium supplemented with 0.6 mg. L^{-1} IBA and 30 or 45 g. L^{-1} sucrose. The same measurements in the previous paragraph were taken after eight weeks of culture. 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:

1.Effect of combination of BA and NAA in seed germination:

Figure 1 shows the germination of seeds of dahlia plant (Fig. 7,A) when cultured on MS medium supplemented with 2.0+0.3 mg. L^{-1} combination of BA and NAA, respectively as the percentage of germination 100%. While the seeds cultured on MS medium supplied one of other combinations (0.0+0.0, 1.0+0.0 or 1.0+0.3 mg. L^{-1} BA and NAA, respectively) did not germinate. That the reason for the high percentage of germination of the seeds cultured on MS medium supplemented with 2.0+0.3 mg. L^{-1} combination of BA and NAA, respectively, is to break the dormancy of seeds in the process and encouraged to germinate.

2.Effect of light in seed germination:

As Figure 2 indicates the seed germination in the darkness when cultured as MS medium supplied with combination of BA and NAA at 2.0+0.3 mg. L^{-1}, respectively, which represented 100%. While not germinate seeds exposed to light when cultured on MS medium supplemented with 2.0+0.3 mg. L^{-1} of BA and NAA, respectively. The reason is due to the inhibition of light in seed germination and entry into dormancy phase.

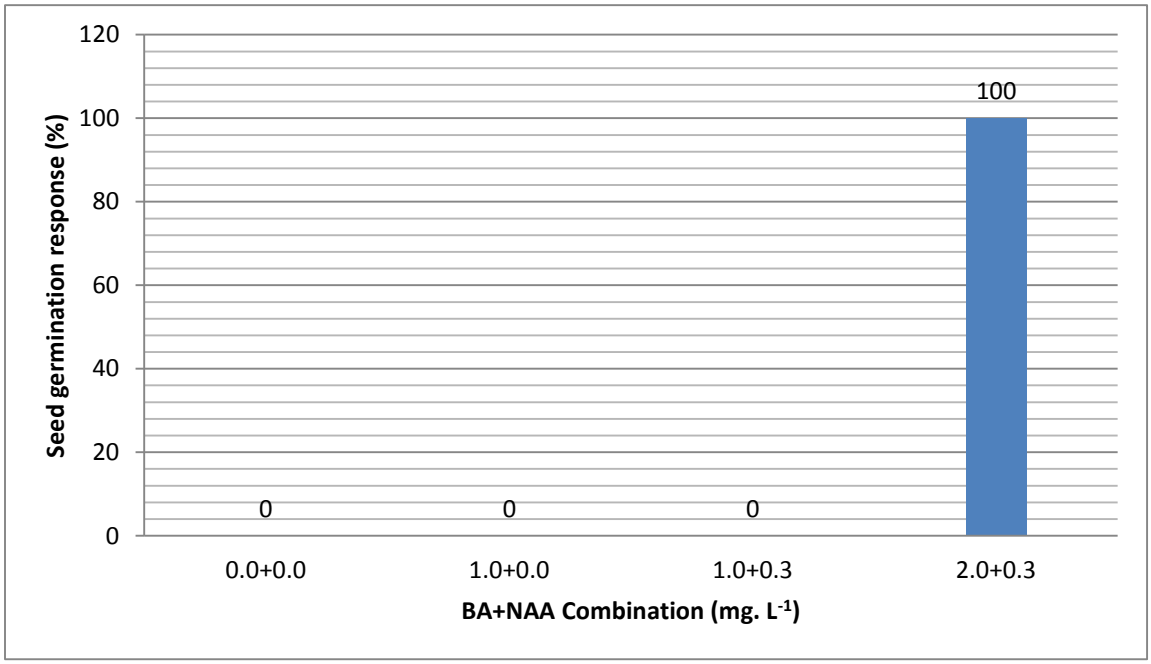


Fig. 1: Effect of BA and NAA combinations on seed germination after 2 weeks from culture.

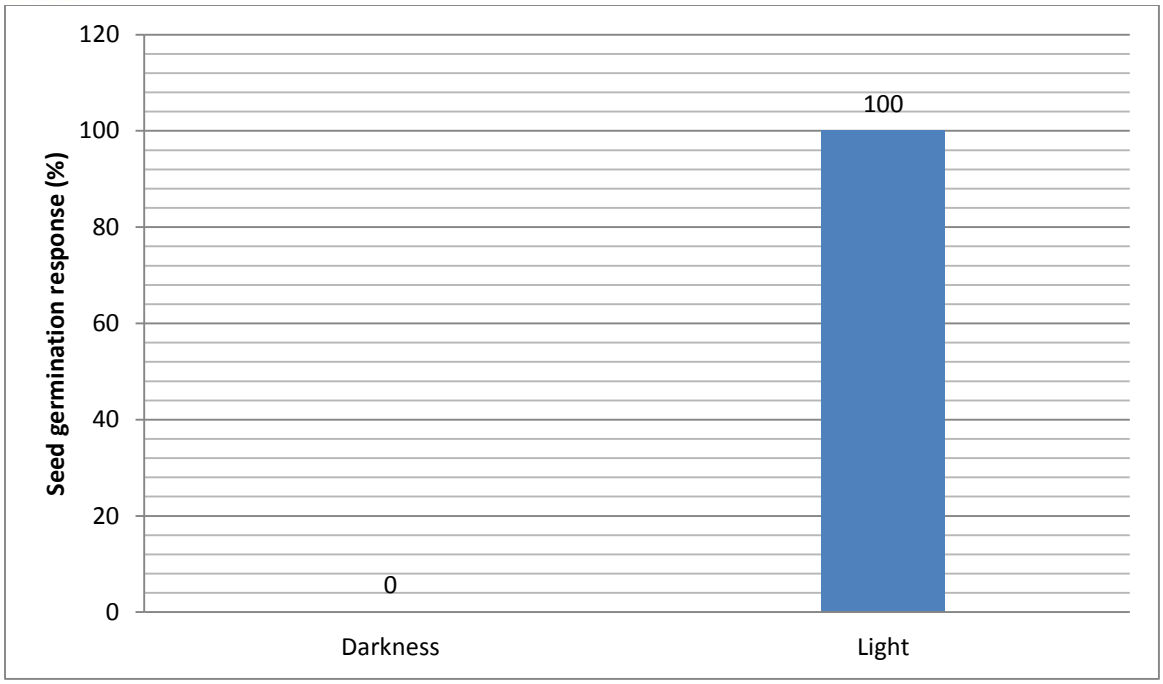


Fig. 2: Effect of light on seed germination after 2 weeks from culture.

3.Effect of source of explant on shoot multiplication:

Results of Figure 3 shows the effect of source of explant supplemented with 2.0+2.0 mg. L^{-1} of BA and NAA, respectively, in the percentage of response to shoot initiation. That shoot tip and hypocotyl showed the highest response to the formation of shoots amounted to 86.67% and 43.33%, respectively. The same figure also shows the significant superiority of the shoot tip on the hypocotyl in the percentage of response to the initiation of shoots. The reason for the high response of shoot tips in the formation of shoots is stimulate cytokinin (BA) to growth and elongation axillary buds when compared with hypocotyls (Fig. 7, B and C). While cotyledon leaf, nodal and root segments did not give any response to shoot formation. These results agreed with the results of many researchers when their studies on micro propagation of dahlia plant (Qasab Bashi, 1998 and Salman *et al*., 2010).

4.Effect of BA and NAA combinations on shoot multiplication:

The MS medium supplemented with 2.0+2.0 mg. L^{-1} of BA and NAA was significantly superiority that the 2.5+2.5 mg. L^{-1} combination of BA and NAA in the percentage of response to shoot tips for shoots multiplication, which amounted to 86.67% and 46.67%, respectively (Figure 4 and Fig. 7, D). While the shoot tips did not give any response to the formation of shoots when cultured on MS medium supplemented with 1.5+1.5 or 3.0+3.0 mg. L^{-1} combination of BA and NAA. The reason may be due to the high response to the formation of shoots is that the combination 2.0 +2.0 mg. L^{-1} BA and NAA is the best of the shoot multiplication when compared to other combinations. Results of the study agreed with Al-Hajaimi (2010), when study on plant micro propagation of *Catharanthus roseus* and Ibrahim *et al*. (2013) on *Fragaria ananassa*.

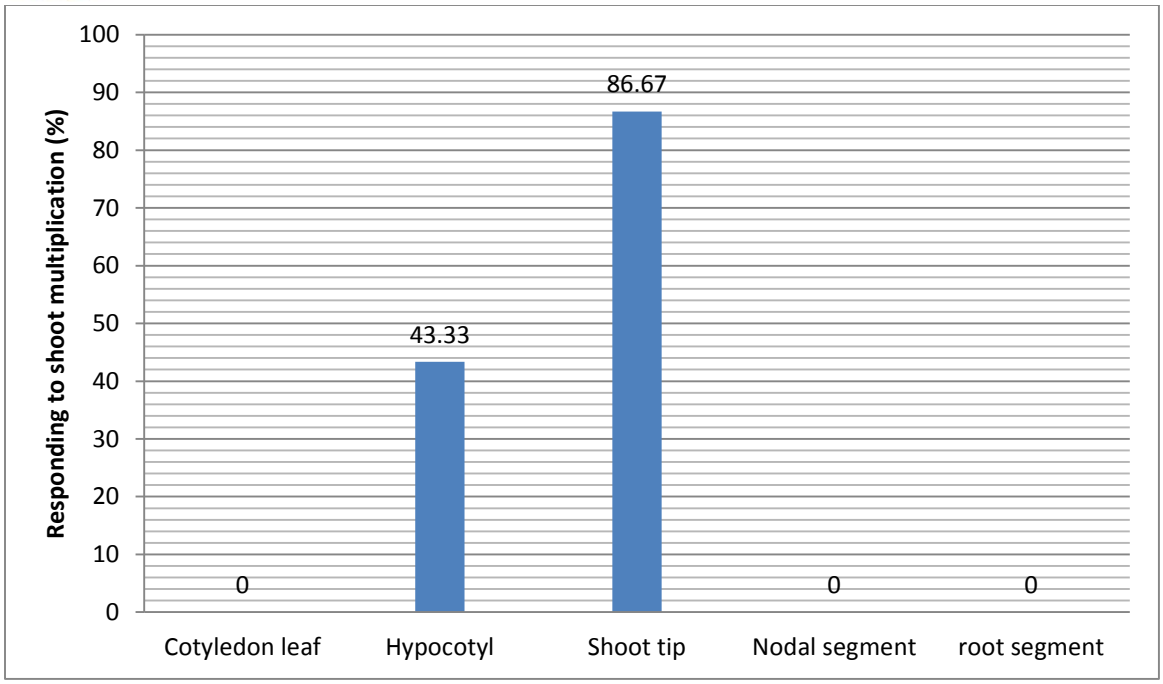


Figure 3: Effect of source of explant on responding to shoot multiplication after eight weeks from culture.

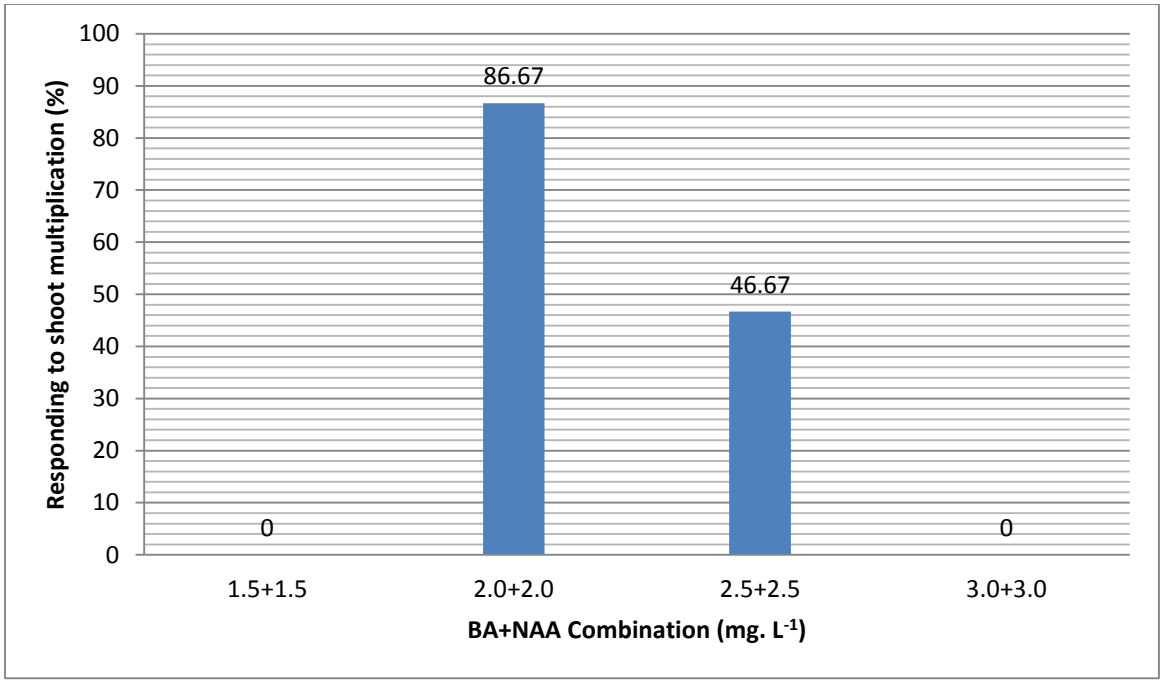
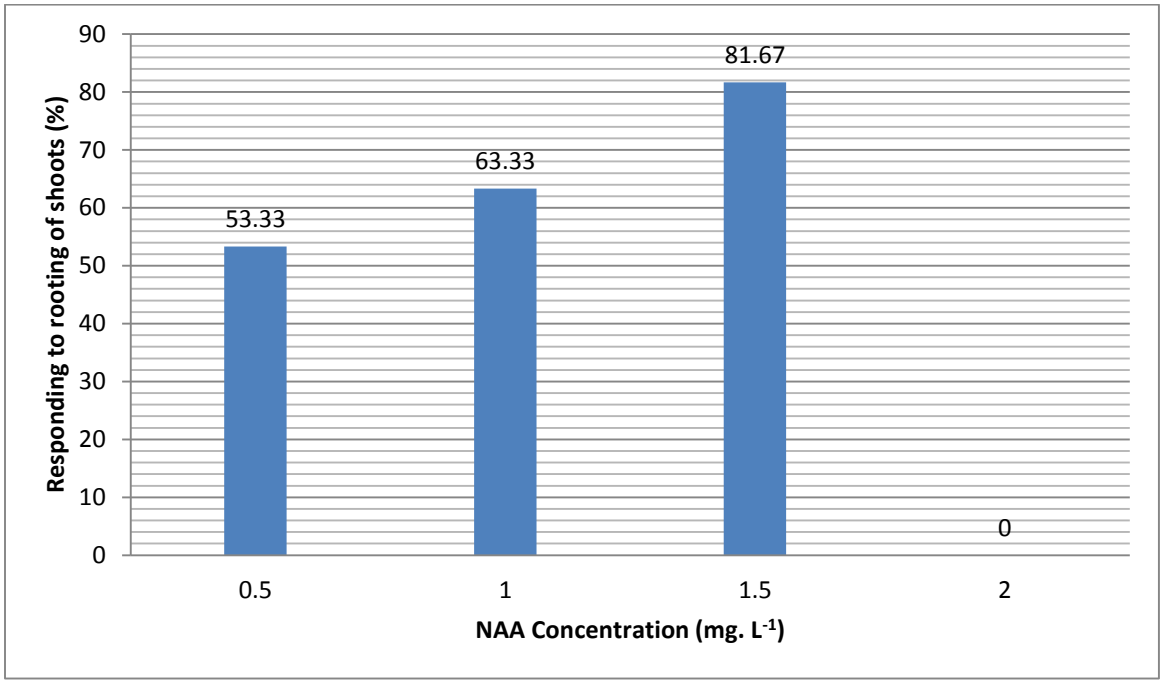


Figure 4: Effect of BA and NAA combinations on responding to shoot multiplication after eight weeks from culture.

5.Effect of NAA on rooting shoots:

The results showed that shoots gave the highest significant response to rooting when cultured on a half strength of MS medium supplemented with 1.5 mg. L^{-1} NAA concentration compared with 0.5 and 1.0 mg. L^{-1} NAA, which reached 81.67%, 63.33% and 53.33%, respectively (Figure 5 and Fig. 7, E). The results of this study agreed with the findings of the Salman *et al*. (2010) in rooting of shoots of dahlia plant *in vitro*. While the shoots did not give the roots when cultured on half strength of MS medium supplemented with 2.0 mg. L^{-1} NAA (Fig. 7, E). Table 1 refers to the significant superiority of MS medium supplied with 1.5 mg. L^{-1} NAA in the number of secondary roots and root length of rooting shoots compared to with 0.5 or 1.0 mg. L^{-1} NAA. The results of this study are similar to what Qasab Bashi (1998) indicated of study on the micro propagation of dahlia plant.



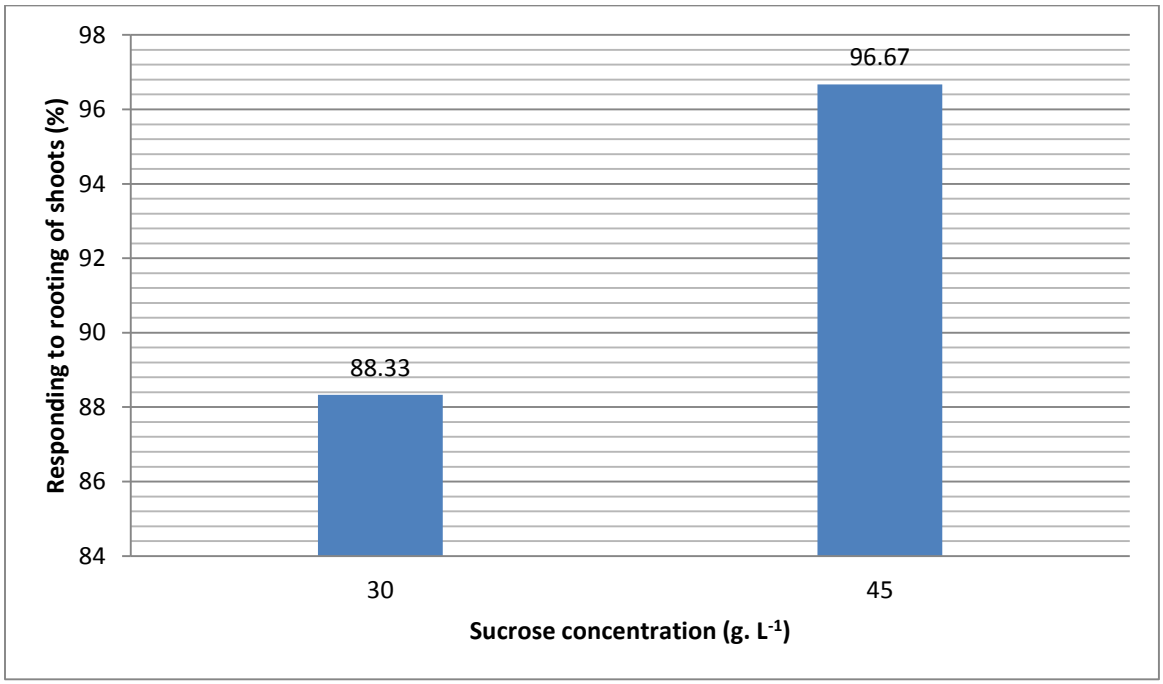
Figu. 5: Effect of NAA concentrations on responding to rooting of shoots after eight weeks from culture.

Table 1 : Effect of different concentrations of NAA on rooting of shoots.

Concentration of NAA (mg. L^{-1})	roots number /plantlet	Secondary roots number /plantlet	Root length (cm)
0.5	2.0	1.70	0.23
1.0	1.7	4.33	0.60
1.5	1.7	10.33	1.47
2.0	0.0	0.00	0.00
R-LSD (0.05)	N.S	4.20	0.42

6.Effect of sucrose on rooting shoots:

Figure 6 indicates that there is no significant difference between the two treatments of 30 and 45 g. L^{-1} sucrose in the shoots of rooting response. But the results of Table 2 show a significant difference between the two treatments of 30 and 45 g. L^{-1} sucrose in the number of secondary roots and root length. Treatment 45 g. L^{-1} sucrose gave highest rate of number of secondary roots and root length when compared to treatment with 30 g. L^{-1} sucrose, which amounted to 11.33 and 6.00 roots/ shoot and 1.63 and 1.00 cm, respectively. The results of this study agreed with the findings of the Salman *et al*. (2010). The 2.0+0.3 mg. L^{-1} of BA and NAA combination add to MS medium and darkness lead to seed germination by *in vitro*. Shoot tip cultured on MS medium supplied with 2.0+2.0 mg, L^{-1} gives the highest response for shoot multiplication. The shoot cultured on half strength of MS medium supplemented with 1.5 mg. L^{-1} NAA gave high response to rooting of shoot.



Figu. 6: Effect of sucrose concentrations on responding to rooting of shoot after eight weeks from culture.

Table 2 : Effect of sucrose concentration on rooting of shoots.

Concentration of sucrose (g. L^{-1})	roots number /plantlet	Secondary roots number /plantlet	Root length (cm)
30	2.00	6.00	1.00
45	3.00	11.33	1.63
Significance	-	+	+

(-): No significant differences between the treatment means at the level of probability 0.05.
(+): There were significant differences between the treatment means at level of probability 0.05.

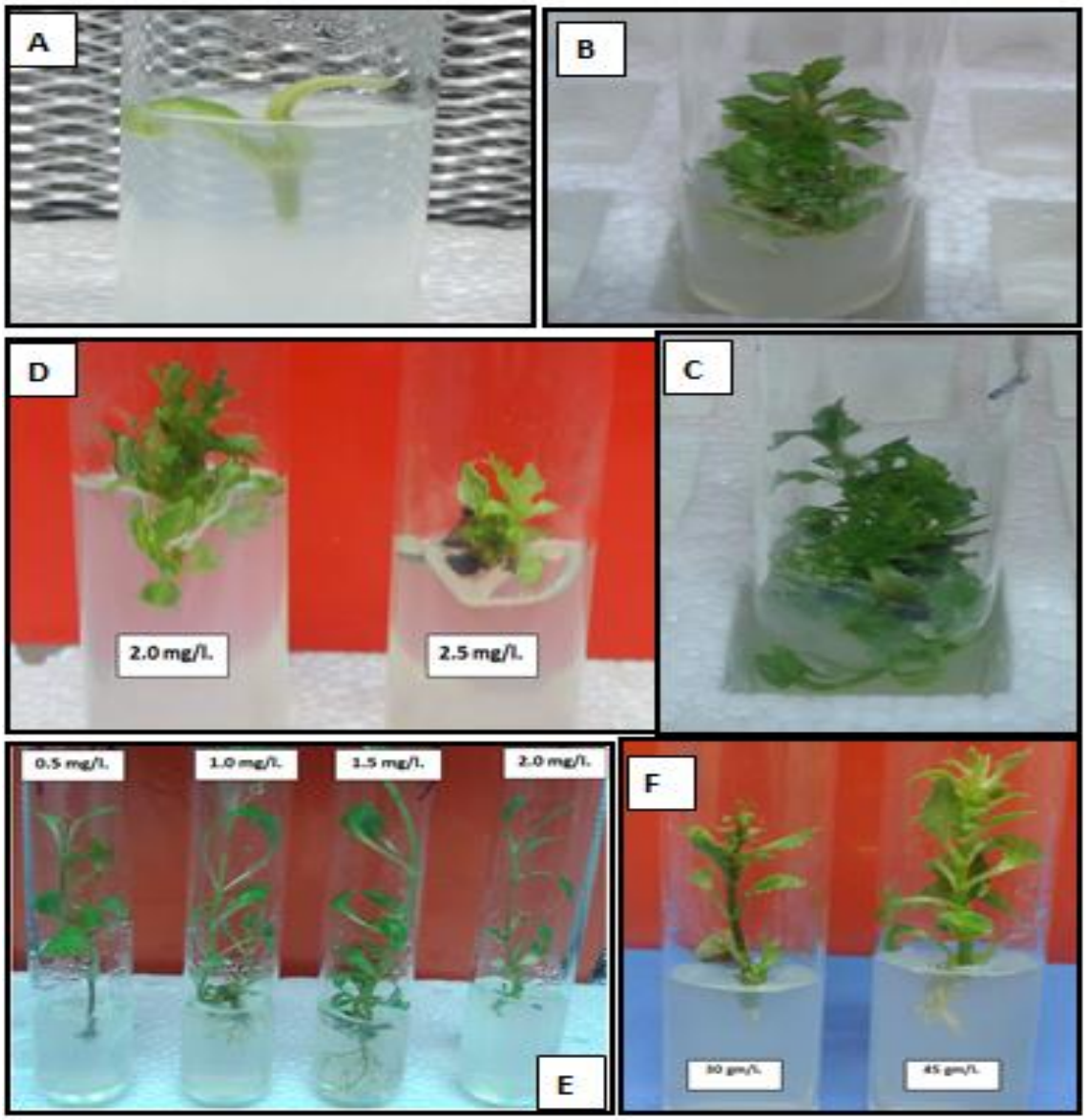


Fig. 7 : Effect of some treatments on seeds germination, shoots multiplication and rooting of dahlia plants via in vitro culture. (A): Seed germination on MS medium supplemented with 2.0+0.3 mg. L^{-1} BA and NAA combination after 2 weeks from culture. Shoot multiplication from shoot tip (B) and hypocotyl (C) cultured on MS medium supplemented with 2.0+2.0 mg. L^{-1} BA and NAA combination after 8 weeks from culture. (D): Shoot multiplication from shoot tips cultured on MS medium supplemented with 2.0+2.0 and 2.5+2.5 mg. L^{-1} BA and NAA combinations after eight weeks from culture. (E): Rooting of shoots which cultured on half strength of MS medium supplemented with 0.5, 1.0, 1.5 or 2.0 mg. L^{-1} NAA after eight weeks from culture. (F): Rooting of shoots which cultured on half strength of MS medium supplemented with 0.6 mg. L^{-1} IBA and 30 and 45 g. L^{-1} sucrose after eight weeks from culture.

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