*J. Agric. Sci. Mansoura Univ., 30 (12): 7955 - 7964, 2005*

# *IN VITRO* DIRECT PLANT REGENERATION OF *Calendula officinalis* L.

**Aboshama H. M. S.**

**Minufiya University -- Genetic Engineering and Biotechnology Research Institute. Plant Biotechnology Department , Egypt.**

## ABSTRACT

Regenerating of whole plants of *Calendula officicnalis*, L. was achieved through direct organogenesis using leaf and cotyledonary nodes as explants. Leaves were taken from 3-week-old *in vitro* plantlets and cultured on MS basal medium supplemented with 200 mg/L glutamine and 500 mg/ L casein hydrolisate. The medium contained BA with different auxins (2,4-D and NAA). Prolific direct adventitious shoot regeneration occurred on most of the tested media which that contained either 2,4-D or NAA. The best response in terms of frequency of shoot regeneration (86.64%), number of shoots per explant (7.60) and bud forming capacity (6.58) were obtained with 1.0 mg/L 2,4-D alone. Cotyledonary node explants were cultured on MS medium,supplemented with various concentrations of Kin and NAA. The highest frequency of regenerated shoots was achieved on MS medium supplemented with 2.0 mg/L Kin and 1.0mg/L NAA (9.20). Regenerated shoots were excised and rooted, the highest number of root/shoot was obtained with half strength MS salt medium supplemented with 1.0 mg/L IBA. *In vitro* rooted plantlets were finally transferred to mixture of peatmoss and vermiculite at equal volume with survival rate95% after 21 days.

**Keywords:** *Calendula officinalis*, organogenesis, leaf culture, direct regeneration, *in vitro*, tissue culture, adventitious shoots, cotyledonary nodes.

**Abbreviations:** MS- Murashige and Skoog; 2,4-D - dichlorophenoxyacetic acid; BA-Benzyladenine ; NAA- α-naphthalene acetic acid; IBA- indole-3-butyric acid; Kin-Kinetin, BFC ; Bud Forming Capacity

## INTRODUCTION

Calendula (*Calendula officinalis* L.) is an Asteraceous plant of industrial and medicinal importance. This herbaceous plant is a native plant of the Mediterranean region (Earle *et al.* 1964), and is grown widely across Europe and North America as an ornamental and medicinal plant.

The flowers are utilized as a source of pigments for food coloring in industry.This species is known to have antiseptic and anti- inflammatory activities (Boucard- Maitre *et al.*1988). *In vitro* regeneration allows the production of pathogen-free uniform plants and conservation of germplasm. Large amount of secondary metabolites can also be produced from *in vitro* cultures.A possible alternative to the conventional approach in crop improvement programmes are somatic hybridization and / or recombinant DNA technology. However, application of either technique is dependent on the availability of a protocol for regeneration (i.e., organogenic or embryogenic). Protocols for regeneration of adventitious shoots from cultured leaves is also of interest that some regenerated shoots may be mutants resulting from somaclonal variation induced *in vitro* (Sandoval *et al.*, 1995., Smolders *et al.*, 1995). *In vitro* shoot regeneration from calendula leaf has

not been reported previously. Here, for the first time this work describe the establishment of a calendula regeneration system (rapid and prolific direct organogenesis from leaf), which represents an excellent tool for work and its genetic manipulation.

## MATERIAL AND METHODES

This study was carried out at Plant Biotechnology Department, Genetic Engineering and Biotechnology Institute, Minufiya University, Egypt, during the years of 2003 – 2004. Seeds (Achenes) of *Calendula officinalis* L. were obtained from the Department of Horticulture, Faculty of Agriculture, Mansoura University. Seeds were surface sterilized in mercuric chloride ($HgCl_2$) at 0.1 % for 4 min. Then the seeds were washed 4 times in autoclaved distilled water to remove all traces of the disinfectant. The sterile seeds were germinated on hormone-free MS (Murashige and Skoog, 1962) medium containing 30 g/L sucrose and solidified with 7.0 g/L agar. The pH was adjusted to 5.8 prior autoclaving at 121°C for 20 min. All cultures were maintained at 25 ± 2°C under florescent light (2000 LUX) and 16-h / 8 dark cycles.

**1. Leaf culture**

Leaf of 3-week-old plantlets were the source of explants for the experiments. Leaf explants were cultured for *in vitro* shoot formations. The leaf explants were placed on MS medium salts, vitamins, 3% (w/v) sucrose and supplemented with 200 mg/L glutamine and 500 mg/L casein hydrolisate. The tested plant growth regulators included various concentrations of $N^6$ benzyladenine (BA) at 0.0, 1.0, and 2.0 mg/L singly or combined with auxins; 2,4 - dichlorophenoxyacetic acid (2,4-D) and α-naphthalene acetic acid (NAA) at 0.0, 0.5 1.0 and 2.0 mg/L. The medium pH was adjusted to 5.8 prior to the addition of 0.7% (w/v) agar. Medium was distributed into culture jars (325 ml) where each jar contained 50 ml of the medium. The jars were capped with polypropene closures and autoclaved at 121°C and 1,2 Kg/Cm air pressure for 20 min. All cultures were incubated at 25 ± 2 C under a 16-h ( 2000 Lux) photoperiod using cool, white fluorescent light. The results were collected four weeks after culture initiation. Explants forming bud percentage and number of buds per explant were counted
For a more realistic determination of the efficiency of a given treatment, the Bud Forming Capacity (BFC) index was calculated according to (Martinez-pulido *et al.* 1992).

$$\text{BFC index} = \frac{(\text{mean number of buds per explant}) \times (\%\text{of explants forming buds})}{100}$$

**2. Cotyledonary nodes culture**

The aim of this experiment was to determine the optimal media for shoot proliferation from cotyledonary node culture. Aseptic cotyledonary nodes explants (from 3-week-old *in vitro* plantlets) were placed on MS medium containing 30 g/L sucrose and solidified with 7.0 g/L agar. This medium was augmented with different concentrations of Kin at 0.0, 1.0 and

2.0 mg/L singly or combined with NAA at 0.0, 0.2, 0.5 and 1.0 mg/L. All cultures were maintained at 25°C under a 16-h photoperiod using cool white fluorescent lights.

After 6 weeks of culture, the number of shoots per explant was counted

All experiments were repeated two times during the years of 2003 and 2004. Experiments were set up in completely randomized block design with five replicates. Each replicate consisted of one jar (325 ml) cultured with three explants. The results were statistically analyzed, according to Gomez and Gomez (1984).

**3. Root formation**

For root induction, the shoots were individually transferred to rooting media. These media consist of half or full strength MS basal salts each of which supplemented with 30 g/L sucrose and two types of auxins IBA or NAA each of which at different concentrations (0.0, 0.5 and 1.0 mg/L). The number of roots per shoot was evaluated 4 weeks later. The cultures were incubated at 25°C day and night. Light was provided by white fluorescent tubes for 16-h per day. The well-developed plantlets were carefully washed in running tap water to remove the substrate, and then transferred to pots containing mixture of peatmoss and vermiculite at equal volume. These pots were covered with plastic bags to maintain high relative humidity around the plants in a greenhouse. A solution of one half strength MS salt was added to the pots to enhance the development of plants. Data was recorded for percentage of survival after 21 days from transplanting.

The experiment was factorial (three factors) in completely randomized block design with five replicates. Each replicate consisted of one jar (325 ml) cultured with three explants. The results were statistically analyzed according to Gomez and Gomez (1984).

## RESULTS AND DISCUSSION

**Leaf culture**

The present investigation show that it was possible to obtain high frequency of shoot bud regeneration directly from leaf explants of *Calendula officinalis*, L. Initially, the leaf explants on the responded treatments of growth regulators enlarged and developed globular like structures on the petioles (end of the leaf from intact stem) within 2 weeks of culturing. After four weeks from culturing, all shoots were directly proliferated from petioles (Fig1). This result was in agreement with Jeong *et al.*, (2001) who reported that in some genera petiole and midrib segments of leaves showed a better response to cultural regimes than lamina segments. In addition, Belaemino and Sasahara (1992) working on *Tagetes erecta* reported that leaf explants gave the best response for plant regeneration.

**Effect of BA and 2,4-D on shoot proliferation from leaves:**

Table (1) shows the main effect of growth regulators on percentage of explant forming shoot- buds, number of shoot- buds per explants and Bud Forming Capacity (B.F.C.). Data show that there was no shoot initiation in

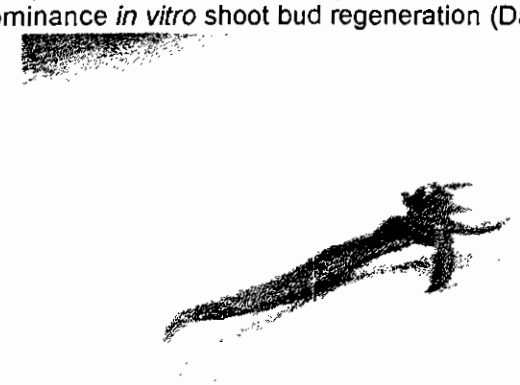
the absence of growth regulators. Data indicated that the main effect of BA show that, BA significantly decreased these characters. On the contrary, 2,4-D significantly increased them.

The same data show the effect of interaction between BA and 2,4 -D on the previous parameters. It is clear that the highest effects on explants forming shoot-buds percentage, number of shoot-buds per explants and BFC (86.64, 7.60 and 6.58 respectively) were found with the medium contained 1.0 mg/L 2,4-D alone, followed by the medium of 2.0 mg/L 2,4-D alone (66.60, 4.40 and 2.93 respectively) when compared with all used concentrations and control. However, BA at 2.0 mg/L alone or when was combined with 0.5 or 1.0 mg/L 2,4-D did not promote regeneration of shoot buds. The same observation was obtained with marigold on the medium contained BA in combination with NAA as mentioned by Vanegas *et al.*, (2002).

**Table (1): Effect of BA and 2,4-D concentrations on direct shoot bud proliferation from Leaf culture of calendula after 4 weeks .**

| Treatments (mg/l) | % explants forming shoot-buds | | | | Mean | Mean Number of shoot-buds per explant | | | | Mean | Bud Forming Capacity (BFC) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2,4-D (B) / BA(A) | 0.0 | 0.5 | 1.0 | 2.0 | | 0.0 | 0.5 | 1.0 | 2.0 | | 0.0 | 0.5 | 1.0 | 2.0 |
| 0.0 | 0.00 | 46.62 | 86.64 | 66.60 | 49.97 | 0.00 | 3.20 | 7.60 | 4.40 | 3.80 | 0.00 | 1.49 | 6.58 | 2.93 |
| 1.0 | 0.00 | 26.64 | 39.96 | 46.62 | 28.30 | 0.00 | 1.40 | 3.00 | 3.40 | 1.95 | 0.00 | 0.37 | 1.19 | 1.58 |
| 2.0 | 0.00 | 0.00 | 0.00 | 39.96 | 9.99 | 0.00 | 0.00 | 0.0 | 3.40 | 0.85 | 0.00 | 0.00 | 0.00 | 1.35 |
| Mean | 0.00 | 24.42 | 42.20 | 51.06 | | 0.00 | 1.53 | 3.53 | 3.73 | | | | | |
| **LSD 5%** | **A = 10.53<br>B = 12.16<br>AxB = 21.07** | | | | | **A = 0.30<br>B = 0.34<br>AxB = 0.59** | | | | | | | | |

The different responses could be due to the varying concentration of growth regulators used in the medium and explant type (Samantary *et al.*, 1995). There was certain regulators action of cytokinin, auxin and apical dominance *in vitro* shoot bud regeneration (Das and Rut,2002).



**Fig(1) :Direct shoot proliferation from leaf cultured on MS medium supplemented with 1.0 mg/L 2,4-D.**

**Effect of BA and NAA on shoot proliferation from leaves:**

Regarding the effect of BA and NAA on explants forming bud percentage, number of shoot -buds per explant and B.F.C., data presented in Table (2) reveal that hormone-free medium was not effective for regeneration

from leaf (0.0%). The main effect of BA indicated that increasing BA concentration decreased the studied parameters significantly. On the other side increasing NAA concentration increased them significantly.

The effect of BA and NAA combination on adventitious shoot formation is shown in Table (2) and Fig (2). A concentration of 2.0 mg/L NAA alone induced the highest percentage of explants forming shoot-buds, number of shoot-buds per explant and B.F.C. (39.96, 3.40 and 1.35 respectively). In addition the lowest concentrations of NAA (0.0 and 0.5 mg/L) did not enhance any regeneration rate when used alone or together with BA. Whereas, BA at all concentrations was effective in this concern when combined with either 1.0 or 2.0mg/L NAA.

**Table (2): Effect of BA and NAA concentrations on direct shoot bud proliferation from leaf culture of calendula after 4 weeks.**

| Treatments (mg/l) | % explants forming shoot-buds | | | | Mean | Mean Number of shoot-buds per explant | | | | Mean | Bud Forming Capacity ( BFC) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NAA ( B) / BA (A) | 0.0 | 0.5 | 1.0 | 2.0 | | 0.0 | 0.5 | 1.0 | 2.0 | | 0.0 | 0.5 | 1.0 | 2.0 |
| 0.0 | 0.00 | 0.00 | 33.30 | 39.96 | 18.31 | 0.00 | 0.00 | 2.20 | 3.40 | 1.40 | 0.00 | 0.00 | 0.73 | 1.35 |
| 1.0 | 0.00 | 0.00 | 19.98 | 33.30 | 13.32 | 0.00 | 0.00 | 1.60 | 2.80 | 1.10 | 0.00 | 0.00 | 0.31 | 0.93 |
| 2.0 | 0.00 | 0.00 | 0.00 | 13.32 | 3.33 | 0.00 | 0.00 | 0.00 | 2.00 | 0.50 | 0.00 | 0.00 | 0.00 | 0.26 |
| Mean | 0.00 | 0.00 | 17.76 | 28.86 | | 0.00 | 0.00 | 1.26 | 2.73 | | | | | |
| LSD 5% | A = 9.22<br>B = 10.64<br>AxB = NS | | | | | A = 0.28<br>B = 0.33<br>AxB = 0.57 | | | | | | | | |

From Tables (1 and 2) it is clear that the presences of auxins either 2,4-D or NAA in culture media were essential for shoot-bud formation from leaves of calendula. This result was in agreement with results of Pierik, (1987) who reported that there is a further group of plants which require exogenous auxin for shoot formation. In addition, Narayanaswamy, (1994) reported that auxins may initiate or promote cell division from tissues cultured *in vitro* and can stimulate shoot growth and inhibit root growth. It is a matter of interest that data of this work showed that the auxins either 2,4-D or NAA were essential for shoot bud formation from leaf culture , while it is well known that cytokinin is the material which help shoot proliferation . This may be due to the high level of endogenous cytokinin in tissues of calendula leaves, so when auxins were added to the proliferation media, a balance between endogenous cytokinin and exogenous auxins may be occur. Subsequently, shoots were proliferated in the presence of auxins. Finally, the presence of glutamine and casein hydrolisate may help shoot proliferation from leaf culture. This observation was supported by Narayanaswamy, (1994) who reported that casein hydrolisate (0.25-1.0g/L) was beneficial for somatic embryogenesis and shoot formation. Also he reported that glutamine and aspartic acid stimulate bud induction in some stem tissues such as that of Tornia. It is worth to mention that the herein data revealed that 2,4-D auxin was more effective in shoot-bud formation than NAA at the same concentration ( 2 mg/L ), since the responded explants was 66.60 % for 2,4-D against 39.96 for NAA and the number of shoot-buds /explant was 4.40 against 3.40, respectively, likewise the shoot-bud forming capacity index was 2.93 against 1.35.

**Fig (2): Direct shoot proliferation from leaf cultured on MS medium Supplemented with 2.0 mg/L NAA.**

**Culture of Cotyledonary nodes**

**Effect of Kin and NAA on shoot proliferation from cotyledonary nodes:**

Data presented in Table (3) and Figs (3 & 4) clearly show the effect of different levels of Kin and NAA on shoot formation of cotyledonary nodes of calendula after 6 weeks from culture. Concerning the main effect of Kin, results in Table (3) show that all Kin concentrations significantly increased the number of proliferated shoots arose from one explant as kin concentration increased. Also, data in Table (3) revealed that NAA significantly affected this parameter only at 1.0 mg/L. Moreover, the results in the same Table indicated that all cotyledonary nodes produced shoots on all tested media, except in the absence of Kin where no shoots were formed (Fig 3).

**Table (3) Effect of Kin and NAA on shoot proliferation from cotyledonary nodes of *Calendula officinalis.***

| NAA(B) / Kin (A) | NO. of shoots | | | | MeanA |
|---|---|---|---|---|---|
| | 0.0 | 0.2 | 0.5 | 1.0 | |
| 0.0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1.0 | 3.80 | 4.60 | 6.60 | 6.40 | 5.35 |
| 2.0 | 7.80 | 7.60 | 5.00 | 9.20 | 7.40 |
| Mean B | 3.86 | 4.06 | 3.86 | 5.20 | |
| **LSD 5%** | **A= 0.50** | | **B= 0.58** | | **AB= 1.01** |

The interaction between NAA and Kin proved that the best combination was 2.0 mg/L kin and 1.0 mg/L NAA which resulted in the highest shoots number (9.20). It was clear that the number of proliferated shoots increased significantly with increasing Kin level. This finding was in agreement with the fact that Kin, as a cytokinin, is active in increasing cell division rate and subsequently enhance axillary branching (Waithaka, *et al.*, 1980). In addition, Çöçü *et al.*, (2004) working on *Calendula officicnalis* mentioned that prolific shoot meristems were observed within 3 weeks on cotyledonary nodes which were later developed into normal shoots after 4-5 weeks of culture initiation.

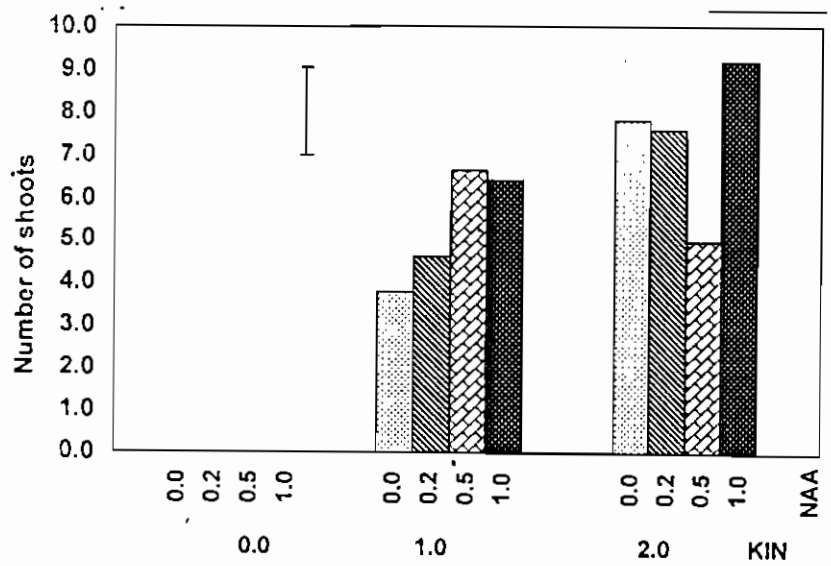



**Fig (3): Effect of Kin and NAA on number of shoots from Cotyledonary nodes of calendula after 6 weeks from culture on MS medium.**

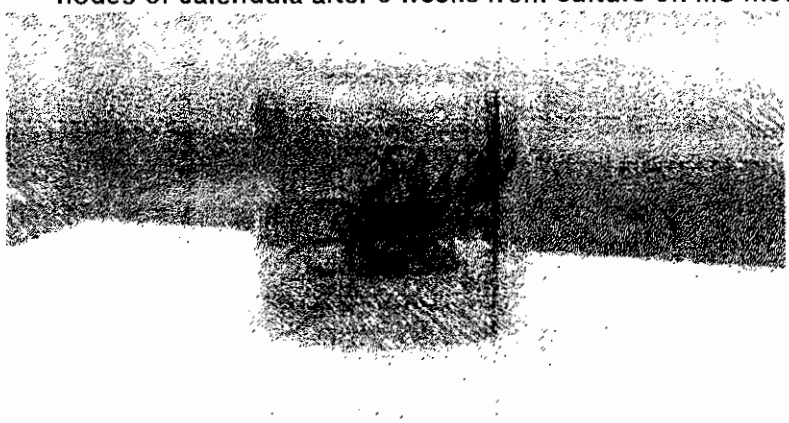


**Fig (4):Proliferation shoots of cotyledonary node cultured on MS medium contained 2.0mg/L Kin + 1.0 mg/L NAA after 6 weeks from culture.**

**Effect of types of auxin and their concentrations and MS strength on the number of roots:**

Data in Table (4) clearly show the effect of different levels of IBA and NAA on the number of roots/shoot after 4 weeks from culture. Evidently, no roots were produced on hormone-free media. Data on the main effect of types of growth regulators showed that IBA and NAA were similar in this concern. Data on the main effect of different used MS strengths showed that

haif-strength of MS was significantly more effective on root formation (2.83) when compared with that of full MS (1.96). Çöçü *et al*,( 2004 ) working on calendula regenerated shoots ( 10-20 mm in length ) that rooted within 3 weeks in half - strength MS medium containing 1.0 mg/L NAA . Results of this study observed that the gradual increases in the concentration regardless auxin type resulted in gradual increases in the number of roots and 1.0 mg/L was more pronounced in this respect (4.25).

**Table (4): Effect of different types of auxin at various concentrations and MS salts strength (1/2 and 1/1) on the number of roots of *Calendula officinalis* shoots after 4weeks *in vitro*.**

| | | Concentrations (C) auxin | | | Means (A x B) | Means of (A) |
|---|---|---|---|---|---|---|
| auxin types (A) | MS strength(B) | 0.0 | 0.5 | 1.0 | | |
| IBA | 1/2 | 0.00 | 3.40 | 5.00 | **2.80** | 2.43 |
| | 1/1 | 0.00 | 3.20 | 3.00 | **2.06** | |
| NAA | 1/2 | 0.00 | 2.60 | 6.00 | **2.86** | 2.36 |
| | 1/1 | 0.00 | 2.60 | 3.00 | **1.86** | |
| **Means of (C)** | | 0.00 | 2.95 | 4.25 | **Means of (B)** | |
| Means of (B x C) | 1/2 | 0.00 | 3.00 | 5.50 | 2.83 | |
| | 1/1 | 0.00 | 2.90 | 3.00 | 1.96 | |
| Means of (A x C) | IBA | 0.00 | 3.30 | 4.00 | | |
| | NAA | 0.00 | 2.60 | 4.50 | | |
| **LSD at 5%** | | **A = NS**<br>**B = 0.44**<br>**C = 0.55**<br>**A x B = NS**<br>**A x C = NS**<br>**B x C = 0.77**<br>**A x B x C = NS** | | | | |

The interaction data revealed that no significant differences were obtained between types of growth regulators and ½ or 1/2 MS strength and between the same growth regulators and the examined concentrations.

In addition, with increasing growth regulations level up to 1.0 mg/L, the number of roots was significantly increased particularly with MS strength at 1/1. Eventually, the same data clearly showed that non significant effect was observed among both types and levels of growth regulators and MS strength.

After 21 days from acclimatization the plantlets grew vigorously and a high percentage of plant survival (95%) was achieved during this phase of culture. Kothari and Chandra (1984) reported that the survived plants percentage after acclimation was 20% in marigold. The plants were normal and developed uniform inflorescences during the flowering season (Fig 5). This result was in agreement with Lee and Phillips, (1988) who reported that normal and developed uniform inflorescences after maturity is of major importance because plants produced by direct organogenesis may exhibit greater genetic stability than those produced from callus.

**Fig (5): Flowering plant obtained from *in vitro* regeneration.**

In conclusion, this study was developed as a protocol for rapid direct regeneration of *Calendula officinalis* from leaf and cotyledonary node explants. The efficiency of morphogenesis was dependent on growth regulators. Auxins (2,4-D or NAA) alone were essential to enhance shoot-bud regeneration from leaf culture, while BA was not effective in this concern. This protocol might be useful for genetic improvement programs of this plant.

## REFERENCES

Belaemino M.M, T. Abe and T. Sasahara (1992).: Callus induction and plant regeneration in African marigold (*Tagetes eracta* L.) J.P.J. Bread. 42: 832-841.

Boucaurd-Maitre, Y., O. Algernon, and J. Raynonrd (1988).: Cytotoxic and antitumoral activity of *Calendula officinalis* extracts. Pharmazie 43: 220-221.

Çöçü S.,S Uranbey,A.Ipek,K.M.Khawar,E.O.Sarihan,M.D.Kaya,I.Parmaksiz and S.özcan (2004),: Adventitious shoot regeneration and micropropagation in *Calendula officinalis* L. Biologia Plantarum 48 (3): 449-451

Das, G., and G.R. Rout (2002).: Direct plant regeneration from leaf explants of *Plumbago* species. Plant Cell Tissue and Orgain Culture. 68: 311-314.

Earle, F. R., K. L. Miklojajczak and I.A. Wdf (1964).: Search for new industrial oils: seed-oils of the Calendula. J. Amer. Oil Chem. Soc. 41: 345-347.

Gomez, K.A. and A.A. Gomez (1984).: Statical procedures for the agricultural researches. John wiley and Son, Inc. New York.

Jeong J.H.,H.N.Murthy and K.Y.Paek (2001). High frequency adventitious shoot induction and plant regeneration from leaves of Statice. Plant cell, tissue and organ culture 65: 123-128

Kothari S.L.and N.Chandra (1984) *In vitro* propagation of African marigold. Hort Scince 19: 703-705

Lee M. and R.L.Phillips (1988) . The chromosomal basis of somaclonal variation. Ann.Rev. Plant Physiol.Plant Mol.Biol. 39:413-437.

Murashige, T. and F. Skoog (1962).: A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant, 15: 473-497.

Martinez-pulido C, I.S. Harry and T.A. Torpe (1992) Optimization of bud induction in cotyledonary explants of Pinus canariensis. Plant Cell Tiss. Org. Cult. 29:247-255

Narayanaswamy S. (1994) Plant cell and tissue culture .Tala McGraw-Hill New Delhi pp 23-50.

Pierik,R.L.M.(1987) *In vitro* culture of higher plants. Kluwer Acad. Publ. Dordrecht.The Netherlands. pp209-212

Samantaray, S., G.R. Rout and P. Das (1995).; An *in vitro* study on organogenesis in *Trema orientalis* (Bluene) Linn. Plant Sci. 105: 87-94.

Sandoval, J., F. Kerbellec, F. Cote and P. Dowmas (1995).: Distribution of endogenous gibberellins in dwarf and giant off type banana (Musa AAA, cv. Grand Nain) plants from in vitro propagation. Plant Growth Reg. 17: 219-224.

Smolders, M.J.M., W. Rus-Kortekas and B. Vosman (1995).: Tissue culture-induced DNA methylation polymorphisms in repetitive DNA of tomato calli and regenerated plants. Theor. Appl. Gen. 19: 1257-1264.

Vanegas, P.E., A.C. Hernández, M.E. Valverde and O. Paredes-López (2002).: Plant regeneration via organogenesis in marigold. Plant Cell Tissue and Organ culture. 69: 279-283.

Waithaka, K, B.E. Struckmeyer and M.N. Dona (1980).: Growth substances and growth of strawberry stolons and leaves. J. Amer. Soc. Hort. Sci. 103: 480-482.

## تكوين افرع مباشره من نبات الاقحوان معمليا

**هارون محمد صالح ابوشامه**

**جامعه المنوفيه – معهد الهندسه الوراثيه والتكنولوجيا الحيويه – قسم البيوتكنولوجيا النباتيه**

تم فى هذا البحث ولاول مره وضع تقنيه لانتاج نباتات عن طريق التكوين العضوى المباشر من الورقه والعقده الفلقيه . يمكن استخدام هذا البرتوكول فى اجراء التحسين الوراثى للاقحوان مستقبلا وبسهوله.

اخذت الاوراق من نبات نامى معمليا بعمر ٣ اسابيع وزرعت فى بيئه موراشيج وسكوج مضاف اليها ٢٠٠ ملجرام / لتر جلوتامين و ٥٠٠ ملجرام/ لتر كازين هيدروليزيت. وكانت منظمات النمو المستخدمه عباره عن بنزيل ادنين مع كل من ٤,٢ داى كلورو فونكسى حامض الخليك او نفتالين حامص الخليك .وقد اظهرت النتائج تكوين الافرع مباشره على منطقه عنق الورقه فى معظم البيئات المحتويه على اى من انواع الاوكسين المختبره على انفراد ، وكانت افضل استجابه كنسبه مئويه ( ٨٦,٦٤ ) وكذلك اعلى معدل لانتاج الافرع على الجزء النباتى الواحد ( الورقه) ( ٧,٦ ) وكذلك اعلى قدره على تكوين الافرع كانت مع استخدام ١,٠ ملجرام / لتر ٤,٢ داى كلورو فونكسى حامض الخليك وحده .

استخدمت العقده الفلقيه كجزء نباتى اخر لانتاج افرع ابطيه منها ، واستخدم لذلك بيئه موراشيج وسكوج مضاف اليها كاينتين و نفتالين حامض الخليك . وكان اعلى معدل انتاج للافرع على بيئه محتويه على ٢,٠ ملجرام /لتر كاينتين و ١,٠ ملجرام / لتر نفتالين حامض الخليك (٩,٢).

فصلت الافرع الناتجه وزرعت على بيئه بغرض تجذيرها وكان اعلى عدد جذور على العقل المنزرعه فى بيئه موراشيج وسكوج عند نصف قوه مضاف اليها اندول حامض البيوتيرك عند تركيز ١,٠ ملجرام / لتر.

نقلت النباتات الى خليط من كميات متساويه حجما من بيتموس و فرموكيوليت وكانت نسبه النباتات الحيه بعد ٢١ يوم من النقل الى الاصص ٩٥%.