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STUDIES ON SOME FACTORS AFFECTING MICROPROPAGATION OF *Eucalyptus gomphocephala* TREES

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ABSTRACT

This work was carried out in Tissue Culture Laboratory in the Vegetable and Floriculture Dept., Fac. of Agric., Mansoura Univ. from 2002 to 2005 years to find out an ideal method of propagation through tissue culture technique for *Eucalyptus gomphocephala* trees. In this concern, shoot tips (terminal part) and basal part (one node from the base of the stem) each explant part was about 1 – 1.5 cm prepared from *E. gomphocephala* trees. After sterilization, the explants were initiated on culture medium i.e. Murashige and Skoog (MS) supplemented with 1.0 mg/L BA (6-benzyl adenine) and 0.1 mg/L IBA (Indol-3-butyric acid) for establishment stage. The newly formed shoots were transferred to the same medium supplemented with BA at the concentration of (0. 0.5, 1, 2 and 4 mg/L) and with or without activated charcoal (2 g/L) through proliferation stage to study the effect of the explant type, activated charcoal and the plant growth regulators on proliferation of shoots and some vegetative charcaters (shoot number and length and number of leaflets). Highest number of formed shoots were regarded when culturing the basal explant part on MS without activated charcoal, supplemented with 0.2 mg/L BA. While, the tallest shoots were resulted from culturing the shoot tip on MS media with 2 g/L activated charcoal. Microshoots were rooted in the same full and half strength media supplemented with IBA at the concentration of (0, 0.5, 1, 2 and 4 mg/L). Full strength MS medium supplemented with 2 mg/L IBA was superior and had the greatest for the rooting growth measurements (mumber of roots/plantlet and average root length). At acclimatization the plantlets were transplanted to (300 ml) plastic pots containing autoclaved transplanting media (sand: peatmoss: vermiculite: perlite) mixed by volume (1:1:1:1) and maintained in greenhouse for four weeks to investigate their effect on survival % per plant during acclimatization stage on media mixed. Produced the highest survival percentage of Eucalypts was 81.25% in case of using (1:1:2:1) by volume, respectively. After transfer of plantlets from *in vitro* cultures to the greenhouse or field to study the effect of acclimatization time (48 h, 7, 14 and 30 days) on leaf structure, substantial changes in leaf morphology and anatomy are observed, above all in epidermal characteristics, leaf thickness, differentiation of leaf mesophyll, and chloroplast number and structure.

INTRODUCTION

Egypt suffers from a shortage in wood-raw materials. The country has focused attention, especially during the last three decades, on establishing forest plantations to meet the acute needs for wood.

Eucalypts (*Eucalyptus gomphocephala*, A.DC.), which belongs to family *Myrtaceae* is not widely planted because seed germination and transplanting of seedlings did not exceed 50% successful establishment? In Egypt, *Eucalyptus* species are presently used as poles, windbreaks, shelterbets and has *a* promise as a low-cost source of hardwood fiber

(Maximous, 2004). The essential oils of this tree can be used for the following purposes: pesticide (Pajni and Gill, 1990), antimicrobial and antibiotic (El-Aziz *et al.*, 1990), weevil control (Gakura and Foua, 1995), antifungal (Baba-Moussa *et al.*, 1997) and weed control (Kohli *et al.*, 1998). Moreover, seasoning well, the wood works nicely and is good for boxcars, construction, fenceposts, stakes, trucks and wagons (Little, 1983). In addition, *Eucalyptus gomphocephala* is described as a highly growth rate than *Casuarina glauca, C. equistifolia, E. citriodora, Taxodium distichum, Swietenia mahogoni* and *Khaya senegalensis*, respectively (Abdel-Khader, 2000).

Murashige and Skoog, (1962) (MS) medium, which contained 4627.91 mg/L and total ionic concentration of 93.3 mM, has been widely used for a range of culture. Lowering of the MS salt concentrations to ½ or ¼ that of the original formulation was found to enhance root formation from culture shoots of *Lilium longiflorum* (Koriesh and Stimart, 1989).

Youssef (1996) on *Robinia pseudoacacia* reported that culturing nodal explants on MS medium increased the shoot proliferation rates to 13.9 and 11.6 – fold higher than on others media.

Several investigators used the full strength or the modified basal nutrient medium supplemented with auxins to encourage root formation in the rooting stage. Lakshmisita and Vaidyanathan (1979) reported that root induction and elongation of *E. citriodora* was obtained on (MS) medium with 2% sucrose, supplemented with 8.0 μM NAA. Kapoor and Chauhan (1992) mentioned that rooting of *in vitro* derived shoots of *E. torelliana x E. citriodora* hybrid was obtained in one-half and one-fifth strength MS media each supplemented with 0.1 and 1.0 mg/L IBA.

Niccol *et al.* (1994) observed that the use of IBA alone with half-strength MS medium was successful in initiating roots from *in vitro* derived shoots of *E. microcorys*. 5 μM IBA was the optimum concentration for root induction and elongation. Moreover, results of Koriesh and Al-Manie (2000) showed that explants cultured on MS medium or reduced MS in salt concentrations produced the highest number of roots.

El-Etriby (2001) found that the rooting of *E. citriodora* was favorable in 0.2 strength of MS medium supplemented with 1 or 2 mg/L IBA.

Joshi *et al.* (2003) on *Eucalyptus* hybrid FRI-6 (*E. tereticornis x E. grandis*) reported that best rooting (75%) was observed in ½ MS supplemented with IBA (1.0 mg/L.).

Concerning plant growth regulators, Sinha *et al.* (1993) cultured the explants of *Albizia* foliataria on a medium supplemented with BA at 2.2, 4.4, 8.9 or 17.8 μM. They observed that the greatest number of shoots was found by using 4.4 and 8.9 μM BA.

Youssef and Helmy (1998) on *Azadirachta indica* reported that the greatest number of shootlets per explant was recorded when BA is applied at high concentration (5.0 mg/L.) compared with the low one (2.5 mg/L.). Rudud *et al.* (1997) on *E. nitens*, examined different concentrations of BA (0.1 – 5.0 μM) and found that media containing 0.1 μM BA alone produced the greatest number of embryos per hypocotyle explant. While, Franca *et al.* (1995) on

Stryphnodenderon polyphythum, stated that medium supplemented with 0.04 µM BA enhanced shoot length comparing with other used concentrations.

On the other hand, the effect of cytoxinin at different concentrations was studied by Ibrahim *et al.* (1992). They used BA at 2.5, 5.0 and 10.0. BA. at 5 mg/L resulted in significant higher number of leaves than the other treatments tested. However, there was no significant difference between BA. At 2.5 and 5.0 mg/L. In addition, Mohammed (2001) found that BA at 1 mg/L increased number of leaves produced on *Calistemon lanceolatas* plants.

Jones and Van-Staden (1994) concluded that the introduction of 1% activated charcoal, and the omission of plant growth regulators from the medium improved both elongation and rooting formed per explants of three *E. grandis x E. urophylla* clones of desirable pulping qualities. Rooted plantlets were hardened off and established in the soil.

The different types and concentrations of plant growth regulators had a clear effect on the number of the roots formed per shootlet as found by Mohamed (2001). Koriesh and Al-Manie, (2000) on *Phillenderon oxycardium* found that addition of 3 mg/L IAA with or without BA produced the longest roots. Also, Magyar *et al.* (2002) on apple rootstocks referred that the rooting percentage, number of roots per shoot and length of roots were the best in the media with 1.0 or 2.0 mg/L IBA.

Fraguas *et al.* (2004) on *Ficus carica*, reported that different proliferation media consisting of combinations of various concentrations of activated charcoal (0.0, 1.0, 2.0, 3.0 and 4.0 mg/L) with benzyladenine (0.0, 0.5, 1.0, 2.0 and 4.0 mg/L). Moreover, HuiMei *et al.* (2005) showed that axillary buds from three-year old seedlings of *Camptotheca acuminate* cultured on MS medium with different concentrations of growth regulators (benzyladenine, BA and thidiazuron, TDZ). They found that the highest levels of BA (1.0 mg/L) or lower levels of TDZ (0.1 mg/L) were effective for shoot regeneration cultured on medium containing sucrose.

The aim of this work was, therefore, to investigate the feasibility of using *in vitro* approaches for the micropropagation of commercially important *Eucalyptus gomphocephala* under the effect of different concentrations of cytokinin and auxin.

MATERIALS AND METHODS

The present study was carried out at the Tissue Culture Laboratory of Vegetable and Ornamental Department, Faculty of Agriculture, Mansoura University during the period from 2002 until 2005. The following experiments were carried out:

1- Establishment stage:

Seeds of *Eucalyptus gomphocephala* were obtained from the Department of Woody Trees, Horticulture Research Institute, Agriculture Research Center, Egypt. Seeds were washed under running tap water for 30 minutes, then they were surface sterilized under aseptic conditions inside Laminar airflow by using Ethyl alcohol 70% for 1 minute, 20% commercial clorox for 20 minutes with two drops of Tween 20. All traces of the used disinfectant were removed by rinsing seeds four times in sterilized distilled

water. After sterilization the seeds were cultured on MS (Murashige and Skoog, 1962) medium supplemented with 3% sucrose; 0.1 mg/L IBA and 1.0 mg/L BA (6-benzyl adenine) then solidified by using purified agar (Bacto-Difco agar) at 0.7%. The pH of the medium was adjusted to (5.6 to 5.8). Then, the medium dispended into 100 ml glass jar contained 25 ml medium and then wrapped with plastic screw cap and sterilized. The media were antoclaved at 121°C for 20 minutes. The jars were incubated under conditions for 25°C ± 2; 16 hours artificial light (fluorescent light at 30 μM/hz/sc) and 8 hours darkness.

2- Shoot multiplication stage:

Plant materials needed for this stage were provided from those proliferated shoots newly emerged throughout the previous stage i.e. establishment "1st stage". Hence, the seedlings produced from the previous germination trial for plants divided into two parts, the first was terminal (such explant used in this work was shoot tips which contained the apical meristem) and the second was basal, each of them was about 1 – 1.5 cm in length. These two parts from the seedlings of both plants were used as explants. Cutting from 8-wk-old seedlings processing two leaf pairs, and shoot tips from 1-yr-old plants were used for shoot multiplication. A total of 320 explants were cultured corresponding to 80 seedling cutting per culture medium (10 cuttings X eight replications). In all experiments test tubes (25 X 150 mm) containing 20 ml of culture medium and covered with plastic caps were used. The multiplication rate was evaluated by the number of cultures formed per tube after three subcultures, at 3-wk intervals in the same culture medium. After surface disinfection, the two explants for each plant were cultured into MS medium supplemented with BA (6-benzyl adenine) at concentration of (0.05, 0.1, 0.2 and 0.4 mg/L), without or with (2 g/L) activated charcoal. The multiplication medium, also, contained nutrients and organics, 2% sucrose, 2.5 g dm^{-3} agar, 2.5 g dm^{-3} phytogel and the pH was adjusted to 5.8 prior to autoclaving. Shoot cultures were maintained in a temperature of 25°C and a 16 hrs photoperiod with an irradiance of 90 μ mol $m^{-2}S^{-1}$. After 5 weeks, averages of number of shoots, leaves/shoot and shoot length (cm) were recorded.

3- Rooting stage:

Proliferated shoots (1.5-2.0 cm length) were taken and separated from each other under aseptic conditions and sub-cultured on full or half-strength (MS) media supplemented with (30 g/L) sucrose and media were, also, pertaining the investigated auxin treatments i.e., IBA at the (0, 0.5, 0.1, 0.2 and 0.4 mg/L). pH was adjusted at (5.6 – 5.8) and the media were autoclaved and cultures were incubated under culture conditions in jars containing (40 ml) of the above mentioned rooting media. After six weeks from incubation, number of rootlets/plantlet and average length (cm) of each were recorded.

4- Acclimatization stage:

Produced *E. gomphocephala* plantlets were washed with tap water and then dipped in Topsin-M 70 (1.0 g/L) as fungicide for (10 min) prior to transplanting in (300 ml) plastic pots and maintained in greenhouse for four weeks. In this stage, the plantlets produced from the best treatments

through previous stage (rooting) were cultured. Pots on a mixture transplanting medium consisting of peatmoss, perlite, vermiculite and sand at (1:1:1:1) ratio by volume as following:

No of mixture	Peatmoss	Perlite	Vermiculite	Sand
1	1	1	1	1
2	0	1	1	1
3	1	0	1	1
4	1	1	0	1
5	1	1	1	0
6	2	1	1	1
7	1	2	1	1
8	1	1	2	1
9	1	1	1	2
10	2	0	1	1
11	0	2	1	1
12	1	1	0	2
13	1	1	2	0

Pots were arranged then covered with polyethylene bags to maintain high relative humidity around the plants in greenhouse under 3000-Lux light intensity using florescent lamps for 16-h. photoperiod, at 25 $\pm$ 2°C. After three weeks, the polyethylene bags were partially removed to allow air circulation. The irrigation was applied depending on the requirement of plantlets. Pests and disease control program was controlled as recommended. After one month from transplanting, data on the highest survival percentage were recorded.

5- Anatomical studies:

Small pieces from the third *E. gomphocephala* leaf from tip were taken when transplanting to acclimatization stage and after 2, 7, 14 and 30 days from acclimatization.

The samples were killed and fixed in (70%) ethanol then washed and dehydrated in serious of ethanol (80%, 90% and 100%), clearing in serious of ethanol: xylene (3:1 – 1:1 – 1:3 and 100% xylene) and embedded in paraffin wax (52 – 54°C melting point). Sections were done at 15 – 20 µm thick using rotary microtome and double stained with saffranin, cleared in Xylene and mounted in Canada balsam (Gerlach, 1977). The sections were examined microscopically. After microscopically examination, the following data were recorded for thickness of midrib, blade, palisade, spongy, upper epidermis, thickness of lower epidermal cells and the thickness of main vascular bundle.

Statistical analysis:

The experimental design employed for this work was factorial experiments in completely randomize design with three replicates were used according to Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Multiplication stage:

Effect of activated charcoal (AC):

As shown in Table (1) and Fig. (1), the presence of activated charcoal (AC) in the medium had a high improving effect in multiplication stage, for instance 2.36 (cm) and 7.99 of length of shoots (cm) and number of leaves, respectively, when AC was added to the medium compared to 1.65 and 7.55 for control. However, the presence of AC in the medium had a significant reduction effect on number of shoots of *E. gomphocephala*. AC may adsorb toxic substances in the medium, thereby improving multiplication parameters. The results were in harmony with those reported by Franclet and Boulay (1983) on Eucalyptus clones and Ahuja (1985) on *E. citriodora*. They found that AC at 0.5 or 1.0% increased shoot elongation and leaf area, but significantly reduced the number of shoots per culture compared with controls. In addition, Fraguas *et al.* (2004) on *Ficus carica* found that addition of AC at the rates of 0.0, 1.0, 2.0, 3.0 and 4.0 mg Litre^{-1} with benzyl adenine resulted in inhibition of shoot proliferation.

Concerning effect of AC on shoot number, this may be due to the adsorption of cytokinins on AC, resulting in reduction of cytokinin concentration in medium as reported by Fridborg *et al.* (1978). They explained that cytokinin plays an important role in formation and development of new shoots. So, it is logic that media not including a relatively higher concentration of cytokinin produced a lower shoot number and lower development. In the case of shoot length, AC adsorbs phenolic compounds from the media which inhibit growth and development of the explant. This was supported by Erig and Schuch (2003).

Table (1): Main effects of activated charcoal (AC), explant part and BA concentrations upon number of shoots, shoot length (cm) and leaves number of *Eucolyptus gomphocephala*.

Factors	Characters		
	Number of shoots	Length of shoots (cm)	Number of leaves
AC concentrations			
Control	4.58	1.65	7.55
2 g/L A.C.	2.33	2.36	7.99
L.S.D. 5%	0.31	0.19	NS
Explant part			
Basal part	3.83	1.91	7.23
Shoot tip	3.08	2.10	8.30
L.S.D. 5%	0.31	0.19	0.61
BA (mg/L)			
0	1.16	2.05	9.50
0.5	2.47	2.45	7.57
0.1	3.78	2.03	7.69
0.2	4.81	2.03	6.99
0.4	5.03	1.45	7.09
L.S.D. 5%	0.49	0.31	0.96

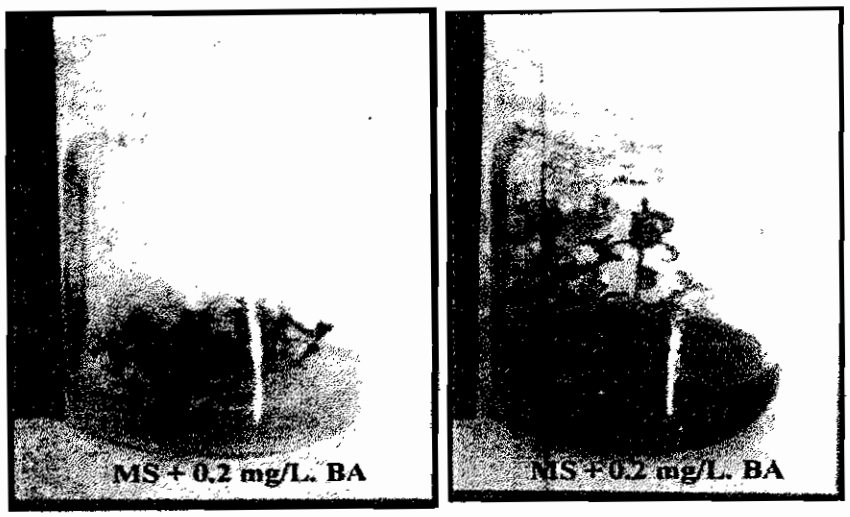

Fig (1): Multiplication of *E. gomphocephala* from basal explant parts using MS supplemented with 0.2 mg/L BA. with or without 2g/L activated charcoal after 5 weeks

Effect of explant part:

The obtained results in Table (1) revealed that the basal part significantly increased multiplication and shoots number as compared to the shoot tip, but significantly decreased number of leaves and shoot length/explant. These results were in agreement with the findings of Koriesh *et al.* (2003) who stated that basal part taken from *E. citriodora* produced multiple shoots were cultivated on MS media than the medium and shoot tip explant. Similarly, Mohamed (2001) on *Bombax malabaricum* and *Callistemon lanceolatus* reported that the basal part enhanced shoot formation than shoot tip. The observed results may be related to the fact that removing the shoot tip inhibited the apical dominance and stimulated the auxiliary shoot formation (Voyiatzi *et al.*, 1995).

Effect of different concentrations of BA:

Data in Table (1) show that, BA at 0.4 mg/L concentration significantly increased, average shoot number as compared to all other concentrations including control. On the other hand, 0.5 mg/L BA significantly enhanced shoot length as compared to the highest one (0.4 mg/L), while the highest number of formed leaves (9.5) was produced from the control. These results were in agreement with the findings of Bunn and Dixon (1992) who stated that the shootlets number of pineapple formed per explant was increased remarkably from 10.2 to 13.9 by increasing BA from 0.5 to 1.0 mg/L. Regarding the effect of BA concentrations on shoot length, the obtained results are in agreement with those found by El-Etriby (2001) and Koriesh *et al.* (2003) on *E. citriodora*. They found that low concentrations of BA induced longer shoots than higher concentrations. From the previous researches about the action mechanism of cytokinin (BA), it was found that BA activate

RNA synthesis and stimulated protein synthesis as well as enzyme activity (Kulaeva, 1980).

Interaction between activated charcoal (AC) and explant part:

Data in Table (2) show a highly significant difference for the interaction between AC and explant part on number and length of shoots and leaves number of *E. gomphocephala*. Culturing the basal part on nutrient media free from AC produced significantly the highest number of shoots/explant (5.18) than all other treatments. However, basal part cultured on media free of (AC) significantly reduced leaves number/explant as compared to the other interactions. These results are in parallel with those of Ahuja (1985), El-Etriby (2001) and Koriesh *et al.* (2003) on *E. citriodora*. They found that shoot multiplication of shoot tip and stem explants of *E. citriodora* on MS medium containing 0.5 mg/L BA and AC promoted the production and elongation of shoots in all explants.

Table (2): Effects of activated charcoal (AC) and explant part interaction on number and length of shoots and leaves number of *Eucolyptus gomphocephala*.

Factors		Characters		
AC Concentration	Explant Part	Number of shoots	Length of shoots (cm)	Number of leaves
Control	Basal part	5.18	1.61	6.58
	Shoot tip	3.98	1.69	8.52
2 g/L A.C.	Basal part	2.48	2.20	7.89
	Shoot tip	2.18	2.51	8.08
L.S.D.	5%	0.44	NS	0.86

Interaction between activated charcoal (AC) and BA concentration:

The obtained results in Table (3) showed that, media free from AC which contained 0.2 mg/L BA produced significantly the highest number of shoots/explant (7.13). While, media containing 2 g/L AC and free from BA produced significantly the tallest shoots (2.66 cm) than most other treatments and produced the highest number of leaves (10.4 g) than all other treatments. These results are in harmony with those of Koriesh *et al.* (2003) on *E. citriodora*, who reported that MS medium supplemented with 0.5 mg/L BA produced the highest number of shoots and leaves, but the tallest shoots were obtained from the media free from cytokinin (BA). As for shoot length, similar results were found by Ibrahim *et al.* (1992) on *Ficus benjamina*, who mentioned that high concentration of BA at 1.0 mg/L decreased shoots length. However, Sharma *et al.* (2003) reported that shoot multiplication of shoot tip and stem explants of peach plants cultured *in vitro* on MS medium containing 0.5 mg/L BA and AC promoted the production of elongation shoot in all explants.

Table (3): Effects of activated charcoal (AC) and BA interaction on average number and length of shoots and leaves number of *E. gomphocephala*.

Factors		Characters		
AC	BA (mg/L)	Number of shoots	Length of shoots (cm)	Number of leaves
Control	0	1.06	1.44	8.75
	0.05	3.06	2.28	7.91
	0.1	5.25	1.99	8.03
	0.2	7.13	1.85	6.74
	0.4	6.38	0.69	6.31
2 g/L A.C.	0	1.25	2.66	10.25
	0.05	1.88	2.63	7.23
	0.1	2.31	2.06	7.36
	0.2	2.50	2.22	7.24
	0.4	3.69	2.22	7.86
L.S.D.	5%	0.70	0.44	1.36

Interaction between explant part and BA concentration:

Dealing with the general interaction effects between explant part and BA, data in Table (4) indicated that the highest number of shoots per explant were (6.00) from culturing the basal part of explants on media supplemented with 0.4 mg/L BA. While, the shoot tip cultured on a media free from BA produced the lowest number of shoots per explant (1.06), but produced the highest number of formed leaves (10.94) than most other treatments with a significant differences. These results were similar to those of Koriesh *et al.* (2003) on *E. citriodora*. These observed increases may be related to the known fact of removing the shoot tip as well as cytokinins inhibiting apical dominance and stimulating auxiliary shoot formation in the media. This was supported by Kulaeva (1980) and Abdel-Kafie (1988).

Table (4): Effects of explant part and BA concentrations on average number and length of shoots and number of leaves of *E. gomphocephala* grown *in vitro*.

Factors		Characters		
Explant part	BA (mg/L)	Number of shoots	Length of shoots (cm)	Number of leaves
Basal	0	1.25	1.75	8.06
	0.05	2.44	2.38	7.09
	0.1	3.81	2.06	8.07
	0.2	5.63	1.96	5.92
	0.4	6.00	1.38	7.03
Shoot tip	0	1.06	2.34	10.94
	0.05	2.50	2.53	8.04
	0.1	3.75	1.99	7.32
	0.2	4.00	2.11	8.06
	0.4	4.06	1.53	7.15
L.S.D.	5%	0.70	NS	1.36

Interaction between activated charcoal (AC), explant part and (BA) concentrations:

The obtained results in Table (5) show that the interaction between the three factors, as affected shoot number was significant, while it insignificantly affected length of shoots (cm) and number of leaves. Culturing the basal part on MS media supplemented with BA at the concentrations of (0.05, 0.1, 0.2 and 0.4 mg/L) significantly increased shoot multiplication as compared to other treatments. These results are confirmed by Rasai *et al.* (1994) and Youssef (1996) who cleared that, MS medium was the best one for some tree species. As for the explant parts, Yang *et al.* (1995) and Rao (1988) pointed out that axillary meristems were used for successful multiplication. Regarding the effect of BA concentration, Rasai *et al.* (1994) and Youssef and Helmy (1998) mentioned that there was a specific BA concentration for multiplication. In *in vitro* multiplication of *Eucalyptus*, BA appears to be necessary for plant cell division, regulates the synthesis of proteins involved in the formation and function of the mitotic spindle apparatus (Centeno *et al.*, 1996). BA, is very effective in promoting shoot initiation. As mentioned before, and as shown in the Tables (3, 4 and 5), BA produced more numbers of shoots or leaves with the longest shoot.

Table (5): Effects of activated charcoal (AC), explant part and BA interaction on number and length of shoots and leaves number of *E. gomphocephala*.

AC g/L	Explant part	BA	Characters		
			Number of shoots	Length of shoots (cm)	Number of leaves
Control (0 g/L)	Basal	0	1.13	1.19	7.50
		0.05	3.38	2.31	6.81
		0.1	5.88	1.94	7.26
		0.2	8.25	1.86	5.53
		0.4	7.25	0.75	5.79
	Shoot tip	0	1.00	1.69	10.00
		0.05	2.75	2.25	9.00
		0.1	4.63	2.04	8.79
		0.2	6.00	1.84	7.96
		0.4	5.50	0.63	6.84
2 g/L A.C.	Basal	0	1.38	2.31	8.63
		0.05	1.50	2.44	7.38
		0.1	1.75	2.19	8.88
		0.2	3.00	2.06	6.31
		0.4	4.75	2.00	8.26
	Shoot tip	0	1.13	3.00	11.88
		0.05	2.25	2.81	7.09
		0.1	2.88	1.94	5.85
		0.2	2.00	2.38	8.16
		0.4	2.63	2.44	7.46
L.S.D.		1%	NS	NS	NS

Rooting stage:

Regarding the response of number of the developed rootlets and their average length to the specific effect of investigated factor i.e., media type and auxin treatments, Table (6) show that MS medium strenght had no statistical effect in this concern.

As for the specific effect of auxin treatments, the results presented in Table (6) and illustrated in Fig. (2) show that the used concentration of IBA significantly increased average roots number and root length as compared to control, at the meantime the highest concentrations of 2 and 3 mg/L significantly increased root number as compared to the lowest one 0.5 and 1.0 mg/L. While, the differences among the used concentration as affected on root length were non-significant.

Table (6): Effects of medium strength and IBA concentration upon roots number and roots length of *E. gomphocephala*.

Media strength	Number of roots	Length of roots (cm)
MS	3.08	1.01
0.5 MS	2.80	1.08
L.S.D. 5%	NS	NS
IBA		
IBA (mg/L)	Number of roots	Length of roots (cm)
0	0.15	0.15
0.5	1.85	0.67
1	2.95	1.34
2	4.95	1.71
4	4.80	1.36
L.S.D. 5%	1.07	0.42

Fig (2): *In vitro* rooting of *E. gomphocephala* using MS with 2mg/L IBA after 6 weeks

In this concern, many investigators try to obtain maximum rooting for different species using definite IBA concentration. Kapoor and Chauhan (1992) reported that rooting of *in vitro* derived shoots of *Eucalyptus* hybrid was obtained in half and one fifth strength MS medium supplemented with 0.1 and 1.0 mg/L IBA. Also, Yang *et al.* (1995) mentioned that, 1, 4, 5 mg/L IBA concentrations were the most effective in inducing root primordial on hypocotyl explants of *Eucalyptus globulus* as compared to control. Besides, *E. hybrid* was successfully rooted in medium with IBA from 0.1 to 10 mg/L. These observed increase may be related to the known fact that (IBA) stimulates axillary root formation in the media. This was in agreement with fidings reported by Zimmerman and Broome (1981) on apple.

Concerning the interaction effect of various combinations between media type and auxin treatments, Table (7) shows that there was non significant differences of studied traits and however, full media strength (MS) supplemented with (2 mg/L) IBA gave the greatest number and tallest rootlets per plantlet.

Table (7): Effects of media strength and IBA interaction on roots number and roots length of *E. gomphocephala*.

Media strength	IBA (mg/L)	Number of roots	Length of roots (cm)
MS	0	0.10	0.10
	0.5	2.20	0.77
	1	2.50	1.40
	2	5.20	1.79
	4	5.40	0.97
0.5 MS	0	0.20	0.20
	0.5	1.50	0.57
	1	3.40	1.27
	2	4.70	1.62
	4	4.20	1.75
L.S.D. 5%		NS	NS

Acclimatization stage:

In this stage, the plantlets produced from the best treatments (rooting media and auxins) through the previous stage (rooting) were chosen and cultivated on transplanting medium consisting of peat moss: sand: vermiculite: perlite) at (1:1:1:1) ratio by volume for acclimatization stage and placed in the greenhouse for 2 weeks under 50% shade. A suitable environment was created by covering young plantlets with tents of polyethylene film and subjecting them to intermittent water misting. During the following 3 weeks the humidity was gradually reduced, and the shade was changed from 50 to 20%. At the end of the 3rd week the polyethylene cover was removed and plantlets were self-supported.

Table (8) and Fig. (3) show the effect of some specific treatments used in rooting stage on survival % and Aresin % during acclimatization stage. The obtained results in this Table, show that, also, the mixture which containing (peatmoss: perlite: vermiculite: sand) by volume, respectively,

produced the highest survival percentage of *E. gomphocephala* which was 81.25% in case of using 1 peat + 1 perlite + 2 vermiculite + 1 sand (by volume), respectively, and with a significant difference with all other treatments.

Table (8): Effects of soil mixture content (peatmoss: perlite: vermiculite: sand) on acclimatization stage on plant survival percentage of *E. gomphocephala*.

Mixture	Survival %	Aresin %
1:1:1:1	56.25	48.75
0:1:1:1	25	30
1:0:1:1	25	30
1:1:0:1	25	30
1:1:1:0	50	45
2:1:1:1	50	45
1:2:1:1	50	45
1:1:2:1	81.25	67.5
1:1:1:2	50	45
2:0:1:1	25	30
0:2:1:1	25	30
1:1:0:2	25	30
1:1:2:0	25	30
L.S.D. 5%		4.84

Fig (3): Acclimatization and establishment of *E. gomphocephala* under greenhouse conditions after 6 weeks from transplanting.

Anatomical studies:

The purpose of these studies was to determine how environmental factors and condition of culture can be modified to produce plants that are more competent for field survival, with a lower mortality and decreased acclimation requirement.

After transfer of *E. gomphocephala* plantlets from *In vitro* cultures to the greenhouse or field, substantial changes in leaf morphology and anatomy were observed, above all in epidermal characteristics, leaf thickness, differentiation of leaf mesophyll, and chloroplast number and structure. Transverse section of leaves from *In vivo* and *In vitro* developed plants (Fig. 4) show that the laminae exhibited a typical dorsiventral character varied with different time treatments. The effect of time on various leaf characteristics of leaves developed under *in vitro* or *In vivo* conditions is shown in Table (9).

As *a* result, cultured plants require an acclimation period during the transition culture in field or greenhouse conditions, which usually consist of a period with a gradual decrease in humidity. In acclimatized *E. gomphocephala* plants, thicker leaves than in *in vitro* grown plantlets, and mesophyll tissue differentiated into palisade and spongy parenchyma were found, the spongy parenchyma had fewer and smaller air-spaces. Similar results were found by Brainerd *et al.* (1981) in *Prunus institia*, Waldenmaier and Schmidt (1990) in *Rhododendron Spp.* and Noé and Bonini (1996) in *Vaccinium corymbosum*.

Data presented in Table (9) show that the progressive increase in thickness of midrib, thickness of blade, thickness of palisade, thickness of spongy and thickness of upper epidermal cells after 14 and 30 days from date of transplantation. Whereas, after 7 days from transplantation a slight of any change (Fig. 4). As compared with control plants after from 48 h transplantation, the thickness of lower epidermal cells was unchanged after 7, 14 and 30 days from the time of transplantation.

Table (9): Effect of time from acclimatization on leaf structure of *Eucalyptus gomphocephala* (by micron).

Time from trans-planting	Thick. of midrib	Thick. of blade	Thick. of pallsade	Thick. of spongy	Thick. of upper	Thick. of lower	Main vascular bundle	
							Length	width
2 days	850	580	110	360	50	60	250	450
7 days	870	560	110	340	50	60	300	520
14 days	900	500	120	240	80	60	320	650
30 days	950	500	130	220	90	60	350	820

In addition, leaves from *in vitro* plantlets of *E. gomphocephala* had a less developed cuticle in contrary to the well developed cuticle in leaves transplanted and field grown plants. However, an increase in cuticle thickness, mass and wax content from young to adult leaves was found in both *in vitro* and *ex vitro* grown *E. gomphocephala* plants.

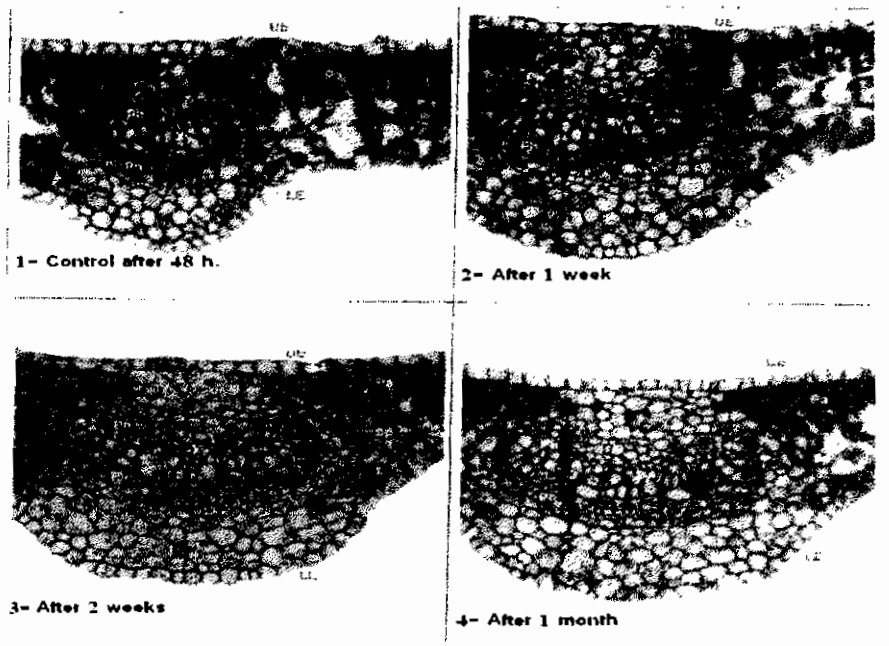

Fig (4): Cross sections in *E. gomphocephala* leaf showing the time of acclimatization (Obj x 10.Oc. x 15).
LE = Lower Epidermis; Pa = Palisade parenchyma; Ph = phloem; Sp = spongy parenchyma; St = Stomata; Xy= Xylem; UE = Upper Epidermis

Conclusions

The derived results from this research showed clearly, the possibility of inducing an incredible number of plants through the application of micropropagation methods in *E. gomphocephala* having a great economical value of ornamental industry. Accordingly, it could be concluded that highest number of formed shoots were regarded when culturing the basal explant part on MS without activated charcoal, supplemented with 0.2 mg/L BA. While, the tallest shoots were resulted from culturing the shoot tip on MS medium with 2 g/L activated charcoal. Full strength MS medium supplemented with 2 mg/L IBA was superior and had the greatest for the rooting growth parameters (number of roots/plantlet and average root length). At acclimatization, the highest survival percentage of plantlets (81.25) was obtained in case of using 1 peat + 1 perlite + 2 vemiculite + 1 sand (by volume) culture media.

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دراسات على بعض العوامل المؤثرة على التكاثر الدقيق لأشجار الكافور جومفوسيفلا

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أجريت هذه الدراسة بمعمل زراعة الأنسجة بقسم الخضر والزينة بكلية الزراعة – جامعة المنصورة خلال الفترة من ٢٠٠٢ حتى ٢٠٠٥ على أشجار كافور *Eucalyptus gomphocephala* بهدف تطوير إكثارها بطريقة زراعة الأنسجة. وفى هذا الصدد تم فصل جزء نباتى من البرعم الطرفى

والجزء القاعدى الذى يتمثل فى العقلة المأخوذة من قاعدة الساق بطول ١ – ٥ر١ سم لكل جزء نباتى منفصل من أشجار الكافور، حيث تم إجراء عدة معاملات خلال المراحل التالية (الأساس – التضاعف – التجذير – الأقلمة) لدراسة تأثيرها كما يلى:

أولاً: مرحلة الأساس

فى هذه المرحلة وبعد إجراء التعقيم للأجزاء النباتية المفصولة تم زراعتها على البيئة الغذائية موراشيج وسكوج (MS) مضافا إلى كل منها ١ ملجم/لتر بنزيل أدينين (BA)، ١ر٠ ملجم/لتر إندول حامض البيوتيرك (IBA) وبعد أربعة أسابيع من الزراعة تم إجراء المرحلة التالية.

ثانيا: مرحلة التضاعف

فى هذه المرحلة تم إعادة الزراعة للفريخات الناتجة من مرحلة الإعداد على نفس البيئة الغذائية السابقة الذكر ذات القوة الكاملة المضاف إليها بنزيل أدينين بنسبة تركيزات هى (٠، ٥ر٠، ١، ٢، ٤ ملجم/لتر) والمحتوية على الفحم النشط (٢ جم/لتر) أو بدون لدراسة تأثيرها على عدد النموات وأطولها وعدد الأوراق الناتجة. وقد أوضحت الدراسة النتائج التالية:

- استخدام بيئة MS الخالية من الفحم كانت أفضل البيئات المستخدمة فى الدراسة حيث سجلت أعلى معدل تضاعف ولكن كانت أطول النموات الناتجة على البيئة المحتوية على الفحم النشط.
- نتج أكبر عدد من النموات الحديثة من زراعة الجزء القاعدى بصفة عامة على أى بيئة وتحت أى مستوى من السيتوكينين.
- أعطى BA بتركيز ٢ر٠ ملجم/لتر أكبر عدد للنموات بالمقارنة بالتركيزات الأخرى.

ثالثا: مرحلة التجذير

تم تجذير الأفرخ الجديدة المتكونة فى مرحلة التضاعف على البيئة السابقة الذكر الكاملة القوة وذات النصف تركيز لأملاحها والمضاف إليها إندول حامض البيوتيرك (IBA) بنسبة تركيزات هى (٠، ٥ر٠، ١، ٢، ٤ ملجم/لتر)، وقد تم دراسة تأثير التفاعل للتراكيب المختلفة على استجابة قياسات التجذير (عدد الجذور المتكونة – متوسط طول الجذور)، وقد أوضحت الدراسة النتائج الآتية:

- سجلت بيئة (MS) الكاملة القوة أعلى قيمة مع عدد الجذور المتكونة ومتوسط طول الجذر والمحتوية على ٢ ملجم/لتر IBA.

رابعا: مرحلة الأقلمة

هذه المرحلة أجريت تحت ظروف الصوبة الزجاجية حيث تم نقل نباتات الكافور الناتجة من أفضل معاملة ناتجة من بيئة (MS) والمستخدمة تحت الدراسة فى مرحلة التجذير لأقلمتها وذلك بغسلها بماء الصنبور ثم تفريدها فى أصص بلاستيك (٣٠٠ مم) مملوءة بمخلوط معقم من الرمل، البيتموس، الفيرمكيوليت والبرليت بنسبة حجمية (١:١:١:١) لمدة أربعة أسابيع لدراسة نسبة بقاء النباتات حية بعد عملية الأقلمة، وقد أوضحت النتائج المتحصل عليها مايلى:

– النباتات المجذرة على البيئة المتكونة من الرمل، البيتموس، الفيرمكيوليت والبرليت بنسب ١:٢:١:١ بالحجم كانت أفضل البيئات وكانت نسبة النجاح ٢٥ر٨١% فى هذا الصدد.

خامساً: مرحلة الدراسة التشريحية

هذه المرحلة كان الهدف منها دراسة التركيب التشريحى للورقة أثناء المراحل المختلفة من عمر النبات وخلال عملية الأقلمة لدراسة التطور الحادث فى النباتات وذلك من خلال التغيرات التشريحية فى الورقة وملائمتها للظروف الجديدة التى نقلت إليها خلال (٤٨ ساعة، ٧، ١٤، ٣٠ يوم) وأثناء ذلك:

– لوحظ من خلال الدراسة التشريحية تطور طبيعى فى تركيب الورقة لملائمة الظروف الجديدة والتى تتمثل فى الصفات الوظيفية للبشرة العليا، وسمك الورقة وحدوث تكشف فى طبقة الميزوفيل وكذلك عدد وتركيب الكلوروبلاست فى الورقة.