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MICROPROPAGATION OF *Eucalyptus grandis* TREES

Maximous, S. L. and M.M. E. Abd El- Kader
Hort. Res. Inst, Forestry Res. Dept., Agric., Res. Center., Giza Egypt.

ABSTRACT

This work aimed to study the effect of medium type, explant part and kinitin concentration on in vitro multiplication as well as in vitro rooting of *Eucalyptus grandis* to obtain the optimum regeneration phase and to develop the propagation method of E, *grandis*

The results indicated that, the highest number of formed shoots was regarded with culturing the basal node on MS medium supplemented with 2mg/litre kin. as compared with WPM medium.

All treatments (½ MS medium supplemented with IBA) induced root formation on *E. grandis* shoots, but IBA at 2mg/litre recorded the best results. The rooted shootlets (plantlets) were removed from rooting medium after seven weeks and cultured in plastic pots 10 cm. diameter, containing peatmoos, clay and sand (1:1:1 by volume) under greenhouse conditions for four weeks.

The survival percentage of *E. gandis* plants recorded 83% . from the above results, the micropropagation techniques strongly recommended to increase the number of *E. grandis* plants in Egypt.

Keyword: Micropropagation, invitro, tissue culture, explants, IBA, Kinetin, media type, MS, WPM, rooting .shooting, media strength, multiplication, *Eucalyptus grandis*.

INTRODUCTION

Egypt and similar arid countries suffer from shortage in wood-raw materials which are necessary for several industrial uses therefor, the country has focussed attention especially during the last three decades, on establishing forest plantations to meet the acute needs for wood in Egypt (Abou- Gazia *et al.,* 1992).

Eucalyptus grandis is used for pulpwood, fuel wood and timber for mining, a 6 -10 years rotation is common. For industrial plantation it is used for production of small wood for domestic purposes by using thinning in most countries. The tree's main nectar value is as a supporting species. The wood has been used for fence posts, building, transmission and telephone poles, boxes and hooks. It is especially used for boat building, flooring, plywood, panelling construction.

It is used as part of an agrofbrestry research project in southeastern Brazil. The mean annual temperature for *E. grandis* (-1 to 40 c°) and mean annual rainfall (100 - 1800 mm). Also perform well on lighter sandy soils, FAO (1979) and Conto *et al.* (1982).

The seeds of E. *grandis* were imported from Guatemala to evaluate this specie under the Egyptian conditions but the percentage of seeds germination did not increase than 30%. For that we needed to use tissue culture techniques to produce the seedling in Egypt especially in Upper Egypt in Tosheka.

Furze and Cresswell (1985) concluded that, the percentage of micropropagated shoots, that formed roots was about 90% for E. *grandis* and about 90% of the rooted shoots survived after hardening off.

Youssef (1986) on *Melaleuca armillaris* reported that, culturing nodal

explants on MS medium increased the shoot proliferation rates to 13.9 and 11.6 fold higher than on WPM and B5 medium, respectively.

Warrag *et al* (1990) found that, the high multiplication rates was obtained on auxin free medium with 0.6 mg/litre BAP. Elongation of shoots was best on media with high auxin (2.5 mg/L of IBA) and cytokinin (1-1.5 mg/L. of Zeatin). Up to 98% rooting was achieved on % MS with 2 mg/L. IBA. Rooted propagules were successfully transferred to mist greenhouse with 82% survival and then to greenhouse conditions of E. *grandis* hybrids.

Ditmer (1991) observed that, results for adventitious bud induction of *Betula pendula* were better on MS medium than on WPBM.ajedium.

Roux *et al.* (1991) revealed that. root initiation was achieved on half - strength modified MS medium with 2 mg/L. IBA. Rooted plants were hardened and established in the field of E. *grandis.*

Warrag et al. (1991) concluded that. hypocotyl calluses of E. *grandis* initiated on 4 mg/litre NAA and 1 mg/litre kinetin formed massive nodular structures which produced shoots and roots after 4 weeks on hormone free medium. Shoots were successfully rooted (98%) rooting and plantalets were transferred to a mist greenhouse and then to greenhouse conditions with 95% survival,

Lubrano (1992) reported that, multiplication of E. *grandis* induced by adding 0.5 mg/litre BA and 0.01 mg NAA/ litre to the basal medium. After 20 days the etiolated shoots were transferred to rooting medium containing 1mg IBA/ litre and placed in the dark.

Rooting percentages ranged between 61% and 93%; survival in the greenhouse and nursery was approximately 90%.

Jones *et al* (1994) revealed that. nodal explants of 3 E. *grandis* clones were successfully multiplied in MS medium containing 0.2 mg/lire of BA and 0.01 mg/litre of NAA. Shoot elongation was achieved in a similar medium containing 0.2 mg/litre kinetin, and root formation was stimulated by 0.1 - 2.0 mg/litre IBA.

Niccol *et al* (1994) found that. use IBA alone with half-strength MS medium was successfully initiating roots from in vitro derived shoots of E. *microcorys*, 5 u M IBA was the optimum concentration for root induction and elongation.

Yang *et al.* (1995) showed that, in vitro multiple shoot production of E. *grandis* was optimum on MS medium containing 0.1 mg/litre BA and 0.1 mg/litre NAA, averaging 13.7 shoots per explant in 4 0 days culture period. Root formation was optimum on medium consisting of full strength MS basal macro elements and vitamins, half strength trace elements, supplemented with 0.3 mg/litre IBA.

Yossef (1996) reported that, using culture media of MS and Ba on *Robinia pseudoacacia* exhibited a clear influence on producing the greatest number of nodes per shootlet. The root length observely increased in case of using MS and WPM as media. The modal and shoot tip explants had similar effect on number of multiplicated shootlets. number of formed roots per shootlet, root length and hardening capacity of the plantlets.

Wachira (1997) indicated that. the optinal shoot regeneration was obtained on Murashige and Skoog medium supplemented with 0.4 mg (BAP)

and 1.0 mg IBA/litre and 3% (W/V) sucrose to shoot multiplication with a pH at 5.8 giving better growth of E. *grandis*.

Cid *et al.* (1999) showed that, the adventitious shoot clusters were greater in number (30 - 50 shoots/callus) and appeared heal their on half - strength MS medium (SP medium) supplemented with 0.5 u M NAA + 5.0 u M BA. For rooting, 50 mm long shoots ware cultured on root induction medium containing 2.5 u M IBA for different period and them transferred to the same medium but without auxin, for 30 days. Pantlets were then successfully transplanted to greenhouse conditions of *E. gandis*

MATERIALS AND METHODS

1. This work was carried out at the Plant Research Department. Atomic Energy Authority, Nuclear Research Center, Naser City, Cairo during 2001 to 2002. Seeds of *Euaclyptus grandis* were imported from Guatimala in 2001. Seeds were sterilizated before beginning this study. Seeds were washed under running tap water for 20 min. , They were surface sterilized under aseptic conditions inside the culture cabinet Laminar air flow by using Ethyl alcohol 70% for 5 min., 15% commercial Clorox for 15 min. and two drops of Tween 20. All traces of the used disinfectant were removed by rinsing seeds five times in sterilize distilled water.

Murashige and Skoog basal salt mixture (MS 1962) and Woody Plant Medium MPM according to Lioyd and Me Cown (1980) were used for in vitro micropropagation.

The pH of the two used media was adjusted to 5.8 by using HCl or KoH, 0.1 M and was solidified by difco bacto agar (7g/L). the medium was cooked and distributed into 200 ml. glass jars containing 30 ml. of medium. Jars were covered and autoclaved at 121 C° at 1.5 kg/cm^2 for 20 min.. Jars of E. *grandis* were incubated in the growth chamber under the following conditions: temperature 25 C° ± 2 C°, photoperiod 16h. Light and 8h. dark controlled automatically and illumination intensity 1500 Lux at top culture vessels by fluorescent lamps (120 cm. Long) for 7 weeks.

Aseptically the in vetro seedlings of 7 weeks old of *E. grandis* were divided to three explant parts as terminal part, middle part and basal part, each explant part was about 1 - 1.5 cm.

This investigation was included a factorial experiments consisted of Medium type (MS and WPM), explant types (terminal, middle and basal), and kinetin (Kin),at different concentrations (0.0, 1, 2 and 3 mg/litre) for shooting. For rooting: MS medium at quarter and half strengths of salt concentrations and Indole butyricacid at the concentrations of 0.0, 1, 2 and 3 mg/ liter were used.

After 7 weeks data were recorded for :-

I - Shooting stage:

(1) Number of shoots / explant.
(2) Shoot length in cm.
(3) Number of leaves / shoot.

II - Rooting stage:

(1) Number of roots / explant.
(2) Root length in cm.

III - Acclimatization stage:

Transferred the ex vitro plantlets from the aseptic culture condition to the free living environment of the greenhouse and ultimately to final location, topsin-M70 fungicide was used to saturate the sterilized soil, mix of plastic pots (10cm)which were of filled with the soil mixture consisted of clay + sand + peatmoss at 1:1:1 by volume and cultured with the plantlets. Pots were kept at high relative humidity place, using plastic sheets for four weeks to protect the plantlets from desiccation. Pots were maintained under 3000 lux light intensity derived from florescent lamps for 16-h. photo period, at 25± 2c

The experimental designs were completely randomized as a factorial experiment according to Gomez and Gomez (1984),. The differences were examined by Tukeys method to reduce the error differences between means

Table (1):- F values for the main effects and their interactions on number of shoots, number of leaves and shoots length of *Eucalyptus grandis* grown in vitro

Source of Variance	d.f.	Observed F			Required F	
		Characters				
		No. of shoots	No. of leaves	Shoots length (cm.)	0.05	0.01
Media type (A)	1	39.50**	142.26**	75.15**	3.75	5.2
Explant part (B)	2	72.14**	4.36**	1.42	3.02	4.30
Kin. (c)	3	54.83**	3.94**	2.98*	2.11	3.65
AXB	2	1.65	4.35*	4.82	3.02	4.30
AXC	3	8.82**	7.38**	8.53**	2.11	3.65
BXC	6	4.02**	1.11	0.80	1.75	2.14
AXBXC	6	3.95**	1.75	1.37	1.75	2.14

RESULTS AND DISCUSSION

I - Shooting stage:

1.1 Effect of media type :-

Data recorded in Table (2) revealed that, effect of media type on number of shoots, number of leaves per explant and shoot length. It is clearly MS medium increased this studied parameters with significant difference. These results are in agreement with those obtained by , Youssef (1986), Ditmer (1991) and Youssef (1996)They mentioned that MS medium was the best medium.

1.2 Effect of explant part:

The obtained results in Table (2) indicated that, the basal part significantly increased multiplication and leaves number as compared to the middle and terminal part. These are in accordance with Yang *et al* (1995) and Gad and Shehata (2003).

1.3 Effct of Kin-Concentrations :-

The results in Table (2) showed that, Kin at 2 mg/L, concentrations significantly increased shoots number, as compared to all other concentrations. On the other hand. 1mg/L. Kin significantly enhanced shoot length as compared to the highest one 3mg/L. While, the other differences were non-significant for the effect of kin concentrations on leaves number.

The results cleared that, all the concentrations significantly increased leaves number as compared to kin free medium with exception of 3mg/L. These results are in harmony with obtained by Warrag *et al* (1990 and 1991) and Jones *et al* (1994)

Table (2) :- Effects of medium type, explant part and kin. concentrations on number of shoots, number of leaves and shoots length for E. *grandis* grown in vitro

Factors	Characters		
	No. of shoots ,	No. of leaves	Shoots length (cm)
Media type			
MS	5.1 a	9.15a	1.852 a
WPM	2.74 b	4.65 b	0.831 b
Explant parts			
Basal	5.82 a	6.26 a	1.321 a
Medial	2.73 b	5.70 b	1.38 a
Terminal	2.13c	5.40 ab	1.432 a
Kin (mg/L.)			
0	1.02c	3.86 b	1.28ab
1	3.62 b	4.98 a	1.83 a
2	5.31 a	5.23 a	1.35 ab
3	4.07 b	4.25 ab	1.25ab

Means with the same letters within a column are not significantly different according to Tukeys Multiple range Test (0.05) in all Tables (2,3,4,5,6 and 7).

1.4- Interaction between media type and explant part:-

The results in Table (3) cleared that the most effective media was MS in combined with the basal part for multiplication and leaves number with significant differences.

Table (3) : - Interaction between medium type and explant part on number of shoots, number of leaves and shoots length of *E. grandis* grown in vitro.

Factors			Characters	
Media type	Explant part	No. of shoots	No. of leaves	Shoots length (cm)
	Basal	6.20 a	9.92 a	1.93 a
MS	Medial	3.72 c	7.43ab	1.97 a
	Terminal	3.43cd	7.02b	1.73 a
	Basal	4.18b	4.74c	0.82 b
WPM	Medial	2.46 e.f	3.60 cd	0.85 b
	Terminal	1.65f	3.18 d	0.80b

On the other hand shoots length didn't recorded significant differences between the three explant parts for the two media types. But there were a significant difference between two media types. These results are confirmed by Jones *et al.* (1994), Yanyefa/. (1995)

1.5 Interaction between media type and kin concentrations :-

Data in Table (4) indicated that. MS media supplemented with kin. at different concentrations significantly increased *E. grandis* studies traits as compared to the anther media supplemented with kin. at the same

concentrations. These are in accordance with Jones *et al.*(1994) and Wachira (1997)

Table (4): Interaction between medium type and kin concentrations on number of shoots, number of leaves and shoots length of E. *grandis* grown in vitro

Factors			Characters	
Media type	Kin (mg/L.)	No.of shoots	No. of leaves	Shoots length (cm)
MS	0	1.80c	8.21 ab	2.75 a
	1	4.75 b	9.13 a	2.31 ab
	2	6.17a	9.98 a	1.92 be
	3	4.15 ab	7.16 b	1.61 cde
WPM	0	0.56d	1.80e	0.32g
	1	1.87c	3.50 d	1.02de
	2	4.62ab	5.52c	1.08de
	3	4.21 ab	4.43 cd	0.87ed

1.6 Interaction between explant part and kin concentrations:

The results in Table (5) cleared that, the interaction between explant parts in combined with kin. concentrations showed nonsignificant effect on either shoot length or leaves number.

Table (5): Interaction between explant part and kin. concentrations on number of shoots, number of leaves and shoots length of £. *grandis* grown in vitro.

Factors		Characters		
Explant part	Kin. (mg/L.)	No. of Shoots	No. of leaves	I Shoots length (cm)
Basal part	0	1.32fg	5.82 a	1.15 a
	1	6.23b	7.20 a	1.49a
	2	7.85 a	7.28 a	1.75a
	3	4.72 be	6.12a	1.36 a
Medial part	0	1.32fg	4.63 a	1.40 a
	1	2.96e	6,26 a	1.34 a
	2	4.75 bed	5,75 a	1.46 a
	3	2,65ef	5.36 a	1.18a
Terminal part	0	1.12g	5.12 a	1.53 a
	1	2.92e	5.80 a	•1.50 a
	2	4.65de	5,97 a	1.60 a
	3	2.92 efg	5.03 a	1.40 a

While, it significantly affect on shoots number. The basal explant part cultured on media supplemented by 2mg/L kin. significantly increased shoot number as compared to other concentrations and other explant parts. These results are in parallel with Lubrano, (1992) and Yang *et al.* (1995)

1.7 Interaction between media type, explant part and kin. concentrations:-

Results in Table (6) showed that, the interaction between the three factors, as affected as on shoot length was insignificant while, it was significantly affected on shoots number and leaves number culturing the

basal part on MS medium supplemented with kin. at the concentrations (1,2 and 3 mg/L) significantly increased shoot multiplication as compared to other treatments. Also, culturing the three explant parts on MS medium supplemented with kin. at different concentrations significantly increased leaves number as compared to the other treatments. These results are confirmed by Roux *et al* . (1954Yang *et al.* (1995)

Table (6):- Interaction between medium type part and kin concentrations on number of shoots, number of leaves and shoots, length of *E. grandis* grown *in vitro*.

Factors			Characters		
Media type	Explant part	Kin (mg/L.)	No. of shoots	No. of leaves	Shoots length (cm)
MS	Basal	0	2.60 h.k	8.65 a-d	1.51 e.j
		1	8.20 ab	9.28abc	2.26 a-e
		2	9.40 a	10.50 a	2.92 a-b
		3	8.30 ab	9.00 a-f	1.94 a-g
	Medial	0	1.92 h-k	7.90 a-f	2.00 a-g
		1	2.98 g-k	8.60 a-d	2.24 a-f
		2	6.20 a-c	9.92 a-b	3.65 a
		3	3.40 g-k	5.12 e-i	1.43 b- i
	Terminal	0	1.32 l-j	7.56 a-g	1.98 a-g
		1	3.61 f-j	8.85 a-f	2.02 a-g
		2	5.12 c-h	9.15 a-f	2.70 a-d
		3	4.95 c-h	6.72 a- i	1.75 b- i
WPM	Basal	0	0.49 k	1.30 l	0.18 i
		1	1.32 i-jk	2.78 i -l	0.60 h- i
		2	6.60 a-c	6.20 b-j	1.20 d- i
		3	2.93 g-k	4.05 g-l	1.08 e- i
	Medium	0	0.49 k	1.83 k-l	0.35 i
		1	1.79 h-k	3.35 h-l	0.83 e- i
		2	3.80 f-j	3.65 g-l	1.30 d- i
		3	1.76 h-k	2.78 i -l	0.80 e- i
	Terminal	0	0.60 j-k	2.4 i -k-l	0.38 h- i
		1	1.15 l-j-k	3.00 i -l	0.88 e- i
		2	2.53 h-k	4.15 g-l	1.22 d-i
		3	1.25 l-j-k	3.02 i-l	0.82 f- i

II- Rooting Stage :

2.1 -Effect of medium strength :-

The results in Table (7) revealed that, MS media at half strength duplicated each of roots number and root length than quarter strength. These results are inagreement with those obtained by Roux *et al.* (1991), Yang *et al.* (1995) and Cid *et al.* (1999)

Table (7) : Effect of media strength and IBA concentration on number of roots and roots length of *E. grandis* based on transformed data

Media strength	No. of roots	Roots length (cm)
¼MS	0.61 b	0.40 b
½MS	1.25 a	0.83 a

IBA (mg/L.)	No. of roots	Roots length (cm)
0	0.05 c	0.05 b
1	0.68 b	0.72 a
2	1.67 a	1.21 a
3	0.85 b	0.93 a

Media strength	IBA (mg/L.)	No. of roots	Roots length (cm)
¼ MS	0	0.05e	0.05c
	1	0.33 de	0.95 ab
	2	1.19 bc	1.02 ab
	3	0.35 cde	0.36 bc
½MS	0	0.05e	0.05c
	1	1.08 bc	1.40 a
	2	2.57 a	1.50 a
	3	1.32ab	0.92 ab

2.2 Effect of IBA concentrations :-

The results in Table (7) showed that, the concentration of IBA significantly increased roots number and root length as compared to control. IBA at the concentration 2mg/L. recorded the significantly increased root number compared the other concentrations. While; the differences were non-significant on root length between all IBA concentrations. These results are in line with those of Site *et al.* (1986), Warrag *et al.* (1990), Rout *et al.* (1991), Lubrano (1992), Jones *et al.* (1994) Yang *et al.* (1995), Wachira (1997) and Cid *et al* .(1999).

2.3 Interaction between medium strength and IBA concentrations :-

The results in Table (7) cleared that, the half strength with 2 or 3mg/L IBA significantly increased rooting as compared to the other concentrations including control. While. 1 and 2 mg/L. IBA significantly increased root length as compared to control only. Besides quarter strength media take the same trend of half strength media. These results are in agreement with Warrag *et al.* (1990) and Roux *etal.* (1991), Yang *et al.* (1995) and Cid *et al.* (1999).

III- Acclimatization of plantlets :-

After the acclimatization stage for plantlets under the experimental condition which recorded the best results cleared that, the survival of seedlings were a bout 83%. These results are in agreement with Furze and Cress Well (1985), Warrag et *al.* (1990) and Lubrano (1992).

DISCUSSION

In this study it is clear that MS medium produced the highest number of shoots per explant, longest shoots and highest number of leaves. These signmcam increase which were observed with MS medium may be due to one or more of the following reasons: -

(1) The total ionic concentrations of MS medium are high as 93.3 but in WPM medium its about 41.5.

(2) Potassium was also increased to 20 mM comparing with WPM that contained 12.6 mM.

(3) Sulphate is present at a higher concentration than WPM medium.
(4) MS medium contained 40 mM of NO3and 20 mM of NH4

On the other hand WPM contains 5 mM of NO3 and 9.7 mM of NH4 . In this concern it is well known that the growth and morphogensis of tissue cultures is markedly influenced by the availability of N and the form in which it is presented Kyte (1987) reviewed that growth is usually most rapid when nitrate and ammonium ions are both available. Reduced N compounds are then often found to have regulatory roles in cultures

Cytokininis tend to promote the shoots formation, some compounds with cytokinin activity have been found to be present in transfer- RNA molecules, but it is not yet clear whether in corporation into t.RNA is necessary before typical cytokinin effects can become apparent. In some circumstances, cytokinin have been shown to activate RNA synthesis and to stimulate protein synthesis and enzyme activity Kulaeva, (1980)

The number of formed leaves per shootlet, explant was not related to the nariations in topophysical origin of explants along the plant stem from basal to terminal position Gosukonda *et al.* (1995).

The rooting behavior might be ascribed to that the metabolic processes leading to adventitious root formation remarkably decreased with increasing the developmental stages of plant tissues, chronologically from juvenility to maturity. These results indicated that, in each one of the studied plant age. The shootlets of different explants position along the stem axis of plant had different rooting capacity and this might be due to that each one of explant position possessed a specific rooting processes which considerably varied from plant age to anther Smith *et al.*, (1974).

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الإكثار الدقيق لأشجار الكافور جراندس

صفوتلبيب مكسيموس – محمود محمد سيد عبد القادر

قسم بحوث الأشجار الخشبية و الغابات – معهد بحوث البساتين مركز البحوث الزراعية الجيزة مصر

يهدف هذا البحث إلى دراسة تأثير نوع البيئة و تركيزات الكينتين المختلفة و كذا الجزء المستخدم و الناتج من إنبات البذور معمليا لإكثار كافور جراندس بإستخدام تكنيك زراعة الأنسجة. و أيضا تأثير تركيز البيئة المستخدمة مع حامض الا ندول بيوتيرك لتكوين الجذور على الأفرع الناتجة و قد أوضحت النتائج المتحصل عليها من خلال هذه الدراسة الآتي:

١- نتج أكبر معدل لتكوين الأفرع من استخدام الجزء القاعدى على بيئة MS مع تركيز ٢ ملليجرام /لتر من الكينتين. و ذلك مقارنة ببيئة <WPM

٢- أعطت بيئة MS و المخففة إلى نصف تركيزها مع إضافة حامض الاندول بيوتيريك الأفضل معدل لتكوين الجذور على الأفرع الناتجة و أن كان تركيز ٢ ملليجرام/لتر الأفضل فى تكوين الجذور.

٣- بلغت نسبة نجاح إنتاج شتلات جيدة حوالى ٨٣% بعد نقل النباتات الناتجة فى المعمل بعد سبعى أسابيع و زراعتها فى أصص بلاستيك صغيرة ١٠سم تحتوى على طمى + بيتموس + رمل بنسبة ١:١:١ حجما و وضعها فى الصوبة البلاستيك لمدة أربعة أسابيع كمحاولة لأقلمتها.

و يتضح من خلال هذه الدراسة هة إمكانية إستخدام تكنيك زراعة الأنسجة لإكثار الكافور جراندس للتوسع فى زراعته فى مصر.