PROPAGATION OF SOME HARD–TO–ROOT ORNAMENTAL PLANTS BY TISSUE CULTURE
III – ROOTING STAGE

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ABSTRACT

Growing *Cupressus macrocarpa* shoots on **Murashige and Skoog (1962)** medium (MS) with IBA at 20 ppm for 7 days before sub-culturing on a medium free of plant growth regulators gave rise to the best possible rooting percentage with only one root/shoot. Rooting percentage of *Conocarpus erectus* rose significantly to its utmost with indolebutyric acid (IBA) at 5 ppm.

Key Words: *Cupressus macrocarpa*, *Conocarpus erectus*, Tissue culture, Indolebutyric acid, Naphthalene acetic acid.

INTRODUCTION

In propagation by stem cuttings, it is necessary that a new root system be formed, since a potential shoot system – a bud – is already present. Production of adventitious roots on these cuttings is the only measure of success, even if the bud developed into shoots and leaves. Unfortunately, cuttings of many plants such as *Cupressus macrocarpa* and *Conocarpus erectus* are somewhat calcitrant. They do not root easily or even do not root at all. Tissue culture technique represents a promising means to solve this problem.

Monterey cypress (*Cupressus macroparca* Hartw. ex Gord.) is a large-sized evergreen tree native to North America and commonly naturalized in warm temperate and subtropical regions worldwide. Button Mangrove (*Conocarpus erectus* L.) is an evergreen tree reaches up to 6 m tall, with a 20 cm diameter trunk and a spreading crown. Flower clusters mostly 3-8 cm long at end of twigs and in leaf axils, of several small heads, about 5 mm in diameter on slender stalks. Flowers are many in each ball, 2 mm long, mostly bisexual. **Pardo *et al.* (2002)** reported that rooting in conventional cuttings of *Conocarpus erectus* was less successful. Cuttings had few leaves and buds, which lasted between 6 and 12 months, but were unable to generate a functional root system.

The need to use auxins, as a means to enhance root formation on shoot explants, is a well-established fact. **Franco and Schwarz (1985)** stated that elongated shoots of *Pinus oocarpa* and *Cupressus lusitanica* were rooted after auxin treatment. **Zoglauer *et al.* (1992)** found that the formation of adventitious roots on *Pseudotsuga menziesii* (Fam. Pinaceae) shoots *in vitro* was enhanced by the addition of 10-25 μM (2-4.5 ppm) NAA. This method also allowed *Cupressus sempervirens* and *Larix species* (Fam. Pinaceae) to be propagated. **George (1993)** reported that auxins have been found to activate enzymes regulating respiration via the pentose phosphate pathway, while enzymes concerned with glycolytic respiration are reduced in activity. Synthetically-

prepared IAA is used as an auxin in plant tissue culture media. IAA has been used with other regulants to induce either callus or direct morphogenesis, including the rooting of microcuttings. **Capuana and Giannini (1997)** claimed that for rooting of cypress (*Cupressus sempervirens*), IBA or IAA treatments were effective, particularly those consisting of 7 days' culture on medium containing 0.1 mM (20 ppm) IBA. However, rooting was the most problematic step of the multiplication cycle. **Spanos *et al*. (1997)** ascertained that following conditioning on a growth regulator-free medium for 28 days, 95% of *Cupressus sempervirens* shoots rooted on half-strength medium containing 1% sucrose and 0.5 mg IBA/litre. **Mala *et al.* (2000)** found that for the induction of rooting of oak (*Quercus robur*, Fam. Fagaceae) shoots, agar medium with high concentration of NAA (14 mg/l) has been used. After 1 week, the microcuttings were transferred to agar media without NAA. **Shahzad *et al.* (2001)** remarked that excised microshoots of *Hibiscus mutabilis* (Fam. Malvaceae) were rooted on one-half strength MS medium + 1 mg IAA/litre. **Aziz *et al.* (2002)** found that individual shoots of *Acacia nilotica*, when transferred onto half strength MS medium with 4.0 mg/l IBA, formed healthy roots in 62.5% of the cultures. **YanJuan *et al.* (2002)** stated that the most suitable medium for rooting of *Michelia* shoots (Fam. Magnoliaceae) was ½ MS + IBA at 3.0 mg/l. **Abdullah *et al.* (2003)** found that a high rooting percentage (up to 98%) of *Gardenia jasminoides* (Fam. Rubiaceae) was obtained as a result of reducing the IAA concentration to 1 mg/litre. **Hatzilazarou *et al.* (2003)** mentioned that the best rooting percentage (41 and 36%) of microcuttings of bougainvillea 'Alexandra' was achieved with the application of 2 μM of either IAA (0.35 ppm) or NAA (0.37 ppm), respectively. **Muller (2003)** found that *in vitro*-grown shoot segments of *Grevillea* (Fam. Proteaceae) started to root 30 days after transplanting to a rooting medium containing IBA at 1 ppm. **Prashanta *et al.* (2003)** claimed that rooting of the induced shoots of *Syzygium jambos* (Fam. Myrtaceae) was obtained on a medium with 6 mg/litre IBA. **Vengadesan *et al.* (2003)** reported that when transferred to half-strength MS medium augmented with 7.4 μM (1.5 ppm) indolebutyric acid, *in vitro*-regenerated shoots of *Acacia sinuate* (Fam. Mimosaceae) produced prominent roots.

However, some plants can root satisfactorily independent of auxins. **Lambardi *et al.* (1995)** mentioned that rooting of *Cupressus sempervirens* occurred spontaneously as adventitious shoots aged and transfer intervals were increased. **Anitha and Pullaiah (2002)** claimed that the microshoots of *Decalepis hamiltonii* (Fam. Asclepiadaceae) were rooted in 1/4 MS media with or without auxins (NAA at 0.1-3.0 ppm or IAA at 0.1-2.0 ppm). **Aswath *et al.* (2003)** mentioned that in the absence of NAA, the initiation of a single root with secondary root formation was observed on shoots of gerbera (cultivars GJ-1, GJ-2 and GJ-3). However, NAA in the media induced the formation of multiple adventitious roots without secondary roots.

On the other hand, situation is not totally in favor of auxin application, as they failed sometimes to induce rooting. **Haralampieva and Atanassova (2000)** carried out a research that involved the most commonly used auxins, IAA and IBA at 0.1, 0.5, 1.0, 2.0 and 5.0 mg/litre, for rooting promotion of microplantlets of *Azalea* (*Rhododendron spp.* Fam. Ericaceae), cv. Doberlug. After adding 2 mg/litre IBA to the basal medium, the rooting percentage

increased and though it was the optimum concentration, the rootability was still too low. **Aziz *et al.* (2002)** found that shoots of *Acacia tortilis* subsp. *Raddiana* did not develop roots in any media tested even when transferred onto half strength MS medium with 4.0 mg/l IBA.

The presence of a cytokinin in the rooting medium together with an auxin was found by a lot of authors to be of great influence on root induction. **QingFeng (2002)** remarked that a rooting rate of *Hylocereus undatus* (Fam. Cactaceae) shoots of over 96% was obtained on half-strength MS medium supplemented with 0.1-0.4 mg NAA/litre and 0-0.3 mg 6-BA/litre. **Karwa (2003)** found that the MS medium without auxins resulted in very poor or no rooting of *Citrus reticulata* Blanco (Nagpur mandarin) shoots. IBA alone induced up to 50% rooting. The highest rooting percentage (78%) and average number of roots per shoot (5.8 ±0.86) were obtained with 4.92 μM (1 ppm) IBA + 1.11 μM (0.25 ppm) BA. **Ramin (2003)** indicated that rooting of 4 sugarcane (*Saccharum officinarum*, Fam. Poaceae) cultivars was obtained in SH medium supplied with 5 mg/litre IBA and 1 mg/litre kinetin. **Shahin (2003)** reported that in the rooting stage, shoots of mango cv. Zebda treated with 4 ppm IAA + 1 ppm BAP and 30 ppm adenine produced the highest rooting percentage and root number and length. **Kumar *et al.* (2004)** mentioned that the regenerated shoots of *Gerbera jamesonii* were rooted on MS medium containing 1 mg/l BAP + 0.1 mg/1 IAA.

George (1993) stated that several highly effective plant growth retardants have been discovered which include a triazole ring in their structure. They are all potent inhibitors of gibberellin synthesis, but can also interfere with sterol and abscisic acid production. The triazole growth retardants have been noted to promote adventitious root formation of several different kinds of plants. Paclobutrazol has been the subject of most *in vitro* experimentation. **Loewe (1990)** mentioned that *in vitro*-produced shoots of walnuts (*Juglans regia*, Fam. Juglandaceae) were transferred to a rooting medium containing 2 mg IBA and 0.5, 1.0 or 2.0 mg paclobutrazol/litre. Cultures were then transferred to a medium containing no growth regulators. Rooting percentages were very low (less than or equal to 13.3%). **Ma *et al.* (1990)** declared that rooting of apple cultivar Fuji was stimulated by the use of IAA or IBA at 1.0-3.0 mg and 0.5 mg/litre, respectively. Number of roots/explant was increased by addition of paclobutrazol (PP333) to the rooting medium. **LiHua *et al.* (2000)** stated that for the rooting of *Gypsophila paniculata* cv. Fairy Bristol adventitious buds were cultured on MS media with different concentrations of PP333 [paclobutrazol] + NAA. The optimum concentrations of NAA and PP333 were 0.01 and 0.10 mg/litre, respectively. **LongQing *et al.* (2000)** reported that stem segments of ground cover chrysanthemum (*Dendranthema grandiflora* [*D. morifolium*] Fam. Asteraceae) cv. Silver Cup, with 2-3 nodes from rosette plants were cultured on rooting medium comprising 0.1 mg NAA/litre and supplemented with 0.01, 0.1 and 1.0 mg PP333 [paclobutrazol]. Results showed that the 3 concentrations of PP333 promoted the rooting, and increased root quantity; the effect of 0.1 mg PP333/litre was best.

The aim of this study was to establish an applicable protocol for the rapid micropropagation of two ornamental plant species important for landscaping purposes and belonging to different genera, *Cupressus macrocarpa* and *Conocarpus erectus*.

MATERIALS AND METHODS

This work was carried out in the Tissue Culture Laboratory of Pomology, Horticulture Department, Faculty of Agriculture, Ain Shams University through three successive years (2001-2004). Different concentrations of certain auxins were added to **Murashige and Skoog (1962)** medium (MS) in the rooting stage according to the layout of the experiment. These auxins were 3-indolebutyric acid (IBA), 3-Indoleacetic acid (IAA) and 1-naphthalene acetic acid (NAA). Half strength MS medium supplemented with the different types of plant growth regulators were used in a series of experimental trials in an effort to induce rooting on the *in vitro*-produced shoots of the two species previously obtained from Part II of this study. The plant growth regulators used with *Cupressus macrocarpa* were:

1 IAA (at 0.5, 1, 1.5, 2.0 and 2.5 ppm)
2 NAA (at 0.5, 1, 1.5, 2.0 and 2.5 ppm)
3 IBA (at 0.5, 1, 1.5, 2.0 and 2.5 ppm)
4 IBA (at 0.5, 1, 1.5, 2.0 and 2.5 ppm) + 2,4-D at 0.1 ppm
5 IBA (at 0.5, 1, 1.5, 2.0 and 2.5 ppm) + PP333 at 1.0 ppm
6 IBA (at 0.5, 1, 1.5, 2.0 and 2.5 ppm) + BA at 0.1 ppm
7 IBA (at 0.5, 1, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 ppm) + AC at 10 g/l
8 IAA (at 15, 20 or 25 ppm) for 7 days, then transferred to PGR-free medium
9 NAA (at 15, 20 or 25 ppm) for 7 days, then transferred to PGR-free medium
10 IBA (at 15, 20 or 25 ppm) for 7 days, then transferred to PGR-free medium

Other experimental trials with *Conocarpus erectus* were

1 IAA (at 2.5, 5, 10, 15 and 20 ppm)
2 NAA (at 2.5, 5, 10, 15 and 20 ppm) + BA at 2 ppm
3 IBA (at 2.5, 5, 10, 15 and 20 ppm) + BA at 2 ppm
4 IBA (at 5, 10, 15 and 20 ppm)

Where, AC = activated charcoal (at 10 g/l.)
PGR = MS medium free of plant growth regulators

Each treatment comprised 4 replicates, with 3 tubes in each replicate. Data obtained were rooting percentage, number of roots/shoot and length of roots.

Explants were inoculated under aseptic conditions using a laminar airflow cabinet. Tubes were incubated at 25/20°C (day/night) ±2°C, 70% relative humidity. Two fluorescent tubes/shelf were installed at 30 cm above explants to provide light intensity of 2200-2400 lux at explant level.

Data of these experiments were statistically analyzed using SAS 1995 computer program, and means were compared by L. S. D. method and all percentages % were transformed according to **Snedecor and Cochran (1980)**.

RESULTS AND DISCUSSION

Effect of auxins on rooting :

Experiment 1. Effect of auxin type and concentration on rooting.

A series of experimental trials on the effect of different auxins on rooting of *Cupressus macrocarpa* and *Conocarpus erectus* were carried out in order to probe for a suitable type and concentration of auxin that can induce rooting.

Negative outcomes were encountered in most of these trials, particularly in case of *Cupressus macrocarpa*.

1- Effect of auxins type and concentration on rooting of *Cupressus macrocarpa* shoots (Table 1)

Trial number 1-10 shows that when *Cupressus macrocarpa* shoots were grown on MS medium supplemented with IBA at 20 ppm for 7 days before subculturing on medium free of plant growth regulators, only one shoot out of 12 shoots (representing the whole treatment) produced a single root. This gave a rooting transformed percentage of 16.78%. As growth of this root is very slow, a distinctive characteristic of conifers, the length of this root at the end of this study was less than 1mm.

Table (1) Treatments and results of rooting experimental trials on *Cupressus macrocarpa*

No.	Treatment	Results
1-1	IAA (at 0.5, 1, 1.5, 2.0 and 2.5 ppm)	No rooting, callus only
1-2	NAA (at 0.5, 1, 1.5, 2.0 and 2.5 ppm)	No rooting, callus only
1-3	IBA (at 0.5, 1, 1.5, 2.0 and 2.5 ppm)	No rooting, callus only
1-4	IBA (at 0.5, 1, 1.5, 2.0 and 2.5 ppm) + 2,4-D at 0.1 ppm	No rooting
1-5	IBA (at 0.5, 1, 1.5, 2.0 and 2.5 ppm) + PP333 at 1.0 ppm	No rooting
1-6	IBA (at 0.5, 1, 1.5, 2.0 and 2.5 ppm) + BA at 0.1 ppm	No rooting
1-7	IBA (at 0.5, 1, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 ppm) + AC at 10 g/l	No rooting
1-8	IAA (at 15, 20 or 25 ppm) for 7 days, then transferred to PGR-free medium	No rooting
1-9	NAA (at 15, 20 or 25 ppm) for 7 days, then transferred to PGR-free medium	No rooting
1-10	IBA (at 15, 20 or 25 ppm) for 7 days, then transferred to PGR-free medium	low rooting % with IBA at 20 ppm

AC = activated charcoal
PGR = medium free of plant growth regulators

Some authors encountered the problem of low percentage of *in vitro* rooting. **Oliveira *et al* (2003)** stated that microshoots of stone pine (*Pinus pinea*) reached incipient rooting after induction with a combination of auxin and hypertonic shock, but their development *in vitro* was not sustained. At this stage, co-culturing plantlets with some fungi isolated from ectomycorrhizas succeeded in overcoming this barrier, enabling satisfactory development in vermiculite and later in soil. **Pereira *et al* (2003)** stated that the percentage of *in vitro* rooting of *Anemopaegma arvense* (Fam. Bignoniaceae) buds was low.

The technique of culturing shoots in a medium supplemented with auxins for some days before moving them into auxin-free one was adopted by some workers. **Capuana and Giannini (1997)** claimed that for rooting of cypress (*Cupressus sempervirens*), IBA or IAA treatments were effective, particularly those consisting of 7 days' culture on medium containing 0.1 μM IBA.

In some instances, instead of root induction, auxins gave rise to callus. **Bravo *et al* (1999)** stated that IBA at 0.25-1.0 mg/l produced roots on shoots of *Aristolochia fimbriata* (Fam. Aristolochiaceae) cultured in half MS medium.

NAA at 0.25-1.0 mg/l only led to callus formation. **Ahmad *et al* (2003)** stated that the best root system was developed on shoots of peach rootstock GF 677 grown on half strength MS media supplemented with 3 mg IBA/l. Higher level of IBA (4.0 mg/l) induced callus and inhibited normal root development.

2- Effect of auxin type and concentration on rooting of *Conocarpus erectus* shoots (Table 2,)

Treatments and results of rooting experimental trials on *Conocarpus erectus* are shown in Table 2. Trial number 2-4 shows that applying IBA alone at different concentrations was successful in inducing roots. Full report of these promising treatments will be discussed in the following chapter.

Table 2. Treatments and results of rooting experimental trials on C*onocarpus erectus*

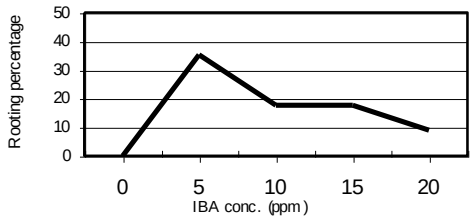
No.	Treatments	Results
2-1	IAA (at 2.5, 5, 10, 15 and 20 ppm)	No rooting
2-2	NAA (at 2.5, 5, 10, 15 and 20 ppm) + BA at 2 ppm	No rooting
2-3	IBA (at 2.5, 5, 10, 15 and 20 ppm) + BA at 2 ppm	No rooting
2-4	IBA (at 5, 10, 15 and 20 ppm)	rooting

2-1- Effect of IBA on rooting percentage of *Conocarpus erectus* (Table 2-1).

The effect of applying IBA at different levels on rooting percentage of *Conocarpus erectus* was statistically significant. This percentage rose significantly from 0% with IBA at 0 ppm to 35.26% when IBA was used at 5 ppm. However, further increase in IBA concentration affected this record negatively, though insignificantly as rooting percentage declined to 17.63% by applying IBA at either 10 or 15 ppm. The highest level of IBA, i.e. 20 ppm resulted in more significant decline in this record to 8.82%.

Table (2-1) Effect of IBA on rooting percentage of *Conocarpus erectus*

IBA conc. (ppm)	% of Rooting
0	00.00 b
5	35.26 a
10	17.63 ab
15	17.63 ab
20	8.82 b
LSD at 5%	21.04



A lot of papers revealed the importance of IBA as a rooting agent. However, suitable level of this growth regulator varies according to the plant species or even the cultivar. **Giridhar *et al* (2003)** mentioned that shoots of *Decalepis hamiltonii*were rooted on a medium containing 2 ppm IBA. **Karwa (2003)** stated that IBA at 0.5-2 ppm induced up to 50% rooting of *Citrus reticulata* Blanco (Nagpur mandarin) shoots. **Mereti *et al* (2003)** found that the highest rooting percentages of microcuttings of the strawberry tree (*Arbutus unedo*, Fam. Ericaceae) were achieved in 10 µM (2 ppm) IBA (92%). **Rogalski *et al* (2003)** stated that percentage rooting of shoots of *Prunus* rootstock "VP417" was highest on medium supplemented with 2.0 mg IBA/litre (64%).

Higher levels of IBA were used in many instances. **Colina *et al* (2002)** found that the MS medium containing 5 mg IBA/litre was most adequate for rooting,

yielding 68% of rooted plants of pawpaw (*Carica papaya*, Fam. Caricaceae) hybrid IBP 42-99. **Baksha *et al* (2003)** found that half MS salt solution supplemented with IBA (at 5.0 mg/l) was found suitable for rooting of the shoots of sugarcane (*Saccharum officinarum*) clone I 273-91. **Isutsa (2004)** achieved rooting *in vitro* of yellow passion fruit (*Passiflora edulis* var. *flavicarpa*) with 62% rooting percentage on 24.5 µM (5 ppm) IBA medium.

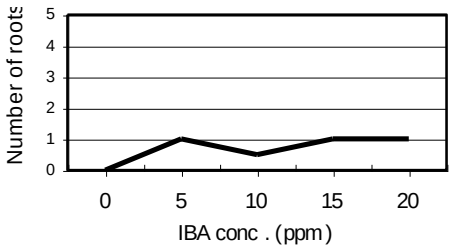
On the contrary, lower concentrations of IBA were sometimes better than the higher ones. **Bravo *et al* (1999)** mentioned that rooting percentage of *Aristolochia fimbriata* were 60, 40 and 13% on half MS medium supplemented with IBA at 0.25, 0.5 or 1.0 mg/l, respectively. **Magyar-Tabori *et al* (2002)** stated that with *in vitro* shoots of apple rootstock M.26, the highest rooting percentage (94%) was obtained with the lowest rate of IBA (1 ppm); the number of rooted shoots decreased with the increase in the IBA concentration (up to 3 ppm).

2.2. Effect of IBA on number of roots/shoot of *Conocarpus erectus* (Table 2-2)

Applying IBA at any level used did not significantly influence the number of roots/shoot. IBA-free medium did not induce rooting at all. The incorporation of IBA in the media at 5, 15 and 20 ppm resulted in the growth of only 1 root/shoot. At 10 ppm IBA the mean number of roots/shoot was 0.5, insignificantly different from the former treatments.

Table (2-2) Effect of IBA on number of roots/shoot of *Conocarpus erectus*

IBA conc. (ppm)	Number of roots/shoot
0	0.00 a
5	1.00 a
10	0.50 a
15	1.00 a
20	1.00 a
LSD at 5%	NS



All percentages were transformed according to Snedecor and Cochran (1980).

The effect of IBA on the number of roots produced *in vitro* depended mainly on the concentration used. **Rogalski *et al* (2003)** cultured shoots of *Prunus* rootstocks on Lepoivre medium supplemented with IBA (0.1, 0.5, 1.0 or 2.0 mg/litre). They stated that the number of roots per shoot was highest in Capdeboscq and GF677 in medium supplemented with 2.0 mg IBA/litre (9.6 and 5.2, respectively). **Isutsa (2004)** achieved rooting *in vitro* of yellow passion fruit (*Passiflora edulis* var. *flavicarpa*) with one root per shoot on 24.5 µM (5 ppm) IBA medium.

Some reports declared that the higher the IBA concentration, the lower the number of roots produced. **Atta-Alla *et al* (2003)** stated that rooting of proliferated shoots of *Bombax malabaricum* (*B. ceiba*, Fam. Bombacaceae) was on MS medium containing 0, 0.5, 1, 2 or 3 mg IBA /litre. IBA at low concentrations resulted in the highest number of developed roots.

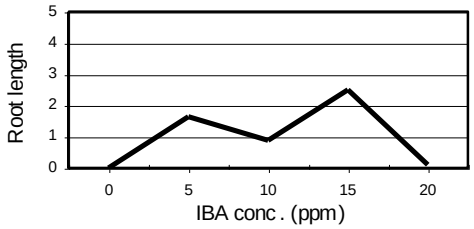
2-3- Effect of IBA on root length of *Conocarpus erectus* (Table 2-3).

Statistically insignificant effects were detected on root length of *Conocarpus erectus* as affected by the different levels of IBA. However, longest roots were

those of shoots grown on a medium supplemented with 15 ppm IBA. At 0 ppm IBA no roots were observed.

Table (2-3) Effect of IBA on root length of *Conocarpus erectus*

IBA conc. (ppm)	Root length (cm)
0	0.00 a
5	1.63 a
10	0.88 a
15	2.50 a
20	0.10 a
LSD at 5%	NS



Nath and Buragohain (2003) mentioned that rooting of the microshoots of the medicinally important herb *Centella asiatica* was obtained in MS basal medium containing 2.0 mg/l IBA with root length of 19.7 cm. On the other hand, **Atta-Alla *et al* (2003)** stated that rooting of proliferated shoots of *Bombax malabaricum* (*B. ceiba*) was on MS medium containing 0, 0.5, 1, 2 or 3 mg IBA /litre. IBA at low concentrations resulted in the highest root length.

Therefore, it is recommended to grow *Cupressus macrocarpa* shoots on MS medium supplemented with IBA at 20 ppm for 7 days before subculturing on medium free of plant growth regulators. For *Conocarpus erectus* rooting is to be induced with IBA at 5 ppm.

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إكثار بعض نباتات الزينة صعبة التجذير بواسطة زراعة الأنسجة
٣ – مرحلة التجذير

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أجريت هذه الدراسة فى معمل زراعة الأنسجة الخاص بالشتلات فى قسم البساتين بكلية الزراعة – جامعة عين شمس خلال المدة من ٢٠٠١ حتى ٢٠٠٤.

بزراعة أفرع السرو الليمونى على بيئة موراشيج وسكوج المزودة بحمض الإندول بيوتيريك بتركيز ٢٠ جزء فى المليون لمدة ٧ أيام ثم نقلها على بيئة خالية من منظمات النمو، أمكن التوصل إلى أفضل نسبة ممكنة من التجذير.

وبلغت النسبة المئوية للتجذير أقصاها بشكل معنوى فى حالة الكونوكاربوس عند إستعمال حمض الإندول بيوتيريك بتركيز ٥ جزء فى المليون.