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In vitro Rooting of Pecan (*Carya illinoensis* Wang - C. Koch)

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Abstract: An efficient method for *in vitro* rooting has been developed for pecan (*Carya illinoensis* Wang-C. Koch). In this study, intense efforts have been made for improvement of pecan *in vitro* rooting treatments. Stem segments of pecan were cultured on Mc Cow's Woody Plant Medium (WPM) containing 3 mg/l 6-benzyl adenine (BAP) for multiplication. After four weeks from culture, elongated shoots were excised and their response to the rooting treatments was investigated. Pecan shoots base were splitted before culture, then shoots were cultured on¼ and ½ strength WPM medium supplemented with 0.0, 1.0, 2.0 and 3.0 mg/l indol-3-butyric acid (IBA) and 1.5 mg/l silver nitrate ($AgNO_3$). In addition, 20 g/l sucrose, 1 g/l activated charcoal (AC) and 3 g/l phytagel were added to the media. The best rooting percentage (75%) was obtained on half strength WPM medium supplemented with 3.0 mg/l IBA+1.5 mg/l $AgNO_3$. Finally, the rooted pecan plantlets were transferred to peat moss and sand (1:2 v/v). Eighty percentage plantlets were successfully acclimatized in greenhouse. This technique can be effectively used for *in vitro* rooting and acclimatization of pecan on a large commercial scale.

Key words: Pecan · *Carya illionensis* · *In vitro* · Rooting

INTRODUCTION

Pecan (*Carya illinoensis* Wang - C. Koch) is a deciduous tree belonging to the family Juglandaceae, it is increasingly important through the world as a horticultural crop [1]. The nuts are a very rich source of proteins, fats, minerals and a concentrated source of energy [2, 3]. Pecan is the only nut which needs low chilling requirements; therefore it can be cultivated in different type of Egypt's lands. Pecan is a temperate nut crop. There is a general tendency to raise pecan by seeds which causes great variability, prolonged the juvenile period, occupying a larger space and are often low in fruit productivity with low quality [4]. In most cases, propagation of pecan is done primarily by budding or grafting of improved cultivars onto seedling rootstocks. Although propagation by cuttings of mature trees, though leads to uniform clonal rootstocks, it is very difficult due to their low rooting ability [5]. Even with the use of hormones, inconsistent results with only limited improvements have been reported [6]. However, these methods suffer from many disadvantages such as considerable time, expense and poor transplanting survival of the plants. Many treatments on difficult-to-root *Juglans* species, have been studied in order to improve rooting efficiency but without significant improvements [7].

Now the expansion of pecan cultivation is limited by the difficult reproduction of budded seedlings and marketable varieties, since budding operations results are complicated and low yield percentage of success [8]. Hence, *in vitro* propagation offers great potential for pecan industry for large scale multiplication of selected clones and enables production of high amount of constant quality plantlets within a short time, but poor adventitious root formation is a major obstacle in micropropagation. Efficient *in vitro* rooting is a critical step in micropropagation of fruit crops. Losses at this step will have vast economic consequences. Therefore, the ability of *in vitro* proliferated shoots to form roots is affected by several factors, including differences between genotypes [9] culture procedures [10] mineral nutrition [11, 12] the level of tissue maturity [13] physiological age [14] and time of explant taken Hassanen [15]. Claudet *et al*. [16] and Hassanen [15] suggested that, the phloem pecan stem continuity of the sclerenchymatous cylinder encircle inhibits rooting or roots emergence and

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this factors, *in vitro* shoots showing a variety of rooting responses. In Egypt, is important to raise trees by cultivating them in different arid and semi-arid regions. There are many trees species grown in various areas of country but cultivation of woody trees (including pecan), has faced different problems amongst which are failure to provide the necessary provision for plantation and lack of interest by farmers and crop growers.

The main objective of this study was to find a reproducible method for successful *in vitro* rooting and acclimatization of pecan trying a wide range of different *in vitro* culture treatments. Since, pecan needs lowest chilling requirements, results obtained from this study will help encouraging its cultivation in different types of Egypt's lands, which could be a promising crop in Egypt.

MATERIALS AND METHODS

Microshoots of pecan were multiplied in McCow's Woody Plant Medium (Mc Cown and Lloyd [17]; Duchefa, Harlem, the Netherlands) supplemented with 3 mg/l 6-benzylaminopurine (BAP), 30% (w/v) sucrose and gelled with 3 g/l phytagel (Duchefa, Haarlem, the Netherlands). For rooting induction, the shoots were cultured on quarter and half WPM medium supplemented with different concentrations of IBA (0.0, 1.0, 2.0, 3.0 mg/l) and silver nitrate ($AgNO_3$) (0.0, 1.0, 1.5, 2.0 mg/l) which the most were tested individually for optimizing rooting of explants. A separate experiment was performed using a promising concentration of $AgNO_3$ (1.5 mg/l) in combination with different concentrations of IBA as described earlier. In addition to 1 g/l activated charcoal and 2% (w/v) sucrose. The pH of the medium was adjusted to 5.7-5.8 before gelling with 3 g/l phytagel and autoclaved at a pressure 1.05 kg/cm^2 and 120°C for 15 min. All shoots were splitted from the base before culture on the rooting media. All cultures were incubated under controlled conditions at a temperature of 23 ±1°C in darkness for 7 days. Then transferred to the light intensity of about 3000 lux. The photo-period was light dark (16h/8h). Rooting percentage, average number of roots/shoot and average root length (cm) was evaluated after 50 days of culture. Pecan plantlets were washed carefully with distilled water and planted in small plastic pots (5 cm diameter) containing peat moss and sand (1:2 v/v). To maintain high humidity level, the pots were covered with polyethylene bags. Plantlets were incubated at 23±2°C with 16 hours photoperiod and irrigated by ½ WPM medium, after four weeks, the plants were transferred to green house (27±1°C and 70-80% relative humidity). After 30 days, covers were removed and plants were exposed to external moisture levels gradually. The plants were transferred to bigger plastic pots (14 cm diameter). The elongation of transplanted shoots and emergency of new leaves were observed after 21 days.

Statistical Analysis: Each experiment was conducted as completely randomized design, with 20 replicates for each treatment. Variance analysis of data was carried out using ANOVA program for statistical analysis. The differences among means for all treatments were tested for significance at 5% level by using Duncan [18] as described by Snedecor and Cochran [19].

RESULTS AND DISCUSSION

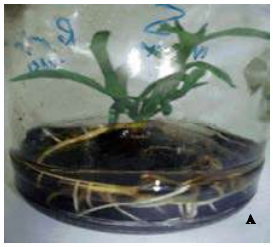
The existence of well-developed and healthy root system of *in vitro* pecan plants was a very important step for subsequent acclimatization in greenhouse conditions. In preliminary studies, no results could be obtained for *in vitro* root formation of pecan when IBA or $AgNO_3$ were used individually except for 1.5 mg/l of $AgNO_3$. Therefore, 1.5 mg/l $AgNO_3$ was used in combination with different concentrations of IBA to induce the rooting of pecan.

Data presented in Table 1 illustrated the effect of two different strengths of WPM medium (¼ WPM and ½ WPM), different concentrations of IBA in addition to 1.5 mg/l $AgNO_3$ mg/l on the rooting percentage, average number of roots per shoots and average length of pecan roots (cm).

From data presented in Table 1 and are shown in Fig. 1A, it is observed that *in vitro* shoots grown on ½ WPM medium supplemented with 3.0 mg/l IBA+1.5 mg/l $AgNO_3$ produced the highest rooting percentage (75%), average roots per shoot (3) and average root length (6.5 cm), followed by ½ WPM medium supplemented with 2.0 mg/l IBA+1.5 mg/l $AgNO_3$, which gave 33% rooting percentage, average root number (2.0) and average roots length (2.7cm). These results are in agreement with those obtained by Caboni *et al*. [20] who reported that, MS medium supplemented with 2.0 mg/l IBA resulted in a good rooting of an apple rootstock (M9 York). As reported by Tougeer *et al.* [21] the maximum number of roots was obtained by using IBA peach rootstock GF 677. Rooting percentage was scored the less rooting percentage, average number of roots and average root length (13%, 1.0 and 1.0 cm, respectively), were scored on ¼ WPM medium + 1.0 mg/l IBA + 1.5 mg/l $AgNO_3$. From the previous results it is clear that ½ WPM medium was

Table 1: Effect of quarter and half strength WPM media supplemented with different IBA concentrations in combination with 1.5 mg/l $AgNO_3$ on rooting of pecan (*Carya illinoensis*)

Strength of WPM	IBA (mg/l)	Percentage of rooted/shoot (%)	Average no of roots/shoot	Average root length (cm)
Quarter	0.0	00 f	0.0 d	0.0 d
"	1.0	13 e	1.0 c	1.0 c
"	2.0	15 d	1.0 c	1.0 c
"	3.0	18 c	2.0 b	1.0 c
Half	0.0	00 f	0.0 d	0.0 d
"	1.0	25 d	2.0 b	2.5 b
"	2.0	33 b	2.0 b	2.7 b
"	3.0	75 a	3.0 a	6.5 a



(A) Rooting of shoots

(B) Acclimatized shoots after one month in greenhouse

(C) Acclimatized shoots after six months outside greenhouse

(D) Acclimatized shoots after twelve months outside greenhouse

Fig. 1: *In vitro* rooting of pecan *Carya illinonises*

better than ¼ WPM medium for rooting induction of pecan. In addition, when increasing the concentration of IBA, the rooting percentage, average shoot number and shoots length were also increased. From the previous results, it is obvious that IBA played an important role and had a significant effect on the rooting of pecan. IBA is commonly used to promote root initiation both i*n vitro* and *ex vitro* [22]. Hausman [23] indicated that in tissue culture media, IBA oxidized slowly (10%). Its slow movement and delayed degradation may be the primary reason for its better performance. IBA may also, enhance rooting *via* increasing internal free IBA or may synergistically modify the action of endogenous synthesis of IAA [24] $AgNO_3$ increased the colour of leaves and thickness of roots. In general $AgNO_3$ (1.5 mg/l) gave healthy plantlets (roots and shoots) and caused good acclimatization *ex vitro*. These results are in harmony with those reported by Bais *et al.* [25] and Reddy *et al.* [26] on *Decalepis hamiltonii*; they found that $AgNO_3$ resulted in good root initiation and elongation. Also, the maximum number of shoots and the highest shoot length were obtained on a medium containing 20µ M $AgNO_3$. Thus, it is clear that $AgNo_3$ don't use to induce shoot multiplication only but also influenced rooting of vanilla explants [27]. Ma *et al*. [28] demonstrated that the use of ethylene inhibitors such as $AgNO_3$ may promote root formation in apple. Silver nitrate also induced rooting *in vitro* of the rare, rhoeophytic woody medicinal plant (*Rotula aquatica*) [29]. The role of silver ions in regulating morphogenesis, it is important to know the aspect of ethylene biosynthesis. In brief, the biosynthesis of ethylene starts with conversion of the amino acid methionine to S-adenosyl-L-methionine (SAM, also called Adoment) by the enzyme met adenosyltransferase. SAM is subsequently converted to 1- aminocyclopropane- 1- crboxylic acid (ACC) by the enzyme ACC synthesase. The final step requires oxygen and involves the action of the enzyme (EFE) [30] Ag^+ ions from ethylene inhibitors such as silver nitrate and aminoethoxy vinyl glycine (AVG) inhibit ethylene action

in a wide variety of ethylene-induced responses in plants. Ethylene inhibiting effect of Ag^+ is believed to be due to an interference with ethylene binding [31]. Whereas, AVG blocks the activity of Amino Cyclopropane Carboxylic acid (ACC, an ethylene precursor) synthesis, which plays a key role in regulating ethylene production [32]. The beneficial effects of the ethylene inhibitors on organogenesis have been widely reported by Bais *et al.* [25]. The early aging of the plants is inhibited by silver Ag^+ present in silver nitrate which blocks the production of ethylene and there by promotes good growth of plant [33]. Thus, $AgNO_3$ may be useful as a media supplement to develop efficient rooting and propagation protocols. The highest survival percentage of acclimatized plantlets (80%) was recorded (Fig. 1C and D).

CONCLUSION

The present study demonstrated a simple and reliable method for *in vitro* rooting of pecan from stem segments explant by using ½ WPM medium supplemented with IBA and $AgNO_3$ due to good roots and healthy shoots. The plantlets were successfully acclimatized in the greenhouse then in the soil. Therefore, *in vitro* propagation protocol offers great potential for the pecan industry for large scale and enables production of high amount of constant quality plantlets within a very short time.

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