

Effect of Auxins and Medium Strength on *In vitro* Rooting of *Populus alba* L. Micro-Shoots

¹Mohammed Hewidy, ¹Mohamed Fathy Ahmed and ²Eman Tawfik

¹Horticulture Department, Faculty of Agriculture,
Ain Shams University, Hadayek Shobra, Cairo, Egypt

²Eman Tawfik, Botany and Microbiology Department, Faculty of Science,
Helwan University, Ain Helwan, Cairo, Egypt

Abstract: *Populus alba* is a promising woody tree in the Egyptian territory for wood and various application. Based on an *in vitro* propagation protocol resulted micro-shoots were introduced to rooting stage. The experiment was performed to study the effect of MS media strength and, auxin type and concentration on rooting performance of micro-shoots of *P. alba*. MS media strength were ($\frac{1}{8}$, $\frac{1}{4}$, $\frac{1}{2}$, $\frac{3}{4}$ and full) supplemented with IBA or NAA at 0.0, 0.1 and 0.5 mg/l. Medium strength reduction gave better leaf number, shoot length plantlet fresh weight and root number. Also, the lower auxin level play important rule in the increase of root number and length. In addition, pigments showed higher values when micro-shoots were cultured on half strength MS medium supplemented with 0.5 mg/l of NAA. Phenolic compounds were measured as estimation of lignin formation and xylem amount in this woody plant. However, in this work, the use of different media power and hormone concentrations resulted in different values in morphological and physiological responses of *P. alba*.

Key words: *P. alba* • Tissue culture • *In vitro* rooting • Auxin • IBA • NAA • Medium strength

INTRODUCTION

The genus *Populus* is widely distributed all over the world especially in the Northern hemisphere. They are dioecious, medium-sized woody tree with simple, glabrous leaves and scale-covered buds. It belongs to family Salicaceae [1, 2]. The genus *Populus* is the main model system for genomic and physiological research in trees [3]. The poplar is an important model for woody perennial biotechnology because it is amenable to *in vitro* culture and genetic engineering through *Agrobacterium*-mediated transformation [4]. It was the first tree which genome has been sequenced [5]. *Populus alba* (White poplar) is a native to the Mediterranean region. It is a fast-growing, deciduous tree. White poplar leaves can be used as bio-monitors of soil pollution [6].

Populus alba was one of the first examples subjected to *in vitro* propagation trials. The explants for *Populus* culture started as follows: cambial, callus improvement, then shoot or root development. The previous studies

founded the vegetative propagation established from a single bud and different originated callus-based plant regeneration. At the beginning, there were difficulties of culture establishment and genetically determined differences between the species. The success of establishment depends on the age of the mother plants. Recently, the development of micropropagation methods of poplars is for commercial purposes with media optimization. Breeding work based on *in vitro* explants started almost parallel with the development of an *in vitro* mass-propagation procedure for poplars. This protocol based on protoplast and cell suspension production followed by plant regeneration [7].

Gifston *et al.* [8] mentioned that phenolic compounds also play a vital role in the plant antioxidant system. Moreover, Amin *et al.* [9] and Gad El-Hak *et al.* [10] mentioned that phenolic compound stimulated vegetative growth, protein content, total carbohydrate, nitrogen, phosphorus, potassium and yield of different plants.

Flavonoids are common in plants, with many functions. Flavonoids are the most important plant pigments for flower coloration. It's responsible for yellow, red or blue pigmentation in petals designed to attract pollinators. In higher plants, flavonoids are involved in UV filtration, floral pigmentation and symbiotic nitrogen fixation. Besides, they are chemical messengers, cell cycle inhibitors and physiological regulators. Flavonoids secreted by the root of their host plant help Rhizobia in the infective stage of their symbiotic relationship with many legumes. Also, some flavonoids have inhibitory activity against pathogenic microorganisms, e.g. *Fusarium oxysporum* [11]. The flavonoids, one kind of polyphenolic compounds, have a broad spectrum of action in plants. They protect against UV-B radiation and pathogen attack, act as attractants for pollinators, act as signal molecules for initiating plant-microbe symbiotic associations [12].

Biometric and physiological variations were reported to occur due to the propagation process with different media compositions. Therefore, it is important to assess physiological effect of *in vitro* rooting treatments. None of these studies were previously investigated for the *in vitro* propagation of *P. alba*.

MATERIALS AND METHODS

Plant Materials: Nodal segments explants of *Populus alba* were brought from Horticulture Research Station, Al-Qalyubia Governorate. Then they were brought to tissue culture lab in of ACGEB (Agriculture Center for Genetic Engineering and Biotechnology (ACGEB), in Faculty of Agriculture, Ain Shams University). *P. alba* stem node segments were sterilized and cultured on free MS media for 2 months. The explants were defoliated and washed carefully in fluid tap water to eliminate all the stacked dust/soil practices. Tailed with surface sterilization of the explants were using 20% Clorox + 0.1% HgCl₂ for 20 minutes and washed 4 to 5 times with double distilled sterile water. Excising and procedure of culture for explants stem nodal was performed under sterilized condition. MS basal medium contains required nutrients of macro- and micro-elements for the *in vitro* cultured plants as described by Murashige and Skoog [13]. The medium was allocated into incubation jars where each jar contained 60 ml. Stem nodal cultures were incubated at 25 ±2°C and satisfactory fluorescent light of 3000 Lux for 16-hour photoperiod provided by cool, white, fluorescent lamps.

Resulted shoots from establishment were excised and transferred into multiplication medium of MS supplemented with 0.075 mg/l of BAP in order to obtain micro-shoots required for the rooting experiment.

Method

***In vitro* Rooting Experiment:** *Populus alba* stem node segments were sterilized and cultured on free MS medium for 2 months. *P. alba* were maintained on MS tissue culture medium according to Murashige and Skoog [13]. For root formation, shoots developed on MS multiplication medium with low levels of 6-Benzylaminopurine (BAP), 0.5 g/L activated charcoal and 20 g/L sucrose then were transferred and cultured in 400 ml jars containing 60 ml MS media. The different treatments are mentioned in experimental design section.

Experimental Design: Design factors are parameters that can influence the performance of a treatment in general and response variables in particular. In this study there are two main factors (media power and Auxins hormone concentration). Four nodal segments/ jar (about 10-15 mm long) were cultured on 1/8, 1/4, 1/2, 3/4 and full-strength MS basic medium supplemented with different concentrations of 3-indolebutyric acid (IBA) or naphthalene acetic acid (NAA) at (0.0, 0.1 and 0.5 mg/l) for each hormone. The shoots were maintained for 4 weeks under the same culture conditions as for development of shoots (16h light/ 8h dark). This design was performed using general factorial design using design expert (DX 12). These treatments resulted in 25 different runs in this block work. Such design can serve several purposes such as: total means and ratio from minimum to maximum values for all model. Also, provide 3D surface histogram representation for each response in this model.

Morphological Measurements: The measured morphological biometric parameters were shoot length, leaf number, plantlet fresh weight, root length, rooting percentage and root number.

Physiological Measurements: The physiological parameters were measured as photosynthetic pigments, total phenolic compounds and total flavonoids of various rooting treatments of *P. alba* plantlets.

Photosynthetic Pigments: Chlorophyll a, b and carotenoids were determined in the fresh leaves of the plantlets according to Metzner *et al.* [14]. A known

weight of fresh leaves was homogenized in 85% acetone. After centrifugation, the supernatant, which contained the pigments, was made up to a definite volume with 85% acetone. The extract was measured against a blank of pure 85% aqueous acetone at three wave lengths of 452, 645 and 664 nm using a colorimeter. The concentration of chlorophyll a, b and carotenoids were calculated as µg/ml using the following equations:

Chlorophyll a = $10.3 E_{664} - 0.918 E_{645}$

Chlorophyll b = $19.7 E_{645} - 3.87 E_{664}$

Carotenoids = $4.3 E_{452} (0.0265 \text{ Chl. a} + 0.426 \text{ Chl. b})$

Then, the fractions were calculated as mg/g fresh weight:

$$\frac{\text{Fraction} \times \text{dilution}}{1000} \text{ mg / g}$$

Total Phenolic Compounds: Air dry powdered *P. alba* (1 g) was extracted with stirring 30 ml methanol (80%) at the room temperature until extraction solvent become colorless. Total phenolic contents were determined according to the method described by Kujala *et al.* [15], using Folin-Ciocalteu reagent and gallic acid as a standard. Briefly, 0.5 ml of filtered extract was added to test tube containing 2.5 mL Folin-Ciocalteu's reagent (diluted with ethanol 1:1), 2 mL of Na₂CO₃ (7.5%) and mixed well. After 15 min incubation at room temperature, the absorbance of mixtures was recorded spectrophotometrically at 765 nm using a Jenway 6405 UV-Vis spectrophotometer. The total phenolic content was calculated from a calibration curve of gallic acid standard solutions and expressed as mg gallic acid equivalent (GAE) per gram of extract (mg GAE/g dry weight of extract).

Total Flavonoids: Total flavonoids content of *P. alba* extract was determined using aluminium chloride colorimetric assay [16]. 0.5 ml of the extract was added to 150 µl of 5% NaNO₃ and allowed to stand for 6 minutes. Then 150 µl of 10% AlCl₃ solution was added and allowed to stand for 6 minutes after which 200 µl solution of 1 M NaOH was added then the mixture was completed to 5 ml with methanol and mixed well. After incubation for 15 min, the absorbance was measured spectrophotometrically against a blank at 510 nm. The total flavonoids content was expressed in milligrams of quercetin equivalents (QE) per gram extract (mg QE/g). Standard curve of quercetin was used for calculation of total flavonoids.

Both total phenolic compounds and total flavonoids content were calculated according to the following equation:

$$\text{Concentration (mg/g)} = \frac{(R - B) * \text{dilution factor} * \text{factor}}{1000}$$

*R: reading of samples at spectrophotometer, B reading of blank at spectrophotometer

Statistical Analysis: The experimental design was performed by design expert (DX 12) using general factorial design. Data were subjected to analysis of variance ANOVA using Co-stat program. Separation among means was performed using Tukey test multi-comparison analysis.

RESULTS

Morphological Measurements: Resulted plants demonstrated in Figure (1) were subjected for various biometric parameters. Result data laid out in Table (1) show that auxin type (IBA or NAA) at various concentration and use of various medium strength and their interaction had significant effects on shoot length, leaf number and fresh weight/young rooted shoot. NAA application at 0.1 mg/l and free medium gave significant increases in shoot length/young rooted shoot (5.47 and 5.59 cm) than remaining treatments. Regardless auxin application, MS medium 1/8 strength showed the tallest shoot length/young rooted shoot (5.97 cm) when compared to other strengths. NAA application at 0.1 mg/l gave significant increases in leaf number/young rooted shoot (9.07) than remaining treatments. Regardless auxin application, full strength MS medium showed the highest leaf number followed by 1/4 strength medium (9.27 and 8.80) when compared to other strengths.

Concerning fresh weight, free auxin media gave the highest fresh weigh (0.211 g/young rooted shoot). Regardless auxin application, 1/2 strength MS medium showed the highest fresh weight followed by 1/4 strength MS medium (0.203 and 0.200 g/young rooted shoot) when compared to other strengths.

About the interaction between auxin and medium strength, free medium of auxin and at 1/4 strength gave the tallest and higher both number of leaves and fresh weight/young rooted shoot (6.93 cm, 11.00 and 6.47 g/young rooted shoot, respectively) when compared to almost all remaining combinations (refer to Table (1) for details).

Table 1: Effect of auxin and medium strength and their interaction after three subcultures on shoot length, leaf number and fresh weight, root length and root number/young rooted shoot of *P. alba* from *in vitro* rooting

Auxin (mg/l)	Medium strength					Mean
	1/8	1/4	1/2	3/4	full	
Shoot length (cm)						
0	6.57 ^{abc}	6.93 ^a	5.47 ^{efg}	4.40 ^m	4.60 ^{j-m}	5.59 ^A
IBA 0.1	6.40 ^{abc}	6.17 ^{bcd}	4.80 ^{h-i}	4.10 ^m	5.17 ^{f-i}	5.33 ^{BC}
IBA 0.5	5.07 ^{fk}	4.90 ^{j-l}	5.67 ^{def}	5.07 ^{f-k}	4.43 ^{lm}	5.03 ^D
NAA 0.1	6.73 ^{ab}	5.53 ^{ef}	5.27 ^{fgh}	5.27 ^{fgh}	4.53 ^{j-m}	5.47 ^{AB}
NAA 0.5	5.10 ^{fj}	5.43 ^{efg}	6.00 ^{cde}	4.90 ^{j-l}	4.47 ^{klm}	5.18 ^{CD}
Mean	5.97 ^A	5.79 ^A	5.44 ^B	4.75 ^C	4.64 ^C	
Leaf number						
0	6.33 ^{gh}	11.00 ^a	8.00 ^{def}	7.67 ^{ef}	9.33 ^{bc}	8.47 ^B
IBA 0.1	8.67 ^{cde}	8.00 ^{def}	9.00 ^{bcd}	6.00 ^h	8.00 ^{def}	7.93 ^C
IBA 0.5	7.67 ^{ef}	8.00 ^{def}	8.00 ^{def}	7.67 ^{ef}	10.00 ^{ab}	8.27 ^{BC}
NAA 0.1	7.33 ^{fg}	10.00 ^{ab}	8.00 ^{def}	10.00 ^{ab}	10.00 ^{ab}	9.07 ^A
NAA 0.5	9.00 ^{bcd}	7.00 ^{fgh}	8.00 ^{def}	8.00 ^{def}	9.00 ^{bcd}	8.20 ^{BC}
Mean	7.80 ^C	8.80 ^B	8.20 ^C	7.87 ^C	9.27 ^A	
Plantlet fresh weight (g)						
0	0.171 ^j	0.384 ^a	0.168 ^k	0.206 ^f	0.125 ^u	0.211 ^A
IBA 0.1	0.105 ^w	0.117 ^v	0.212 ^e	0.132 ⁱ	0.217 ^d	0.157 ^E
IBA 0.5	0.152 ⁿ	0.140 ^f	0.165 ^l	0.186 ^g	0.173 ⁱ	0.163 ^D
NAA 0.1	0.283 ^c	0.216 ^d	0.172 ^j	0.176 ^h	0.157 ^m	0.201 ^B
NAA 0.5	0.135 ^s	0.142 ^q	0.297 ^b	0.146 ^o	0.144 ^p	0.173 ^C
Mean	0.169 ^C	0.200 ^B	0.203 ^A	0.169 ^C	0.163 ^D	
Root length (cm)						
0	1.13 ^j	3.30 ^{fgh}	3.37 ^{fg}	4.03 ^e	7.53 ^c	3.87 ^C
IBA 0.1	5.33 ^d	4.03 ^e	1.60 ^j	11.10 ^a	8.00 ^b	6.01 ^A
IBA 0.5	2.97 ^{fi}	2.77 ^{ghi}	2.63 ⁱ	3.20 ^{fi}	3.47 ^{ef}	3.01 ^E
NAA 0.1	4.03 ^e	6.80 ^d	2.73 ^{hi}	5.07 ^d	5.57 ^d	4.84 ^B
NAA 0.5	5.03 ^d	2.97 ^{fi}	3.37 ^{fg}	3.13 ^{fi}	3.53 ^{ef}	3.61 ^D
Mean	3.70 ^D	3.97 ^C	2.74 ^E	5.31 ^B	5.62 ^A	
Root number						
0	6.67 ^{a-d}	7.00 ^{abc}	4.67 ^{fi}	3.00 ^{hi}	4.00 ^{fgh}	5.07 ^A
IBA 0.1	5.00 ^{d-g}	4.00 ^{fgh}	8.33 ^a	2.00 ⁱ	5.00 ^{d-g}	4.87 ^{AB}
IBA 0.5	6.33 ^{b-c}	5.33 ^{c-g}	5.33 ^{c-g}	5.00 ^{d-g}	3.00 ^{hi}	5.00 ^A
NAA 0.1	7.33 ^{ab}	5.67 ^{b-f}	4.67 ^{fi}	2.00 ⁱ	2.00 ⁱ	4.33 ^{BC}
NAA 0.5	5.33 ^{c-g}	3.67 ^{ghi}	4.00 ^{fgh}	4.33 ^{fgh}	2.00 ⁱ	3.87 ^C
Mean	6.13 ^A	5.13 ^B	5.40 ^B	3.27 ^C	3.20 ^C	



Fig. 1: Plantlet of *Populus alba* after *in vitro* rooting 1/8 strength of MS supplemented with 0.1 mg/l NAA

IBA application at 0.1 mg/l gave significant increases in root length/young rooted shoot (6.01 cm) than remaining treatments. Regardless auxin application, full strength MS medium showed the longest root length (5.62 cm) when compared to other strengths. About the interaction between auxin and medium strength, IBA at 0.1 mg/l auxin and at 3/4 strength gave the tallest root length/young rooted shoot (11.10 cm) when compared to all remaining combinations. IBA application at 0.1 and 0.5 mg/l beside free auxin medium gave significant increases in root number/young rooted shoot (4.87, 5.00 and 6.01) than remaining treatments. Irrespective auxin application, 1/8 strength MS medium showed the highest root number (6.13) when compared to other strengths.

About the interaction between auxin and medium strength, MS medium at 1/4 strength and free of auxin medium gave the higher number of roots/young rooted shoot (8.33) when compared to almost all remaining combinations.

For the two responses, number of branches and rooting percentage, there is no change in values 1 and 100% respectively. As they didn't change with the different 25 treatments of the two factors.

Physiological Parameters

Shoots Pigments: Chlorophyll a and b and carotenoids content showed significant effect due to medium strength and auxin application and their interaction (Table 2). Concerning MS medium strength, half strength MS medium gave the highest significant values of chlorophyll a and b and carotenoids content (0.425, 67, respectively 54.832) mg/g. Whereas, the lowest values were obtained at 1/4 for chlorophyll a and at full strength for chlorophyll B and carotenoids content (0.402, 0.481 and 3.305 mg/g, respectively). For auxin application, NAA at 0.5 mg/l gave the highest chlorophyll a, b and carotenoids (0.427, 0.627 and 4.417 mg/g, respectively) while the lowest values of chlorophyll a, b and carotenoids were obtained when medium was supplemented with IBA at 0.5 mg/l (0.403, 0.562 and 4.417 mg/g, respectively). Concerning the interaction, it revealed that half strength MS medium + NAA at 0.5 mg/l (Fig. 2) gave the highest significant values of chlorophyll a, b and carotenoids (0.515, 0.861 and 7.366 mg/g, respectively).

Plantlet Total Phenolic and Flavonoids Contents: Phenolic content demonstrated in Table (2) show significant increase when micro-shoots were cultured on 1/8 strength of MS medium whereas the lowest value

was obtained from 3/4 strength of MS medium (70.90 and 34.98 mg/g, respectively). For auxin application, NAA application at 0.1 mg/l gave the highest value but the lowest value was registered at 0.5 mg/l of NAA. Flavonoid content showed significant increase when micro-shoots were cultured on 1/4 strength of MS medium whereas the lowest value was obtained from full strength of MS medium (5.59 and 4.51 mg/g, respectively).

Concerning the interaction, phenolic content gave the highest value when microshoots cultured on 1/8 strength of MS medium supplemented with 0.1 mg of NAA (11.62 mg/g). Whereas, the highest flavonoids content was assimilated on one quarter of strength of MS free auxin medium.

There are some correlations between the measured responses. The results of correlations obtained from SPSS statistical multivariate analysis. The shoot length is correlated more to root number, chlorophyll b, carotenoids and phenolic. Number of roots is quite high correlated to both shoot length and phenolics. Chlorophyll a is highly correlated with chlorophyll b and carotenoids. Chlorophyll b is correlated with shoot length, chlorophyll a and carotenoids. Carotenoids is highly correlated to shoot length, chlorophyll a and b. Total phenolic compounds are correlated with shoot length and number of roots. Both number of branches and rooting percentage responses aren't correlate to any other response. While, the other responses like number of leaves, fresh weight, root length and flavonoids have low and negative correlations to other responses.



Fig. 2: Plantlet of *Populus alba* resulted from *in vitro* rooting on half strength MS medium supplemented with 0.5 mg/l of NAA

Table 2: Average measurements of different physiological reactions within treatments of *Populus alba* (mean ± SD)

Auxin (mg/l)	Medium strength					Mean
	1/8	1/4	1/2	3/4	Full	
Chlorophyll A (mg/g)						
0	0.402 ^q	0.402 ^s	0.406 ^j	0.405 ^k	0.412 ^h	0.405 ^D
IBA 0.1	0.414 ^g	0.398 ^u	0.403 ^o	0.382 ^w	0.461 ^b	0.412 ^B
IBA 0.5	0.403 ⁿ	0.399 ^t	0.402 ^p	0.404 ^l	0.409 ^j	0.403 ^E
NAA 0.1	0.402 ^q	0.414 ^f	0.398 ^u	0.420 ^c	0.418 ^d	0.410 ^C
NAA 0.5	0.402 ^r	0.396 ^v	0.515 ^a	0.404 ^m	0.416 ^c	0.427 ^A
Mean	0.405 ^C	0.402 ^E	0.425 ^A	0.403 ^D	0.423 ^B	
Chlorophyll B (mg/g)						
0	0.618 ^k	0.602 ^l	0.626 ⁱ	0.546 ^r	0.441 ^v	0.566 ^C
IBA 0.1	0.673 ^c	0.600 ⁿ	0.586 ^p	0.367 ^y	0.592 ^o	0.563 ^D
IBA 0.5	0.502 ^t	0.584 ^q	0.661 ^d	0.647 ^e	0.420 ^x	0.562 ^E
NAA 0.1	0.601 ^m	0.696 ^b	0.643 ^g	0.626 ^j	0.440 ^w	0.601 ^B
NAA 0.5	0.647 ^f	0.634 ^h	0.861 ^a	0.478 ^u	0.513 ^s	0.627 ^A
Mean	0.608 ^C	0.623 ^B	0.675 ^A	0.533 ^D	0.481 ^E	
Carotenoids (mg/g)						
0	4.541 ^f	3.853 ⁿ	4.060 ⁱ	3.353 ^r	2.514 ^v	3.664 ^D
IBA 0.1	5.120 ^b	3.782 ^o	3.922 ^m	1.626 ^y	4.668 ^d	3.824 ^C
IBA 0.5	3.095 ^t	3.737 ^q	4.650 ^e	4.201 ^h	2.335 ^x	3.604 ^E
NAA 0.1	4.017 ^k	5.026 ^c	4.163 ⁱ	3.764 ^p	2.476 ^w	3.889 ^B
NAA 0.5	4.433 ^g	3.982 ^l	7.366 ^a	3.044 ^u	3.259 ^s	4.417 ^A
Mean	4.241 ^B	4.075 ^C	4.832 ^A	3.197 ^D	3.050 ^E	
Total phenolics (mg/g)						
0	67.41 ^b	50.36 ^{ef}	52.16 ^d	33.02 ^m	40.86 ^{hi}	48.76 ^B
IBA 0.1	62.89 ^c	51.82 ^{de}	41.99 ^{gh}	37.78 ^l	38.29 ^{kl}	46.56 ^C
IBA 0.5	48.97 ^f	51.17 ^{de}	38.38 ^{kl}	39.59 ^{ijk}	39.32 ^{jk}	43.49 ^D
NAA 0.1	113.62 ^a	40.04 ^{ji}	32.67 ^m	32.36 ^m	42.98 ^g	52.33 ^A
NAA 0.5	61.62 ^c	43.07 ^g	40.54 ^{hij}	32.13 ^m	32.44 ^m	41.96 ^E
Mean	70.90 ^A	47.29 ^B	41.15 ^C	34.98 ^E	38.78 ^D	
Total flavonoids (mg/g)						
0	4.988 ^d	7.645 ^a	4.547 ^{hij}	4.448 ^{ij}	4.050 ^k	5.14 ^A
IBA 0.1	4.689 ^{igh}	5.812 ^b	4.774 ^{ef}	5.783 ^b	4.007 ^k	5.01 ^B
IBA 0.5	4.576 ^{hij}	5.315 ^c	4.547 ^{hij}	4.661 ^{gh}	4.434 ^{ij}	4.70 ^D
NAA 0.1	4.618 ^{ghi}	4.988 ^d	4.860 ^{de}	4.036 ^k	5.684 ^b	4.84 ^C
NAA 0.5	4.888 ^{de}	4.178 ^k	4.618 ^{ghi}	4.519 ^{hij}	4.39 ^{ij}	4.52 ^E
Mean	4.75 ^B	5.59 ^A	4.67 ^C	4.69 ^C	4.51 ^D	

DISCUSSION

As the use of micro-propagated plantlets became more and more popular and require further understood for react to each growing stage. Rooting represent a critical stage in term of quality and resource consumption. Therefore, morphological description and physiological measurements performed to estimate the extent of change in plant structure due to response to change in growth media composition.

IBA at lower concentration showed a better performance than NAA on root number and length per young rooted shoots. When rooting trial was carried out to *in vitro* root shoots of *Populus alba*, results

here clearly demonstrated that both MS medium strength and auxin added had no effect at all on the rooting percentage of shoots. Their effects were mainly on leaf number, shoot length, number of roots, root length and fresh weight/young rooted shoot. Interestingly enough, MS medium at half strength supplemented with IBA at 0.1 mg/l produced the highest number of roots/ young rooted shoot in *Populus alba*. This result on agree with the result obtained by Khattab [17] on *P. alba*.

On the obvious trend, other *in vitro* rooting results were obtained elsewhere in which IBA was provided at 0.5 to 1.5 mg/l and NAA was added too at 0.5 to 2 mg/l to rooting media so as to root shoots of *Populus spp.* [7, 18-22].

Auxins are well identified to encourage root formation on stem cuttings and also induce the development of branch roots in vegetative propagation and specifically in tissue culture [23]. Auxins could promote rooting by encouraging cell elongation and/or division during *in vitro* differentiation of roots. They stimulate differentiation of vascular bundles in cells as they take part in differentiation of roots [24]. Early stages of lateral root formation are regulated by polar auxin transport [25, 26]. In specific detail, auxins cause cell elongation probably by: 1) increasing osmotic solutes of cells, 2) reducing cell wall pressure, 3) increasing permeability of cell to water, 4) increasing cell wall synthesis and 5) inducing synthesis of specific DNA new mRNA and specific enzymic proteins [27].

Both, lower strength of medium and lower auxin level are responsible for the increase of flavonoids and phenolic content. Flavonoids are essential compound in the response of plants to variable factors and they are related to pigmentation in plants and have a role in carotenoids formation and percentage. Hence, we measure the total flavonoids to correlate them to pigmentation in *P. alba* response to different media treatments. Total flavonoids are phenolic compound. Lignin is a responsible for the formation of plant cell wall especially in xylem. So, it could be an indication for intensity of formed xylem. According to Lebo *et al.* [28], lignin is cross-linked phenolic polymer. As lignin is phenolic compound, we measure the total phenolics in each treatment to estimate which treatment increase or decrease the xylem formation amount.

Further study can be performed on the genetic response of micro shoot for various auxins and medium strengths in order to understand the relation between them. Further correlation could be established to correlate between phenolic and flavonoids compound in relation to plantlet acclimatization.

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