



ANATOMICAL STRUCTURE AND MICROPROPAGATION ABILITY OF *POPULUS ALBA* L. UNDER EFFECT OF DROUGHT STRESS

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Abstract

The objective of this study was to evaluate the *in vitro* propagation ability, photosynthetic pigments contents and anatomical structure (leaf and stem) of *Populus alba* L. under various drought stress levels using different concentrations of polyethylene glycol (PEG 6000 sigma at 0.0, 5, 10, 20, 30 and 40 g/l) and alleviate the output damage of drought stress using Paclobutrazol (PBZ) at 0.5 and 1.0 mg/l. Some morphological characters such as survival percentage, number and length of shootlets / explants, number of leaves /shootlets, rooting percent, number and length of roots /shootlets were increased significantly with low concentration of PEG (5g/l), while the concentration 10 g/l PEG gave the best results for chlorophyll a, b and total carotenoids content. MS culture medium supplemented with PEG at 10g/l + PBZ at 0.5mg/l was significantly superior than other treatments for *in vitro* growth ,chlorophylls content, stem structure and caused notable enhancement in leaf blade thickness. The anatomical study showed that drought stress have a negative effect on stem and leaf anatomical structure of *Populus alba* L. plants. Also, treating plants with 0.5 mg/l PBZ under drought stress caused a relatively remercable increase in stem characters and leaf thickness and all their internal tissue.

Key words : *Populus alba* L., polyethylene glycol, paclobutrazol, *in vitro* propagation, pigments and anatomical structure.

Introduction

Forest trees are great environmental and economic importance and also display remarkable developmental traits (Groover *et al.*, 2004). Among tree species, poplar is considered model species for its phytoremediation ability of heavy metals polluted soil (Giachetti and Sebastiani, 2006 and Sebastiani *et al.*, 2004) because of its high growth rate and extensive root system (Marmiro *et al.*, 2011).

Drought is a major abiotic factor that limits agricultural crop production, to improve agricultural productivity within limited land and water resources, it is imperative to ensure high crop yield against unfavorable environmental stresses (Zhang *et al.*, 2007 and Fletcher *et al.*, 2010).

Uniconazole and paclobutrazol (Triazole) are plant growth regulators having important role in production of crops, towered to manipulation of plant growth and yield and effect in variety of morphological and biochemical responses in ornamental plants (Fletcher and Hofstra,

1990).

Simulation of drought stress underneath *in vitro* conditions throughout the regeneration method constitutes convenient thanks to study the consequences of drought on the morphogenic responses (Sakhivelu *et al.*, 2008). Tissue culturing is additionally to check the impact of abiotic stress on the cell metabolism (Das *et al.*, 1990 and Misra *et al.*, 1990). Fathy *et al.* (2019) mentioned that, the attempts to improve tree tolerant in the coming years and additional protection measures are required to maintain growth and wood production of *Populus* trees.

The aim of this work was to evaluate some morphological characters, chemical changes and anatomical structure in *Populus alba* L .cultured *in vitro* under the effect of drought stress levels.

Materials and Methods

This work was conducted at Tissue Culture Technique lab., Central laboratories, Department of Ornamental Plants and Woody Trees, National Research Centre (NRC) and Agricultural Botany Department, Faculty of

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Agriculture, Cairo University, Egypt during years 2017-2018 to evaluate some morphological, chemical changes and anatomical structure of *Populus alba* L. cultured *in vitro* under the effect of drought stress levels.

Explant source and surface sterilization

Seedling of *Populus alba* L. (two years old) were collected from nursery of Timber Trees Department Horticulture Research Institute-Agriculture Research Centre, Giza, Egypt. The shoot tips were washed then sterilized using ethanol 70% (v/v) for 30 seconds then immersed in 15% of sodium hypochlorite (Clorox) for 7 min then 1% Hg₁₂ (MC) solution (w/v) for 10 minutes and rinsed three times in sterile water.

Culture medium

After surface sterilization, nodal explants (two nodes) were cultured for one month on MS free of hormones (Murashige and Skoog, 1962) supplemented with 0.2 ppm of 6-benzylamino-purine (BAP) and 0.1 ppm indole butyric acid (IBA), 2.5% sucrose and 0.7% agar. The pH of the medium was adjusted to 5.6-5.8 then autoclaved at 121°C and 15 minutes. The *in vitro* obtained shootlets were used as explant source for two experiments.

First experiment : The micropropagation ability of *Populus alba* L. explants was examined under various concentrations (0.0, 5.0, 10, 20, 30 and 40 g/l) of polyethylene glycol (PEG 6000 sigma).

Second experiment : Alleviation of drought stress on the micropropagation of plant using two concentrations (0.5 and 1.0 mg/l) of pacbutrazole (PBZ) supplemented to each concentration of PEG that above mentioned (0.0, 5.0, 10, 20, 30 and 40 g/l).

Culture conditions

Cultures were incubated in growth chamber at 22°C under white cool florescent lamps with light intensity of 3k lux at 16 hr photoperiod. The culture period for each experiment (first and second) took two months after start of culture then, the following data were recorded:

Shooting behavior : Survival %, number of formed shootlets per explant, shootlet length (mm) and number of leaves per shootlet.

Rooting behavior : Percentage of roots formation (%) number of roots/ shootlet and root length (mm).

Photosynthetic pigments

Photosynthetic pigments (chlorophyll a and b) as well as carotenoids were determined in shootlets tissues as mg 100g fresh weight using spectrophotometer, according to the procedure achieved by Saric *et al.* (1967).

Anatomical structure

Specimens of the anatomical investigations were chosen from the median internode of the main stem and its leaf. Specimens were cut, killed and fixed in F.A.A. (10 ml formalin, 5 ml glacial acetic acid and 85 ml ethyl alcohol 70%). Then, these specimens were washed in 50% ethyl alcohol, dehydrated in normal butyl alcohol series, embedded in paraffin wax of 56 (melting point) and cut with a rotary microtome. Finally samples were stained with crystal violet and erythrosine, mounted in Canada balsam (Nassar and El-Sahhar, 1998). The slides examined with a photo-microscope, counts and measurements (μ) of the different tissues were calculated.

Statistical analysis

The average of data recorded for different parameters statistically analyzed using randomized complete block design with three replicates per treatment. The treatments means were compared for significance by Duncan's New Multiple Range test at 0.05% level of probability (Duncan, 1955) using COSTATV-63.

Results and Discussion

In vitro shooting and rooting behaviors of *Populus alba* under effect of drought stress degrees

The effect of various concentrations of PEG (0, 5.0, 10, 20, 30, and 40g/l) on *in vitro* propagation behavior of *Populus alba* L. plantlets is illustrated in table 1. For evaluation of drought stress, it was observed that the low concentrations of PEG (5.0 and 10 g/l) supplemented to the culture medium increased the percentages of both survived explants and rooting of shootlets as well as the length of shootlets and roots. While, using PEG at 40g/L led to the lowest survival percent and shortest shootlets as compared to control treatment. However, the highest numbers of shootlets formed per explant, leaves and roots per shootlets were in the highest values due to using the low concentrations of PEG.

These results are similar to those reported for *in vitro* drought screening of other plant species (Bidabadi *et al.*, 2012). It is interesting to note that the intensity of multiplication rate and shoot vigour reduction under certain levels of PEG treatment was not genotype dependent, as this contrasts with the results reported by Sakthivelu *et al.* (2008) on soybean shoot vigour that was also declined with increasing concentration of PEG. This may be a consequence of hampered water and nutrient absorption due to a decreasing water potential of the medium or cell elongation (Jaleel *et al.*, 2009). Drought stress is a major abiotic stress factor affecting significantly success of plant

Table 1 : *In vitro* shooting and rooting behaviors of *Populus alba* L. under effect of drought stress

Characters Treatment (g/l)	Survival	Number of shoolets/ explant	Shoot length (mm)	Leaves number/ shoolet	Rooting %	Number of Root/ shoolet	Root length (mm)
Control	100.00 a	1.33a	27.90a	9.20 a	100.00 a	1.78 a	41.73ab
PEG 5.0	100.00 a	1.33 a	18.10 b	7.73a	33.30 b	0.50b	46.67 a
PEG 10	88.87ab	1.11a	16.80 b	8.83 a	22.30b	0.50b	46.67 a
PEG 20	66.60 bc	0.60b	13.30bc	6.25ab	11.10b	0.17b	15.0ab
PEG 30	44.40c	0.50b	11.70bc	5.97ab	11.10b	0.17b	5.00 ab
PEG 40	11.10d	0.11c	6.67c	3.00b	0.00	0.00	0.00

Means within a column having the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level.

Table 2 : *In vitro* shooting and rooting behaviors of *Populus alba* L. under effect of drought stress levels and paclobutrazole.

Characters Treatment (g/l)	Survival	Number of shoolets/ explant	Shoot length (mm)	Leaves number/ shoolet	Rooting %	Number of Root/ shoolet	Root length (mm)
Control	100.00 a	4.33 a	46.36 a	24.66 bc	100.00 a	6.00 a	54.43 a
PEG (5.0g/l) + PBZ (0.5 mg/l)	88.86 a	3.00 ac	37.76 b	13.00 d	22.20 bc	1.66 bc	16.23 b
PEG (5.0g/l) + PBZ (1.0 mg/l)	88.86 a	3.66 ab	35.0bc	24.00 bc	11.10 c	0.66 c	10.66 b
PEG (10g/l) + PBZ (0.5 m/l)	100.00 a	4.33 a	40.00 ab	32.66 a	44.40 b	4.30 ab	16.66 b
PEG (10g/l) + PBZ (1.0 mg/l)	100.00 a	3.00 ac	27.00 cd	26.89 ab	0.00	0.00	0.00
PEG (20g/l) + PBZ (0.5 mg/l)	55.50 b	1.66 c	20.94 de	17.33 cd	0.00	0.00	0.00
PEG (20 g/l) +PBZ (1.0 mg /l)	77.73 ab	2.66 bc	19.88 de	17.11 cd	0.00	0.00	0.00
PEG (30 g/l)+PBZ (0.5 mg /l)	100.00 a	3.00 ac	18.69 df	22.33 bc	0.00	0.00	0.00
PEG (30 g/l) +PBZ (1.0 mg /l)	77.73 ab	2.00 c	16.08 ef	17.33 cd	0.00	0.00	0.00
PEG (40 g/l) +PBZ (0.5 mg /l)	88.86 a	2.60 bc	15.33 ef	16.66 cd	0.00	0.00	0.00
PEG (40 g/l) +PBZ (1.0 mg /l)	88.86 a	3.00 ac	9.95 f	12.76 d	0.00	0.00	0.00

Means within a column having the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level.

production (Hassanein and Dorion, 2006).

***In vitro* shooting and rooting behaviors of *Populus alba* under effect of drought stress levels and paclobutrazole**

The results in table 2 showed that the micropropagation ability of *Populus alba* L. cultured on MS medium supplemented with PEG(10g/l) and PBZ (0.5 mg/l) was significantly superior than the other treatments and gave the highest shoot number per explant, highest values of shooting and rooting characters comparing with other treatments, while increasing the concentrations of PEG and PBZ in the medium decreased these characters. The results are in agreement with those obtained by Anchalee and Bodhipadma (2012) on some species of *Curcuma*, pointed out that Triazole compounds protect plants from various environmental stresses including chilling, drought, heat, water logging, air pollutants and

heavy metals.

Effect of drought stress levels on chlorophylls (a,b) and total carotenoids content

Data in Fig. 1 observed that adding PEG at 10g/l to the culture medium significantly increased chlorophyll a content to the highest value, while using PEG at 40 g/l declined this value to the lowest one and significantly reduced chl. a as compared to control. Decreasing the concentration of PEG to 10g/l in the culture medium significantly increased chl. b as well as carotenoids contents giving the highest values, whereas using PEG at 40 g/l significantly decreased these values as compared to control.

Water stress adversely impacts many aspects of the physiology of plants, especially photosynthetic capacity (Osakabe *et al.*, 2014). Water stress directly affects rates

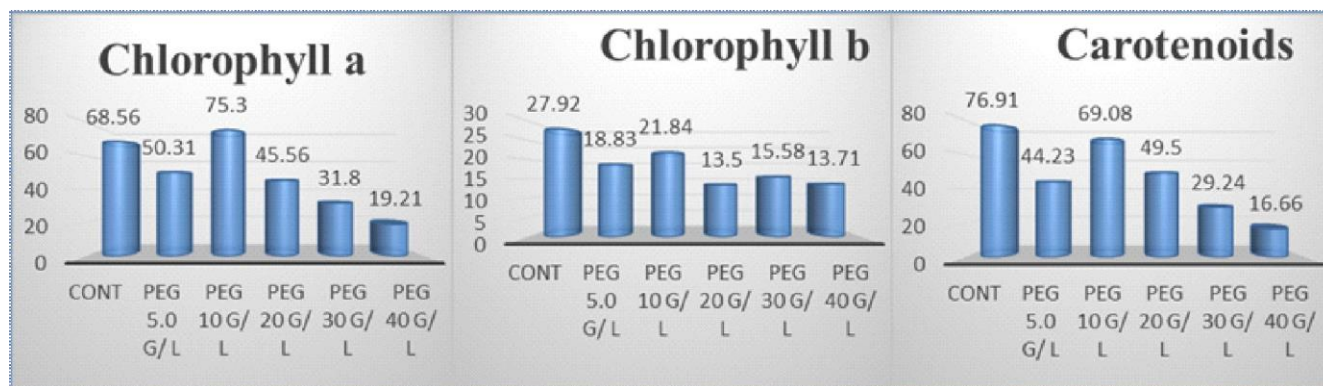


Fig. 1 : Effect of drought stress levels on Chlorophyll a, b and total carotenoids content of *Populus alba* L.

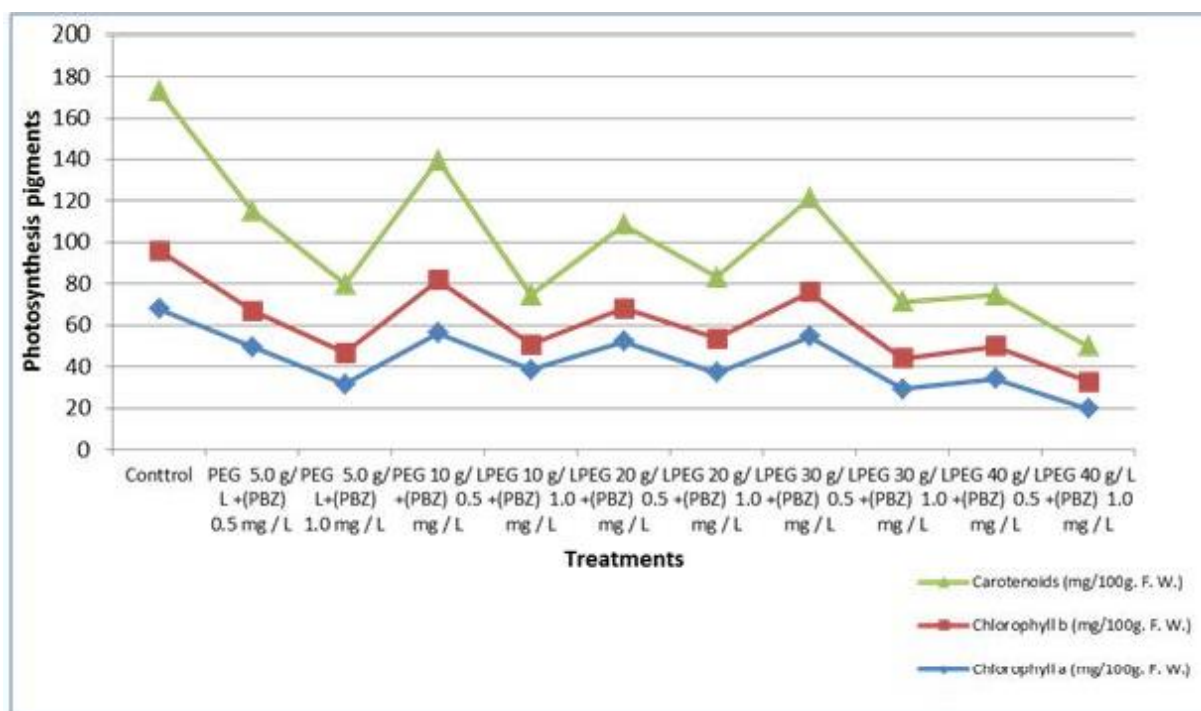


Fig. 2 : Effect of drought stress levels and paclobutrazole on photosynthesis pigments contents of *Populus alba* L.

of photosynthesis due to the decreased CO₂ availability resulted from stomata closure and/ or from changes in photosynthetic metabolism (Chaves *et al.*, 2009).

Drought has a negative effect on photosynthesis when the rates of photosynthesis are reduced by water stress (Osakabe and Osakabe, 2012).

Effect of drought stress levels and paclobutrazole interaction on Chlorophylls(a,b) and total carotenoids content

The influence of PEG and paclobutrazole at various concentrations is shown in fig. 2 recorded that treating *Populus alba* L. plant with PEG 10g/l and PBZ (0.5 mg/l) increased chlorophyll a content to the highest value. While, using PEG (40 g/l) and PBZ (1.0 mg/l) declined this value and significantly reduced chl. a but increased

chl.b contents (giving the highest value) as compared to control and other treatments. While, the highest content of carotenoids were found at the concentration of PEG 10g/l +PBZ 0.5 mg/l comparing with other treatments.

Our results were confirmed with those found by Pinhero and Fletcher (1994) on maize seedlings. Aboelfazl *et al.* (2013) reported that treating plants of banana with PBZ increased the total leaf chlorophyll content when compared with the control treatment.

Our results showed that PBZ concentration had significant effect on leaf chlorophyll content. PBZ as one in triazol group stimulates cytokinin synthesis that enhanced chloroplast differentiation, chlorophyll biosynthesis and prevents chlorophyll degradation (Fletcher *et al.*, 2000).

Table 3: Microscopic measurements (μ) and counts of certain anatomical features in transverse sections through the median portion of the main stem of *Populus alba* L. grown under drought stress (PEG, 0, 10, 30 g/L) and treated by 0.5 mg/l paclobutrazol.

Histological characters	Control	10g/l PEG	% of 10g/l PEG to control	30g/l PEG	% of 30g/l PEG to control	10g/l PEG +0.5PBZ	% of 10g/l PEG +0.5 PBZ to control	% of 10g/l PEG +0.5 PBZ to 10g PEG
Av. Stem diameter	1290.00	1185.00	-10.20	1140.00	-11.63	1890.00	+46.51	+59.49
Av. Epidermis thickness	18.40	16.00	-13.04	12.80	-30.43	19.20	+4.30	+20.00
Av. Cortex thickness	195.00	200.00	+2.56	170.00	-12.82	352.50	+80.76	+76.52
Av. Phloem tissue thickness	62.50	50.00	-20.00	31.25	-50.00	125.00	+100.00	+150.00
Av. Xylem tissue thickness	87.50	62.50	-28.57	43.75	-100.0	150.00	+71.42	+140.00
Av. Vessel diameter	28.00	24.00	-14.28	22.00	-21.42	24.00	-14.28	0.00
Av. Pith diameter	587.50	562.50	-4.25	450.00	-30.55	600.00	+2.13	+6.67

Table 4: Microscopic measurements (μ) and counts of certain anatomical features in transverse sections through the median portion of the *Populus alba* L. leaf grown under drought stress (PEG 6000 sigma, 0, 10, 30 g) and treated by 0.5 mg/l paclobutrazol

Histological characters	Control	10g/l PEG	% of 10g/l PEG to control	30g/l PEG	% of 30 g/l PEG to ontrol	10 g/l PEG+ 0.5 PBZ	% to control	% to 10g PEG
Av. Lamina thickness	125.00	125.00	0.00	162.50	+30.00	212.50	+70.00	+70.00
Av. Upper Epidermis thickness	24.00	22.00	-8.33	18.00	-25.00	28.00	+16.67	+27.27
Av. Palisade tissue thickness	50.00	81.25	62.5	75.00	+50.00	112.50	+125.00	+38.46
Av. Spongy tissue thickness	75.00	75.00	0.00	100.00	+33.33	162.50	+116.67	+116.67
Av. midvein thickness	537.50	500.00	-6.97	375.00	-30.23	475.00	-11.63	-5.00
Av. Xylem thickness	96.00	80.00	-16.67	56.00	-41.67	76.00	-20.83	-5.00
Av. Xylem vessel diameter	20.00	12.00	-40.00	8.00	-60.00	16.00	-20.00	33.33

Effect of drought stress on stem anatomical structure

Microscopic measurements of the stem transverse sections of *Populus alba* L. plant as affected by drought stress and treated with paclobutrazol (0.5mg/l) are given in table 3 and fig. 4. Data showed that there was a difference between the control and PEG 10 g/l treatments in all characters; Whereas, there was a highly decrease in these characters between control and 30 PEG treatments. Furthermore, the stem diameter and epidermis thickness were decreased with increasing drought stress compared to control. It might be due to the shrinkage and malformation in included tissue with increasing drought stress. Additionally, the cortex tissue thickness and the average vascular cylinder diameter were also decreased due to all PEG treatments. Moreover, drought stress induced structural changes in xylem tissue as the diameter of xylem vessels were decreased. Relative to the control, Xylem vessel diameter was decreased by 14.28 and 21.42% at 10 g/l and 30g/l PEG, respectively. The results of the present study are in agreement with those obtained by Lovisolo and Schubert

(1998) as well as Burnett *et al.* (2005). They found that the stem and phloem diameter and xylem cross-sectional area were typically decreased with increasing PEG concentrations. In this work it is noticed that *Populus alba* L. plants were micropropagated under 10g PEG drought stress with paclobutrazol (0.5mg/l) caused a relatively remarkable increase in stem diameter (46.51%), cortex thickness and all its internal components. The increases that occurred in stem diameter due to the applied paclobutrazol (0.5 mg/l) were linked with another increase in thickness of vascular cylinder diameter as well as pith diameter. It is observed also that, both xylem and phloem tissue thickness of the plants grown under drought stress when interacted with paclobutrazol (0.5 mg/l) showed remarkable increase in thickness, but the averages of vessels diameter were decreased as compared to their respective control.

Effect of drought stress on leaf anatomical structure

The obtained results in table 4 and fig. 5 showed that, there was a negative impact on most leaf anatomical characters of *Populus alba* L. plant as a result of



Fig. 3 : *In vitro* shooting induction of *Populus alba* (A): Rooting of shootlets cultured on PEG 10g/l; (B): Prepared rooted plantlets for the acclimatization stage and (C, D): Acclimatized plants to greenhouse.

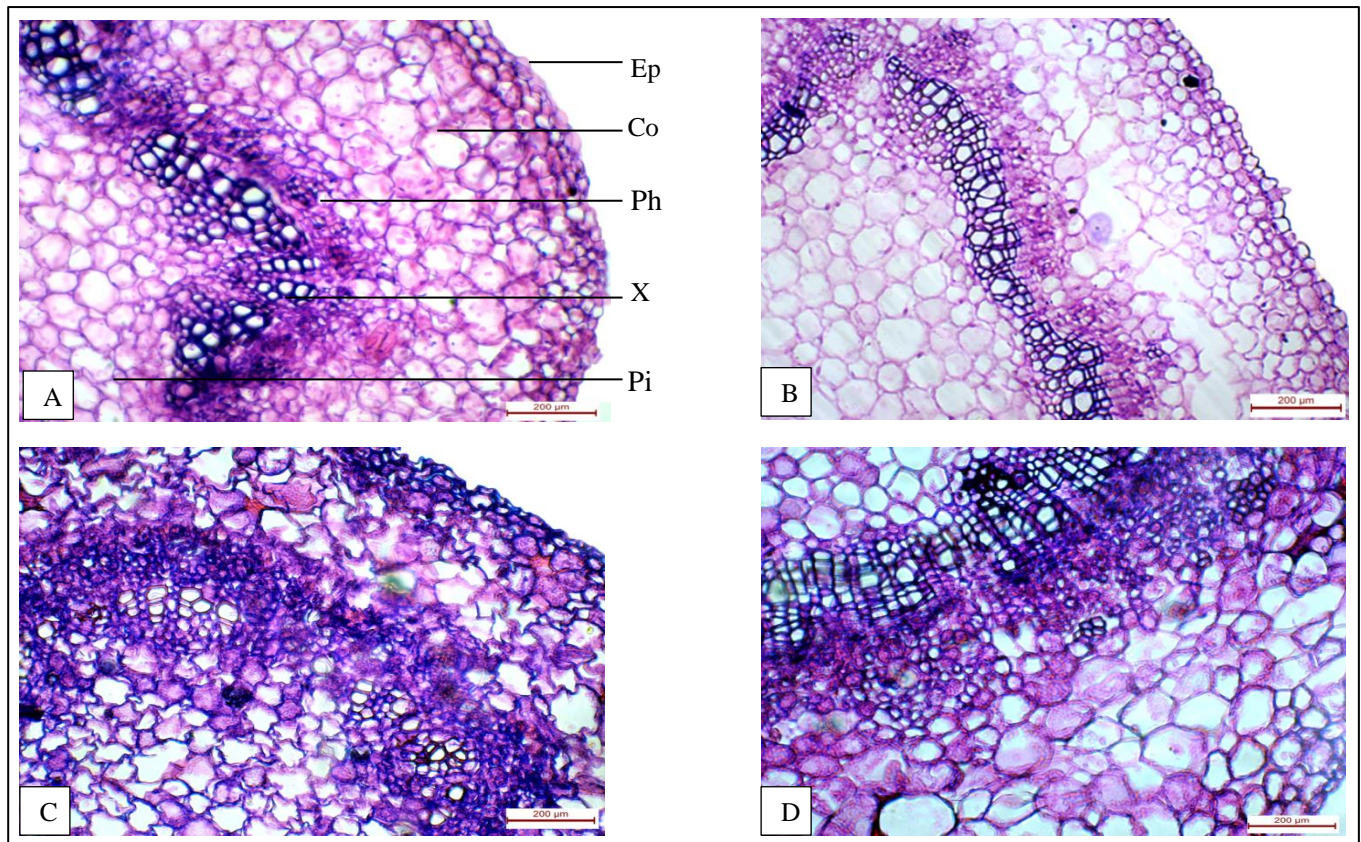


Fig. 4 : Transverse sections of the median portion of the main stem of *Populus alba* L. plant as affected by drought stress and treated with paclobutrazol.

A. Control plant. B. Plant grown under 10g PEG 6000 sigma drought stress. C. Plant grown under 30g PEG 6000 sigma drought stress. D. Plant grown under 10 PEG 6000sigma drought stress and treated with PBZ (0.5 mg/l).

Details: co, cortex; ep, epidermis; ph, phloem; pi, pith; x, xylem.

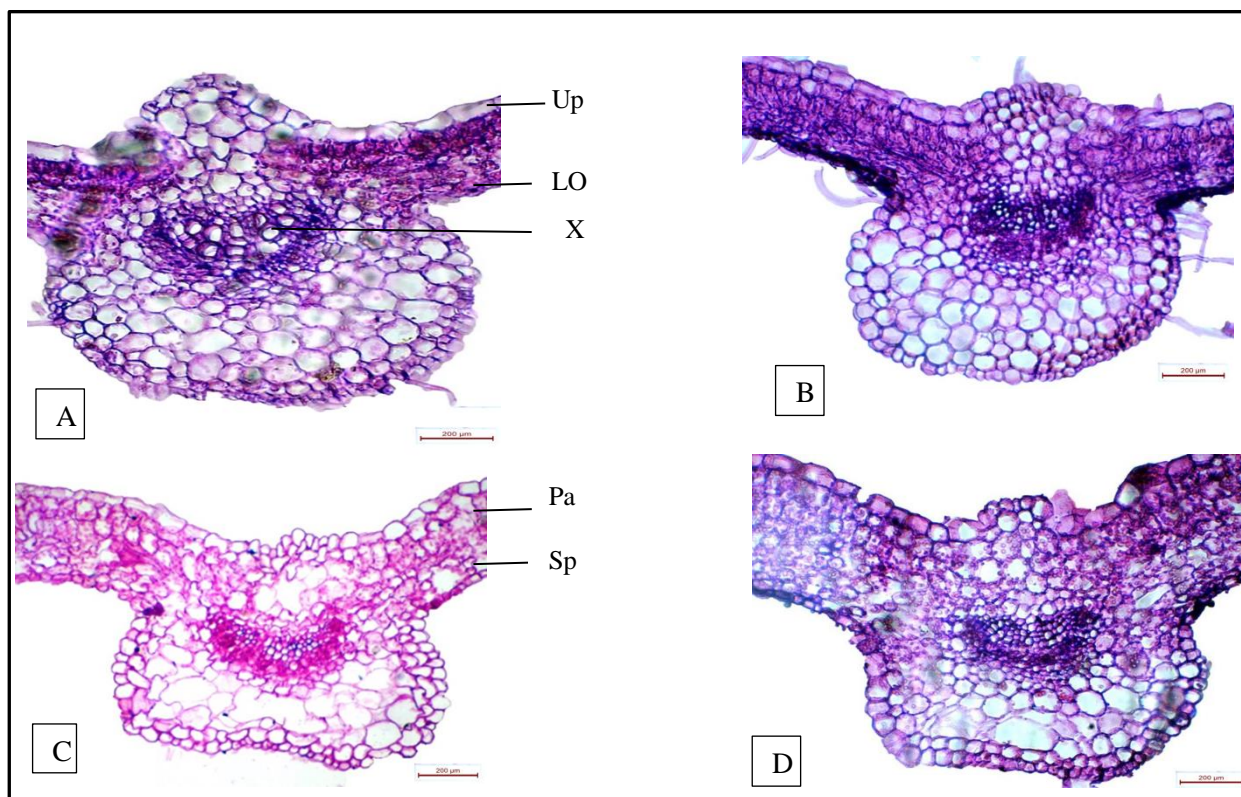


Fig. 5 : Transverse sections of the median portion of median leaf on the main stem of *Populus alba* L. plant as affected by drought stress and treated with paclobutrazol.

A. Control plant

B. Plant grown under 10g PEG 6000 sigma drought stress.

C. Plant grown under 30g PEG 6000 sigma drought stress.

D. Plant grown under 10g PEG 6000 sigma drought stress and treated with PBZ (0.5 mg/l).

Details: Lo: Lower epidermis; Pa: Palisade tissue; Sp: Spongy tissue; Up: Upper epidermis; X: Xylem

increasing PEG levels. The reduction of these values were obtained in thickness (μ) of midvein, lamina, upper epidermis, mesophyll tissue, xylem tissue and xylem vessels diameter for *Populus alba* L. plant. The minimum reduction was detected with drought treatment 10g/l PEG, while the maximum reduction was achieved at 30g/l of PEG. Additionally, the average bundle diameter was also decreased due to both applied PEG treatments. By increasing the drought level, the midvein thickness comparing with the control was decreased. Palisade tissue was appeared to be more compact and the epidermal cells had dense trichomes on plants grow under 10g/l PEG compared to control and 30g/l PEG. The thickness of the midvein was remarkably reduced with increasing drought stress. Furthermore, the thickness of the epidermis was decreased by increasing the drought stress either at 10, 30g/l of PEG over the unstressed plants that were 8.33 and 25.00%, respectively.

The obtained results showed decrease in average xylem tissue thickness and xylem vessel diameter of the main vascular bundle of the leaf midvein. The average

Of xylem tissue thickness and xylem vessel diameter recorded 56.00 and 8.00 μ under 30g /l PEG comparing with 96.00 and 20.00 μ under control treatment. The reduction in leaf anatomical measurements as a result of harmful effect of drought stress that was due to decreasing of morphological characters, *i.e.* leaf area, physiological characters, water potential, photosynthetic rate, transpiration rate, and stomatal conductance (Klamkowski and Treder, 2008). Moreover, reduced water availability induces numerous physiological and biochemical changes in all plant organs. Stomatal conductance and photosystem in leaves is limited, which in turn reduces leaf and shoot photosynthesis rate (Zhao *et al.*, 2010). In addition, increasing PEG level or treatment with PBZ increased in the mesophyll layer thickness comparing with control treatment. It is clear that the application of PEG (0.5mg/l) under 10g PEG drought stress caused enhancement in leaf blade structure compared to control. Hence, the palisade and spongy tissues of mesophyll were well differentiated, and the cells became wider and longer at PBZ (0.5mg/l) under 10g/l of PEG

as compared to the shorter palisade and spongy parenchyma of control.

The number of the palisade and spongy layers were increased with PBZ (0.5mg/l) treatment as compared to PEG treatments and unstressed plants. Treated plants with PEG led to much narrow xylem vessels when compared to control plants and 10g/l PEG treatments. The same trend was found with the average xylem vessel diameter, recorded 16 μ . These results were in agreement with the findings of Sankar *et al.* (2014) on peanut plants.

Conclusion

The results of this study indicated that *Populus alba* L. explants could be put out drought stress *in vitro* using PEG (5g/l) alone and PEG at 10g/l + PBZ at 0.5mg / l treatments and they were significantly superior than other treatments for *in vitro* growth, chlorophylls content and anatomical structure of stem and leaf.

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