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INFLUENCE OF CALCIUM CARBONATE AND KAOLIN ON GROWTH, DROUGHT RESISTANCE INDEX AND PHYSIOLOGICAL TRAITS OF PAULOWNIA (*PAULOWNIA TOMENTOSA,* THUNB.) SEEDLINGS UNDER DROUGHT STRESS CONDITIONS

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ABSTRACT

Two pot experiments were carried out at lath house in the Nursery of Ornamental Plants, Hort. Dept., Zagazig Univ., Egypt during the two spring consecutive seasons of 2018/2019 and 2019/2020 to study the effect of water stress (irrigation at 100, 75 and 50 % from field capacity), antioxidants type [(distilled water as control, 4 g/l of calcium carbonate (CC) and 30 g/l of kaolin (Ka)] as foliar applications and their interaction treatments on growth parameters, physiological traits, drought resistance percentage and some chemical constituents of paulownia seedlings. Plant height, stem diameter, leaf number per plant, dry weight of leaves per plant, drought resistance index, wood density and total chlorophyll content decreased significantly with increasing drought treatments from 100% to 50 % field capacity. However, water stress significantly increased soluble sugar content and proline content in leaves compared to full irrigation treatment. Furthermore, using each calcium carbonate or kaolin significantly increased all abovementioned parameters compared to control (sprayed with distilled water) in both seasons. Generally, it could conclude that 30g/l Ka showed a uniform influence in alleviating of *Paulownia tomentosa* growth inhibition and its wood density under moderate water stress condition with increasing in drought resistance percentage to reach 105.68 and 102.09 % in the first and second seasons, respectively.

Keywords: *Paulownia tomentosa,* drought stress, antioxidants, growth, wood density, salt resistance, chlorophyll

INTRODUCTION

Princess tree (*Paulownia tomentosa*, Thunb.) belongs to the family Scrophulariaceae (Hu, 1959). Paulownia, a hardwood tree, is described with very fast-growing and short-rotation plant with large leaves coordinated in obverse pairs on the stem. It is growing found in several parts of the world including Japan, Southeast Asia, China and North and Central America as well as Australia and Europe (Rahman *et al*., 2013). Several Paulownia species are planted in various temperate zones worldwide due to their fast growth and timber market value (Yadav *et al*., 2013). The Paulownia wood can avail as a kindly material for furniture making, chests, boxes, lumber, composting and coal (Rafighi and Tabarsa, 2011).

For the time being, Egypt faces a problem in the irrigation amounts water because the Egyptian water budget is specified, thus the prime procedure of the Egyptian strategy is enhancing crops productivity from unit area with the lowest water of irrigation (Tamer, 2014 and Ramadan and Omar, 2017). Water stress is one of the most serious environmental limitations impacting the plant development, growth and productivity (Fereres and Soriano, 2007 and Geerts and Raes, 2009).

Foliar spray with anti-transpirants may do minimize the transpiration rate. Calcium carbonate can hold stomata from opening perfectly by influencing stomatal guard cells, lessening losses of water vapor (Steinberg *et al*., 1990). The nature of particles as a rule includes minerals of high reflectivity, among them calcium carbonate ($CaCO_3$) is alternatives of relative minimum cost, safe utilize, low abrasion, decreased particle size and water prevalence capability (Glenn *et al*., 2003). Moreover, kaolin is a white nonabrasive fine-grained and contains alumino silicate mineral [$Al_4 Si_4O_{10}(OH)_8$] that has been purified and sized so that it acts as an anti-transpirant and easily disperses in water as well as reducing drought stress on plants (Puterka *et al*., 2000). Kaolin as a particle film has long been utilized to limit the impact of heat and water stress on several plants (Azizi *et al*., 2013). In addition, it enhanced the plant height (cm), total plant dry mass (g) and water utilized efficiency in water-stressed *Physalis peruviana* seedlings (Segura-Monroy *et al*., 2015). Furthermore, Ramadan and Omar (2017) pointed out that plant growth and yield as well as N, P and K contents increased with evaporation replenishment at 80% level and with the application of antitranspirants (especially by using $CaCO_3$ and kaolin) under water stress (60%).

The goal of this work is an effort to enhance paulownia seedlings growth, drought resistance index, wood density and pigments under water stress during the summer season using some anti-respirations as calcium carbonate and kaolin to minimize water losses under Sharkia Governorate conditions.

MATERIALS AND METHODS

Two field experiments were carried out during the two consecutive winter seasons of 2018/2019 and 2019/2020 at lath house in the Nursery of Ornamental Plants, Hort. Dept., Zagazig Univ., to study the effect of water stress (irrigation at 100, 75 and 50 % from field capacity), anti-respiration types as foliar applications [(distilled water as control, 4 g/l of calcium carbonate (CC) and 30 g/l of kaolin (Ka)] and their interaction treatments on growth parameters, physiological traits, drought resistance percentage and some chemical constituents of paulownia seedlings. This experiment included 9 treatments, which were arranged in a split-plot design with three replicates. The water stress treatments were randomly arranged in the main plots; also, the anti-respirations (ATs) types were randomly distributed in the sub plots.

Paulownia tomentosa plantlets were obtained from Plant Tissue Culture Laboratory, Hort. Depart., Fac. Agric., Zagazig Univ., Egypt. The symmetrical and healthy of 6 months old seedlings were selected as plant material for this study. The irrigation treatments (water stress levels) consisted of three replicates, each replicate consisted of five seedlings. Paulownia seedlings were planted in pots 30 cm filled with soil mixture of clay and sand (1/1, v/v), on the 8st October during both seasons. The physical and chemical properties of the used soil mixture are shown in Table 1 according to Chapman and Pratt (1978). The paulownia seedlings were irrigated with tap water for 15 days before starting water stress treatments. The irrigation treatments were executed by weighing the 5 pots contained soil mixture only without seedlings planting per water stress level every three days and adding the consumed amount of water during the full period of experiment to get the percentage of moisture in the treatment. Field capacity was calculated according to Kulte (1986).

75 days from planting date. The untreated plants (control) were sprayed with distilled water.

Data recorded

Growth parameters

Plant height (cm), stem diameter (cm), number of leaves per plant and leaves dry weight/plant (g) were recorded after 90 days from planting date. Three plants were randomly chosen from each experimental unit in the two seasons.

Physiological parameters

Leaf relative water percentage, drought resistance index and wood density were tabulated.

Leaf relative water percentage

Leaf relative water percentage (LRW%) was determined by measuring leaf fresh weight (FW), hydrated (TW) (24h, at 4°C in the dark on contact with a moist filter paper) and oven dry weights (DW) of leaf disks (Barrs and Weatherley, 1962).

The LRW % is determined as follows: LRW% = (FW – DW)/ (TW – DW) × 100.

The drought resistance index

The drought resistance index (DRI %), as a real indicator for drought stress tolerance was calculated from the equation mentioned before by Bidinger *et al.,* (1982) on pearl millet: DRI (%) = Mean total dry plant (stem + leaves) of the water stress treated plants/mean total dry plant of control one × 100.

Wood density (g/cm^3)

Samples were cut transversely into disks and diameter and thickness were measured to calculate wood density. Each sample disk was oven-dried at 70˚C to a constant

			Physical analysis						Soil texture
Clay (%)				Silt (%)			sand (%)		Sandy
22.87				8.43			68.70		

Time	pH	E.C. (dsm^{-1})	Soluble cations (m.mol/l)					Soluble anions (m.mol/l)		Available (ppm)			
								Chemical analysis					
		Ca^{++}	Mg^{++} / Na^{+}	Zn^{++}		Mo^{++}	Cl^{-}	HCO_3^{-} / SO_4^{--}	N	P		K	
Before planting	**7.70**	0.58	1.80	0.95	0.30	1.10	1.32	3.04	1.12	0.84	127	46	51

Table 1. Physical and chemical properties of experimental farm soil (average of two seasons)

The basal doses of nitrogen (N), phosphorous (P2O5) and potassium (K2O) were applied in each pot at the rate of 140 mg/kg, 60 mg/kg and 40 mg/kg through ammonium sulphate, single superphosphate and potassium sulphate respectively at 30, 50 and 70 days of planting date. Moreover, calcium carbonate and kaolin rates were added as foliar application. The first application began after 30 days after planting and repeated every two weeks until

weight and then weighed (g). The sample volume (cm^3) was estimated by displacing water from a graduated glass beaker. Wood density was calculated by dividing sample dry weight by volume (Oh-uchi, 1989).

Leaves chemical constituents

Total chlorophyll content (SPAD unit), soluble sugars content (mg/g f.w.) and proline acid content (mg/g f.w.)

were determined.

Total chlorophyll

In fresh leaf samples of existing paulownia seedlings after 75 days from planting date during both seasons, it was measured by using SPAD- 502 meter as described by Markwell *et al.,* (1995).

Soluble sugars content in leaves (mg/g as fresh weight)

The amounts of soluble sugars were measured by phenol-sulfuric acid method (Robyt and White, 1987). The absorbance was noticed by a spectrophotometer at a 640 nm wavelength and sugars contents were calculated utilizing a standard curve of glucose.

Proline content in leaves (mg/g as fresh weight)

Proline was determined calorimetrically as ninhydrine complex in toluene (Troll and Lindesly, 1955). The photometric absorbance of the Toluene extract was executed utilizing a spectrophotometer at a 528 nm wavelength of utilizing a standard curve of proline.

Statistical Analysis

Data of the present study were statically analyzed and the differences between the means of the treatments (water stress levels and anti-respiration types) were considered significant when they were more than the least significant differences (L.S.D) at the 5% levels by using computer program of Statistix Version 9 (Analytical Software, 2008).

RESULTS AND DISCUSSION

Growth parameters:

It is quite clear from data in Tables 2 and 3 that, paulownia seedlings that received 75 and 50 % of field capacity significantly decreased plant height, stem diameter, number of leaves per plant and leaves dry weight compared to control (100 % of field capacity) in both seasons. In general, paulownia growth traits were decreased with the decreasing of irrigation levels to reach its minimum by using that of 50 % of field capacity. Furthermore, using anti-respiration treatments (calcium carbonate and kaolin) significantly increased paulownia height, stem diameter as well as leaf number and leaf dry weight per plant compared to untreated plants in 2018/2019 and 2019/2020 seasons. In most cases, using treatment of 30 g/l Ka significantly increased growth parameters compared to control and 4 g/l CC in both seasons. Moreover, the increases in dry weight of leaves per plant was about 11.88 and 21.92 % for kaolin treatment, with significant difference between this treatment and control (unsprayed plants) in the 1st and 2nd seasons, respectively. Furthermore, the combination between drought stress and CC or Ka decreased abovementioned parameters comparing to control (except that of 100 % of field capacity plus Ka or CC).

The ability of paulownia seedlings on noticed lower height, stem diameter, leaf number and weight per plant at all water stress levels than those of full irrigation one may be elucidated according to Webster *et al.,* (2000), they pointed out that water stress inhibited the cytokinin transport from root to shoots and enhanced the amount of leaf abscisic acid which reflected in decreasing growth parameters. These results may be due to the evasion mechanism through modification of plant growth rate or reduced rate of photosynthesis. Reduction in terms of growth parameters were obtained for *Populus kangdingensis* (Yin *et al*., 2005), *Populus davidiana* (Zhang *et al*., 2005) and *Populus cathayana* (Xiao *et al*., 2009). However, Hamdy *et al.,* (2016) reported that the maximum values of vegetative growth parameters of pomegranate seedlings were achieved when the seedlings were irrigated with 100 % of field capacity, while the minimum values in this concern were recorded at 40 %

Drought stress (Field capacity %)	antioxidants type							
	0.0	$CaCO_3$	Kaolin	Mean (A)	0.0	$CaCO_3$	Kaolin	Mean (A)
	2018/2019 season				2019/2020 season			
	Plant height (cm)							
100	62.40bc	63.80ab	65.00a	**63.73A**	61.97c	65.13b	71.40a	**66.17A**
75	58.43e	60.97cd	61.83cd	**60.41B**	60.17d	60.47d	62.73c	**61.12B**
50	57.47e	60.63d	61.13cd	**59.74B**	57.37f	58.67e	59.90de	**58.64C**
Mean (B)	**59.43B**	**61.80A**	**62.66A**		**59.83C**	**61.42B**	**64.68A**	
	Stem diameter							
100	1.17bc	1.27a	1.28a	**1.24A**	1.22c	1.25b	1.30a	**1.26A**
75	1.09e	1.16c	1.20b	**1.15B**	1.13e	1.19d	1.24bc	**1.19B**
50	0.99f	1.11de	1.13d	**1.07C**	0.88h	0.98g	1.06f	**0.97C**
Mean (B)	**1.08C**	**1.18B**	**1.20A**		**1.08C**	**1.14B**	**1.20A**	

Table 2. Effect of water stress (A), antioxidants type (B) and their interaction (A×B) treatments on plant height (cm) and stem diameter (cm) of *Paulownia tomentosa* seedlings during the two seasons of 2018/2019 and 2019/2020

of field capacity. Drought in summer in southern China negatively influences the growth of oil tea seedlings(Dong *et al*., 2017).

Drought stress (Field capacity %)	antioxidants type							
	0.0	$CaCO_3$	Kaolin	Mean (A)	0.0	$CaCO_3$	Kaolin	Mean (A)
	2018/2019 season				2019/2020 season			
	Number of leaves/plants							
100	11.22cd	11.78b	12.44a	**11.81A**	11.56d	12.34b	12.78a	**12.22A**
75	10.44e	11.00d	11.56bc	**11.00B**	10.89e	11.78cd	12.11bc	**11.59B**
50	9.22 g	9.56fg	9.89 f	**9.56C**	9.44 g	9.89 f	10.22f	**9.85C**
Mean (B)	**10.30C**	**10.78B**	**11.30A**		**11.70A**	**11.34B**	**10.63C**	
	Leaves dry weight / plant (g)							
100	3.30b	3.79a	3.80a	**3.63A**	3.86cd	4.21b	4.73a	**4.27A**
75	2.93c	3.31b	3.33b	**3.19B**	3.34e	3.67d	4.10bc	**3.70B**
50	2.85c	3.02c	3.03c	**2.97C**	2.79f	3.25e	3.36e	**3.13C**
Mean (B)	**3.03B**	**3.37A**	**3.39A**		**3.33C**	**3.71B**	**4.06A**	

Table 3. Effect of water stress (A), antioxidants type (B) and their interaction (A×B) treatments on number of leaves per plant and dry weight of leaves per pant (g) of *Paulownia tomentosa* seedlings during the two seasons of 2018/2019 and 2019/2020

Kaolin and calcium carbonate treatments resulted in higher plant height, stem diameter and plant dry weight, which could be attributed to the decreased water loss due to the reduction of leaf temperature and vapor pressure deficit (Dordas, 2009; Roussos *et al*., 2010), resulting in lower transpiration and higher water use efficiency. Since, calcium carbonate ($CaCO_3$) treatments significantly affect the fresh and dry weights of *Satureja hortensis* herb which were increased by applying 5 t ha^{-1} $CaCO_3$ (Babalar *et al*., 2010). Likewise, Kaolin treatment improved the *Physalis peruviana* seedlings plant height and total plant dry mass in water-stressed plants (Segura-Monroy *et al*., 2015). Moreover, Hagagg *et al.,* (2019) showed that spraying Kalamata and Manzanillo olive cultivars with Calcium Carbonate at 7% on was the promising treatment for increase vegetative growth (shoot length and diameter) compared with other treatment and control.

Physiological parameters

Data recorded in Table 4 suggest that, leaf relative water content, drought resistance index percentage and wood density were significantly decreased with 75 and 50 % levels of field capacity compared with full irrigation level in both seasons. In other words, the decreases in wood density were about 10.83 and 8.48 % for the 75% of field capacity level as well as 18.41 and 18.11 % for the 50 % of field capacity level, with significant difference between these treatments and control (100 % irrigation level) in the 1st and 2nd seasons, respectively. *Paulownia tomentosa* drought resistance index (%) was significantly increased by using $CaCO_3$ or Kaolin as anti-respiration compared to control in both seasons. In the same time, drought resistance index, leaf relative water percentage and wood density of paulownia seedlings was increased as a result of the treatments of kaolin combined with most of water stress levels compared to the combination between anti-respiration types and the highest level of water stress in the two seasons. Gindaba *et al.,* (2004) found that severe water stress resulted in dehydration and dieback of both *Eucalyptus camaldulensis* and *Eucalyptus globulus* seedlings within 7 days. Husen (2010) indicated that relative water content decreased significantly with increasing drought treatments. Glenn *et al.,* (2010) noticed that in well-watered vines, kaolin film application enhanced leaf water potential conserved water and increased water use efficiency under non limiting soil moisture conditions. AbdAllah (2019) stated that kaolin under deficit irrigation effectively enhanced the physiological activities of *Solanum lycopersicum*, particularly fewer than 80% depletion of available water (DWE), but it did not recompense the negative influences under 60% DWA.

Leaves chemical constituents

From data presented in Table 5 it is obvious that, using water stress level treatments (except 100 % of field capacity) decreased total chlorophyll content of paulownia compared to control in both seasons. Such decrease was significant by using the level of 50 % of field capacity. In other words, proline content and soluble sugar content were significantly increased with 75 and 50 % of field capacity levels compared with full irrigation level in both seasons. Calcium carbonate at 4 g/l and kaolin at 30 g/l significantly increased total chlorophyll content (SPAD unit), soluble sugars content (mg/g as fresh weight) and proline acid content (mg/g as fresh weight) compared to control in the first and second seasons. the increases in total chlorophyll content was about 9.30 and 6.99 % for

Drought stress (Field capacity %)	antioxidants type							
	0.0	$CaCO_3$	Kaolin	Mean (A)	0.0	$CaCO_3$	Kaolin	Mean (A)
	2018/2019 season				2019/2020 season			
	Draught resistance index (%)							
100	100.00d	112.10b	120.88a	**110.99A**	100.00C	113.323b	117.84a	**110.36A**
75	88.16e	96.86d	105.68c	**96.90B**	89.16e	96.43d	102.09c	**95.89B**
50	75.91f	87.24e	89.02e	**84.06C**	80.45f	88.96e	89.12e	**86.18C**
Mean (B)	**88.02C**	**98.73B**	**105.19A**		**89.87C**	**99.54B**	**103.02A**	
	Leaf relative water (%)							
100	38.57cd	42.87b	44.37a	**41.93A**	37.17e	42.47b	45.30a	**41.64A**
75	35.07f	38.03d	39.07c	**37.39B**	34.40f	39.23d	40.70c	**38.11B**
50	32.30h	33.73g	36.60e	**34.21C**	30.50g	34.70f	37.10e	**34.10C**
Mean (B)	**35.31C**	**38.71B**	**39.51A**		**34.02C**	**38.80B**	**41.03A**	
	Wood density (g/cm^3)							
100	0.402e	0.449bc	0.480a	**0.444A**	0.433d	0.461b	0.475a	**0.456A**
75	0.416de	0.432cd	0.458ab	**0.435A**	0.409f	0.418ef	0.446c	**0.424B**
50	0.372f	0.393ef	0.413de	**0.392B**	0.367h	0.392g	0.426de	**0.395C**
Mean (B)	**0.396C**	**0.425B**	**0.450A**		**0.403C**	**0.424B**	**0.449A**	

Table 4. Effect of water stress (A), antioxidants type (B) and their interaction (A×B) treatments on draught resistance index (%), leaf relative water (%),and wood density (g/cm3) of *Paulownia tomentosa* seedlings during the two seasons of 2018/2019 and 2019/2020

Drought stress (Field capacity %)	antioxidants type							
	0.0	$CaCO_3$	Kaolin	Mean (A)	0.0	$CaCO_3$	Kaolin	Mean (A)
	2018/2019 season				2019/2020 season			
	Total chlorophyll (SPAD)							
100	42.87e	46.75b	48.36a	**45.99A**	43.80de	45.51b	46.86a	**45.92A**
75	42.53e	45.10c	47.10b	**45.01B**	43.21e	44.77c	46.19ab	**44.72B**
50	42.40e	43.89d	44.22d	**43.50C**	41.73f	44.35cd	44.72c	**43.60C**
Mean (B)	**42.60C**	**45.35B**	**46.56A**		**42.92C**	**44.87B**	**45.92A**	
	Soluble sugar content (mg/g as fresh weight)							
100	15.12d	18.21bc	18.63bc	**17.32B**	14.52d	15.97c	16.11c	**15.53B**
75	15.59d	17.57c	19.31b	**17.49B**	16.06c	18.35ab	18.81a	**17.74A**
50	17.53c	19.13b	21.08a	**19.25A**	16.47c	17.52b	19.00a	**17.66A**
Mean (B)	**16.08C**	**18.30B**	**19.67A**		**15.68C**	**17.28B**	**17.97A**	
	Proline content (mg/g as fresh weight)							
100	4.61f	5.19ef	5.77de	**5.19C**	5.13g	5.63f	6.23e	**5.66C**
75	6.26de	6.58d	9.04c	**7.29B**	6.92e	7.76d	9.86c	**8.18B**
50	8.15c	10.24b	11.72a	**10.04A**	7.77d	11.28b	12.04a	**10.36A**
Mean (B)	**6.34C**	**7.53B**	**8.65A**		**6.61C**	**8.22B**	**9.38A**	

Table 5. Effect of water stress (A), antioxidants type (B) and their interaction (A×B) treatments on total chlorophyll (SPAD), proline content (mg/g as fresh weight) and soluble sugar content (mg/g as fresh weight) of *Paulownia tomentosa* seedlings during the two seasons of 2018/2019 and 2019/2020

kaolin treatment, with significant difference between this treatment and CC treatment and control in the 1st and 2nd seasons, respectively. Also, the combination treatment between drought stresses at 50 % of field capacity 30 g/l Ka significantly increased soluble sugars content and proline acid content (mg/g as fresh weight) of *Paulownia tomentosa* seedlings compared to control during the two consecutive seasons. However, the increases in proline content were about 20.25 and 15.36 % compared to control in the 1st and 2nd seasons, respectively.

The contents of photosynthetic pigments in plants objected to drought stress are recognized to degenerate (Nabil *et al*., 2008). Also, Husen (2010) suggested that increasing drought treatments from 5 to 20 days significantly decreased chlorophyll a, b, total chlorophyll and soluble protein content compared to control. The decrease in irrigation water levels led to increase the proline content of pomegranate seedlings (Hamdy *et al*., 2016). Qu *et al.,* (2019) found that under moderate drought stress, the contents of amino acids (such proline content) were significantly enhanced; however, under severe drought stress, the synthesis ability of amino acids was increased.

The main influence of kaolin application was the lowering, albeit small, of photosynthesis, which seemed to be regarding to the shading of the leaves (Rosati *et al*., 2006). The highest total chlorophyll content (SPAD units) of olive cultivars was found under each of calcium carbonate as well as kaolin as foliar spray compared to control (Khaleghi *et al*., 2015; Hagagg *et al*., 2019). Deficit irrigation treatments significantly increased leaf proline content of apple cultivars compared to control. In the first year, kaolin treatments increased leaf proline but in the second year, leaf proline was not significant (Faghih *et al*., 2021).

CONCLUSION

Using a sustained deficit irrigation strategy (moderate drought stress) will save water. The results of this study showed that irrigation application at 100% of field capacity followed by 75% of field capacity compared to 50% of field capacity plus 30 g/l kaolin application will improve growth and wood density of *Paulownia tomentosa* seedlings as well as total chlorophyll and proline content.

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