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Research Article

Effects of Different Times of Cutting Soaking and Concentrations of IAA on Morphological features of *Robinia pseudoacacia* Stem Cuttings

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Abstract: This research was conducted in the open field of the Department of Biology, Faculty of Science and Health, Koya University, Kurdistan region, located at 36º N, 44º E and 570m of altitude above sea level**.** A factorial experiment with randomized complete block design (RCBD) with three replications was conducted to study the combined effect of different times of cutting soaking and concentrations of IAA. In this study four times of cutting soaking 0, 10, 30 and 60m (t_0, t_1, t_2 and t_3), four concentrations 0, 500, 800 and 1000 ppm (C_0, C_1, C_2 and C_3) and distilled water was used as control. When *Robinia pseudoacacia* was exposed to various concentrations of IAA and times of cutting soaking, it was found that IAA concentration and times of cutting soaking in *R. pseudoacacia* had significant effects on the shoot percentage, shoot height, number of leaves, number of main branches, diameter of main branches, leaf area, chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid content. Showed significant effects such as an increase in some vegetative growth, leaves chemical content as compared with control. Overall, the results show that *R. pseudoacacia* stem cuttings are superior for a time of cutting soaking with ten minutes and IAA concentration with 500 ppm.

Keywords: IAA, Times, Vegetative propagation, Stem cuttings, *Robinia pseudoacacia*.

1. Introduction

Black locust (*Robinia pseudoacacia*) belongs to the family Fabaceae and subfamily Faboideae, it is a native tree of North America. Black locust is grown throughout the world and is well suited for growth under a wide variety of ecological conditions. (Xiu *et al*., 2016; Rédei *et al.*, 2013; Swearingen, 2009; Zhang *et al*., 2007).

Robinia pseudoacacia grows well in full sun and well-drained soils. It is drought tolerant and fast-growing plant. It grows well on poor sites or acidic soil (pH 4.8). It is growing at altitude: over 800m, annual temperature: -35 to 40°C, annual rainfall: 1000-1500mm, soil type: found on a wide range of soils but does well on calcareous and well-drained loams. It cannot grow in the shade. Black locust does not do well on compacted, clayey or eroded soils (Li *et al.*, 2013; Cierjacks *et al.*, 2013; Rice *et al.*, 2004; Orwa *et al.*, 2009; Sekabembe *et al.*, 1994; Huntley, 1990).

R. pseudoacacia is a medium-sized tree, 25m tall, 60cm dbh, trunk irregular, crown open, branches short, brittle, the persistent stout spines on young shoots are found on mature wood, the smooth bark is reddish-brown and deeply furrowed with age. Leaves are deciduous, alternate, pinnately compound, composed of 7-19 leaflets on a central stalk, 20-30cm long, 2 spines at the base of each leaf, leaflets oval, 30-50mm long, dull green, bristle tipped, smooth margined. Flowers showy, white, pea-like, fragrant, in loose, drooping clusters, about 14cm long, arising from leaf axils near the tip of a new shoot. Fruits are pods, 7-10cm long, flat; husk thin-walled, smooth, dark to reddish-brown, seeds dark, beanlike, 3-5mm long, 5-8 per pod, with a hard impermeable coat. It can be easily propagated by seed or cuttings and this allows for easy reproduction of the plant. Cultivars may also be grafted as this ensures that the parent and daughter plants are genetically identical. The pea-like, bisexual flowers, borne in long dense racemes, are cross-pollinated by insects (Orwa *et al.*, 2009; Huntley, 1990).

R. pseudoacacia is considered an excellent plant for growing in highly disturbed areas as an erosion control plant and for soil enrichment. Black locust has been planted widely for its nitrogen-fixing abilities and as a source of nectar for honeybees. It has all aspects of biology; its ecological characteristics, structure and physiology, response to biotic factors and environment. It is an attractive ornamental tree that is often planted

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along streets and in parks, especially in large cities, because it tolerates pollution well and also has numerous commercial uses. It is also planted for firewood because it grows fast, wood is extremely hard, being one of the hardest woods in Northern America. Despite the presence of toxins in the leaves, the fodder is considered highly attractive to many animals. In traditional medicine of India, different parts of *R. pseudoacacia* are used as laxative, antispasmodic, and diuretic (Orwa *et al.*, 2009; Huntley, 1990).

Plant cutting is one of the most common methods of vegetative reproduction, extensively used today due to low cost. Stem cuttings are the most important types of cutting based on the nature of the wood. Cuttings are divided into four groups: hardwood, semi-hardwood, softwood and herbaceous cuttings. Success of propagation via stem cuttings is usually affected by a number of factors, including the status of mother plant or cutting source, type of culture medium, type of cutting, rooting hormones and environmental conditions (light, temperature, air humidity and soil moisture). The chemical composition of upper and lower cuttings of a plant is different. This has been observed when the production of root from the cuttings of different parts of shoot (Khudhur and Omar, 2015; Sevik and Guney, 2013; Hassanein, 2013; Rahbin *et al.*, 2012; Pijut *et al.*, 2011; Swamy *et al.*, 2002).

Auxins are a class of plant growth substances (phytohormone or plant hormone). Play an important role in coordination of numerous growth and behavioral processes in the life cycle of a plant. Auxins are first of the main plant hormones to be discovered and are a major coordinating signal in plant development. Auxin enters cuttings primarily via the cut surface. Indole-3-acetic acid (IAA) synthesized and examined for their stimulatory effects on adventitious root formation in stem cuttings of *R. pseudoacacia* as well as on subsequent growth and survival of these cutting raised plantlets, commonly applied to stimulate root growth when taking cuttings of plants. However, high concentrations of auxin inhibit root elongation and instead increase adventitious root development. (Khudhur and Omar, 2015; Gehlot *et al*., 2014; Sevik and Guney, 2013; Pijut *et al*., 2011; Pop *et al*., 2011; Kenney *et al.*, 1969).

The aim of this experiment was to evaluate the effects of different times of cutting soaking and concentrations of IAA on the vegetative growth, chemical characteristics and some stomata characteristics of *R. pseudoacacia.*

2. Material and Methods

2.1 Collection of Stem Cuttings and Auxin Treatments

This research was conducted in the open field of the Department of Biology, Faculty of Science and Health, Koya University, Kurdistan region in 2016-2017. A factorial experiment with randomized complete block design (RCBD) with three replications was conducted to study the combined effect of different times and concentrations of IAA, with four times of cutting soaking 0, 10, 30 and 60m, denoted as (t_0, t_1, t_2 and t_3) and four concentrations 0, 500, 800 and 1000 ppm, denoted as (C_0, C_1, C_2 and C_3). Preparation of *R. pseudoacacia* cuttings was collected from three years old and 4m high mother plants, planted in the garden of Koya University, Kurdistan Region, in 2014. The stem cuttings of these trees were collected on February 28, 2017. Stem cuttings without lateral shoots by removing leaves and leaving intact leaf buds in each cutting. For this study, the cuttings were chosen to be kept in water until planting time.

Auxin solutions were prepared by dissolving each of 1g of IAA with 5 drops of ethanol in 100ml distilled water to obtain concentrations (0, 500, 800 and 1000 ppm). 30cm long stem cutting with 1cm diameter were treated by dipping their basal 3.0cm portions for different times of cutting soaking 0, 10, 30 and 60m, control cuttings were dipped in distilled water. The treated carried out in the late evening to avoiding auxin reaction. After treating the stem cuttings were planted vertically in polythene bags sized 10x10x30cm filled with loamy soil taken from the research center Koya city as growth medium on March 1, 2017. The cuttings were watered regularly when needed. After 62 days of planting observations, some parameters were recorded.

2.2 Meteorological data

Maximum and Minimum temperature, the relative humidity and the amount of rainfall in the open field during the planting season are shown in table (1), as recorded by Agro-Meteorological Station, Koya city.

Table 1: Maximum and minimum temperature, the relative humidity and the amount of rainfall during the growing season (2017).

Month (2017)	Air Temperature (C°)		Relative Humidity (%)		Rainfall (mm)
	Maximum	Minimum	Maximum	Minimum	
March	17.77	10.23	67.42	57.45	6.81
April	24.23	13.97	60.93	52.53	4.65

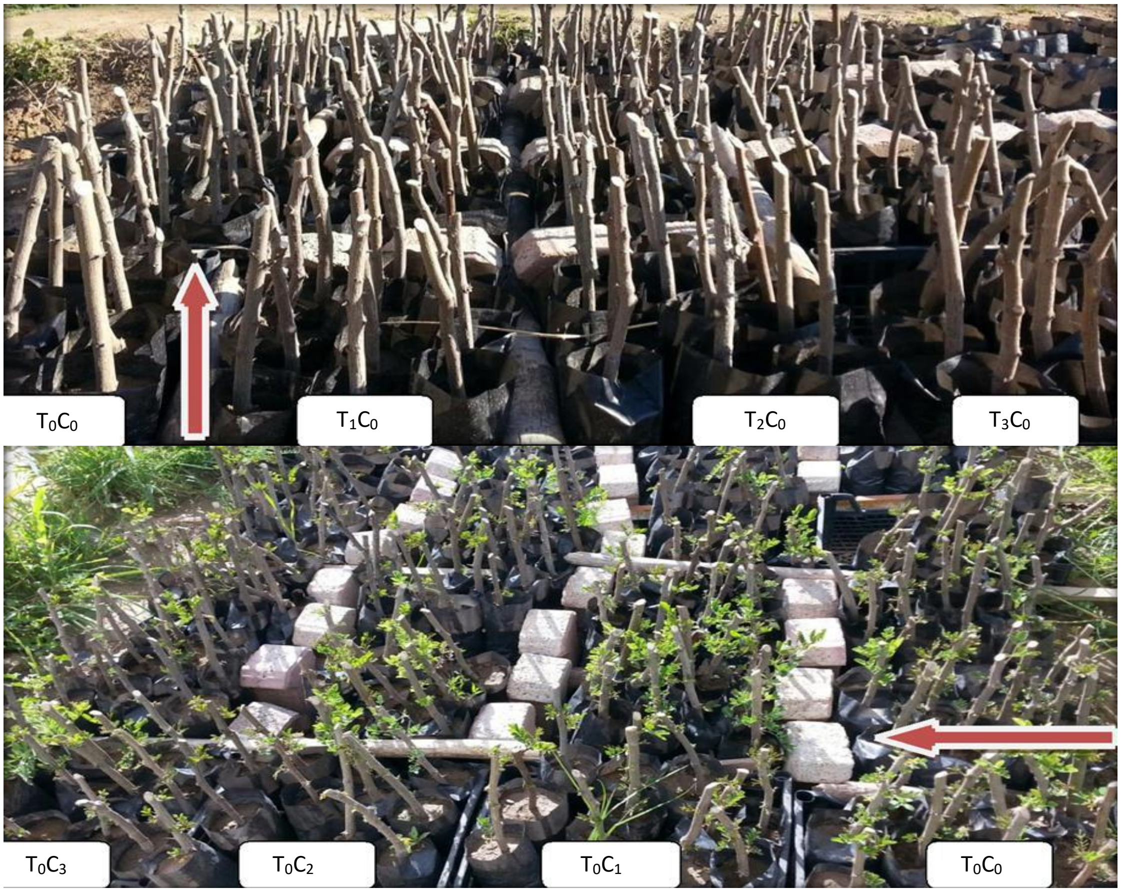

Fig. 1: Stem cuttings of *Robinia pseudoacacia*.

On May 2, 2017, the following characteristics were studied:

A. Vegetative growth included

Shooting percentage: Total number of the survival percentage of stem cuttings was counted, including those that can be seen by naked eyes.
Shoot height (cm): It was measured from the point of stem attachment with soil to the apical point of the main shoot by using metric tapeline.
Number of leaves/stem cutting: Total number of leaves was counted, including those that can be seen by naked eyes.
Number of main branches/cutting: Total numbers of main branches was counted, including those that can be seen by naked eyes.
Diameter of main branches ($cm.cutting^{-1}$): Diameter of main branches was measured by vernier micrometer.
Leaf area ($cm^2.stem\ cutting^{-1}$): Five leaves were selected randomly from plants, after measured comprise leaf length (L) from lamina tip to the connected place petiole and width (W) from tip to tip at the widest of lamina, by ruler, leaf area calculated by the formula described by Thomas and Winner, 2000:

$$La\ (cm^2) = Length * Width * 0.95$$

B. Chemical characteristics of leaves

Chlorophyll content (mg/100g fresh weight): The amount of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid was estimated by spectrophotometry of samples prepared by 80% acetone extraction. 0.2g of fresh leaves of each experimental unit were taken, mixed extracting solution (acetone : alcohol : distilled water = 4.5 : 4.5 : 1), kept for about 24h at room temperature in the dark until green leaf turn to white, then use spectrophotometer PD-303 to measure the optical density (OD) values at 663nm (maximum absorptions wavelength for chlorophyll a), 645nm (maximum absorptions wavelength for chlorophyll b), 440nm (maximum absorptions wavelength for carotenoid) respectively. Calculating chlorophyll, chlorophyll a, chlorophyll b and carotenoid concentration according to following formula:

Chlorophyll a (mg/L) = $9.784OD_{663} - 0.990OD_{645}$;
Chlorophyll b (mg/L) = $21.426OD_{645} - 4.650OD_{663}$;
Chlorophyll (mg/L) = $5.134OD_{663} + 20.436OD_{645}$;
Carotenoid (mg/L) = $4.695OD_{440} - 0.268$ (Chlorophyll a + Chlorophyll b);

The following formula is used to convert chlorophyll content from mg/liter to mg/100g fresh weight:

$$\text{mg chlorophyll}/100\text{ g fresh weight} = \frac{\text{mg}/\text{l}}{1000} \text{ X } \frac{100\text{ g}}{\text{g sample}}$$

C. Number, length and width of stomata in upper and lower leaf surface

Three leaves were selected from different plants and plucked carefully from each experimental unit and kept in polythene bags on May 2, 2017, and brought to the laboratory. The leaf epidermal peel slides were made by the method of lasting impressions. In this method, at least one square centimeter on leaf surface was painted by a thick patch of clear nail polish. Allowed the nail polish to dry completely, then taped a piece of clear cellophane tape to the dried nail polish patch. Gently, peeled out the nail polish patch by pulling a corner of the tape and the fingernail polish along with the leaf peel. This leaf impression which was taped on slides and labeled as adaxial and abaxial surface. Leaf impression was examined under at least 400x magnifications by light microscope. Stomata numbers are counted per square millimeter area. Length and width of stomata guard cells of a leaf were measured in μm (micron) with ocular micrometer under high power magnifications with the help of "Stage - Ocular micrometer" (Rai and Mishra, 2013).

2.3 Statistical analysis

Comparisons between means were made by using Duncan's Multiple Range Test at 5% level (Reza, 2006). The statistical analysis was carried out by using SAS Program (2000).

3. Results and Discussion

3.1 Effect of different times and concentrations of IAA on survival percentage of stem cuttings (shoot percentage) and vegetative growth of *Robinia pseudoacacia*

Table (2) showed significant response of different times of cutting soaking to vegetative growth, including; shoot percentage, shoot height, number of main branches, diameter of main branches and leaf area from treatment. The ten minutes of cutting soaking time caused an increase in plant vegetative growth as compared to control treatment. Highest values were recorded in T_1 (95.8%, 3.4cm, 4.6, 2.4mm and 1.3cm^2) for the parameters respectively, while the lowest values were recorded in T_0 (92.1%, 2.0cm, 3.5, 2.2mm and 1.1cm^2) respectively. There were no significant differences between different concentrations with IAA on their parameters. IAA concentration and their interactions with different times of cutting soaking had significant effect on the shoot height, number of leaves, diameter of main branches and leaf area. Which records the highest values in T_1C_1 (4.2cm, 5.4, 2.9mm and 1.7cm^2) for the parameters respectively, while the lowest values were recorded in T_0C_1 (1.7cm, 2.8, 2.0mm and 1.0cm^2) respectively. Showed significant effect and increasing some vegetative growth as compared with control.

Table (2) showed that the time of cutting soaking of ten minutes with IAA concentration with 500 ppm increased all growth parameters significantly as compared to control. Auxins are the most widely used plant hormones for vegetative propagation, this effect may be attributed to the physical properties of the plants. The growth of plant decreased with increasing the IAA concentration because IAA can also affect plant growth (plant physiological, morphological, and biochemical processes), but higher concentration of growth hormones showed inhibitory effect on rooting of the cuttings is presumed to be dedicated through its effect on mobilizing the reserve food material by enhancing the activities of hydrolytic enzymes. The results agreed partially with (Mbagwu *et al.,* 2017; Khudhur and Omar, 2015; Alagesaboopathi, 2012; Park & Han, 2003).

Results in table (3) showed significant differences between different times of cutting soaking on chlorophyll a, b, total and carotenoid. Which records the highest values in T_1 (4.6, 7.1, 11.9 and 5.4mg/100g) for the parameters respectively, while the lowest values were recorded in T_0 (3.3, 5.6, 9.5 and 4.3) respectively. While IAA concentration has non-significant effect on the chlorophyll a, b, total chlorophyll and carotenoid as compared with control. The interaction between different times of cutting soaking and IAA concentrations had significant effects on the chlorophyll a, b, total and carotenoid characteristics. The highest values (4.8, 7.4, 12.1 and 5.6mg/100g) were obtained from T_1C_1, while, the lowest values (3.3, 5.6, 9.5 and 4.3 mg/100g) was obtained from T_0C_0 with control.

The result showed that chlorophyll a, b, total and carotenoid of leaf content significantly superior in 10 minutes of cutting soaking and IAA concentration with 500 ppm as compared to other treatments, plant leaf area is an essential component to estimate plant growth through its incidence on crop physiology mechanisms, and determinant of light interception and consequently of transpiration, photosynthesis, evapotranspiration and plant productivity studies. Leaf photosynthesis can be influenced by many plant factors such as leaf position and age, as well as environmental factors such as light, temperature, nutrition, water availability and photosynthetic pigments. Chlorophyll content is an important experimental parameter for research in agronomy and plant biology. Amount of chlorophyll shows alteration depending on many factors such as light, leaf position and plant age. The results agree with (Khudhur & Omar, 2015; Al-Barzinji *et al*., 2016).

Table 2: Effect of different times of cutting soaking and concentrations of IAA and their interaction on survival shoot percentage and vegetative growth characteristics of *Robinia pseudoacacia* stem cuttings.

Treatment		Survival of cuttings (%)	Shoot height (cm)	No. of Leaves /cutting	No. of main branches /cutting	Diameter of main branches (cm)	Leaf area (cm)²
Different times of cutting soaking (Minutes)							
Control	(T_0)	92.1 b	2.0 b	22.0 a	3.5 a	2.2 b	1.1 a
10 minutes	(T_1)	95.8 a	3.4 a	25.6 a	4.6 a	2.4 ab	1.3 b
30 minutes	(T_2)	94.9 a	3.3 a	17.1 a	3.7 ab	2.3 a	1.2 a
60 minutes	(T_3)	89.6 a	3.0 a	26.0 a	3.3 b	2.3 ab	1.2 a
IAA Concentration (ppm)							
Control	(C_0)	94.2 a	2.5 a	17.1 a	4.4 a	2.4 a	1.2 a
500	(C_1)	95.8 a	3.1 a	23.0 a	3.9 ab	2.3 a	1.1 a
800	(C_2)	93.8 a	3.2 a	23.0 a	4.0 ab	2.3 a	1.4 a
1000	(C_3)	91.7 a	3.0 a	19.0 a	3.1 ab	2.3 a	1.1 a
Interaction between different times & IAA Con.							
T_0C_0		93.3 a	1.9 d	20.3 a	5.3 ab	2.0 cd	1.0 ab
T_0C_1		100 a	1.7 d	22.0 a	3.5 abc	2.1 cd	1.5 b
T_0C_2		91.7 a	2.0 d	24.0 a	3.5 abc	2.3 abcd	1.2 ab
T_0C_3		83.3 a	2.4 dc	21.7 a	2.8 c	2.3 abcd	1.4ab
T_1C_0		91.7 a	2.5 bcd	18.3 a	4.1 abc	2.1 dc	1.1 ab
T_1C_1		100 a	4.2 a	29.9 a	4.8 ab	2.9 a	2.0 a
T_1C_2		91.7 a	3.6 ab	35.3 a	4.4 a	2.2 abcd	1.ab
T_1C_3		100 a	3.2 abcd	18.9 a	4.0 abc	2.3 abcd	1.1 ab
T_2C_0		91.7 a	3.1 abcd	15.0 a	4.1 abc	2.8 ab	1.3 ab
T_2C_1		100 a	3.5abc	21.9 a	4.1 abc	2.5 abcd	1.1 ab
T_2C_2		100 a	3.8 ab	14.8 a	3.7 abc	2.6 abc	1.3 ab
T_2C_3		100 a	3.0 abcd	16.2 a	3.0 c	2.2 abcd	1.1 ab
T_3C_0		100 a	2.6 bdc	14.8 a	4.2 abc	2.5 abcd	1.2 ab
T_3C_1		83.3 a	2.7 bcd	25.7 a	3.2 c	2.1 d	1.1 ab
T_3C_2		91.7 a	3.4 abc	17.2 a	3.2 bc	2.4 abcd	1.7 a
T_3C_3		83.3 a	3.2 abcd	19.0 a	2.7 c	2.4 abcd	1.2 ab

*Means followed by the same letters within columns are not significantly different at $p \leq 0.05$ according to the Duncan test.

Table 3: Effect of different times of cutting soaking and concentrations of IAA on leaf content of chlorophyll a, b, total and carotenoid of *R. pseudoacacia*.

Treatment		Chlorophyll a	Chlorophyll b	Total Chlorophyll	Carotenoids
		Mg/100g fresh weight			
Different times of cutting soaking (Minutes)					
Control	(T_0)	3.3 ab	5.6 b	9.5 b	4.3 b
10 minutes	(T_1)	4.6 a	7.1 a	11.9 a	5.4 a
30 minutes	(T_2)	4.1 ab	6.4 ab	10.5 ab	5.1 ab
60 minutes	(T_3)	3.4 b	6.8 a	10.1 b	4.4 b
IAA Concentration (ppm)					
Control	(C_0)	4.1 a	6.7 a	10.6 a	4.8 a
500	(C_1)	3.7 a	6.4 a	10.2 a	4.8 a
800	(C_2)	3.9 a	6.3 a	10.1 a	4.8 a
1000	(C_3)	4.2 a	6.5 a	10.8 a	4.9 a
Interaction between different times & IAA Con.					
T_0C_0		2.9 cd	4.6 ab	8.7 bc	4.1 b
T_0C_1		4.2 abcd	6.2 ab	10.7 abc	4.7 ab
T_0C_2		3.4abcd	4.7 b	8.1 c	4.5 ab
T_0C_3		4.7 a	5.6 ab	10.7 abc	4.9 ab
T_1C_0		4.2 abcd	6.7 ab	10.2 abc	4.8 ab
T_1C_1		4.8 abcd	7.4 a	12.1 ab	5.6 ab
T_1C_2		4.7 ab	6.5 ab	11.4 abc	5.1 ab
T_1C_3		4.7 abc	7.3 a	11.2 a	5.1 a
T_2C_0		4.6 abc	7.3 a	11.7 abc	5.4 ab
T_2C_1		3.4 abcd	5.7 ab	9.1 cd	4.4 ab
T_2C_2		4.3 abcd	7.0 ab	11.4 abc	5.3 ab
T_2C_3		4.1 abcd	5.6 ab	9.1 bc	4.5 ab
T_3C_0		4.6 abc	7.0 ab	11.1 abc	4.7 ab
T_3C_1		3.0 d	6.1 ab	9.1 bc	4.2 b
T_3C_2		3.0 cd	7.1 ab	10.1 bc	4.2 b
T_3C_3		3.2 cd	7.0 ab	10.1 abc	4.2 b

*Means followed by the same letters within columns are not significantly different at $p \leq 0.05$ according to the Duncan test.

The results in table (4) and figs. 2, 3, 4 and 5 showed that the stomata structure of *R. pseudoacacia* has both adaxial and abaxial epidermis. The anatomical study shows there were no significant differences between times of cutting soaking on this parameter. However, no significant effects were observed with different concentrations of IAA. The interaction between different times of cutting soaking and IAA concentrations had no significant effect on each stomata number, stomata length and width on upper and lower surface. As a result, high concentration of IAA non-significantly reduced the growth of the *R. pseudoacacia* stem cutting. Auxin generally promotes rooting at lower concentrations but inhibits at higher concentrations. It varies with species, physiological, anatomical and environmental conditions. IAA directly effects on the photosynthetic apparatus in dependent of stomatal closure, might be responsible for the reduction in photosynthetic rate. Stomata characteristics like number, length and width are affected by genetic constitution, season, leaf position and leaf surface (upper or lower). These results partially agreed with (Khudhur & Omar, 2015; Alagesaboopathi, 2012; Zan, *et al.*, 2011).

Table 4: Effect of different times of cutting soaking & concentrations of IAA and their interactions on some stomata characteristics for *R. pseudoacacia* stem cuttings.

Treatment	Stomata Number (mm^2)		Stomata Length (micron)		Stomata Width (micron)	
	Upper leaf surface	Lower leaf surface	Upper leaf surface	Lower leaf surface	Upper leaf surface	Lower leaf surface
Different times of cutting soaking (Minutes)						
Control (T_0)	189.3 a	289.9 ab	1.6 ab	1.7 a	1.1 ab	1.0 a
10 minutes (T_1)	166.2 a	292.1 ab	1.8 a	1.6 a	1.2 a	2.4 a
30 minutes (T_2)	208.0 a	319.4 ab	1.6 a	1.4 a	1.3 ab	1.1 a
60 minutes (T_3)	187.2 a	350.3 ab	1.6 ab	1.5 a	1.3 a	1.1 a
IAA Concentration (ppm)						
Control (C_0)	193.8 a	291.1 a	1.7 a	1.5 a	1.3 a	2.3 a
500 (C_1)	168.8 a	325.5 a	1.7 a	1.5 a	1.2 a	1.2 a
800 (C_2)	181.3 a	308.1 a	1.7 a	1.5 a	1.3 a	1.0 a
1000 (C_3)	206.7 a	327.1 a	1.6 a	1.6 a	1.3 a	1.1 a
Interaction between different times & IAA Con.						
T_0C_0	182.0 ab	177.3 a	1.6 ab	1.5 ab	1.3 ab	1.1 b
T_0C_1	144.0 ab	291.0 a	1.3 ab	1.4 ab	1.1 ab	1.0 b
T_0C_2	171.7 ab	333.0 a	1.8 ab	1.7 ab	1.1 ab	1.1 b
T_0C_3	259.7 ab	358.3 a	1.6 ab	1.8 ab	1.2 ab	1.0 b
T_1C_0	155.3 ab	304.0 a	1.7 ab	1.4 ab	1.3 ab	1.0 a
T_1C_1	151.0 ab	290.7 a	1.9 ab	1.6 ab	1.4 ab	1.8 b
T_1C_2	148.7 ab	275.3 a	1.9 ab	1.6 ab	1.4 ab	1.0 b
T_1C_3	209.7 ab	291.0 a	1.6 ab	1.7 ab	1.3 ab	1.1 b
T_2C_0	235.0 ab	322.0 a	1.7 ab	1.5 ab	1.3 ab	1.1 b
T_2C_1	203.0 ab	357.3 a	1.7 ab	1.4 ab	1.3 ab	1.1 b
T_2C_2	201.0 ab	304.3 a	1.6 ab	1.5 ab	1.2 ab	1.1 b
T_2C_3	193.0 ab	294.0 a	1.6 ab	1.4 ab	1.3 ab	1.0 b
T_3C_0	203.0 ab	361.0 a	1.6 ab	1.5 ab	1.2 ab	1.1 b
T_3C_1	177.3 ab	363.0 a	1.7 ab	1.7 ab	1.4 ab	1.2 b
T_3C_2	204.0 ab	319.7 a	1.6 ab	1.3 ab	1.3 ab	1.0 b
T_3C_3	164.3 ab	357.3 a	1.6 ab	1.6 ab	1.4 ab	1.2 b

*Means followed by the same letters within columns are not significantly different at $p \leq 0.05$ according to the Duncan test.

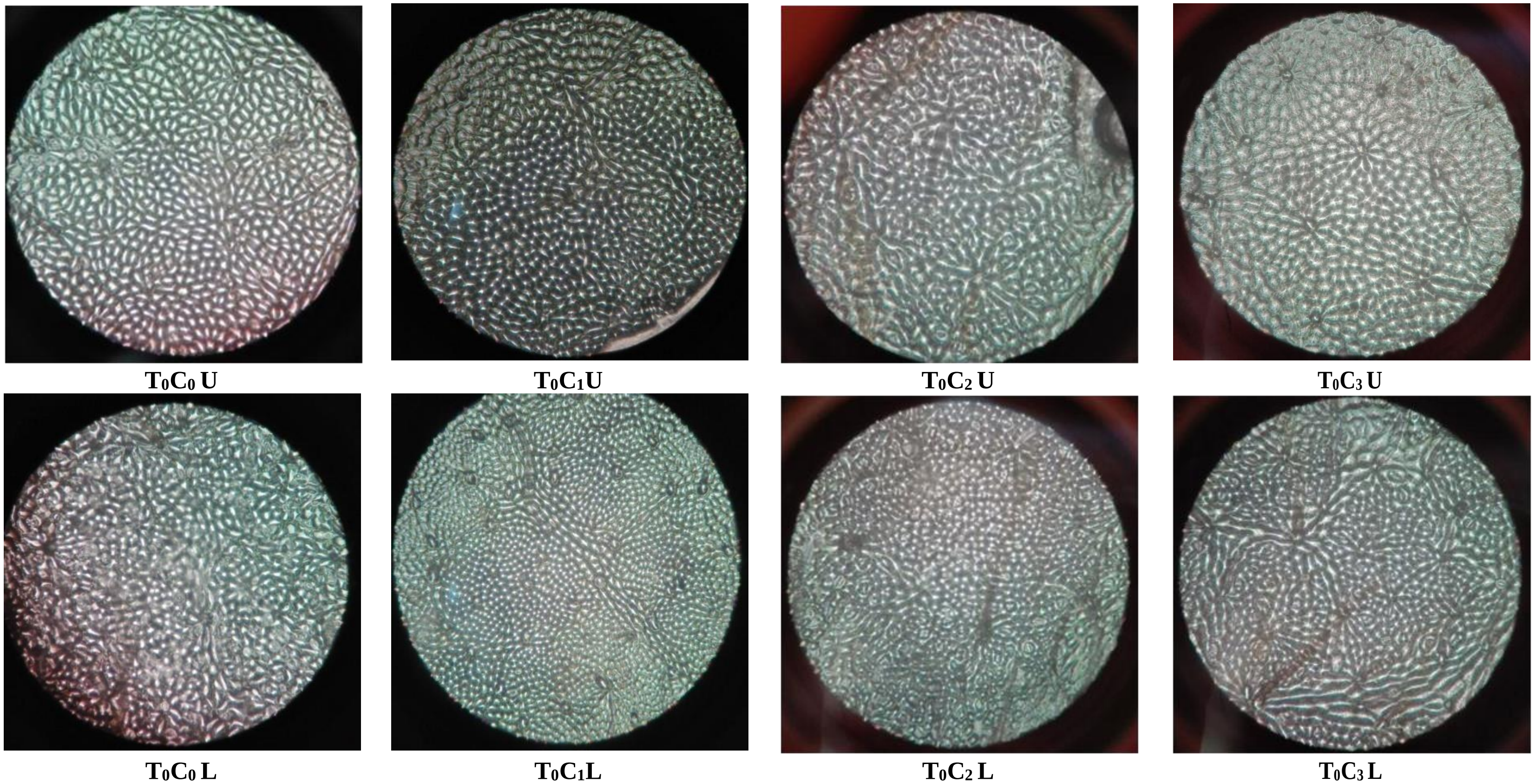

Fig. 2: Upper (adaxial) and Lower (abaxial) stomata of *R. pseudoacacia* leaves surfaces 400X. (T_0C_0U & L) stomata in control time of cutting soaking and no concentration of IAA, (T_0C_1U & L) stomata in control time of cutting soaking and concentration with 500 ppm, (T_0C_2U & L) stomata in control time of cutting soaking and concentration with 800 ppm, (T_0C_3U & L) stomata in control time of cutting soaking and concentration with 1000 ppm.

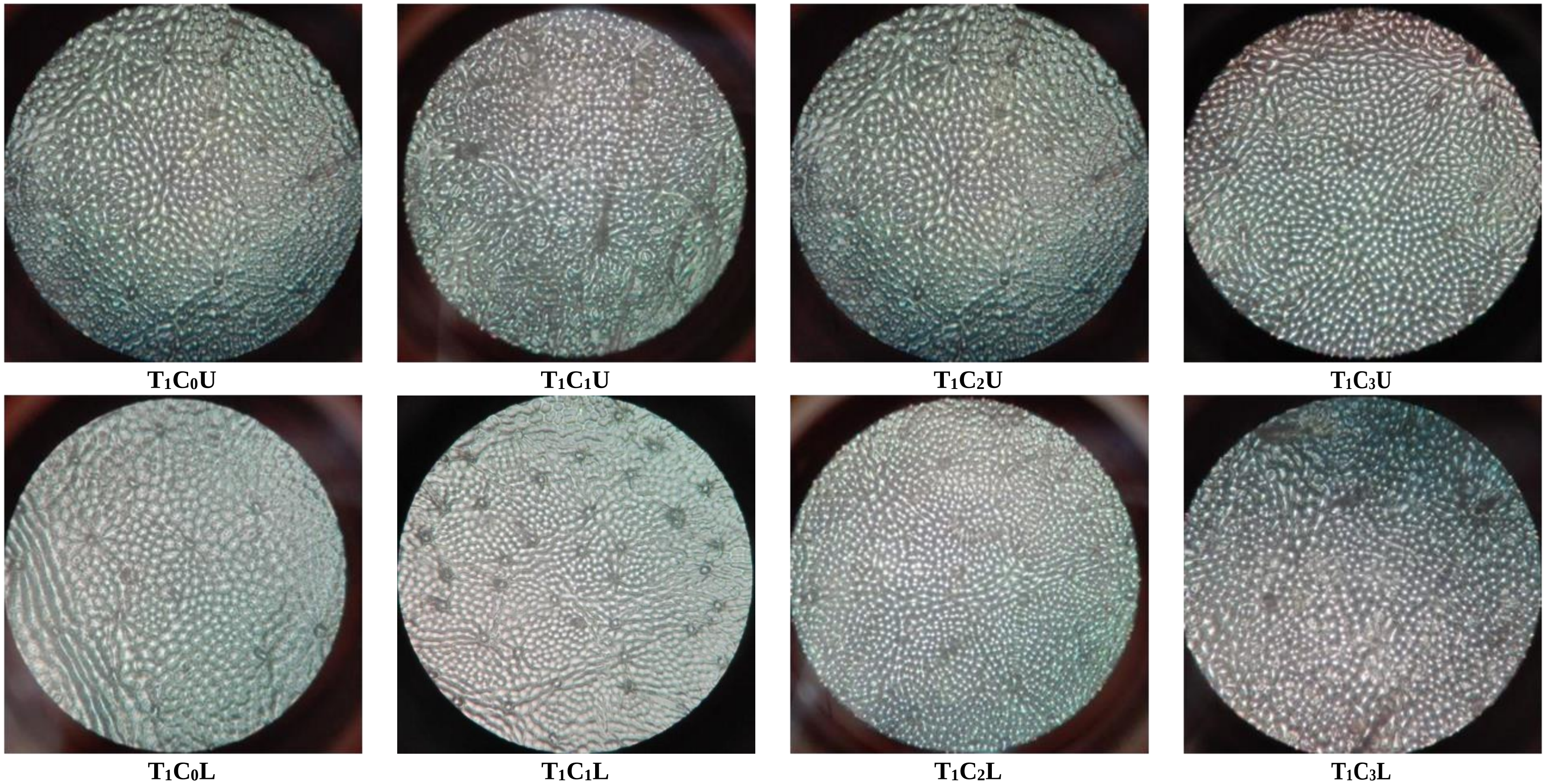

Fig. 3: Upper (adaxial) and Lower (abaxial) stomata of *R. pseudoacacia* leaves surfaces 400X. (T_1C_0U & L) stomata in ten minutes and no concentration of IAA, (T_1C_1U & L) stomata in ten minutes and concentration with 500 ppm, (T_1C_2U & L) stomata in ten minutes and concentration with 800 ppm, (T_1C_3U & L) stomata in ten minutes and concentration with 1000 ppm.

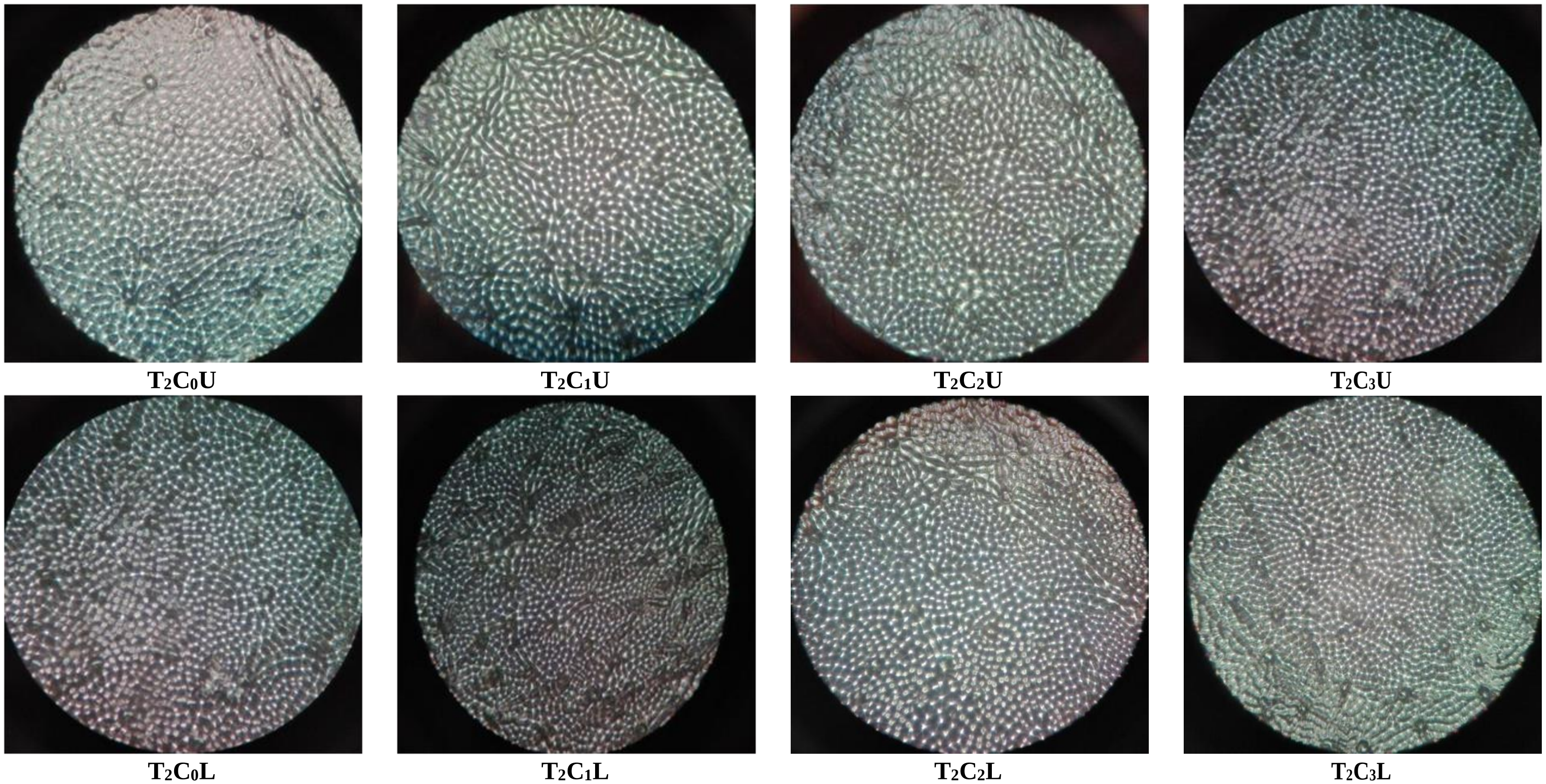

Fig. 4: Upper (adaxial) and Lower (abaxial) stomata of *R. pseudoacacia* leaves surfaces 400X. (T_2C_0U & L) stomata in thirty minutes and no concentration of IAA, (T_2C_1U & L) stomata in thirty minutes and concentration with 500 ppm, (T_2C_2U & L) stomata in thirty minutes concentration with 800 ppm, (T_2C_3U & L) stomata in thirty minutes and concentration with 1000 ppm.

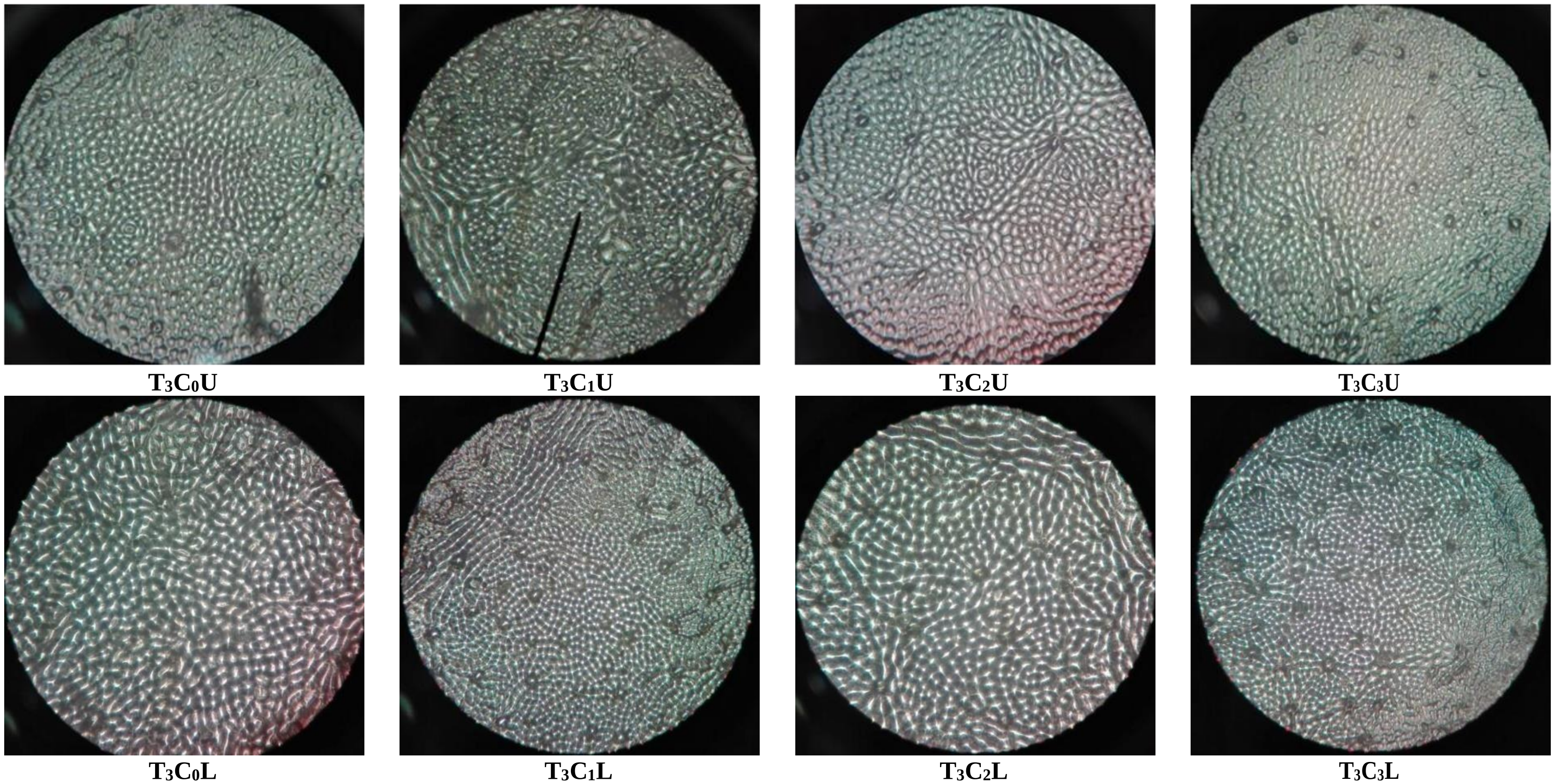

Fig. 5: Upper (adaxial) and Lower (abaxial) stomata of *R. pseudoacacia* leaves surfaces 400X. (T_3C_0U & L) stomata in sixty minutes and no concentration of IAA, (T_3C_1U & L) stomata in sixty minutes and concentration with 500 ppm, (T_3C_2U & L) stomata in sixty minutes concentration with 800 ppm, (T_3C_3U & L) stomata in sixty minutes and concentration with 1000 ppm.

4. Conclusion

From the results of this work, it can be concluded that times of cutting soaking with ten minutes and IAA concentration with 500 ppm significantly affected the vegetative growth characteristics as well as chemical characteristics of plant, in the open field. Therefore, the interaction between 10 minutes of cutting soaking and 500 ppm gave high value respectively.

References

[1]. Alagesaboopathi, C. (2012). Influence of indole acetic acid and indole butyric acid on root development and status of *Andrographis elongata* (Vahl) T. and – an endemic medicinal plant of India. *Int. J. Biosci.*, 2(4): 75-81.

[2]. Al-Barzinji, I.M., Khudhur, S.A. & Abdulrahman, Y.A. (2016). Non-Destructive Method for Prediction Leaf Area and Chlorophyll Content for Some Horticultural Plants Cultivated in Erbil City. *Journal of University of Duhok*, 19(1): 339-347

[3]. Al-Sahaf, F.H. (1989). Applied Plant Nutrition. Ministry of Higher Education and Scientific Research, University of Baghdad. Al-Hikma House, Iraq, pp 260. (In Arabic).

[4]. Cierjacks, A., Kowarik, I., Joshi, J., Hempel, S., Ristow, M., von der Lippe, M. & Weber, E. (2013). Biological Flora of the British Isles: *Robinia pseudoacacia*. *J. Ecol.*, 101(6): 1623–1640. doi: 10.1111/1365-2745.12162.

[5]. Dick, J.McP., Leakey, R.R.B., McBeath, C., Harvey, F., Smith, R.I. & Woods, C. (2004). Influence of nutrient application rate on growth and rooting potential of the West African hardwood *Triplochiton scleroxylon*. *Tree Physiol.*, 24(1): 35–44. doi: 10.1093/treephys/24.1.35.

[6]. Gehlot, A., Gupta, R.K., Tripathi, A., Arya, I.D. & Arya, S. (2014). Vegetative propagation of *Azadirachta indica*: effect of auxin and rooting media on adventitious root induction in mini-cuttings. *Adv. For. Sci.*, 1(1): 1–9. doi: 10.34062/afs.v1i1.1357.

[7]. Hassanein, A.M.A. (2013). Factors Influencing Plant Propagation Efficiency via Stem Cuttings. *J. Hort. Sci. & Ornamen. Plants*, 5(3): 171-176. doi: 10.5829/idosi.jhsop.2013.5.3.1125.

[8]. Huntley, J.C. (1990). *Robinia pseudoacacia* L. black locust. In: Burns RM and Honkala BH (Tech. Coords). Silvics of North America, Vol. 2: Hardwoods. Agriculture Handbook 654. U.S. Department of Agriculture, Forest Service, Washington, DC. pp. 755-761.

[9]. Kenney, G., Sudi, J. & Blackman, G.E. (1969). The Uptake of Growth Substances: XIII. DIFFERENTIAL UPTAKE OF INDOL-3YL-ACETIC ACID THROUGH THE EPIDERMAL AND CUT SURFACES OF ETIOLATED STEM SEGMENTS. *J. Exp. Bot.*, 20(4): 820–840. doi: 10.1093/jxb/20.4.820.

[10]. Khudhur, S.A. & Omar, T.J. (2015). Effect of NAA and IAA on Stem Cuttings of *Dalbergia Sissoo* (Roxb). *Journal of Biology and Life Science*, 6(2): 208–220. doi: 10.5296/jbls.v6i2.7445.

[11]. Li, H., Li, J., He, Y., Li, S., Liang, Z., Peng, C., Polle, A., Luo, Z.-B. (2013). Changes in carbon, nutrients and stoichiometric relations under different soil depths, plant tissues and ages in black locust plantations. *Acta Physiol. Plant.*, 35(10): 2951–2964. doi: 10.1007/s11738-013-1326-6.

[12]. Mbagwu, F.N., Ogbonnaya, C.I., Umeoka, N. and Edoki, N. (2017). Effects of indole-3-acetic acid (IAA) on the Vegetative propagation and Phytochemical properties of Bushbuck (*Gongronema latifolium* Benth.). *J. Food Process. Technol.*, 8(1): 1–3.

[13]. Orwa, C., Mutua, A., Kindt, R., Jamnadass, R. & Anthony, S. (2009). Agroforestree Database: A Tree Reference and Selection Guide Version 4.0. World Agroforestry Centre, Kenya.

[14]. Park, S. & Han, K.-H. (2003). An auxin-repressed gene (RpARP) from black locust (*Robinia pseudoacacia*) is posttranscriptionally regulated and negatively associated with shoot elongation. *Tree Physiol.*, 23(12): 815–823. doi: 10.1093/treephys/23.12.815.

[15]. Pijut, P. M., Woeste, K.E. and Michler, C.H. (2011). Promotion of Adventitious Root Formation of Difficult-to-Root Hardwood Tree Species. In: J. Janick (Ed.), Horticultural Reviews, Vol. 38. doi:10.1002/9780470872376.ch6.

[16]. Pop, T.I., Pamfil, D. & Bellini, C. (2011). Auxin Control in the Formation of Adventitious Roots. *Not. Bot. Horti. Agrobot. Cluj-Napoca*, 39(1): 307–316. doi: 10.15835/nbha3916101.

[17]. Rahbin, A., Abdolhossein, A. & Hamed, H. (2012). Study on the effect of cutting location on shoot and IBA on rooting of 'Night Jessamine' (*Cestrum nocturnum*) stem cuttings. *Intl. Res. J. Appl. Basic Sci*., 3(11): 2345-2348.

[18]. Rai, P. & Mishra, R.M. (2013). Effect of urban air pollution on epidermal traits of road side tree species, *Pongamia pinnata* (L.) Merr. *IOSR J. Environ. Sci. Toxicol. Food. Technol.*, 2(6): 4-7.

[19]. Rédei, K., Keserű, Z., Csiha, I., Rásó, J., Kamandiné Végh, Á. & Antal, B. (2013). Juvenile Growth and Morphological Traits of Micropropagated Black Locust (*Robinia Pseudoacacia* L.) Clones under Arid Site Conditions. *Acta Silvatica et Lignaria Hungarica*, 9(1): 35-42. doi: https://doi.org/10.2478/aslh-2013-0003.

[20]. Hoshmand, A.R. (2006). Design of Experiments for Agriculture and the Natural Sciences. 2nd Edition. Chapman and Hall (CRC Press). pp. 456.

[21]. Rice, S.K., Westerman, B. & Federici, R. (2004). Impacts of the exotic, nitrogen-fixing black locust (*Robinia pseudoacacia*) on nitrogen-cycling in a pine–oak ecosystem. *Plant Ecol.*, 174(1): 97–107. doi: 10.1023/B:VEGE.0000046049.21900.5a.

[22]. Ssekabembe, C.K., Henderlong, P.R. & Larson, M. (1994). Soil moisture relations at the tree/crop interface in black locust alleys. *Agrofor. Syst.*, 25(2): 135–140. doi: 10.1007/BF00705673

[23]. Sevik, H. & Guney, K. (2013). Effects of IAA, IBA, NAA, and GA3 on Rooting and Morphological Features of *Melissa officinalis* L. Stem Cuttings. Scientific World Journal, vol. 2013, Article ID 909507, 5 pages. doi: 10.1155/2013/909507.

[24]. Swamy, S.L., Puri, S. & Kanwar, K. (2002). Propagation of *Robinia pseudoacacia* Linn. and *Grewia optiva* Drummond from rooted stem cuttings. *Agrofor. Syst.*, 55(3): 231–237. doi: 10.1023/A:1020579516144.

[25]. Swearingen, J. (2009). WeedUS - Database of Plants Invading Natural Areas in the United States: Black Locust (*Robinia pseudoacacia*). http://www.invasive.org/weedus/ subject.html.

[26]. Taiz, L. and Zeiger, E. (1998). Plant Physiology. 2nd Edition, Sinauer Associates Publishers, Sunderland, Massachusetts. pp. 792.

[27]. Thomas, S.C. & Winner, W.E. (2000). Leaf area index of an old-growth Douglas-fir forest estimated from direct structural measurements in the canopy. *Can. J. Forest Res.*, 30(12): 1922-1930.

[28]. Xiu, Y., Iqbal, A., Zhu, C., Wu, G., Chang, Y., Li, N., Cao, Y., Zhang, W., Zeng, H., Chen, S. & Wang, H. (2016). Improvement and transcriptome analysis of root architecture by overexpression of *Fraxinus pennsylvanica DREB2A* transcription factor in *Robinia pseudoacacia* L. 'Idaho'. *Plant Biotechnol. J.*, 14(6): 1456–1469. doi: 10.1111/pbi.12509.

[29]. Zan, W., Xi, Y., Xue-Min, W. & Hong-Wen, G. (2011). Growth and physiological response of tall oat grass to salinity stress. *African Journal of Biotechnology*, 10(37): 7183–7190.

[30]. Zhang, J., Liu, Y. & Wang H. (2007). Micropropagation of Black Locust (*Robinia Pseudoacacia* L.). In: Jain S.M., Häggman, H. (eds), Protocols for Micropropagation of Woody Trees and Fruits. Springer, pp. 193–199. doi: 10.1007/978-1-4020-6352-7_18.