

Role of Paclobutrazol on Root Stem and Leaf Inner Structure of *Arabidopsis Thaliana* L.0 Grown Under Different Light Intensities

Wassan F. Abdul Hussain, Luma H. Abdul Qadir



Abstract: Light is one of the most important environmental factors affecting plant growth and development. Paclobutrazol (PBZ) is one of the members of Triazole compounds that possess the qualities of growth regulators. The study aimed to investigate the possible changes in anatomical structures of the root, stem, and leaf of *A. thaliana* grown under different light intensities and the role of pbz in these circumstances. The anatomical characteristics of the root, stem, and leaves of *A. thaliana* col.0 were measured after treating its seedlings at two weeks of age with Hock land's solution at different light intensities (3000, 6000, 9000, 12000 Lux) for four weeks. The results showed changes in anatomical characteristics due to light stress represented by a significant moral decrease in the diameter of the root, vascular cylinder, and thickness of the epidermis and cortex. It also led to a reduction in the width of the stem, the thickness of the xylem, and an increase in the thickness of the epidermis and phloem. Light stress caused a significant decrease in leaf thickness and the ratio of palisade tissue thickness to spongy tissue thickness. They addition the growth regulator PBZ causes a substantial increase in all of the anatomical characteristics of the stem, root, and leaves.

Keywords: *Arabidopsis Thaliana*, Anatomical Characteristics, Light Intensities, PBZ, Plants.

I. INTRODUCTION

Light is one of the most important environmental factors affecting plant growth and development, as changes in light intensity, light quality, and photoperiod affect plant morphology and metabolism [1]. It is the least studied and most complex of the other types of biotic stresses that plants experience repeatedly during their life cycle [2]. Exposure plants to a quantity of light exceeding the light saturation limit (high light intensity) or insufficient illumination puts them under the influence of photo stress that affects various physiological processes including photosynthesis, antioxidant mechanisms, and anatomical characteristics [3, 4]. Paclobutrazol (PBZ) is one of the members of Triazole compounds that possess the qualities of growth regulators and are called the term compounds protecting from stresses Plant multi-stress protectants. The treatment PBZ improves

the grain and dry weight of stressed plants [5]. As pointed out, PBZ increases the thickness of the cuticle layer and its content of waxy substances, in addition to causing an increase in the number of cells [6, 7]. Our study aimed to investigate the possible changes in anatomical structures of the root, stem, and leaf of *A. thaliana* grown under different light intensities and the role of pbz in these circumstances.

II. METHODS

Exposing the plant to lighting stress and testing the role of the growth regulator PBZ in mitigating the potential impact of stress on the internal anatomical structure of the plant. Seeds were planted in the pores that were placed in the growth room and under the intensity of lighting (3000, 6000, 9000, and 12000 Lux) with fluorescent LED lamps and a light period, of 8:16 (light/darkness), and the average temperature of 2 ± 25 degrees Celsius and relative humidity 65% and after two weeks of exposure of the plant to light stress, the seeds were treated with the interference of the growth regulator PBZ concentrations of (1.5, 1, 0.5 mg / l) with the intensity of lighting used (3000, 9000, 6000 and 12000 Lux). After the end of the experiments, the following measurements were taken: Internal dissection of leaves, roots, and stems using the paraffin wax method Anatomical Measurements: Collection of plant samples Roots, leaves, and stem samples were collected and the following steps were performed. The cut parts were fixed in the stabilizer (FAA), which was prepared from (5 ml) of formalin 5 ml of ice acetic acid, and 90 ml of ethyl alcohol for -24 hours, then the sections were washed with ethyl alcohol concentration of %70 to remove the effects of the stabilizer. Did passed the cut parts in an ascending series of ethyl alcohol (80,70 and 95%) for an hour and then to %100 absolute alcohol for a full night with alcohol replaced after 6 hours. The models were placed in bottles containing the absolute alcohol mixture: xylene in a ratio (1:3,1:1,3:1) for 30 minutes in each mixture, then left in pure xylene for 30 minutes, then placed in a mixture of xylene and paraffin wax in a furnace temperature 65-60 (m O) for four hours, then transferred to paraffin wax and left for a whole night at the same temperature. Prepare paraffin at the previous temperature and pour it into special plastic cubes then put the models inside these cubes after they worked and then leave to cool under cold water for a whole night and thus become ready for cutting. Models were cut by the rotating morgue with a thickness of (10 - 15)micrometers and placed in xylene for a whole night, then passed a series of waivers of %50,%70,%80,%90,%100, and 30.%then to distilled water for 5 minutes in each of them and then put with safranin dye for 60-30 minutes.

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I washed with distilled water to remove the excess dye, I then went through an escalating series of ethyl alcohol 90, 80, 70, 50%, 30%, and 100% and then put in a quick green dye for (30-15) seconds. Did washed them with absolute alcohol to remove the excess dye, then passed xylene three in a row for 5 minutes after that transferred them to xylene for 2 minutes added a drop of D.P.X. to the slides then placed covers on. The prepared slides were examined under a light microscope. The sizes of different anatomical structures were recorded and their mean was calculated by the Motic image plus 2 program.

III. RESULT AND DISCUSSION

The results in Table (1) showed that increasing the light intensity from 3000 Lux (control treatment) to 6000 Lux did not lead to a significant increase in the thickness of the cortex and that the addition of (1.5-1) mg/L of pbz led to a significant difference in the thickness of the cortex, reaching) 0.633 mm. It achieved the highest rate of cortex thickness when compared with the rest of the other treatments and the control treatment with a value of (0.223 mm). The addition of pbz a significant difference in cortex thickness in plants exposed to 9000 Lux light intensity. If a substantial increase in the thickness of the cortex was observed when treating (1 pbz + 9000) as it reached (0.606) mm and achieved the highest rate of skin thickness when compared with the luminous intensity coefficients LUX 9000 followed by the treatment 0.5 (pbz + 9000 Lux) at a rate of 0.540(mm). The thickness of the skin showed a significant increase with the increase in the intensity of light, 12000 Lux as it reached the highest rate at (1.5 pbz + 12000) (0.463) mm compared to other treatments and control treatments. The results of the statistical analysis showed that increasing the luminous intensity from (3000 Lux control treatment) to 6000 Lux did not lead to a significant reduction in the diameter of the root and that the addition of (0.5, 1) mg/L of pbz led to a significant increase in the root diameter of 1.797 mm. It achieved the highest rate of root diameter when compared with the rest of the other coefficients. The addition of pbz did not make a significant difference in root diameter in plants exposed to 9000 Lux light intensity. As if a significant increase was observed in the diameter of the root at the treatment (0.5 pbz) 12000 + as it reached (1.666) mm has achieved the highest rate of root diameter when compared with the coefficients of lighting intensity 12000 Lux followed by the treatment (1.5 pbz + 12000 Lux) at a rate of 1.477 mm. In general, a significant increase was observed in the diameter of the root with an increase in the intensity of lighting, as the highest rate was at (0.5 pbz) 12000 + 1.666(mm) compared to other treatments and control treatments. The results of the statistical analysis revealed that the increase in luminous intensity from 3000 Lux (control treatment) to 9000 Lux did not lead to a significant difference in the diameter of the vascular cylinder and that the addition of (0.5- 1) mg/L of pbz led to a significant increase in the diameter of the vascular cylinder amounting to (0.907) mm. It achieved the highest rate of the diameter of the vascular cylinder when compared with the rest of the other coefficients. The pbz treatment made a significant difference in the diameter of the vascular cylinder in plants exposed to 6000 Lux light intensity. A significant increase was observed in the diameter of the vascular cylinder when the treatment (0.5 pbz + 6000 Lux) as it reached (1.240) mm achieved the

highest rate of the diameter of the vascular cylinder when compared with the coefficients of lighting intensity 6000 Lux followed by the treatment 1(pbz + 6000 Lux) at a rate of (0.963 mm). The increase in the intensity of illumination led to a significant increase in the diameter of the vascular cylinder, as the highest rate was (0.5 pbz + 12000 Lux (1.117 mm) compared to other coefficients and the control treatment that was recorded (0.800 mm). The results of the statistical analysis indicated that the increase in light intensity from (3000 Lux control treatment) to 9000 Lux led to a significant increase in epidermis thickness, as the treatment (mg. L) 0.5 1-of pbz has the highest value of (0) 580 mm when compared with the control coefficient of (0.177 mm). No significant difference in epidermis thickness was observed when pbz was added in plants exposed to 6000 Lux light intensity. If a significant increase in the thickness of the epidermis was observed when treating (0.5 pbz) + 12000 as it reached (0.320 mm) and achieved the highest rate of epidermis thickness when compared with the coefficients of light intensity Lux 12000 followed by the treatment (1pbz +12000 Lux) at a rate of 0.250 (mm). (Figure 1) Results were recorded in Table (2) that the increase in light intensity from 3000 Lux (control treatment) to 6000 Lux did not constitute a significant increase in epidermis thickness, and that the addition of growth regulator pbz at concentration (1 mg.L⁻¹) with a light intensity of 6000 Lux caused an increase in the thickness of the epidermis, reaching (0.240 mm). It achieved the highest average epidermis thickness when compared with other treatments. No significant increase in epidermis thickness was served when pbz was added in plants exposed to 9000 Lux light intensity of the results of the statistical analysis as there was a significant increase in the thickness of the epidermis when the treatment (0.5 pbz +12000 Lux) as it reached (0.240 mm) has achieved the highest rate of epidermis thickness when compared with the treatment of light intensity 12000 Lux followed by the treatment (1 pbz + 12000 Lux) at a rate of (0.183 mm). There was a significant increase in epidermis thickness with increasing light intensity, as the highest rate was 1.5 pbz +12000 Lux (0.433 mm) compared to other treatments and the control treatment was recorded at 0.163 mm. The results of the statistical analysis showed that the increase in light intensity from 3000 Lux (control treatment) to 6000 Lux did not see any significant difference in vessel xylem thickness and that the treatment with PBZ with intensity light 6000 Lux caused a significant increase in vessel xylem thickness at a concentration (1.5 pbz mg.L⁻¹) achieved the highest value (0.440 mm) followed by the treatment at a concentration of (1 pbz mg.L⁻¹) was recorded (0.393 mm) while the control treatment (0.357 mm) was recorded. The results of Table (2) showed that the light intensity 9000 Lux has achieved the highest value in vessel xylem thickness with a significant difference of (0.470 mm) and that the addition of growth regulator PBZ also achieved significant superiority at a concentration (1.5 pbz mg .L⁻¹) as a record (0.400 mm) compared to other transactions No significant difference in vessel xylem thickness was observed with the increase in light intensity at 12000 Lux and even when pbz was added in plants exposed to the light intensity 12000 Lux, no significant increase was recorded in the thickness of the xylem.

3000 Lux from intensity light in increase that the revealed analysis statistical of the thickness the difference significant to a led 6000 Lux (control treatment) to a with recorded treatment control to compared (0.267 mm (reached it as, phloem significant a to led (1 mg.L⁻¹) of pbz treatment the that and (0.243 mm) of value phloem of rate highest the achieved It (0.417 mm) phloem the of thickness the increase regulator growth of addition.

The treatments other with compared when thickness exposed plants in the phloem of thickness the difference significant a made pbz with (0.5 mg.L⁻¹) of pbz recorded was the intensity light 9000 Lux to compared (0.263 mm) thickness bark of value intensity highest 9000 Lux in the thickness of the phloem decrease a significant if As treatment the of rest the results the while,12000 Lux at intensity light increase in a with observed was in increase significant a to led pbz regulator growth of the addition the that showed of intensity light a with (1.5mg. L) concentration at cortex the of thickness the light a with (1 mgL.-1) concentration by followed(0.330 mm), then you see that the concentration was (1 mgL.-1) pbz with the light intensity of 12000 Lux, as it recorded (0.280 mm) compared to the other parameters.

The results of the statistical analysis showed that increasing the light intensity from 3000 Lux (treatment control) to 6000 Lux caused a significant increase in the width of the stem, the interaction factor between the light intensity and the PBZ (+6000 Lux + 1.5 mg. L pbz) recorded the highest value as (2.033 mm) when compared with the rest of the other parameters and the control treatment at value reached (1.257 mm). (Figure 2).

The results of the statistical analysis showed that the increase in the light intensity from 3000 Lux (control treatment) to 12000 Lux led to a significant increase in the measurement of leaf stacking (thickness of the palsied tissue / thickness of the leave) as the intensity of light 0.353 (12000 Lux) compared to the control treatment that was recorded (0.233) It was followed by the intensity of light 9000 Lux , as it recorded a significant increase in the measurement of the stacking of the leave with a value of (0.313), followed by the intensity of lighting 6000 Lux with a value of (0.290) The results showed that the addition of the growth regulator pbz led to a significant increase in the measurement of paper stacking, as the treatment of light

intensity and growth regulator 9000 Lux +1.5 pbz recorded a rate of (1.187) which represents the highest value in the stacking of the leave, while the transaction recorded (6000 Lux + 0.5pbz) the lowest value in the stacking of the paper at a rate of (0.240). The results indicated that the increase in the intensity of lighting led to a significant increase in the percentage that represents (the palisade tissue/sponge tissue) as the intensity of the light 12000 Lux recorded the highest value of (0. 287) compared to other lighting intensities followed by the intensity of lighting 9000 Lux with a value of (0.477) then followed by the intensity of lighting6000 Lux (0.447) compared to the control treatment (0.360). The results also showed in Table (3) that the addition of the growth regulator led to a significant increase in the high percentage of leaves representing the thickness of the (sponge tissue/palisade tissue) as the treatment of growth regulator and light intensity (9000 Lux+ 1.5 pbz)was recorded at a rate of (1.85) which represents the highest value in the ratio (spongy tissue/palisade tissue)while the transaction recorded (6000 Lux +0.5 pbz) 0.393 which represents the lowest value compared to other transactions. The results showed that the increase in the intensity of light from (3000 Lux control treatment) to 6000 Lux led to a significant reduction in the number of stomata and that the addition of the growth regulator pbz led to a significant increase in the number of stomata, as the treatment of the growth regulator and the intensity of light was recorded 6000Lux+ 1 PBS)at a rate of (104.666) which represents the highest value in the number of stomata, followed by the transaction6000 LUX 1.5(+pbz) which was recorded at a rate of (100.000). In general, the increase in light intensity from 9000 Lux to 12000 Lux led to a significant decrease in the number of stomata, as the intensity of lighting at 9000 Lux (90.666) was recorded, while the intensity of 12000 Lux recorded an average in the number of stomata (74.333) The addition of the growth regulator pbz led to a significant increase in the number of stomata, as the recorded (12000 Lux+ 1 pbz) value of (83.333) which represents the highest value compared to other transactions, while the transaction recorded 9000 Lux + 1 pbz) the lowest value in the number of stomata at a rate of (41.333) compared to other as shown in Figure 3.

Table- I: Effect of Light Stress and PBZ on Anatomical Characteristics of A. Thaliana Col.0 Roots

Cortex Thickness	Epidermis Thickness	Vascular Bundles Diameter	Root Diameter	Treatment
0.223 ± 0.023	0.177 ± 0.032	0.800 ± 0.010	1.117 ± 0.015	3000
0.343 ± 0.119	0.203 ± 0.101	0.897 ± 0.042	0.997 ± 0.518	6000 LUX
0.500 ± 0.026	0.266 ± 0.090	1.240 ± 0.010	1.797 ± 0.032	6000LUX+(0.5 mg . L ⁻¹ pbz)
0.034 ± 0.070	0.127 ± 0.025	0.963 ± 0.049	1.413 ± 0.110	6000LUX+(1 mg . L ⁻¹ pbz)
0.633 ± 0.049	0.257 ± 0.124	0.950 ± 0.036	1.647 ± 0.015	6000LUX+(1.5 mg . L ⁻¹ pbz)
0.200 ± 0.034	0.390 ± 0.442	0.700 ± 0.121	0.977 ± 0.136	9000LUX
0.540 ± 0.040	0.580 ± 0.105	0.907 ± 0.015	1.630 ± 0.06	9000LUX +(0.5 mg . L ⁻¹ pbz)
0.606 ± 0.059	0.180 ± 0.044	0.843 ± 0.015	1.563 ± 0.020	9000LUX +(1 mg . L ⁻¹ pbz)
0.357 ± 0.072	0.193 ± 0.042	0.833 ± 0.566	1.623 ± 0.035	9000LUX +(1.5 mg . L ⁻¹ pbz)
0.330 ± 0.030	0.223 ± 0.030	0.860 ± 0.020	1.477 ± 0.051	12000LUX
0.397 ± 0.074	0.320 ± 0.020	1.117 ± 0.011	1.666 ± 0.049	12000LUX +(0.5 mg . L ⁻¹ pbz)
0.350 ± 0.104	0.250 ± 0.117	0.710 ± 0.100	1.250 ± 0.017	12000LUX +(1 mg . L ⁻¹ pbz)
0.463 ± 0.060	0.433 ± 0.248	0.623 ± 0.032	1.477 ± 0.099	12000LUX +(1.5 mg . L ⁻¹ pbz)
0.406 ± 0.141	0.277 ± 0.179	0.880 ± 0.211	1.433 ± 0.291	Mean
0.039	0.081	0.060	0.122	LSD

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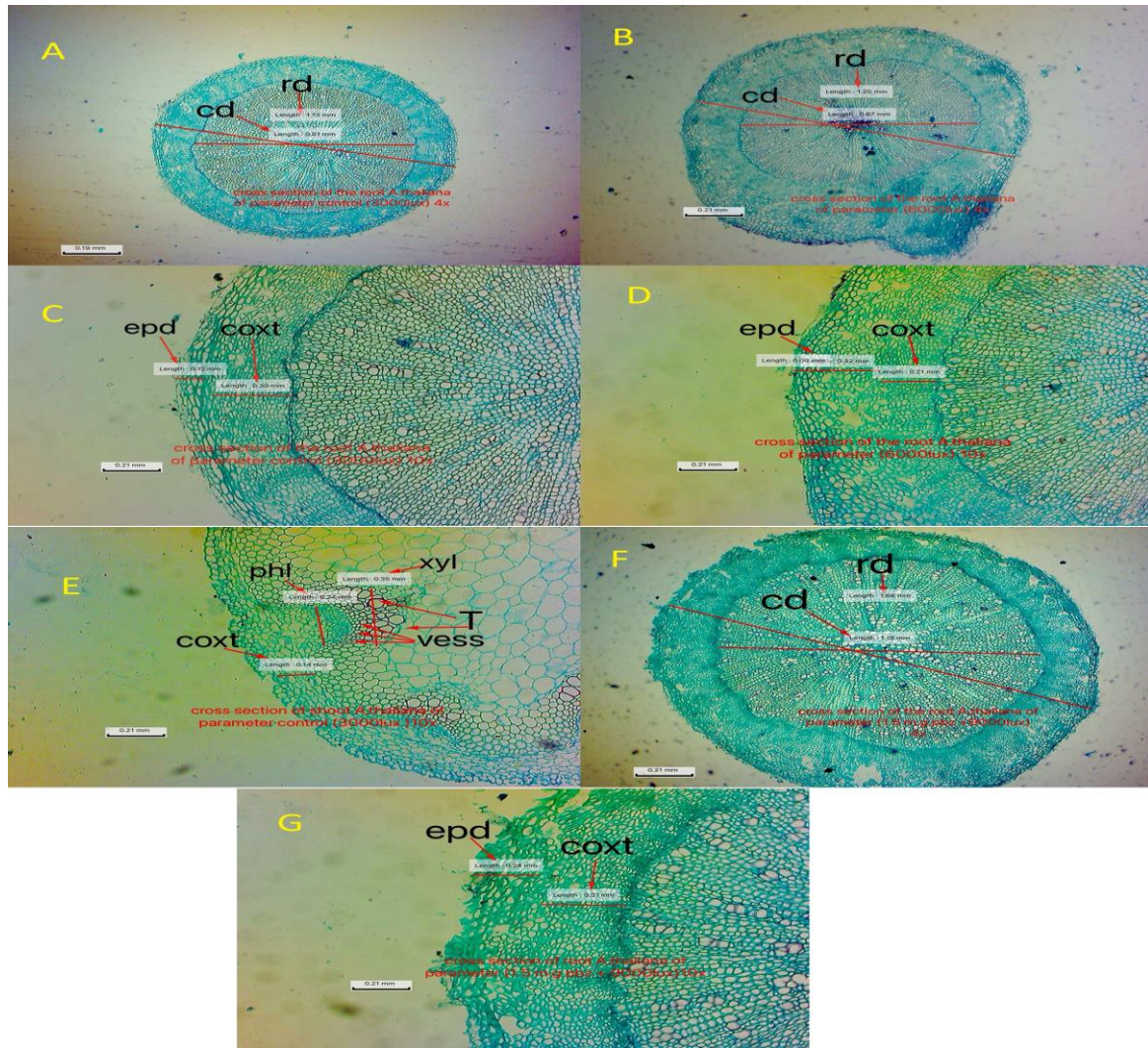


Fig. 1. Cross Section of the Root of *Arabidopsis Thaliana*, (Cd – diameter vascular; rd – diameter root; epd - epidermis; cox -cortex; phl – phlegm; xyl – xylem; vess – vessels; T -Tracheid)

Table- II: Effect of Light Stress and PBZ on Anatomical Characteristics of *A. thaliana* Col.0 Stem

Phloem Thickness	Xylem Thickness	Epidermis Thickness	Stem Width	Treatment
0.243 ± 0.025	0.357 ± 0.030	0.163 ± 0.322	1.257 ± 0.066	3000 LUX
0.267 ± 0.051	0.323 ± 0.159	0.143 ± 0.045	1.603 ± 0.025	6000 LUX
0.290 ± 0.020	0.367 ± 0.041	0.193 ± 0.066	1.883 ± 0.045	6000LUX+(0.5 mg . L ⁻¹ pbz)
0.417 ± 0.120	0.393 ± 0.078	0.260 ± 0.034	1.960 ± 0.062	6000LUX+(1 mg . L ⁻¹ pbz)
0.370 ± 0.044	0.440 ± 0.079	0.170 ± 0.045	2.033 ± 0.035	6000LUX+(1.5 mg . L ⁻¹ pbz)
0.290 ± 0.050	0.470 ± 0.030	0.247 ± 0.055	2.027 ± 0.045	9000LUX
0.263 ± 0.035	0.303 ± 0.020	0.227 ± 0.025	1.847 ± 0.201	9000LUX +(0.5 mg . L ⁻¹ pbz)
0.257 ± 0.042	0.273 ± 0.030	0.250 ± 0.062	1.773 ± 0.161	9000LUX +(1 mg . L ⁻¹ pbz)
0.233 ± 0.059	0.400 ± 0.060	0.217 ± 0.030	1.607 ± 0.047	9000LUX +(1.5 mg . L ⁻¹ pbz)
0.313 ± 0.071	0.333 ± 0.035	0.193 ± 0.035	1.767 ± 0.046	12000LUX
0.247 ± 0.015	0.303 ± 0.015	0.240 ± 0.026	1.353 ± 0.032	12000LUX +(0.5 mg . L ⁻¹ pbz)
0.280 ± 0.069	0.237 ± 0.090	0.183 ± 0.051	1.257 ± 0.032	12000LUX +(1 mg . L ⁻¹ pbz)
0.330 ± 0.044	0.337 ± 0.100	0.157 ± 0.050	1.990 ± 0.055	12000LUX +(1.5 mg . L ⁻¹ pbz)
0.292 ± 0.069	0.349 ± 0.087	0.203 ± 0.053	1.719 ± 0.283	Mean
0.016	0.039	0.039	0.130	LSD

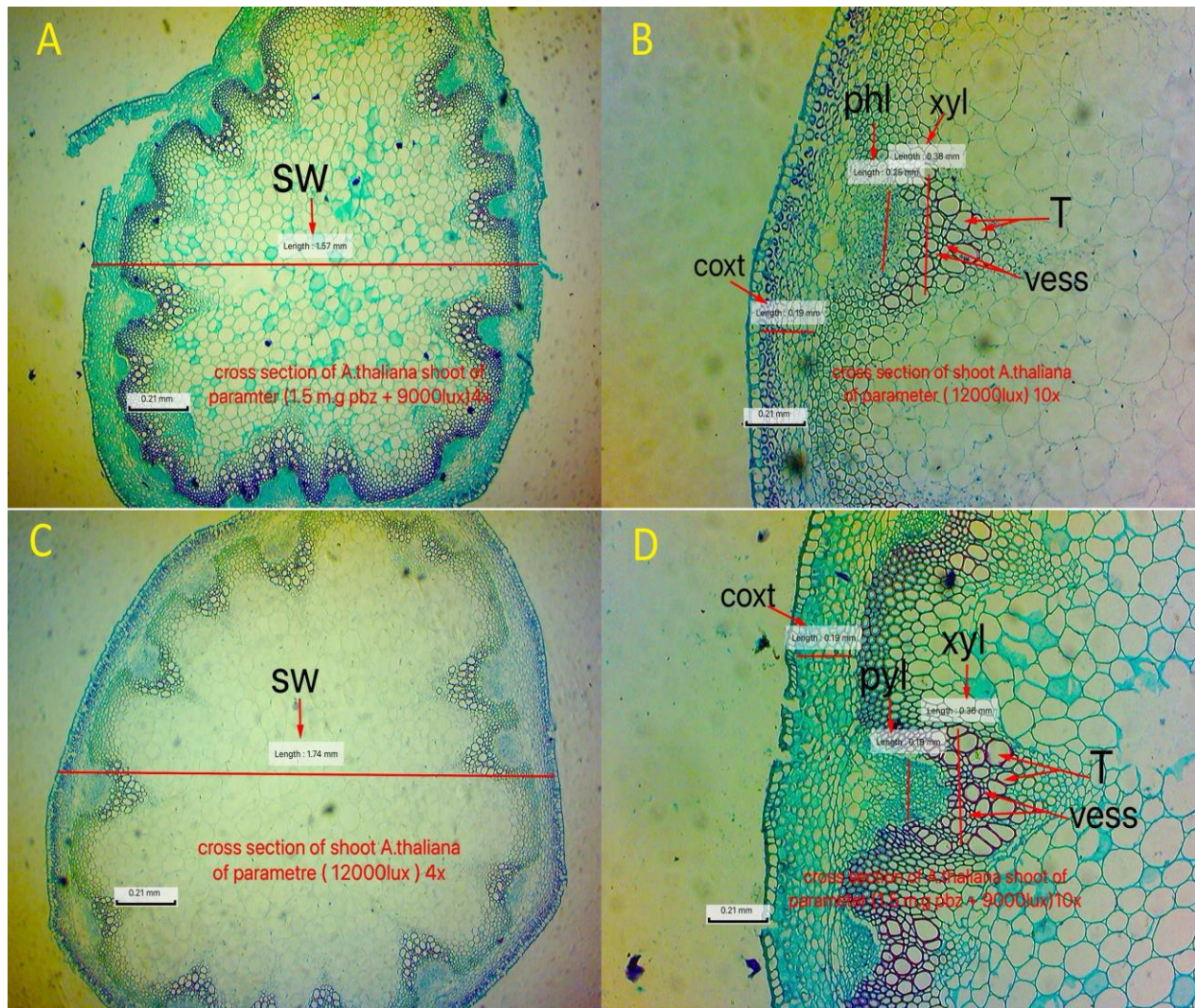


Fig. 2. Cross Section of Shoot of *Arabidopsis thaliana*, (coxt -cortex; phl – phlem; xyl – xylem; vess – vessels; T – Tracheid; Sw – shoot Wight)

Table- III: Effect of Light Stress and PBZ on Anatomical Characteristics of *A. thaliana* Col.0 Leaves

Stomata Number	Ratio	Leaves Stacking	Treatment
54.333 ± 14.640	0.360 ± 0.080	0.233 ± 0.031	3000
42.000 ± 9.539	0.447 ± 0.111	0.290 ± 0.020	6000 LUX
40.333 ± 14.977	0.393 ± 0.032	0.240 ± 0.044	6000LUX+(0.5 pbz)
104.666 ± 10.214	0.420 ± 0.105	0.243 ± 0.021	6000LUX+(1 pbz)
100.000 ± 6.557	0.423 ± 0.129	0.263 ± 0.057	6000LUX+(1.5 pbz)
90.666 ± 14.640	0.477 ± 0.057	0.313 ± 0.021	9000LUX
57.333 ± 13.051	0.410 ± 0.076	0.277 ± 0.060	9000LUX +(0.5 pbz)
41.333 ± 4.041	0.463 ± 0.123	0.287 ± 0.038	9000LUX +(1 pbz)
75.333 ± 16.623	1.850 ± 2.300	1.187 ± 1.485	9000LUX +(1.5 pbz)
74.333 ± 15.143	0.587 ± 0.107	0.353 ± 0.035	12000LUX
62.000 ± 6.557	0.730 ± 0.184	0.407 ± 0.038	12000LUX +(0.5pbz)
83.333 ± 29.091	0.697 ± 0.154	0.393 ± 0.067	12000LUX +(1pbz)
72.000 ± 15.099	0.523 ± 0.050	0.287 ± 0.046	12000LUX +(1.5pbz)
69.051 ± 24.337	0.599 ± 0.657	0.367 ± 0.421	Mean
10.5	0.036	0.033	LSD

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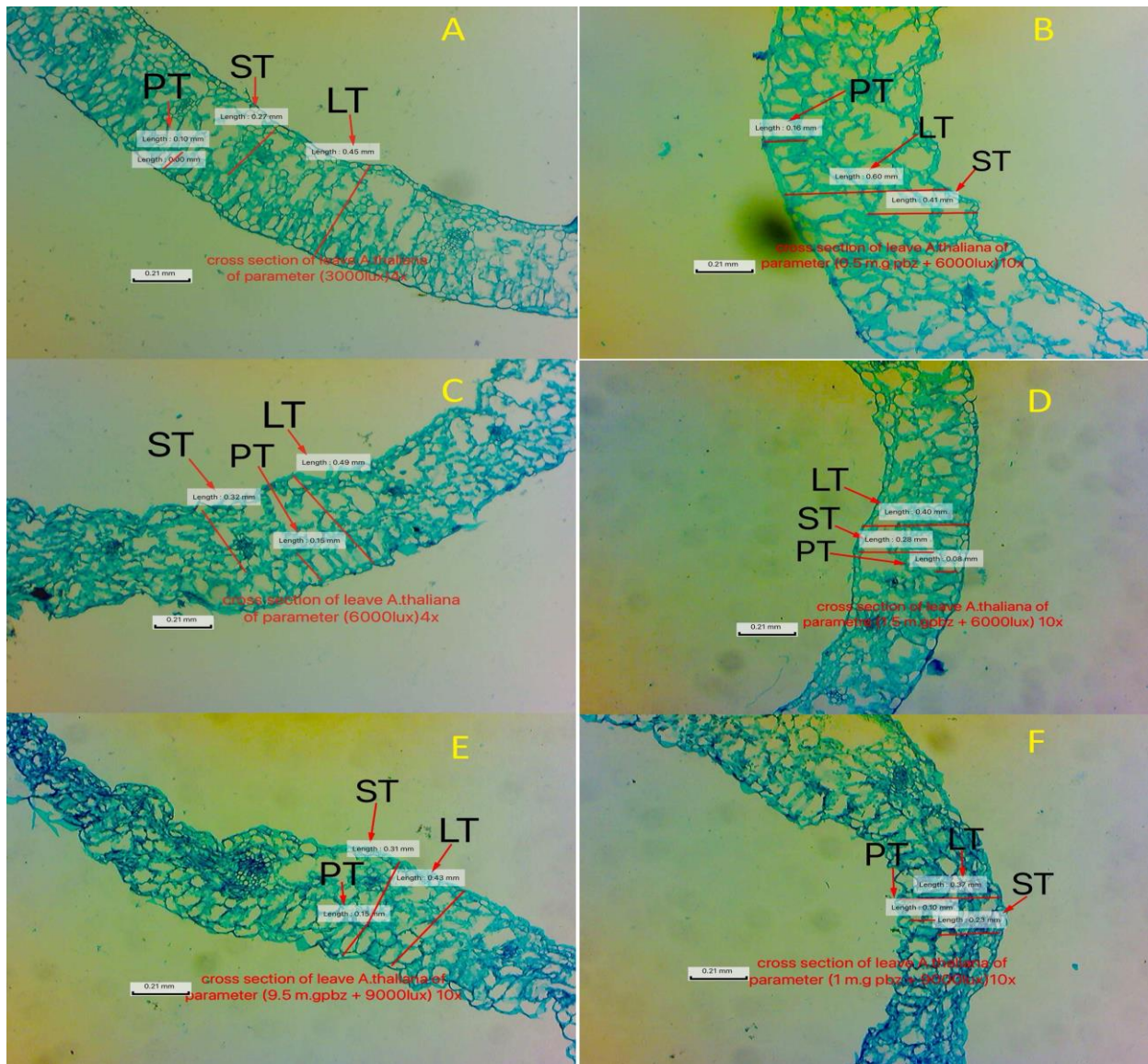


Fig. 3. Cross Section of Leaves of *Arabidopsis thaliana*, (PT – palisaded tissue; LT – Leaf tissue; ST – Spongy Tissue)

The results showed that the increase in light intensity led to a decrease in each of the anatomical structures of the root (root diameter, diameter of the vascular cylinder, thickness of the epidermis, and thickness of the cortex) and the reason for this is that photosynthetic sugars are signals to promote root growth over long distances and sucrose along can enhance the growth of primary roots in the dark [8], as the majority of studies conducted on the *Arabidopsis* plant use Petri dishes that allow plant growth in Under certain light conditions, high illumination on the roots generates a wave of reactive oxygen species (ROS) and alters cytokinin balance [9]. Light stimulates the synthesis of auxin in young leaves, which is polarly shifted to the roots, which promotes primary root growth [10-12]. The results also showed that the treatment with the growth regulator pbz led to improve the root characteristics of the plant *A. thaliana* under study, because pbz is a plant growth regulator of the trazole type, which is known as anti-gibberellins, as pbz can prevent the conversion of ent- Kaurene to ent-Kaurenoic acid during the biogenesis pathway of gibberellins by inhibiting Kaurene oxidase [13, 14], can therefore be attributed to the way PBZ inhibits gibberellin synthesis, which reduces the level of gibberellin and slows cell division and elongation without causing cell toxicity, increases cytokinin content as well as increased root activity and delayed aging [15, 16]. Through the results of

our study, the control treatment led to a significant decrease in the thickness of the stem, while the intensity of the light led to a significant increase in the thickness of the stem, and the reason for this is that during the low light intensity, more carbohydrates are used to increase the length of the stem instead of increasing the thickness of the stem and increasing the length of the plant leads to an increase in the amount of light received by the leaves [17], low light intensity increases plant length, reduces stem thickness and ultimately increases plant dormancy, hindering the transport of nutrients, water and photosynthetic products [18]. The results of the study agreed with the results of Tsegaw et al [19] as it confirmed that the treatment with pbz on tomato plants led to an increase in the diameter of the stem and the reason for this is attributed to the role of pbz in stimulating the thickness of the vascular bundle and increasing the thickness of the cortex tissue and the thickness of the diameter of the vascular tissues. The increase in stem thickness can also be attributed to radial enlargement of cells due to reduced gibberellin activity in response to the stem during PBZ treatment [20] [31] [32] [33].

The results of this study by Fan et al [21] confirmed the results of our current study that the increase in light intensity led to a significant increase in (leaf thickness and thickness of the palisade and spongy tissue) as the leaf is the main part in the process of photosynthesis and any changes in the anatomy of the leaves positively or negatively affect the process of photosynthesis, and these results agreed with the results of the study [21, 22]. They confirmed the improvement of leaf structure during high light intensity, due to the increase in mesophyll tissues and the decrease in light intensity resulting from leaves with a large gap in cells and cell arrangement, and therefore the thickness of spongy and palisade tissues may decrease due to low cell growth and the number of cell layer in the columnar tissues [23]. Furthermore, the increase in light intensity increased the process of cell elongation in the columnar tissues that enhanced the chloroplastic region through which CO₂ enters, and thus the increase in leaf tolerance and photosynthesis capacity was significantly enhanced in soybean plants [18, 24]. From the results of the current study, it appears that a decrease in light intensity leads to a decrease in the thickness of the leaf and the thickness of the palisade and spongy tissue. The reason for this is that the internal structure of the leaf is affected by environmental factors, as many studies indicated that there is a flexible change inside the leaf before the change of external conditions, especially at a low light intensity, which represents one of the adaptive qualities indicated by many previous studies, such as the *Datura stramonium* plant [25] and *Brassica napus* L [26] as chloroplasts are concentrated in palisade cells more than spongy cells until chlorophyll appears with a higher content in the first layer, allowing less incident light to be transferred into the leaf, and thus more light always penetrates the first layer of palisade cells. There are also many air spaces between the spongy cells that reflect the incident light and break it randomly [27], so the larger mesophyll cells can increase the surface area of the inner leaf and thus increase the efficiency of photosynthesis. The results of the current study indicated that the treatment with pbz led to a significant increase in the thickness of the leaf and the measurement of the left terrace (the ratio of the palisade tissue / spongy tissue) and these results agreed with the results of Jaleel et al [15], which confirmed that the treatment with pbz led to an increase in the thickness of the leaves compared to the treatment of control and the reason for this is due to the increase in the size of the mesophyll cells (cells of the palisade and spongy tissue) and the epidermis cells increased in thickness and the cells of the palisade layer suffer from elongation and increase. In spongy cells grow larger and your name from the epidermis layer, as the leaves have a low light intensity will be thinner and this leads to an increase in light interception by the leaves, and also leads to a decrease in the thickness of the leaves, which is a form of adaptation to increase light transmission. About stomata, the results of the statistical analysis showed that the increase in light intensity led to an increase in the number of stomata, as stomata are modified plant cells whose function is to enter Co₂ and absorb water and oxygen (transpiration) and because stomata cells are associated with photosynthesis, so the density of stomata may lead to an increase in the probability of entering Co₂ and increasing the process of photosynthesis [28]. The results of our current study are consistent with the results of Yao et al [26], which confirmed that increasing the intensity of light led to an increase in the density of stomata and a change in their shape. The treatment of growth

regulator pbz led to a significant increase in the number of stomata, as the treatment (6000 Lux + 1 pbz) recorded the highest value compared to the control treatment and this is consistent with the study Tari, [29] and the reason for the increase in the number of stomata is attributed to the fact that the growth regulator pbz works to accumulate the number of stomata per unit area of leaves and the increase is due to the negative impact of pbz on the area of the leaves [30].

IV. CONCLUSION

Light stress caused a significant decrease in leaf thickness and the ratio of palisade tissue thickness to spongy tissue thickness. Adding the growth regulator PBZ causes a substantial increase in all of the anatomical characteristics of the stem, root, and leaves.

DECLARATION STATEMENT

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Authors Contributions	Wassan F. Abdul Hussain; Conceptualization; Data Curation; Investigation; Methodology; Project administration; Resources; Software; Validation; Visualization; Writing – original draft and Writing – review & editing. Luma H. Abdul Qadir; Conceptualization; Data Curation; Resources; Supervision; Validation; Visualization and Writing – review & editing.

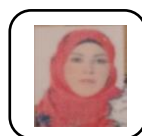
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