A comparative anatomical study of effect of light intensity and growth regulator PBZ on some characteristics of leaves of *Arabidopsis thaliana* L.

Wassan F. Abdul Hussain[1] Luma H. Abdul Qadir[2]

[1,2]Department of Biology, College of Education for Pure Science, Basrah, Iraq.

[1]Corresponding author: Wasansad114@gmail.com

[2]luma.abdulqadir@uobasrah.edu.iq

Abstract

In this paper, some anatomical characteristics of *Arabidopsis thaliana* L. leaves were studied. Measurements of leaf thickness, palisade and spongy tissue after statistical analysis showed that the light intensity of 12,000 lux recorded the largest value in all characters compared to the rest of the stresses. The addition of growth regulator Paclobutrazol (PBZ) at different concentrations ranging from 0.5, 1 and 1.5 mg / L for each light intensity showed varying significant differences, as the treatment of the leaf with the growth regulator at a concentration of 1.5 mg/ L and a light intensity of 9000 lux recorded high significant differences when compared with other concentrations 0.5, 1, 1.5 mg/ L in 6000 lux, 0.5 and 1 mg/ L in 9000 lux and 0.5, 1 and 1.5 mg/ L in 12000 lux .

Keyword: anatomical characteristics, Leaf, *Arabidopsis thaliana*, light intensity, PBZ.

1. Introduction

Organisms, including plants, face periods of extremes in environmental conditions known as plant stress, which is a physiological disorder that occurs as a result of plant exposure to the natural environmental factors that affect plant growth, and the change of these conditions and their differences affect the plant in one way or another. Plant stress is of two types, either biological stress, which affects the environment of living organisms such as plant fungi and pathological stresses, or abiotic stress, which affects both the physiological and environmental state of the organism and includes many physical – chemical factors that occurs naturally, such as acid stress, temperature stress, water stress, salinity stress, elemental deficiency stress, light intensity and heavy element stress [1].

Light is an essential source of energy and is one of the most important environmental factors for plant growth [2], light intensity and quality are essential for plant growth and other physiological responses [3; 4; 5]. It is noteworthy that changes in light intensity affected the anatomy, physiology and morphology of leaves [6 ; 7], it was shown that the blue light spectrum increased the thickness of the epidermis and mesophyll cells, while

the red light spectrum led to a decrease in the thickness of spongy tissue [8 ; 7].

The growth regulator Paclobutrazol is one of the triazole compounds, which have the properties of regulating plant growth and are called plant protection substances from threatening stresses due to their ability to stimulate abiotic stress tolerance by increasing antioxidant enzymes and molecules in plants affected by stress [9].

Studying the changes caused by these stresses and enhancing the ability of plants to tolerate them is one of the most important matters that have occupied and still occupy scientists and researchers, because of the importance of plants in the food chain as productive organisms and for their role in achieving a balance between oxygen and carbon dioxide, many studies have been conducted on different plant species used to determine the mechanisms by which plants can become tolerant or to increase their tolerance to abiotic stresses, and in recent decades, scientists and researchers have resorted to using a plant that is like a Drosophila insect for Zoology, it is a plant *Arabidopsis thaliana*, which is considered one of the most important plants that use as a model plant, it is studied by many researchers because it has easy to study qualities on the one hand and because it is similar enough to other organisms on the other hand so that experiments can be applied and results can be deduced from it, as the plant has become a standard source of information, especially after the publication of its genetic sequence, as it has become a source for the study of floral plants [10].

Therefore, the research aimed to study the effect of light intensity and growth regulator PBZ on some anatomical parameters of leaves of the plant *A. thaliana* L. Col. 0 using a light microscope.

2. Materials and methods

2. 1. Sample preparation

Five seeds bring from Carolina Biological supply company in American United States were planted for each perforated plastic anvil with a diameter of 20 cm containing 2 kg of a mixture of bitumen and sand in a ratio of 1 : 2 and placed in the growth chamber for germination and after two weeks the plant were exposed to the following lighting intensities (3000, 6000, 9000 and 12000 LUX) with LED lamps (16 : 8) light / dark and a temperature rate of ± 20 C^{o} and relative humidity 65 % and the plants were also watered with Haukland nutrient solution to which the growth regulator PBZ was added at concentrations of 0.5, 1, and 1.5 mg/L [11].

2. 2. Anatomical study

Fresh leaves of *A. thaliana* were fixed in fixative F.A.A. (Formalin, glacial Acetic acid and Ethyl alcohol) for 24 hour then washed with 70% ethanol and dehydrated using serial concentrations of ethanol (80% , 90% , 100%) for two hours for each concentration after that transferred to mixture of 3:1, 1:1 and 1:3 ethanol : xylene for 45 minute per mixture then placed in pure xylene for 30 minute after

that transferred to mixture of xylene and paraffin wax in oven at 60 ºC for 3 hours then placed in pure xylene for 30 minutes. after that, they were transferred to a mixture of xylene and paraffin wax in oven for 3 hours at 60 Cº then placed into pure paraffin wax overnight period at same temperature, next the specimens were embedded in melted paraffin wax, which adhered to wooden blocks after hardening then sectioning using a rotary microtome adjusted at 10 microns. The sectioned wax ribbons were placed on glass slides and they were ready for staining. Putting slides in coupling jars containing xylene overnight is the first step in the staining process. Next, they are transferred to serial descending concentrations of ethanol (100% , 90% , 80% , 70%) for 10 minutes for each concentration then putting them in safranine stain for 10 minutes, after that, transfer them to serial ascending concentrations of ethanol (70% , 80% , 90% and 100%) then placing them in fast green stain for 5 seconds then washing them in absolute ethanol after that is transferred to xylene twice consecutively for 5 minutes each time. Finally, add a drop of DPX (Dibutyphthalate Polystrene Xylene) to the slides then covering them [12]. The prepared slides were examined using a light microscope with an ocular micrometer. The size of few anatomical structures were recorded and their mean were calculated.

2. 3. Statistical analysis

The results were statistically analyzed according to the one-way ANOVA variance analysis using the SPSS version 23 program and after obtaining the Anova table variance analysis Table, the averages for each of the studied characteristics were compared using the LSD least significant difference test and below the probability level of 0.05 [13].

3. Results and discussion

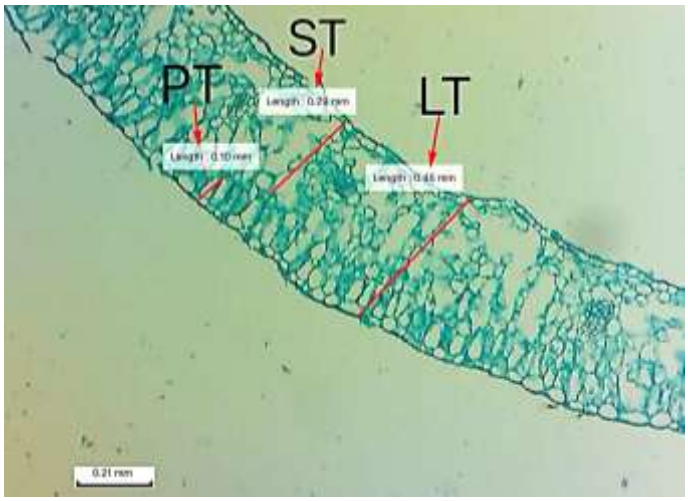
The results of measurements of leaf thickness, palisade and spongy tissue exposed to light intensities 6000, 9000 and 12000 LUX as in plate 1, 2 and 3, which were compared with the leaf exposed to light intensity 300, which represents the control after statistically analyzed as shown in Table (1), showed the presence of significant differences in the studied qualities under the influence of light intensities used in the current study, as the light intensity 12000 recorded the largest value of the spongy tissue to the palisade tissue reached 0.353 ± 0.035 mm and 0.587 ± 0.107 mm, respectively, compared with the rest, and this result is consistent with Feng *et al*. [14] and Swiad [15], which proved that increasing the intensity of light led to a significant increase in the thickness of the leaf and the thickness of palisade and spongy tissue transactions, and the increase of the leaf and the average tissue is one of the adaptations made by plants to avoid exposure to the high amount of light that may affect the existing pigments, especially chlorophyll, and thus affect the process of photosynthesis.

The results showed that the addition of the growth regulator PBZ at the three light

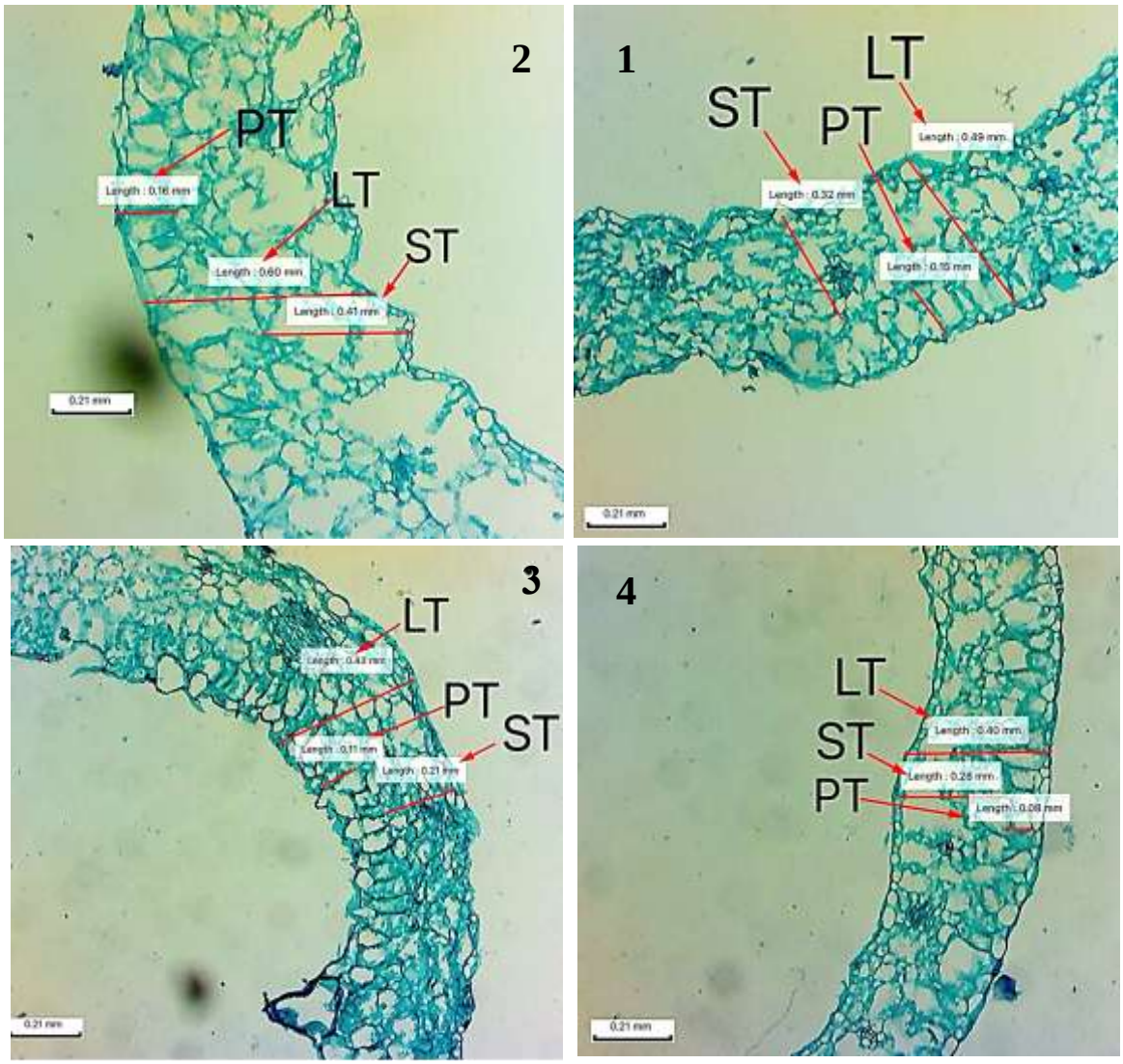
intensities led to a significant increase in thickness of leaf and the ratio of palisade tissue to spongy tissue in control treatment. The results also showed significant differences between the different concentrations of growth regulators at the lighting intensities used, It was found that treating leaves with growth regulator PBZ at a concentration of 1.5 mg/L and a light intensity of 9000 lux showed highly significant differences when compared with the rest of the treatments, as the ratio of palisade tissue thickness to total leaf thickness as well as the ratio of spongy tissue thickness to palisade tissue thickness is 1.187 ± 1.485 mm and 1,850 ± 2,300 mm, respectively, while the lowest significant difference was recorded for the ratio of palisade tissue thickness to the total leaf thickness, as well as the ratio of spongy tissue thickness to palisade tissue thickness at a concentration of 0.5 mg/L at a lighting intensity of 6000 lux is reached 0.240 ± 0.044 mm and 0.393 ± 0.032 mm. Respectively, these results agreed with Jaleel *et al*. [16].

(Table 1) the effect of light intensities and growth regulator PBZ on the anatomical qualities of the leaves of *A. thaliana*

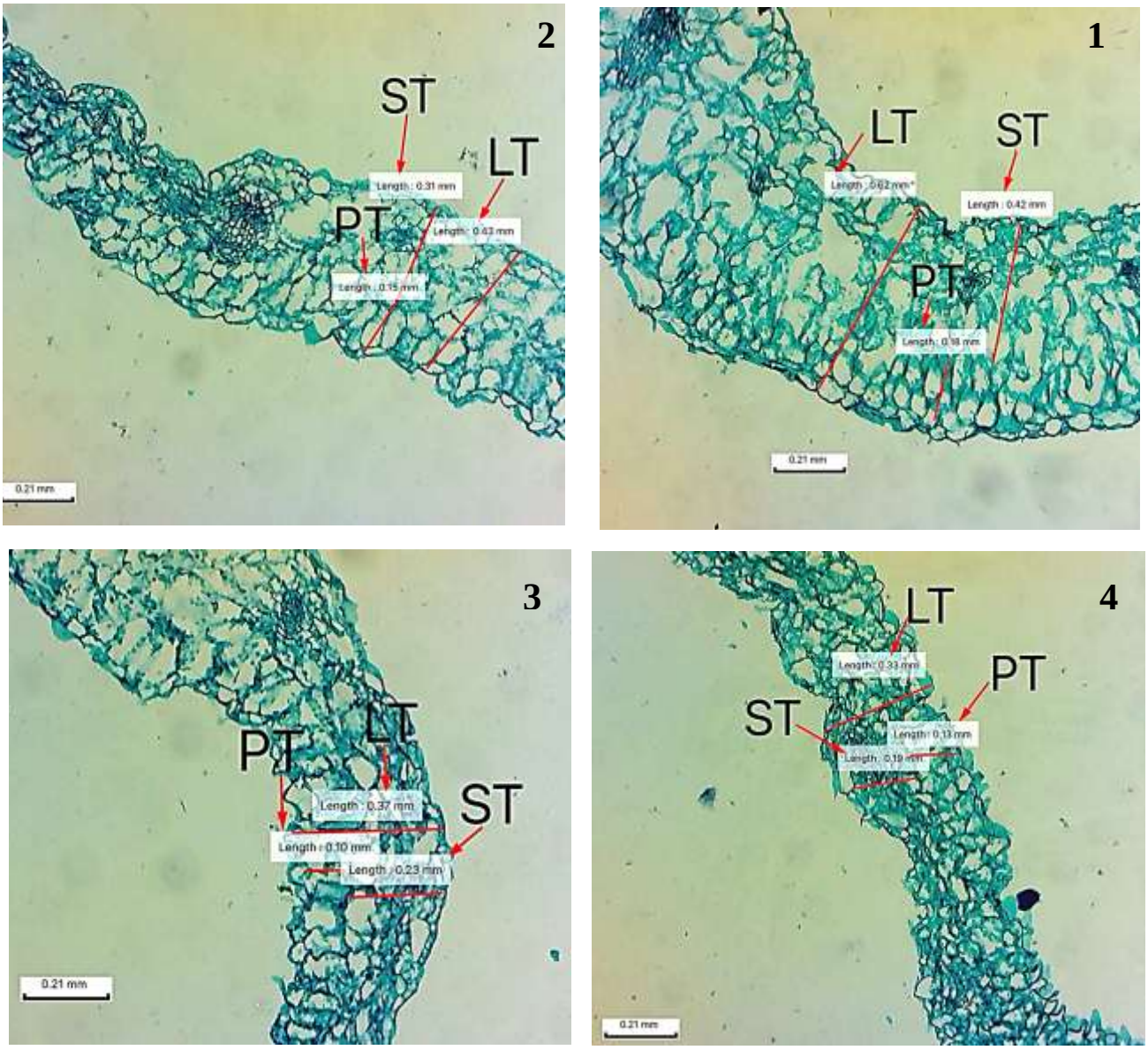
Sponge tissue thickness / Plaisade tissue thickness (mm)	Plaisade tissue thickness / leaf thickness (mm)	Light intensities (lux) and PBZ (mg.L-1) parameters
0.360 ± 0.080	**0.233 ± 0.031**	**3000**
0.447 ± 0.111	**0.290 ± 0.020**	**6000**
0.393 ± 0.032	**0.240 ± 0.044**	**+(0.5PBZ) 6000**
0.420 ± 0.105	**0.243 ± 0.021**	**+(1 PBZ) 6000**
0.423 ± 0.129	**0.263 ± 0.057**	**+(1.5 PBZ) 6000**
0.477 ± 0.057	**0.313 ± 0.021**	**9000**
0.410 ± 0.076	**0.277 ± 0.060**	**+(0.5 PBZ) 9000**
0.463 ± 0.123	**0.287 ± 0.038**	**+(1 PBZ) 9000**
1.850 ± 2.300	**1.187 ± 1.485**	**+(1.5 PBZ) 9000**
0.587 ± 0.107	**0.353 ± 0.035**	**12000**
0.730 ± 0.184	**0.407 ± 0.038**	**+(0.5 PBZ) 12000**
0.697 ± 0.154	**0.393 ± 0.067**	**+(1 PBZ) 12000**
0.523 ± 0.050	**0.287 ± 0.046**	**12000 +(1.5 PBZ)**
0.599 ±0.657	**0.367** ± 0.421	**MEAN**
0.036	**0.033**	**LSD**



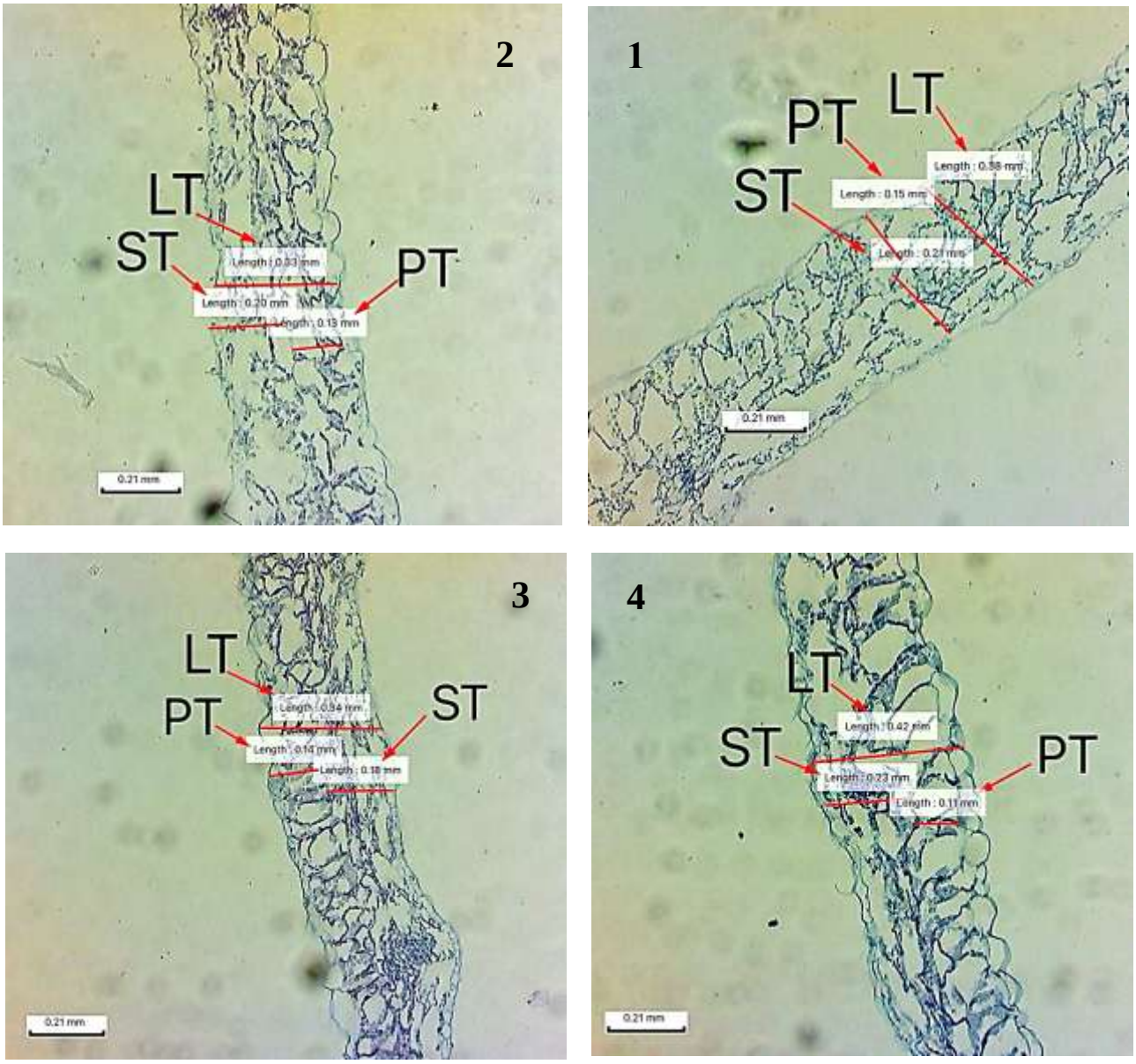
(Picture 1) cross-section of a leaf of *A. thaliana* is under the control of 3000lux



(Plate 1) cross-section of a leaf of *A. thaliana* at llight intensity of 6000 lux and growth regulator pbz at (1) no regulator, (2) concentration of 0.5 mg/ L, (3) Composition of 1 mg/ L and (4) concentration of 1.5 mg/ l, representing PT = palisade tissue thickness, LT = leaf thickness and ST = spongy tissue thickness



(Plate 2) cross-section of a leaf of *A. thaliana* at light intensity 9000 lux and growth regulator PBZ at(1) no regulator, (2) concentration 0.5 mg/ L, (3) concentration 1 mg/ L and (4) concentration 1.5 mg/ l, representing PT = Palisade tissue thickness, LT = leaf thickness and . ST = spongy tissue thickness



(Plate 1) cross-section of a leaf of *A. thaliana* at light intensity 12000 lux and growth regulator PBZ at(1) no regulator, (2) concentration 0.5 mg/ L, (3) concentration 1 mg/ L and (4) concentration 1.5 mg/ l, representing PT = Palisade tissue thickness, LT = leaf thickness and ST = spongy tissue thickness

Conclusion

The present study confirmed that the values of leaf anatomical parameters (leaf thickness and ratio of thickness of palisade tissue to spongy tissue) increased significantly in response to increasing light intensity. Addition of the growth regulator PBZ also contributed positively to increasing the values of leaf anatomical parameters

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References

[1] Singh , D.P. 2014. Stress physiologh. School of Environmental science B.B. Ambedkar university Lucknow(U.P.) 216.

[2] Naovya, F.; Mitsuko, F.; Yoshitaka, O.; Sadanori, S.; Shigeo, N. and Hiroshi, E., 2008. Directional blue light irradiation triggers epidermal cell elongation of abaxial side resulting in inhibition of leaf epinasty in geranium under red light condition. Scientia Horticulturae, 115(2): 176-182. https://doi.org/10.1016/j.scienta.2007.08.006.

[3] Rajapakse, N. C.; Pollock, R. K.; McMahon, M. J.; Kelly, J. W. and Young, R. E. 1992. Interpretation of light quality measurements and plant response in spectral filter research. HortScience, 27(11): 1208-1211. https://hortsci-article-p1208.pdf

[4] Fukuda, N.; Fujita, M.; Ohta, Y.; Sase, S.; Nishimura, S. and Ezura, H. 2008. Directional blue light irradiation triggers epidermal cell elongation of abaxial side resulting in inhibition of leaf epinasty in geranium under red light condition. Scientia Horticulturae, 115(2): 176-182. https://doi.org/10.1016/j.scienta.2007.08.006

[5] Li, Q. and Kubota, C. 2009. Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. Environmental and experimental botany, 67(1): 59-64. https://doi.org/10.1016/j.envexpbot.2009.06.011.

[6] Hogewoning, S. W.; Trouwborst, G.; Maljaars, H.; Poorter, H.; van Ieperen, W. and Harbinson, J. 2010. Blue light dose–responses of leaf photosynthesis, morphology, and chemical composition of Cucumis sativus grown under different combinations of red and blue light. Journal of experimental botany, 61(11): 3107-3117.

https://doi.org/10.1093/jxb/erq132

[7] Macedo, A. F.; Leal-Costa, M. V.; Tavares, E. S.; Lage, C. L. and Esquibel, M. A. 2011. The effect of light quality on leaf production and development of in

vitro-cultured plants of *Alternanthera brasiliana* Kuntze. Environmental and experimental botany, 70(1): 43-50. https://doi.org/10.1016/j.envexpbot.2010.05.012

[8] Sæbø, A.; Krekling, T. and Appelgren, M. 1995. Light quality affects photosynthesis and leaf anatomy of birch plantlets in vitro. Plant Cell, Tissue and Organ Culture, 41: 177-185. https://doi.org/10.1007/BF00051588

[9] Jaleel, C. A.; Manivannan, P.; Sankar, B.; Kishorekumar, A.; Gopi, R.; Somasundaram, R. and Panneerselvam, R. 2007. Water deficit stress mitigation by calcium chloride in *Catharanthus roseus*: Effects on oxidative stress, proline metabolism and indole alkaloid accumulation. Colloids and surfaces B: Biointerfaces, 60(1): 110-116. https://doi.org/10.1016/j.colsurfb.2007.06.006

[10] Meinke, D.W.; Cherry, J.M.; Dean, C.; Rounsley, S.D. and Koornneef, M. 1998. Arabidopsis thaliana: A Model Plant for Genome Analysis‖. Science. 282 (5389): 662–682 . https://doi.org/10.1126/science.282.5389.662

[11] Hussain W. M., 2021. Experiments on the effect of agricultural media and salt stress on the growth, physiochemical and anatomical characters of Arabidopsis ThalianaL. PhD thesis in Biology. Univ. of Basrah, college of Education for Pure Science. 137pp**.**

[12] Johansen, D. A. 1968. McGraw-Hill New York.

[13] Al-Rawi, K. M. and Abdul Aziz, M. K. 2000. Design and analysis of agricultural experiments. Dar Al-Kutub for Printing and Publishing, College of Agriculture and Forestry. University of Mosul. Ministry of Higher Education and Scientific Research. Iraq. 488p.

[14] Feng, L.; Raza, M. A.; Li, Z.; Chen, Y.; Khalid, M. H.; Du, J. and Yang, F. 2019. The influence of light intensity and leaf movement on photosynthesis characteristics and carbon balance of soybean. Frontiers in plant science, 9: 1952. https://doi.org/10.3389/fpls.2018.01952

[15] Swiad, S. Y. 2020. Effect of UV-irradiation on morphological, anatomical and biochemical characteristics of palm seedling. PhD thesis in Biology. Univ. of Basrah, college of Education for Pure Science.162 p

[16] Jaleel, C.; Gopi, R.; Manivannan, P. and Panneerselvam, R. 2007. Responses of antioxidant defense system of *Catharanthus roseus* (L.) G. Don. to paclobutrazol treatment under salinity. Acta Physiol. Plant. 29: 205–209. https://doi.org/10.1007/s11738-007-0025-6

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