Effect of sulfur and glutathione treatment on some anatomical characteristics of the tissue date palm leaves Phoenix dactylifera L. Barhi cultivar under heat stress

Imad Hamid Abdel Samad Al Arab [1]
Aqil Aboud Suhim Al Khalifa [2]
Iman Abdel Ali Hantoush [3]

[1,2,3] Department of Horticulture and Gardening Engineering, College of Agriculture, University of Basra, Iraq

emadhmeed0@gmail.com

amad.abdalsamad@uobasrah.edu.iq

Abstract:

The study was conducted in one of the orchards located in the south of Basra Governorate / Iraq. In the growing season of 2021 to show the effect of adding sulfur at a concentration of (0, 1, 2) kg / seedling and glutathione at a concentration of (0, 100 and 200) mg / liter and their interactions in reducing the damage caused by heat stress on the anatomical characteristics of the leaves of the palm tree cuttings of the cultivar Barhi, and the results showed the superiority of the treatment (2 kg S × (200 mg/L GSH) in the average length and width of the vascular bundle and thickness of wood and bark and on the comparison treatment which recorded (252.1, 177.7, 84.8 and 65.6) µm, respectively, while the comparison treatment recorded the lowest values for the mentioned traits and it was (216.0, 140.8 and 68) .4 and 54.4 µm, respectively, and no treatment recorded a significant superiority for the characteristics of upper and lower epidermal layer thickness and cuticle thickness for both skins, and the widest spread of tannin cells in the control treatment, while the treatment (2 kS x 200 mg/L GSH) recorded the least spread of tannin cells.

Key words: sulfur, glutathione, anatomical characteristics, heat stress.

Number: 10.14704/nq.2022.20.7.NQ33162 **Neuro Quantology 2022; 20(7):1322-1331**

Introduction:

Abiotic stress is one of the major constraints to crop production and food security worldwide. Crop growth is adversely affected by abnormal temperatures due to physical damage, physiological disturbances and biochemical changes reducing crop yield by an estimated 50% annually.(Hasanuzzaman et al. 2012a; Fahad, et al. 2017).

Heat stress is often defined as a rise in temperature above the levels necessary for plant growth and development for a period of time sufficient to cause irreparable damage. In general, an increase in temperature, (10-15) degrees Celsius above the required rate is considered heat stress (Sharma, et al, 2017).Heat stress causes a defect in some plant functions and leads to oxidative stress, and thus increases the levels of reactive oxygen species, which in turn affects the events of cellular toxicity and causes the deterioration of the fatty membranes of cell walls, which affects the regulation of exchange between cells and plant growth, photosynthesis, pollen development, and reproduction. Protein denaturation and alteration of several enzyme activities (Hussain et al. 2019).

The date palm, Phoenix dactylifera L., is an evergreen fruit tree belonging to the Arecaceae family. It is a monocotyledonous plant. Its cultivation is spread in tropical and subtropical areas between latitudes 15-30 north of the equator. The Arab Gulf region is home to the palm tree and considers it a symbol of the desert environment, as it is one of the most adapted plants to the desert environment because it bears high temperatures, drought and salinity

El-Shibli and Korelainen, 2009) ; Dransfield, Govarets, 2005. Temperature plays an important role in plant growth. Although date palms can withstand harsh climatic conditions, excessive and widespread levels of environmental stresses, including sudden rise in temperature and drought, may cause severe damage, significant reduction in yield and quality. Fruits and reduce physiological and metabolic processes in plants (Patankar et al. 2019).

Palm trees resulting from plant tissue cultivation are affected by high or low temperatures as they are the result of laboratory conditions and their leaves are thin and the amounts of protective wax on their leaves differ from what it is in the seedlings produced by their mothers in the field (Al-busaidi, 2002). Between (Hadrami et al., 2011) tissue palm offshoots were affected by abiotic stresses such as drought , salinity and heat. studying the effect of these stresses is one of the challenges facing plant growth and development. The palm shoots resulting from tissue culture exposed to the effect of high temperatures between 53-58 were affected by photosynthesis and leaf characteristics, oxidation complications occurred and the production of reactive oxygen species, as well as the effect of plant pigments and high levels of antioxidants (Khair & Karim, 2015; Al-busaidi, 2002).

To improve the performance of crops under heat stress, various techniques such as mineral feeding have been introduced into the agricultural system to process various nutrients for plants. The use of agricultural sulfur and glutathione (GSH) is one of the effective ways to enhance plant tolerance against high temperature stress (Ihsan et al. 2019).

Treating al-Halawi palm trees with plant hormones led to an increase in the leaves and fruits content of hormonal antioxidants, which are one of the mechanisms of plant resistance to oxidative stress resulting from the high temperature (Al Khalifa and. Al-Meer.2018).

Sulfur is an important nutrient required by plants under stress conditions. Sulfur compounds play an important role as structural components of important cellular molecules within the cell. Moreover, it has an important role as a signaling factor for intercellular communication, and the importance of sulfur for plant growth lies in the fact that it enters into protein formation through the formation of many amino acids and compounds responsible for environmental response such as cysteine (Cys), methionine (Met) and glutathione (GSH). Plants need these sulfur biomolecules to develop effective defense mechanisms against various stresses (Rennenberg & Herschbach, 2012; Khan et al, 2014).

(Abbas et al., 2015) found that sulfur is one of the factors that reduce stress on plants, as it was used to reduce the damages of salt stress on date palm shoots. (Ihsan et al., 2019) showed that sulfur is one of the available methods as a heat stress reliever. He also showed the important role of sulfur as it is part of the components of vitamins and some amino and fatty acids and is included in the composition of some antioxidants.

Glutathione (GSH) is a tripeptide in (c-glutamyl-cys teinyl-glycine; c-Glu-cys-Gly). The structure of GSH includes a bond between the carboxyl group of the glutamate side chain (Glu) and the amine group of cysteine (Cys) that is linked to glycine by a peptide bond. However, the thiol group is the main chemical reaction group with respect to its biological and biochemical functions (Zagorchev et al. 2013).

Glutathione is the most important part of a plant's stress system that is largely dependent on sulfur supply to plants. In the case of sulfur deficiency, the level of GSH in the cell drops rapidly while the use of sulfur fertilizers has returned to its original level (Nazar et al., 2011)..

Glutathione has multiple functions in plant development and growth that other antioxidants cannot perform. For example, it protects membranes by maintaining a reduced state of both a-tocopherol and zeaxanthin, preventing oxidative denaturation of proteins under stress conditions by protecting their thiol groups. Glutathione also helps chelate toxic metals such as metalloids and then transport and isolate them in the vacuole. Apart from detoxifying ROS, GSH is involved in methylglyoxal (MG) detoxification.

Glutathione detoxifies, biosynthesis and stabilizes oxidative stress within the stress-affected plant cell. Glutathione enhances plant tolerance to various abiotic stresses including salinity, drought, high temperature (HT), low temperature and toxic mineral stress. Glutathione regulates cell proliferation, and death. Apoptosis, fibrosis, growth, development, cell cycle, gene expression, protein activity, and immune function (Noctor et al. 2012; Nahar et al. 2015a; Hasanuzzaman et al. 2017)

Materials and methods:

The study was conducted in the season 2021 in one of the private orchards in Siba district, Basra Governorate, southern Iraq. 27 seedlings of date palm cultivar Al-Barhi cultivar, aged 5 years, were selected. To study the changes in the anatomical structure of leaves under heat stress conditions and the effect of treatment with agricultural sulfur and glutathione in mitigating the harmful effects.

The treatments of the experiment were carried out as agricultural sulfur was added at a concentration (0-1-2 kg/vessel) and the amino acid glutathione was sprinkled on the leaves at a concentration (0-100 mg/L-200 mg/L) as well as the interaction treatments.

Paper samples were taken for anatomical examinations, as leaf samples were collected from the middle row and five sheets were taken from each duplicate and the fixation process was performed on them in A.A.F solution for 48 hours, then the samples were passed with ascending concentrations of ethyl alcohol. Samples were immersed in paraffin wax at a temperature of 58-60°C, and leaf models were cut by Microtome Rotary with a thickness of 10 µm, and loaded onto glass slides. To stain the samples, they were placed in Safranin dye for 30-60 minutes and then placed in Fast green dye for 15 seconds, after which the slide cover was placed with drops of DPX to prepare the slide for microscopy (Tomlinson, 1990; Thomas and Franceschi, 2013). Then the slides were studied and the measurements were taken in µm meter micro by a micrometer ocular lens in an Olympus type optical microscope equipped with a camera connected to a computer

Experiment design and statistical analysis.

The experiment was designed using randomized complete blocks (CRBD) and the results were analyzed using analysis of variance for anatomical structure, the SPSS statistical program. Also, the averages were analyzed and significance was tested according to the least significant difference test (RLSD) at the probability level (0.05) (Snedecor and Cochran, 1986).

Results :

The results of Table (1) showed the effect of treatment with sulfur and glutathione on the length of the vascular bundles of the leaves of the tissue date palm variety Barhi. Which recorded the largest length of the bundle, which amounted to (252.1 µm), with a significant superiority over the comparison treatment, which recorded the lowest length of the vascular bundle, which amounted to (216.0 µm).

Table (2) shows the extent to which the width of the vascular bundle was positively affected by the addition of sulfur and glutathione under high temperature stress, as the bandwidth in the interaction treatment reached ((2 kg S x 200 mg/L GSH (177.7 µm), recorded the highest values with significant superiority than the comparison treatment Which recorded lows value of (140.8 µm).

The results in Table (3) showed the effect of adding sulfur and glutathione on the average thickness of wood, leaves, palm trees, cultivar Barhi under high temperature stress, as it was the interference treatment.

((2 kg S x 200 mg/L GSH had the most effect on the amount of wood thickness as it reached) 84.87 (µm) with a significant superiority than the comparison treatment which recorded the lowest value of wood thickness which was (78.40 µm).

As for the thickness of the bark, the results of Table (4) showed the effect of adding sulfur and glutathione on the average thickness of the bark leaves of the palm tree tissue variety Barhi under high temperature stress. (65.60 (µm) with a significant difference from the comparison treatment which recorded the lowest thickness of the bark was (78.40 µm).

Regarding the thickness of the epidermis and the upper and lower cuticle layers, the results of the table (5, 6, 7 and 8) showed the effect of treatment with sulfur and glutathione on the thickness of the upper and lower epidermis and the cuticle layers for both skins. . The pictures in panel (2 and 3) showed the upper and lower epidermal layers and the two cuticle layers in the study parameters.

Plate (1) showed the proliferation of tannin compounds in tissue cells of date palm leaves of Barhi variety treated with sulfur and glutathione, as histological sections showed the proliferation of dense tannin in the comparison treatment, and it was noted that the proliferation of tannin cells varied among other treatments, and that the treatment (2kgS×200mg\ l GSH) had the lowest prevalence of tannin in comparison with other treatments including the control treatment. The proliferation of tannin cells was observed around the large and small vascular bundles.

treatment	0	100mg/l GSH	200mg/lGSH	average
0	216.0	212.6	235.7	221.4
1kgS	219.4	242.6	246.4	236.1
2kgS	237.8	248.2	252.1	246.0
average	224.4	234.4	244.7	LSD=18.59

Table (1) Effect of sulfur and glutathione on average length of vascular bundles (µm) of leaves of the tissue date palm cultivar Barhi.

treatment	0	100mg/l GSH	200mg/lGSH	average
0	140.08	146.27	149.33	145.46
1kgS	149.47	159.93	166.80	158.73
2kgS	155.87	169.33	177.77	167.65
average	148.71	158.51	164.63	LSD=8.519

Table (2) Effect of sulfur and glutathione on the average width of the vascular bundles (µm) of the leaves of the tissue date palm cultivar Barhi

treatment	0	100mg/l GSH	200mg/lGSH	average
0	78.40	80.93	81.53	80.60
1kgS	81.30	82.47	82.63	81.70
2kgS	83.80	82.80	84.87	83.38
average	81.16	82.12	82.95	LSD=1.864

Table (3) Effect of sulfur and glutathione on average wood thickness (µm) of leaves of the tissue date palm cultivar Barhi

treatment	0	100mg/l GSH	200mg/lGSH	average
0	54.40	55.47	58.00	57.88
1kgS	56.80	59.47	63.77	58.09
2kgS	62.83	63.67	65.60	64.03
average	58.01	58.77	62.63	LSD=1.864

Table (4) Effect of sulfur and glutathione on average phloem thickness (µm) of leaves of the tissue date palm cultivar Barhi.

treatment	0	100mg/l GSH	200mg/lGSH	average
0	4.83	4.93	5.10	4.95
1kgS	5.12	5.13	5.23	5.16
2kgS	5.21	5.21	5.36	5.99
average	5.05	5.09	5.70	LSD=0.726

Table (5) Effect of sulfur and glutathione on the average thickness of the upper epidermis (µm) of leaves of the tissue date palm cultivar Barhi.

treatment	0	100mg/l GSH	200mg/lGSH	average
0	2.80	2.82	2.94	2.85
1kgS	2.86	2.91	2.97	2.91
2kgS	2.96	2.93	3.08	2.99
average	2.87	2.88	2.99	LSD=0.714

Table (6) Effect of sulfur and glutathione on the average thickness of the lower epidermis (µm) of leaves of the tissue date palm cultivar Barhi

treatment	0	100mg/l GSH	200mg/lGSH	average
0	2.00	2.17	2.46	2.21
1kgS	2.30	2.43	2.66	2.46
2kgS	2.46	2.59	2.63	2.48
average	2.25	2.43	2.65	LSD=0.67

Table (7) Effect of sulfur and glutathione on the average thickness of the lower epidermal cuticle (µm) of leaves of the tissue date palm cultivar Barhi

treatment	0	100mg/l GSH	200mg/lGSH	average
0	2.40	2.46	2.53	2.46
1kgS	2.48	2.53	2.55	2.52
2kgS	2.55	2.57	2.57	2.57
average	2.47	2.52	2.55	LSD=0.205

Table (8) Effect of sulfur and glutathione on average thickness of the upper cuticle (µm) of leaves of the tissue date palm cultivar Barhi

Discussion:

The reduction in the size of the vascular bundles may be attributed to their effect on abiotic stress, which caused a reduction in the number and size of the transport vessels (wood and phloem), or the deterioration of the transporting elements. Sandalio et al., 2001; Vollenweider et al., 2006) and this was confirmed by the current study and it agreed with the study (Neama et al., 2017). The decrease in the diameter of the vascular bundles is directly related to the reduction in the area of the woody vessels, which is responsible for nutrient delivery (Ortega et al. 2006). The effect of abiotic stress on reducing the thickness of the vascular bundles may be due to the plant's adaptation to environmental stress by the mechanism of uptake and transport of water and nutrients from the roots, which is associated with increased stress (Ceccoli et al. 2011.) Abiotic stress stimulates the plant to make some tissue modifications in order to Maintaining the most important water content within the tissues and reducing the evaporation process by increasing the thickness of the epidermis and cuticles (Hameed et al. 2013).The increase in the number of epidermal cells or the decrease in the volume of the median tissue is due to the influence of the tissue inside the leaf by stress, which reduces the length and diameter of the bundles and reduces Photosynthesis (Atabayeva et al. 2013) The results of the study (Alnajjar, et al. 2020) indicated that the use of antioxidants led to the thickness of the epidermal layer, the upper and lower epidermis and the cuticle layers of the epidermis, the length and width of the vascular bundles, and The diameter of the primary and secondary xylem, and the thickness of the bark of the leaf. The upper two properties of the epidermis play an important role in isolating reactive oxygen species (ROS) away from mesenchymal cells important in photosynthesis processes (2011. (Gomes et al.. A study (Shareef and Sweed.2020) showed that heat stress is the most harmful factor for the anatomical characteristics of the plant than other stress factors, as abiotic stress led to an increase in the thickness of the upper epidermis of leaves and an increase in the thickness of the acuticle in July compared to other treatments. The reason is due to some changes in the anatomical structure of the date palm leaves as a result of their exposure to heat stress, as it is considered as a defense method to reduce water loss by increasing the thickness of the cuticle layer (Challa and Van Beusichem. 2004).The presence of environmental stress led to a significant decrease in the thickness of the epidermis with a decrease The number of epidermal cells from the upper and lower epidermis thus reduces leaf thickness (Duarte et al. 2013).

Tannin compounds are naturally present in plant tissues in small quantities, and the plant's production of these compounds increases when exposed to oxidative stress. (Abass et al. 2016; Dalcoros et al. 2013) Stresses, including oxidative stress, lead to an increase in the build-up and accumulation of phenolic compounds, including tannin, in plant tissues as a non-enzymatic defense mechanism to mitigate the harmful or toxic effects of free radicals and the active oxygen species ROS formed by stress. This is in view of the fact that these compounds have antioxidant properties (Agati et al. 2012; Zhang et al. 2015) and the result of this study is similar to the results of many studies that showed that the accumulation of reactive oxygen species ROS is a common feature of all stresses to which plants are exposed, and this is what Explains the increased production of tannins that are an adaptive response as a non-enzymatic defense mechanism to counteract the harmful and toxic effects of oxidative stress.(Constabel and Lindroth 2010).

The results of the current study showed that the increase in temperatures to extreme levels led to changes in the anatomical structure of the leaves of date palm trees, which may be part of the plant's defense mechanisms to reduce the harmful effect of heat stress, or to its effect in increasing the concentrations of the active oxygen species ROS with toxic effects on plant cells.

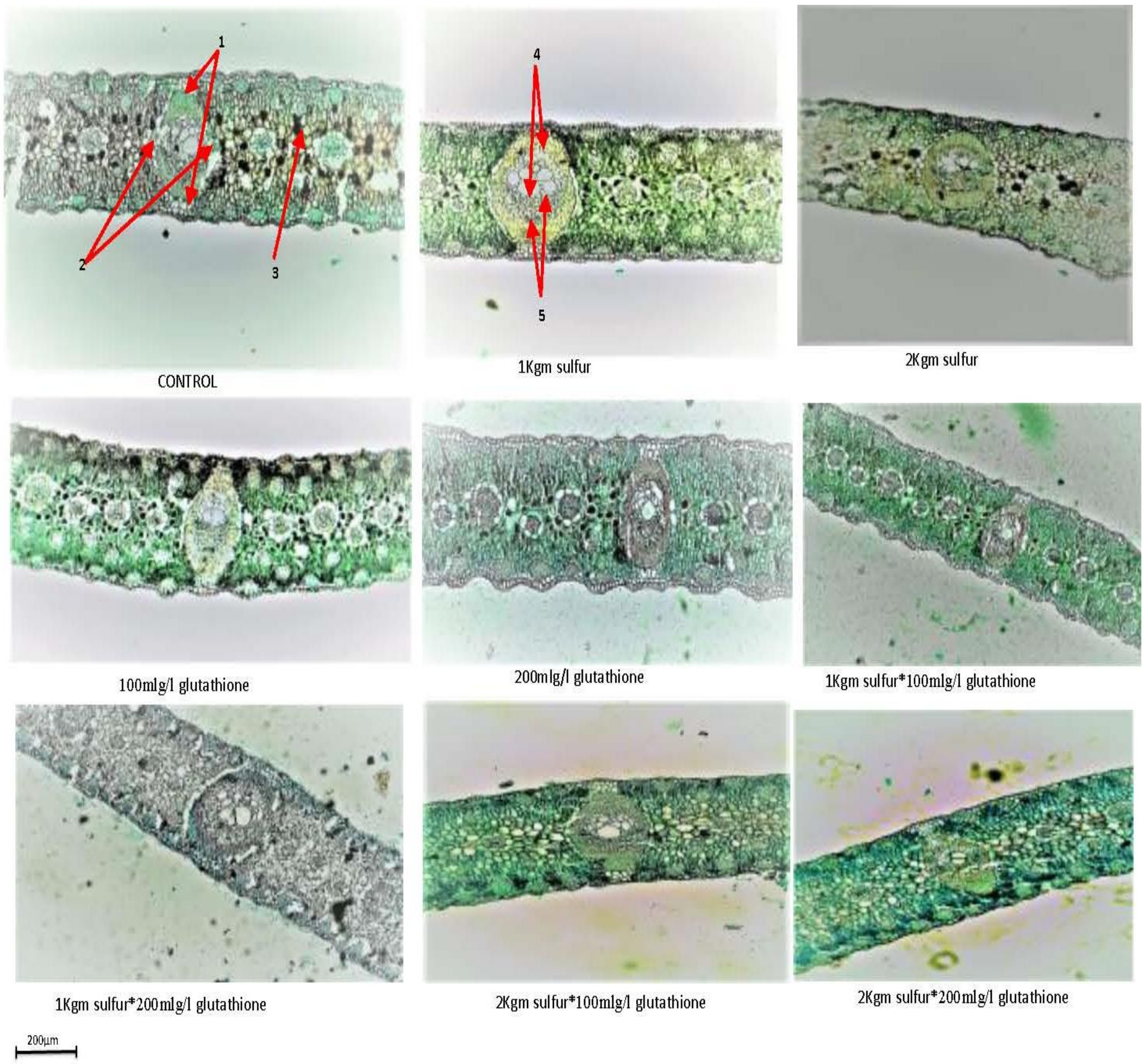

Plate (1): Effect of sulfur and glutathione supplementation on the length and width of the vascular bundle, the thickness of wood and phloem, and the proliferation of tannin cells.

1- length vascular 2- width vascular 3- tannin cells 4- thickness of wood 5- thickness of phloem

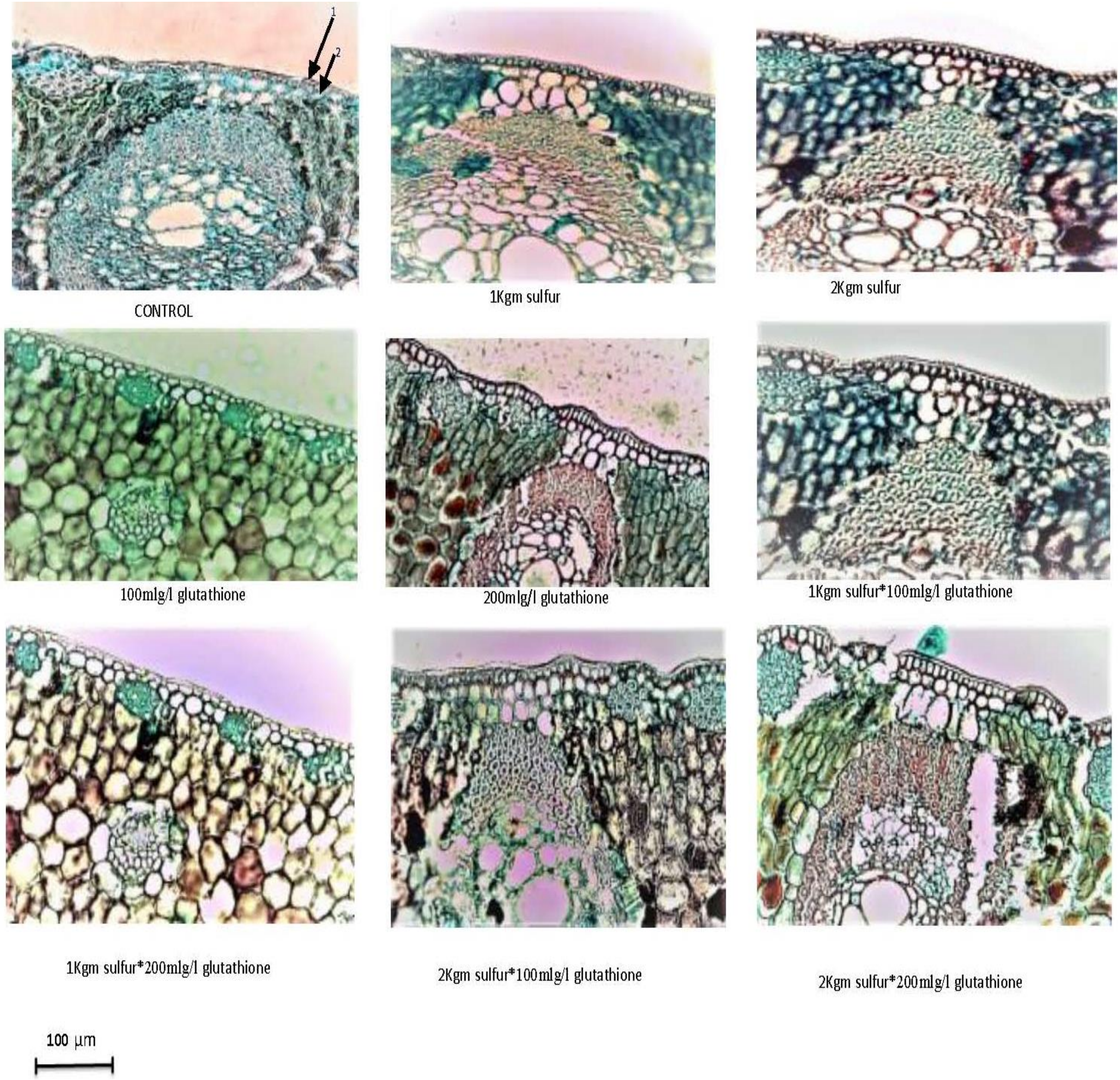

Plate (2) Effect addition of sulfur and glutathione in the thickness of the upper epidermis and the upper cuticle

1-cuticle 2- epidermis

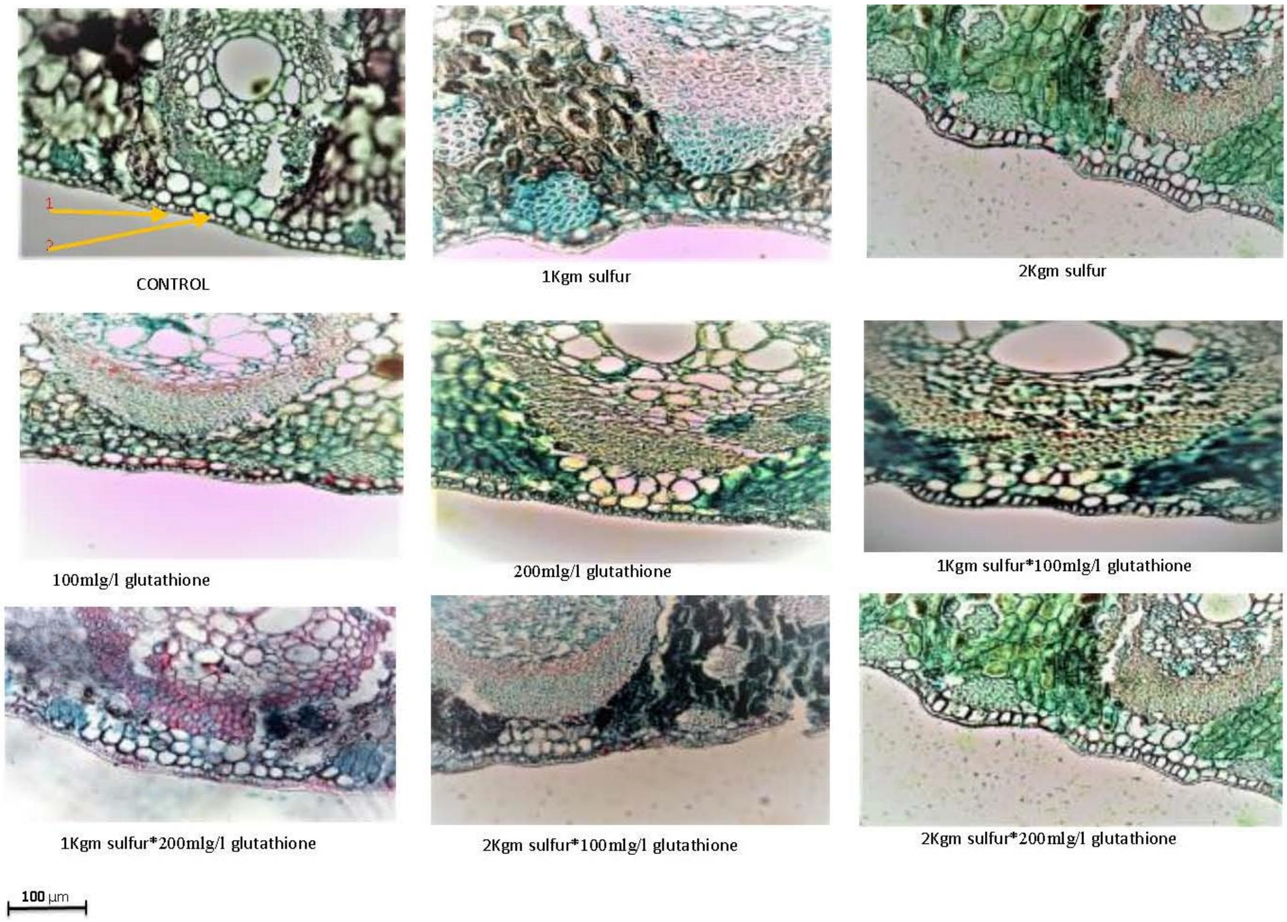

Plate (3) Effect of adding sulfur and glutathione on the thickness of the lower epidermis and the lower cuticle under heat stress

References:

1. Abbas, M. F., Jasim, A. M., Shareef, H. J., Design, L., & Palm, D. (2015). Role of Sulphur in salinity tolerance of Date Palm (Phoenix dactylifera L .). ISSN 2249-8516 Original Article 5(3), 92–97.
2. Abass, M.H.; J.D. Neama; K.M.A. Al-Jabary, 2016, Biochemical responses to cadmium and lead stress in date palm Phoenix dactylifera L. plant. AAB Bioflux 8(3):92-110.
3. Agati , G.; Azzarello, E.; Pollastri, S. and Tattini , M. (2012) . Flavonoids as antioxidants in plants: location and functional significance. Plant Science, 196 : 67–76 .
4. Al-busaidi, K. T. S. (2002). Studies on Salt and Heat Stresses Tolerance of DatePalm Plants Regenerated by Tissue Culture
5. Alnajjar, Mohammed & Fawzi, Wasen & Ibrahim, Majid. (2020). EFFECT OF AMINO ACID PROLINE TREATMENT ON ANATOMICAL CHARACTERISTICS OF LEAVES AND ROOTS OF DATE PALM SEEDLINGS PHOENIX DACTYLIFERA L. DEVELOPED UNDER SALINE STRESS CONDITIONS. Plant Archives. 20. 755-760.
6. Al Khalifa,A.A.S. and Osama N.J. Al-Meer,O.N.J.(2018)The effect of date palm trees spraying with different plant growth regulators on Leaves and Fruits PlantHormones Content, Antioxidant Enzymes status and Improvement of Production in Basra Province
7. Asseng, S; Ewert, F; Martre, P; Rotter, R. P; Lobell, D. B; Cammarano, D; (2015).Rising temperatures reduce global wheat production. *Nat. Clim. Chang* 5,143–147. doi: 10.1038/nclimate2470

8. Brosnan, John T.; Brosnan, Margaret E. (2006). The Sulfur-Containing Amino Acids: An Overview. The Journal of Nutrition, 136(6), 1636S 1640S.
9. Constabel , C.P. and Lindroth, R.I. (2010). The impact of genomics on advances in herbivore defense and secondary metabolism in Populus. In ; Jannson S, Bhaleaero RP, Grover A (eds) Genetics and genomics of Populus. Springer, New York, NY, pp 279 - 305 .
10. Dalcorso, G.; A. Manara.; A. Furini,2013, An overview of heavy metals challenge in plants: from root to shoot. Metallomics, 5(9):1117-1132.
11. El-Shibli S. and Korelainen H. (2009): Biodiversity of date palm (Phoenix dactylifera L.) in Sudan: Chemical, morphological and DNA polymorphism of selected cultivars. Plant Genet. Resour. (7), 194-203.
12. Fahad Shah, Bajwa Ali A., Nazir Usman, Anjum Shakeel A., Farooq Ayesha, Zohaib Ali, Sadia Sehrish, Nasim Wajid, Adkins Steve, Saud Shah, Ihsan Muhammad Z., Alharby Hesham, Wu Chao, Wang Depeng, Huang Jianliang(2017) Crop Production under Drought and Heat Stress: Plant Responses and ManagementOptions . Frontiers in Plant Science. VOL ,8. P,1147.
13. Freney, J.R. and Stevenson, F.J., 1966. ORGANIC SULFUR TRANSFORMATIONS IN SOILS .Govaerts, R., & Dransfield, J. (2005). *World checklist of palms*. Royal Botanic Gardens.
14. Hasanuzzaman M, Hossain MA, Teixeira da Silva JA, Fujita M (2012a) Plant response and tolerance to abiotic oxidative stress: antioxidant defense is a key factor. In: Venkateswarlu B, Shanker AK, Shanker C, Maheswari M (eds) Crop stress and its management: perspectives and strategies. Springer, New York, pp 261–315
15. Hasanuzzaman, M., Nahar, K., Anee, T. I., & Fujita, M. (2017). Glutathione in plants: biosynthesis and physiological role in environmental stress tolerance. *Physiology and Molecular Biology of Plants*, *23*(2), 249-268.
16. Ihsan, M. Z., Daur, I., Alghabari, F., Alzamanan, S., Rizwan, S., Ahmad, M., & Shafqat, W. (2019). Heat stress and plant development: role of sulphur metabolites and management strategies. *Acta Agriculturae Scandinavica, Section B—Soil & Plant Science*, *69*(4), 332-342.
17. Khan NA, Khan MIR, Asgher M, Fatma M, Masood A, Syeed S. 2014. Salinity tolerance in plants. revisiting the role of sulfur metabolites. J Plant Biochem Physiol. 2:120–131.
18. Naema, J. D; Abass,M.H; Al- Jabary, K. M. A.(2017). Effect of treatment with cadmium and lead on some anatomical characteristics of date palm leaves of Phoenix dactylifera L. Barhi cultivar. Basra Journal of Date Palm Research, Vol. 16, p.1, p. 55-74.
19. Nahar K, Hasanuzzaman M, Alam MM, Fujita M (2015a) Glutathione-induced drought stress tolerance in mung bean: coordinated roles of the antioxidant defence and methylglyoxal detoxification systems. Plants.doi:10.1093/aobpla/plv069
20. Noctor, G., Mhamdi, A., Chaouch, S., Han, Y. I., Neukermans, J., Marquez-Garcia,B. E. L. E. N., ... & Foyer, C. H. (2012). Glutathione in plants: an Integrated overview. *Plant, cell & environment*, *35*(2), 454-484
21. Patankar, H.V.; Al-Harrasi, I.; Al Kharusi, L.; Jana, G.A.; Al-Yahyai, R.; Sunkar, R.; Yaish, M.W. (2019) Overexpression of a Metallothionein 2A Gene from Date Palm Confers Abiotic Stress Tolerance to Yeast and Arabidopsis Thaliana Int. J. Mol. Sci., 20, 2871.
22. Sandalio, L. M., Dalurzo H. C., Gomez M., Romero-Puertas M.C. and del Rio L.A. (2001). Cadmium induced changes in the growth and oxidative metabolism of pea plants. J. Exp. Bot. 52:2115-21. Soil Sci., 101: 307-316.
23. Shareef, H., J. and Sweed, S.Y. (2020). Leaf and root anatomical changes Of date palm Phoenix dactylifera L. Khadrawi cultivar under abiotic stress. Basrah J. of Date palm Research. Vol. (19), (2): 23-33.
24. Sharma, I. P., Kumar, N., & Bhatt, S. (2017). Heat stress in agriculture–the problem. *Indian Farmers Digest*, *50*, 13-14.

25. Snedecor, G. M. and W. G. Cochran (1986). Statistical Methods. 9th ed., The Iowa State University, American Press, Iowa, U.S.A., pp. 507.
26. Thomas, R. and D. Franceschi (2013). Palm stem anatomy and computer-aided identification: The Coryphoideae (Arecaceae). Amer. J. Bot., 100(2) : 289-313.
27. Tomlinson, P. B. (1990). The Structural Biology of Palms. Clarendon press. Oxford, UK. 477 pp.
28. Freney, J.R. and Stevenson, F.J., 1966. ORGANIC SULFUR TRANSFORMATIONS IN SOILSSoil Sci., 101: 307-316.
29. Gomes , M.P.; de Sa e Melo Marques , T.C.L.L.; Goncalves Nogueira , M.O.; de Castro, E.M. and Maria Soares, A. (2011) . Ecophysiological and anatomical changes due to uptake and accumulation of heavy metal in Brachiaria decumbens Sci. Agric. (Piracicaba, Braz.) 68: 566-573 .
30. Vollenweider P., Cosio C., Gunthardt-Goerg M. S. and Keller C. (2006). Localization and effects of cadmium in leaves of a cadmiumtolerant willow (Salix viminalis L). Part II Microlocalization and cellular effects of cadmium. Environ. Exp. Bot. 58: 25–40.
31. Rennenberg, H., & Herschbach, C. (2012). Sulfur Metabolism in Plants. *Sulfur Metabolism in Plants*. https://doi.org/10.1007/978-94-007-4450-9
32. Hadrami, A. El, Daayf, F., Elshibli, S., Jain, S. M., & Hadrami, I. El. (2011). Date Palm Biotechnology. *Date Palm Biotechnology*, 183–203.
33. Zagorchev L, Seal CE, Kranner I, Odjakova MA (2013) Central role for thiols in plant tolerance to abiotic stress. Int J Mol Sci 14:7405–7432
34. Zhang , B.; Cai, J.; Duan , C. Q.; Reeves, M. J. and He, F. (2015). A review of polyphenolics in oak woods. International Journal of Molecular Sciences, 16 : 6978 - 7014 .