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Evaluating the Effect of Abiotic Stresses on Levels of H2O2 and Ascorbate Peroxidase in *Eruca sativa* mill

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Abstract

A field experiment was conducted in the greenhouse conditions in the Department of Biology at the College of Education - University of Samarra. In this experiment, we evaluated the effect of a number of stresses and their effect on the levels and activity of H2O2 and ascorbate peroxidase enzyme, The stresses that were dealt with were, drought, salinity, temperature and light. H2O2 and Ascorbate peroxidase levels were affected by drought stress and increased to reached 2.9320 µmol / mg fw, 2.2570 mol ASC/min*mg protein respectively This indicates a clear increase in concentrations and a significant response, The effect of salinity stress is also evaluated and the result of H2O2 and Ascorbate peroxidase is also increased significantly reaching to 2.3257 µmol / mg fw, 1.13967 ASC/min*mg protein respectively, In temperature stress we used variable levels of temperatures 4c°, 30c°, 35c° and 40c° and the result were com as flow , H2O2 content were increased in 4c° and reached to 1.2530 µmol / mg fw after 6-hours of exposure but Ascorbate enzyme witnessed a clear fluctuation in concentrations when treated with different levels of temperature. As for the light intensity stress a variable levels of light were used 10µE, 370µE and 780µE were the result com as flow, H2O2 content were increased in 10µE and reached to 1.1500 µmol / mg fw after 12-hours of exposure, Ascorbate enzyme witnessed an increased especially 370µE in 24-hours and it was 1.8700 mol ASC/min*mg protein

Keywords

Environment Stress, H2O2, Ascorbate peroxidase, Eruca sativa

1. INTRODUCTION

Eruca sativa plant Rocket is very well known and widespread extensively all over the world and is usually consumed fresh for its typical favorable taste, It is mentioned in traditional pharmacopoeia and ancient literature for several therapeutic properties, and it does contain a number of health promotinfg agents including carotenoids, vitamin C, fibers, flavonoids, and glucosinolates GLs [1]. Biotic stresses and abiotic stresses affect in a bad way on plant growth and productivity worldwide, The individual genes steading cannot be dependent approach for the understanding of tolerance mechanisms, since these stresses are frequent and often in combination with each other, and a huge genes are involved in these mechanisms [2]. When plants are exposed to stressful environmental conditions, the production of reactive oxygen species (ROS) increases and can cause significant damage to the cells. Antioxidant defences, which can detoxify ROS, are present in plants. A major hydrogen peroxide detoxifying system in plant cells is the ascorbate-glutathione cycle, in which, ascorbate peroxidase (APX) enzymes play a key role catalyzing the conversion of H2O2 into H2O, using ascorbate as a specific electron donor. The expression of APX genes is regulated in response to biotic and abiotic stresses as well as during plant development and the APX responses are directly involved in the protection of plant cells against adverse environmental conditions [3]. This enzyme is consider as one of the most important H2O2 eliminating enzymes involved in un limited biological processes, Its metabolize hydrogen peroxide (H2O2) into water (H2O) and oxygen (O2) within the plant on the cellular level [4].

2.MATERIALS AND METHODS

2.1 Standard growth conditions of plant

It is important to take specific controlled conditions was used to growth the plant (Eruca sativa Mill), we can summarized this conditions by the following : (14h light with~54µE, and 21 °C / 10h and 20 °C, 55-60 % relative humidity) on a 2:1:1 petamoss, perlite and vermiculite. Flat containers with high edge with cultured pots were transferred to controlled environmental conditions. After 6 weeks, the plants were directly exposed to one by one to the following abiotic stresses (drought, salinity, light and temperature) with different time points of exposure depend on the method and type of abiotic stress, on other hand specific groups were left or kept at growth chamber conditions without any changes.

2.2 *Salinity treatment*

Sodium chloride solution NaCl (100 mL) was used to irrigate 6-week-old (42-day-old) plants, we did this three times a week for up to 14 days. Samples collected in inner leaves were started at 2d, 6d, 10d and 14d in the growth chamber as described by [5]. Controlled samples were continued three times a week for 2 days, 6 days, 10 days and 14 days with tap water under controlled growth conditions.

2.3 *Drought*

For drought treatment, [6] described the following method to get the most accurate results in this field, the plants were irrigated with tap water three times a week for a period of six weeks of growth in controlled conditions, and then the plants was subjected to a gradual water shortage depending on three levels (3 days, 6 days and 9 days) with holding water. At the end of each specific drought exposure, plant sample leaves collected directly in liquid nitrogen plant growth chamber and stored at -80°C until further analysis. Drought treatment was applied Controlled samples were continued irrigated three times a week for further)3 days, 6 days or 9 days(after the plant reached to a period of six weeks of growth in controlled conditions.

2.4 Light quantity treatment

Initially 6-week-old (42) days plants were grown at ≈54 µmol photon/m2s light quantity, as (control). To calculate the quantity treatment, plants were shifted to grow under 10 µmol photon/m2s, 100 µmol photon/m2s, and 370 µmol photon/m2s respectively, leaves samples collecting started after 2h, 6h, 12h and 24h of exposure to growth desired light quantity as described by [7].

2.5 Heat shock

Initially 6-week-old (42) days plants were grown in 21 °C as control temperature. For heat shock we started with transferring the plants to grow at specific temperatures, 4°C (cold), 25°C, 30°C and 35°C. leaves samples collecting started after 2h, 4h and 6h of incubation at desired temperature as described by [8].

2.6 Measurement of H2O2

Method was followed in calculating its results of hydrogen peroxide content of control and treated samples, It was done by homogenizing (~0.1g) frozen leaf material on ice with 0.1% (w/v) trichloroacetic acid (TCA). Then the homogenate was centrifuged at 15,000 g for 15 min at 4 °C. the supernatant was transferred to new Eppendorf tube and 0.5 ml of the supernatant was added to 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0) and 1ml of 1M KI and mixed gently, The absorbance of assay mixture was read at 390 nm and the content of H_2O_2 was calculated based on a standard curve of known concentrations of H_2O_2 [9].

3. RESULTS AND DISCUSSIONS

3.1 Effects of drought on H2O2 and Ascorbate peroxidase content.

As shown in Figure .1 The results show a significant but sequential increase in the concentrations of H2O2, as the increase on D1 was slight and almost insignificant, but on D2 the concentration was (2.2547µmol / mg fw), and on D3 the concentration was (2.9320µmol / mg fw) if compared to the control group, which was (0.2887 µmol / mg fw), this clearly shows the significant increase in H2O2 concentrations, and this is an expected result as the plant responded to producing more free radicals with any biological stress rising.

Ascorbate peroxidase or H2O2-scavenging enzyme, It is one of the most important enzymes that play a key role in protecting the plant cell from damage caused by free radicals resulting from exposure of the plant to various stresses, The results in figure No.1 showed a perfect match between ascorbate on the one hand and H2O2 on the other, and this match is logical because the high level of the enzyme is linked to the high level of free radicals in the plant in general. The results indicated that the concentration on the D1 was (1.6817 mol ASC/min*mg protein) and on the D2 and D3 was (1.8870 mol ASC/min*mg protein) and (2.2570 mol ASC/min*mg protein) respectively but in the control group it was (1.4220 mol ASC/min*mg protein) and this confirms that the action of the enzyme is related to the increase of free radicals, which are shown in the table, The results matched [10,11].

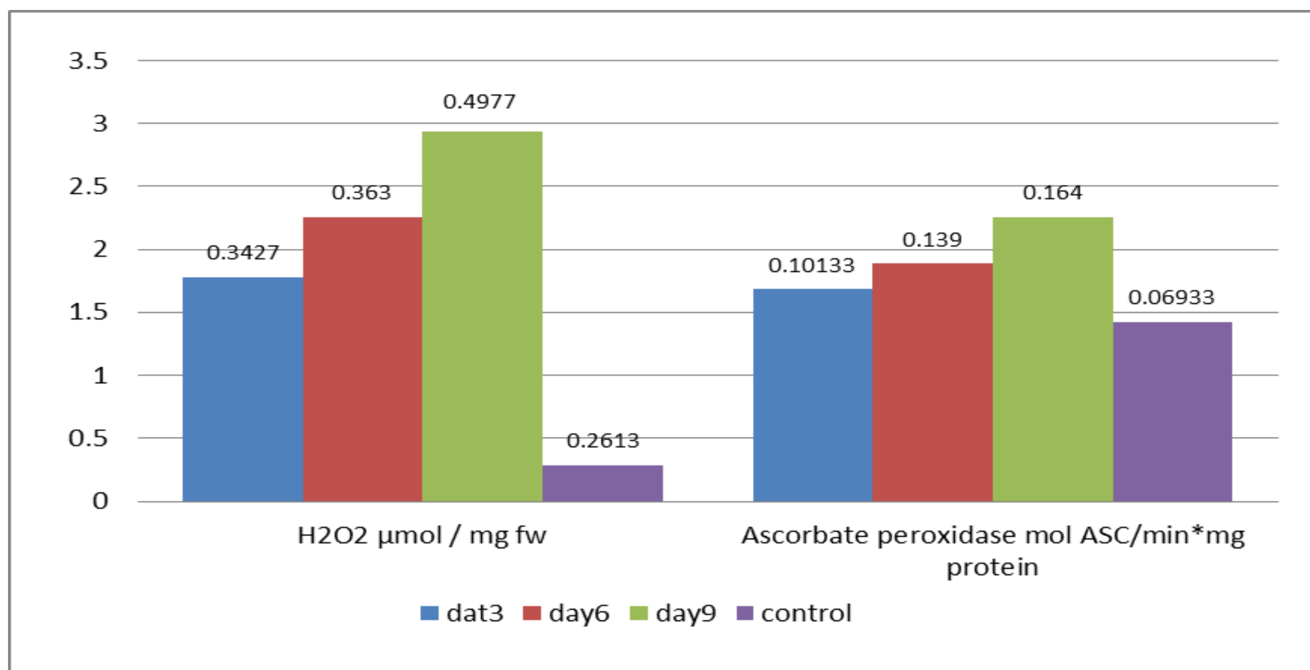

Figure .1: Effect of drought stress on H2O2 and Ascorbate peroxidase In an different stress period 3days, 6days and 9days.

The results showed a sharp increase in the concentrations of H2O2 on the third day of treatment and it increases with increasing exposure period, reaching its maximum on the ninth day, as the plant initiates the formation of H2O2 in response to the stress it is exposed to, At the same time we notice an equal increase of ascorbate peroxidase concentrations which works on dismantling the H2O2 into simpler compounds that are easier for the plant to get rid of, So we note a match in the results between the enzyme and H2O2 with the difference in levels, H2O2 accumulation in plant known as the oxidative burst is one of early event of plant defense response to abiotic and biotic stresses. Where drought induces H2O2 accumulation in linear manner with the severity of drought stress since chloroplasts, mitochondria and peroxisomes are the seats as well as first target of reactive oxygen species produced under drought stress [12, 13].

3.2 Effects of Salinity on H2O2 and Ascorbate peroxidase content.

As shown in figure No.2, The results show a significant but sequential increase in the concentrations of H2O2, and the highest concentration was in s4 it was (2.3257µmol / mg fw) While the results of both s1, s2 and s3 were (0.9197µmol / mg fw) , (1.5923µmol / mg fw) and (2.0250µmol / mg fw) respectively. All results have witnessed an increase if compared to the control group, and this confirms the response of the plant and its secretion of free radicals during exposure to salinity stress, which is matches our hypothesis. The effect of salinity stress on the H2O2 content in leaves were agreed with pervious reporting [14].

Ascorbate peroxidase or H2O2-scavenging enzyme, It is one of the most important enzymes that play a key role in protecting the plant cell from damage caused by free radicals resulting from exposure of the plant to various stresses, The results indicated that the concentration on the s1 was (0.56967mol ASC/min*mg protein) its paper the minimum concentration and on the s2 and s3 was (1.03667mol ASC/min*mg protein) and (1.08667mol ASC/min*mg protein) respectively but in the control group it was (1.42233mol ASC/min*mg protein) and this confirms that the action of the enzyme is related to the increase of free radicals, all the result show significant decrease in concentration of enzyme which are shown in the figure No.2, The results matched [15].

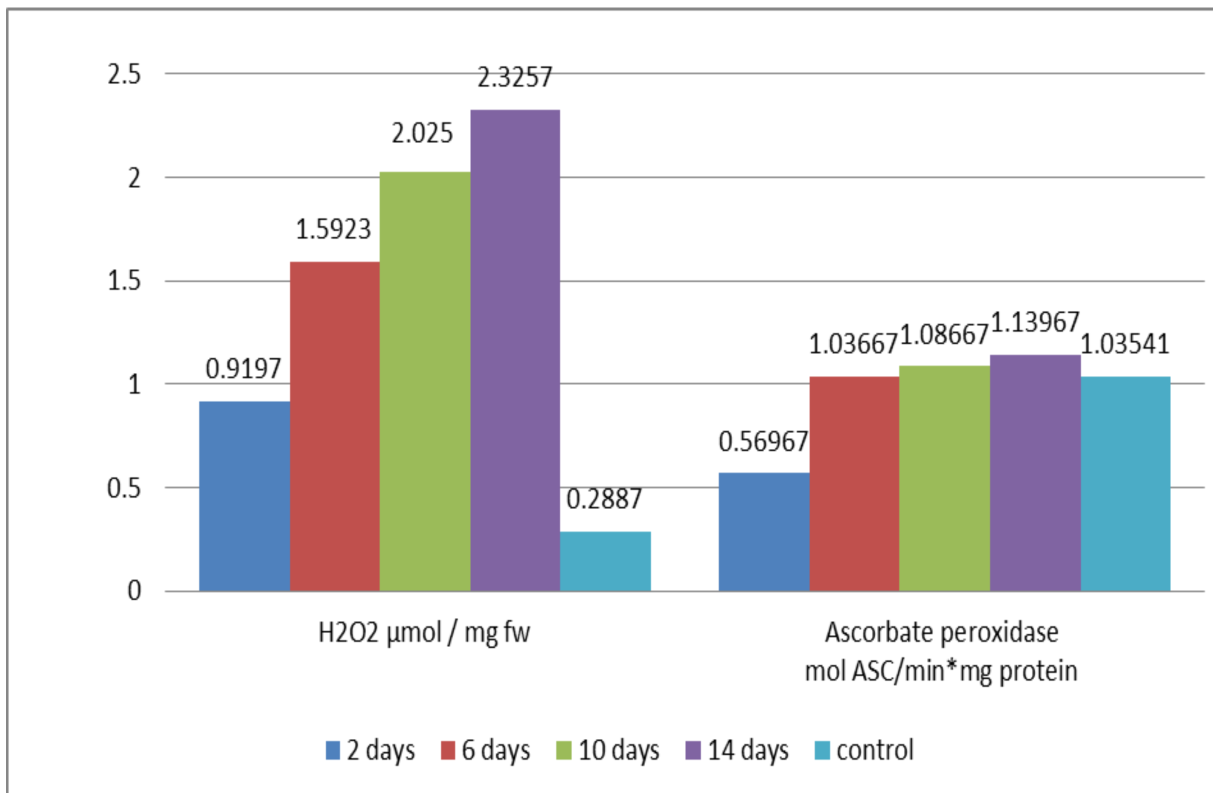

Figure.2: Effect of Salinity stress on H2O2 and Ascorbate peroxidase In different stress period 2days, 6days, 10days and 14days.

The results show a sharp and significant increase in the concentration of H2O2. This increase is affected by the increase in the treatment period, as the fourteenth day witnessed the highest increase, and the results decreased until reaching the second day of the treatment. The ability of induction might be linked to the induction. H2O2 to induce antioxidant enzymatic defenses to reduce the salinity deleterious effects, since H2O2 is a probable signaling molecule that mediates crosstalk between signaling pathways contributes to protection towards another stresses [16]. The enzyme Ascorbate peroxidase showed a significant increase compared to the control group, and the highest percentage of the increase came on day14 of the treatment, followed by day 10, but on the sixth day, there was no significant change occurred, The work of the enzyme is related to the work of H2O2, as its

activity is limited to scavenging free radicals and mitigating the damage they cause at the cellular level. You speak it at the cellular level. Previous study about the influence of salt stress reported that there was increased total and specific PRX activity that might be to reduced stress severity as acclimation response which could be associated with lignification and recovery of cell membrane damage by salt stress [17].

3.3 Effects of Temperature on H2O2 content.

In H2O2 content a significant differences showed in flowing results and an sharp increase happened especially in (T1c) Figure No. 3a, Show that T1a which was (0.6210 µmol / mg fw), And at the level of T1b, T1c, it was (0.9047 µmol / mg fw) and (1.2530 µmol / mg fw) which was the highest increase on H2O2 concentration in (T1c) , wail other level of temperature is increased also, In the T2a, b, c concentration, it was (0.2887 µmol / mg fw). The increase on T1c concentration is for the extreme condition of exposure low temperature and the length of the treatment period. The result of T3a, T3b and T3c (0.3017 µmol / mg fw) , (0.3893 µmol / mg fw) and (0.5077 µmol / mg fw) respectively, These results is fluctuate in comparison with control group which was T2a,b,c concentration was (0.3157 µmol / mg fw). The result of T4a, T4b and T4c (0.2197 µmol / mg fw) , (0.3230 v and (0.4773 µmol / mg fw) respectively, it's also fluctuat than control group, maybe that belongs to tolerance activity of plant. In T5 result a, b and c it was (0.2313 µmol / mg fw) , (0.3317 µmol / mg fw), (0.2993 µmol / mg fw) respectively, The concentration levels of H2O2 was significantly increased in first 2h and rise again in next 4 and 6 hours.

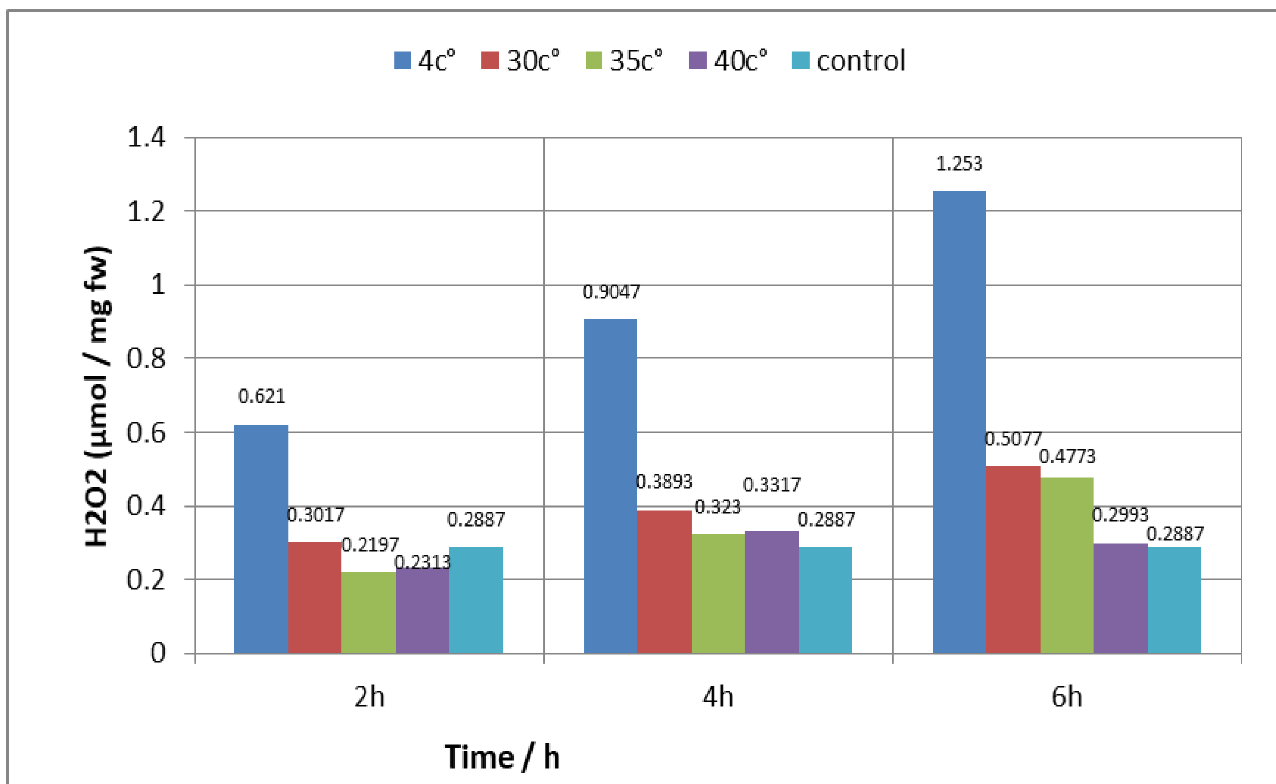

Figure 3.: Effect of Temperature stress 4c°, 30c°, 35c° and 40c° on H2O2 content In different period of time 2h, 4h, 6h.

It is clear that the H2O2 levels increased significantly when the plant was treated with a temperature of 4°C, especially after 6 hours of treatment, This is a kind of severe response to low temperatures and its effect on the plant. While the results of other temperatures of 30°C, 35°C and 40°C were clearly fluctuating, except for 30°C, which increased after 6 hours of treatment. It seems that 40°C led to a decrease in the biological activity of the

plant, which led to a clear lack of response at that temperature, These results match [18, 19].Several early studies discovered that one of the initial reactions of plant cells to abiotic stress was an increase in the formation of reactive oxygen species (ROS) and about the ROS, a variety of plant responses has been found to be triggered by H2O2 such as acclimation to stresses and developmental stages [20]. The response of plants with regard to the formation of free radicals varies due to the effect of any type of stress, but in general, the H2O2 ratios rise with a decrease in temperature to the level of 4 °C.

4. CONCLUSION

In conclusion, environmental stress impacts H2O2 and APX content in plants by increasing ROS production and triggering antioxidant defense mechanisms. Understanding these responses is crucial for developing strategies to enhance plant tolerance to environmental stressors and improve agricultural productivity in challenging environments.

5.REFERENCES

1. Andreia, Ispas. (2012) . Conceptual approach of leadership and employees' individual performance relationship. The Journal of the Faculty of Economics 1.1 1021-1026.

2. Ashrafi-Dehkordi, E., Alemzadeh, A., Tanaka, N., and Razi, H. (2018). Meta-analysis of transcriptomic responses to biotic and abiotic stress in tomato. PeerJ, 6, e4631.

3. Barillari, J., Canistro, D., Paolini, M., Ferroni, F., Pedulli, G. F., Iori, R., and Valgimigli, L. (2005). Direct antioxidant activity of purified glucoerucin, the dietary secondary metabolite contained in rocket (Eruca sativa Mill.) seeds and sprouts. Journal of Agricultural and food chemistry, 53(7), 2475-2482.

4. Chin, D. C., Kumar, R. S., Suen, C. S., Chien, C. Y., Hwang, M. J., Hsu, C. H., ... and Yeh, K. W. (2019). Plant cytosolic ascorbate peroxidase with dual catalytic activity modulates abiotic stress tolerances. Iscience, 16, 31-49.

5. Christou, A., Manganaris, G. A., Papadopoulos, I., and Fotopoulos, V. (2013). Hydrogen sulfide induces systemic tolerance to salinity and non-ionic osmotic stress in strawberry plants through modification of reactive species biosynthesis and transcriptional regulation of multiple defence pathways. Journal of experimental botany, 64(7), 1953-1966.

6. Egert, A., Keller, F., Peters, S. (2013). Abiotic stress-induced accumulation of raffinose in Arabidopsis leaves is mediated by a single raffinose synthase (RS5, At5g40390). BMC Plant Biology, 13:218.

7. Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., and Basra, S. M. A. (2009). Plant drought stress: effects, mechanisms and management. In Sustainable Agriculture (pp. 153-188).Springer Netherlands.

8. Gondim, F. A., Miranda, R. D. S., Gomes-Filho, E., and Prisco, J. T. (2013). Enhanced salt tolerance in maize plants induced by H2O2 leaf spraying is associated with improved gas exchange rather than with non-enzymatic antioxidant system. Theoretical and Experimental Plant Physiology, 25(4), 251-260.

9. Gülen, H., Çetinkaya, C., Kadıoğlu, M., Kesici, M., Cansev, A., andEriş, A. (2008).Peroxidase activity and lipid peroxidation in strawberry (Fragaria X ananassa) plants under low temperature. J. Biol. Environ. Sci, 2(6), 95-100.

10. Lateef, O. S., Al-Badri, M., Al-Badri, K. S. L., & Mohammed, S. A. (2023). Polarization-insensitive Archimedes'-spiral-shaped ultrathin metamaterial absorbers for microwave sensing application. Scientific Reports, 13(1), 19445.

11. Jajic, I., Sarna, T., andStrzalka, K. (2015).Senescence, Stress, and Reactive Oxygen Species. Plants, 4(3), 393-411.

12. Kwon, M. Y., and Woo, S. Y. (2016). Plants' responses to drought and shade environments. African Journal of Biotechnology, 15(2), 29-31.

13. Liu, T., Ye, X., Li, M., Li, J., Qi, H., and Hu, X. (2020). H2O2 and NO are involved in trehalose-regulated oxidative stress tolerance in cold-stressed tomato plants. Environmental and Experimental Botany, 171, 103961.

14. Mittler, R., and Zilinskas, B. A. (1994). Regulation of pea cytosolic ascorbate peroxidase and other antioxidant enzymes during the progression of drought stress and following recovery from drought. The Plant Journal, 5(3), 397-405.

15. Nishizawa, A., Yabuta, Y., and Shigeoka, S. (2008). Galactinol and raffinose constitute a novel function to protect plants from oxidative damage. Plant physiology, 147(3), 1251-1263.

16. Neill S, Desikan R, Hancock J (2002). Hydrogen peroxide signaling. Current Opinion in Plant Biology 5:388-395.

17. Mohammed, S. A., Albadri, R. A. K., & Al-Badri, K. S. L. (2023). Simulation of the microwave five-band a perfect metamaterial absorber for the 5G communication. Heliyon, 9(9)..

18. Oelze, M. L., Vogel, M. O., Alsharafa, K., Kahmann, U., Viehhauser, A., Maurino, V. G., and Dietz, K. J. (2011). Efficient acclimation of the chloroplast antioxidant defence of Arabidopsis thaliana leaves in response to a 10-or 100-fold light increment and the possible involvement of retrograde signals. Journal of experimental botany, 63, 1297–1313.\

19. Mahdi, W. M., Al-Badri, K. S. L., & Alqaisi, M. R. (2021). Effect of Microwave Radiation on Bacteria, Fungi and Some Growth Characteristics of Cowpea Vigna unguiculata L. Gesunde Pflanzen, 73(2), 161-167.

20. Sharma, P., and Dubey, R. S. (2005). Drought induces oxidative stress and enhances the activities of antioxidant enzymes in growing rice seedlings. Plant growth regulation, 46(3), 209-221.