The Effect of Plant Extracts and Nano-Selenium on the Protein Profile of Mango seedling (Mangifera indica L.) Under Stress Conditions

Noor Abdulrazzaq Abdullah1, Wasen Fawzi Alpresem2, Ahmed Yousef Lafta Hzaa3
1Department of Horticulture and Landscape Design, College of Agriculture, University of Basrah, Basrah, Iraq
2Unit of Medicinal and Aromatic Plants, College of Agriculture, University of Basrah, Basrah, Iraq
3Department of Marine Biology, Marine Science Centre, University of Basrah, Iraq
*Corresponding Author email: wasen.fadel@uobasrah.edu.iq

Abstract

This study was conducted at the Agricultural Research Station and Laboratories of the College of Agriculture, University of Basrah, Department of Horticulture and Landscape Design. Two year old seedlings of mango were planted in 25 Kg pots. The study involved a factorial experiment with two factors and six replications, resulting in 54 experimental units. The first factor consisted of three treatments: Moringa leaf extract (0,100 mg L-1), Damas leaf extract (100 mg L-1), and control. The second factor was nanoselenium at concentrations of 0, 25, and 50 ppm. The protein pattern of the leaves was analyzed using protein transfer technique on polyacrylamide gel using denaturing slab electrophoresis (SDS). The results showed that the protein profiles on the polyacrylamide gel varied in size, area, and height depending on the type of treatment. The treatments affected the properties of the protein bands, resulting in differences in protein quality due to changes in gene expression. The number of protein bands ranged from 4 to 9, depending on the type of treatment. Four protein bands were observed among the treatments: Moringa extract + Selenium 25 mg•L-1, Selenium 50 mg•L-1, and Selenium 25 mg•L-1. The highest number of protein bands with nine bands were recorded with the Damas extract + Selenium 50 mg L⁻ 1 treatment. Five protein bands were observed in the control, Moringa extract, and Damas extract. The stress conditions induced by the different treatments significantly affected. The molecular weight of the first protein band in all conditions ranged from 201.656 to 225.00 kilodaltons, indicating that the trees belonged to the same species and were from a common source.

Keywords: anti-transpiration, nano-selenium, protein pattern, mango, stress .

Introduction

The mango tree, Mangifera indica L., belongs to the Anacardiaceae family and is cultivated in tropical, subtropical, and temperate regions [1]. Mango trees originated in South Asian, particularly in eastern India. Mango is the second most produced tropical crop globally [2,3]. Mango fruits are highly valued for their nutritional, economic, and medicinal value due to their chemical composition, which includes dietary fibers, proteins, ascorbic acid, carbohydrates, fats, and essential nutrients. [4-6]. The optimal climate for mango tree growth is hot and humid, with a temperature range of 24 to 27°C. Mango trees require a cool and dry or cold and dry climate before flowering, and a dry climate during flowering and fruit set [7,8]. Studies have shown that high temperatures exceeding 35°C in mango trees lead to decreased pollen vitality and reduced fruit set [9,10]. Most plants grown in direct sunlight experience increased temperature and intense light, which leads to drought stress [11]. Water stress, a type of abiotic environmental stress, has a negative effect on plant growth and development and causes significant damage to physiological processes, enzyme activity, and hormone levels [12]. To reduce the effects of high temperatures and lack of water, researchers are looking for alternatives to reduce these damages. This includes treating plants with anti-evaporation agents or substances that reduce transpiration in agricultural fields and horticultural crops. [13,14]. Foliar spraying is used to achieve maximum benefit for the plant [15]. Plant extracts contain numerous biologically active compounds that enhance physiological processes, stimulate plant growth and development, and increase nutrient utilization efficiency [16]. Notably, Moringa leaf extract and Damas leaf extract have shown significant benefits. Proteins. which constitute about 50% They are characterized by their high molecular weight and their impermeability to permeable membranes. Depending on the needs of the plant and its response to certain treatments, changes in gene transcription and translation processes can form new proteins through gene expression [17]. Protein analysis is essential to reveal the extent to which plants interact with abiotic stressors [18.[

Material and Methods

The investigation was conducted at the Agricultural Research Station and Laboratory, Department of Horticulture and Landscape Engineering, Faculty of Agriculture, University of Basrah. Two year old seedlings of mango were gathered from private nurseries and planted in 25 kilograms of potted plants that were comprised of sand and peat moss in a 3:1 ratio. The pots were randomly distributed within sectors. The study employed a factorial experiment with two factors and six replicates, resulting in 54 experimental units. The first factor involved plant extracts: Moringa leaf extract (0,100 mg. L^{-1}), damas leaf extract (100 mg. L-1), and a control treatment. The second factor was nano selenium in concentrations of 0, 25 and 50 ppm. Selenium treatment was used at the beginning of the experiment and then one week after the antiperspirant treatment. The second treatment was applied one month after the initial treatments.

Study of the Protein Profile of Leaves

Leaf samples were freeze-dried at a temperature of -26°C. The protein extraction process was then followed [19]. One gram of leaves was incorporated into a ceramic mortar that was 3 ml in volume and contained Phenyl methane sulfonyl fluoride PMSF at a concentration of 4°C. The mixture was then centrifuged at 4 degrees Celsius and 18,000 revolutions for 30 minutes. Next, 40 ml of the supernatant were transferred to a polyacrylamide gel and electrophoresed. The migration of proteins was accomplished using the slab-electrophoresis method with SDS as the denaturing agent, as described by [20]. Promega's broad range of protein molecular weight markers were employed to estimate and document the molecular weights of the proteins using the PhotoCapt Mw (version 17) software.

The treatments were numbered as follows

-1Marker,2- Control treatment,3- Moringa extract, 4- Damas extract, 5- Moringa extract + selenium 50 mg.L^{-1}, 6- Moringa extract + selenium 25 mg.L^{-1}, 7- Selenium 50 mg.L^{-1}, 8- Damas extract + selenium 50 mg.L^{-1}, 9- Selenium 25 mg.L^{-1}, 10- Damas extract + selenium 25 mg.L^{-1}.

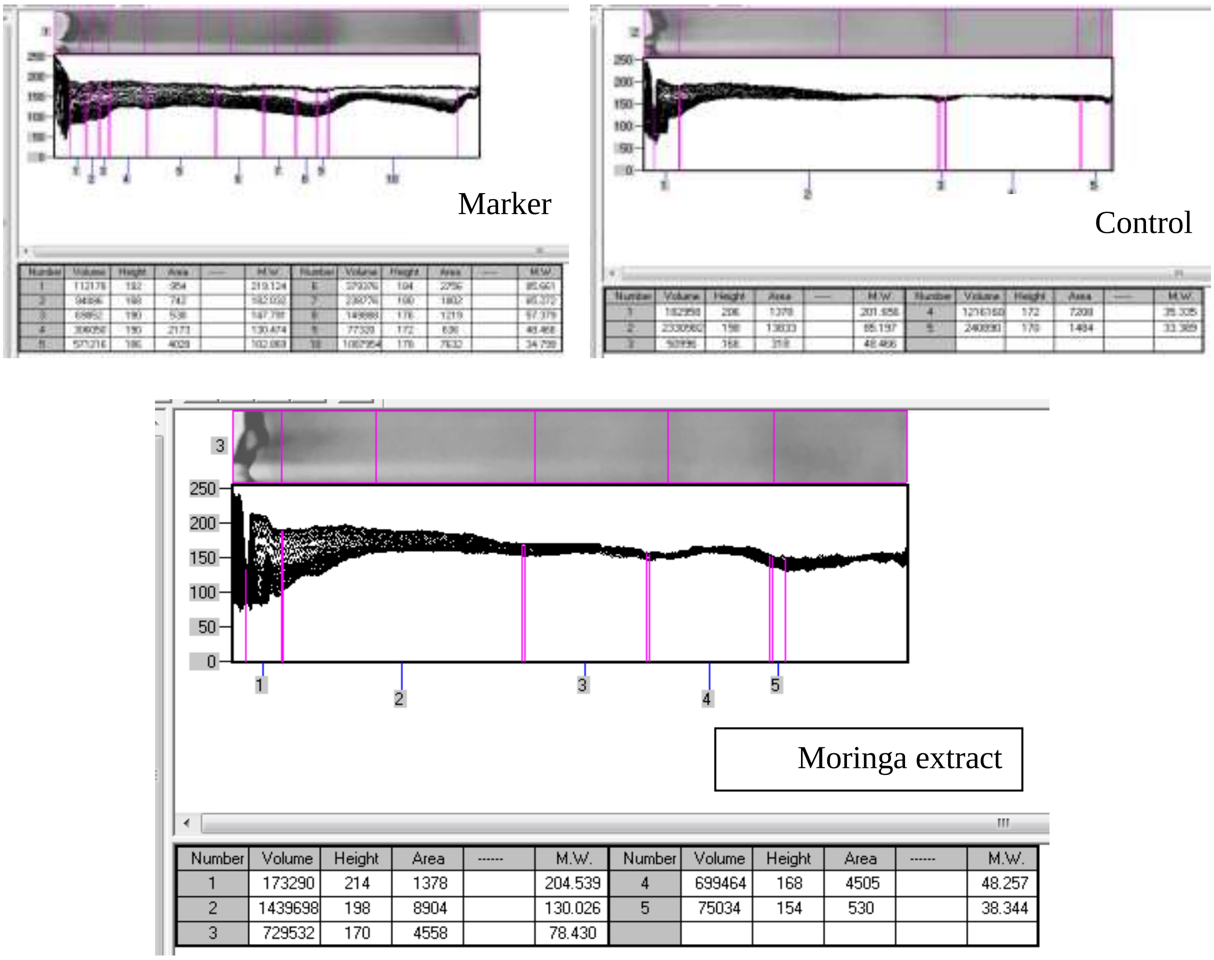
Results and Discussion

Observation of the protein pattern of mango leaves under different treatments Figure (1: a, b, c, and d), revealed significant differences in the protein bands on the polyacrylamide gel.

ISSN 2072-3857

The characteristics of the protein bands, including size, area and height, varied depending on the type of treatment. The parameters studied affected these characteristics, resulting in gene expression leading to differences in protein quality. Figure (2) shows that there were differences in the number, position and specifications of protein bands on the polyacrylamide gel between the different treatments. The number of protein bands varied between 4 and 9 depending on the type of treatment, with 4 protein bands observed in three treatments: Moringa extract + Selenium 25 mg.L-1, Selenium 50 mg.L-1 and Selenium 25 mg.L-1. No significant gene expression occurred in these treatments. This could be because the mango trees were not exposed to drought or heat or because nano-Selenium and plant extracts improved the resistance of mango trees to drought stress. The Damas extract + Selenium 50 mg L-1 treatment had the highest number of protein bands recorded on the polyacrylamide gel, with a total of 9 bands. In addition, five protein bands appeared in the three treatments: control, Moringa extract, and Damas extract. The stress conditions caused by the different treatments significantly changed the positions of the protein bands in all treatments. Table (1) shows that the molecular weight of the first protein band was always similar in all studied conditions, ranging from 201.656 to 225.00 kDa. This indicates that the trees belong to the same species and come from a common source (asexual reproduction). The second, third, fourth, and fifth bands showed similar positions and molecular weights in the Moringa extract and Damas extract treatments, indicating that the two treatments alone had the same effect. When treated with Moringa extract + 50 mg.L-1 Selenium, a sixth protein band with a molecular weight of 34.396 kDa appeared, which may be due to gene expression triggered by drought or heat stress. Damas extract + 50 mg•L-1 Selenium treatment increased gene expression and produced 4 new protein bands compared to the control treatment, for a total of 9 protein bands with molecular weights between 219.124 and 34.295 kDa. These bands may help plants resist drought and heat stress. The protein pattern results showed that Moringa extract treatment and Damas extract treatment alone had the same effect, while their combination with nano-selenium produced different effects. These findings suggest that exposure to stress conditions reduces native protein synthesis and leads to changes in transcription and translation processes, as well as the plant and the type of stress. This mechanism ensures the resistance of plants to adverse conditions [21]. Drought or heat stress significantly affects the growth and development of mango trees, especially under abnormal conditions such as low humidity and high temperature. This type of stress causes water stress and reduces the ability of plants to absorb water. In general, most stressors lead to a significant reduction in protein synthesis and nucleic acid production in plant tissues, the appearance of new protein bands, and the disappearance of other protein bands [22,23]. In order to determine stress resistance, it is necessary to identify the influential molecules that trigger adaptive mechanisms in plants grown under stress conditions. These influential molecules include proteins and metabolites produced through gene expression. They regulate important processes within the plant to cope with stress, ensuring resistance to adverse conditions [24,25.[

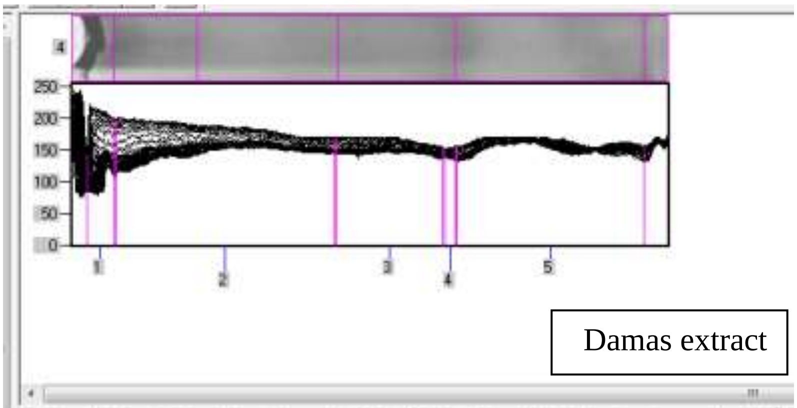
ISSN 2072-3857



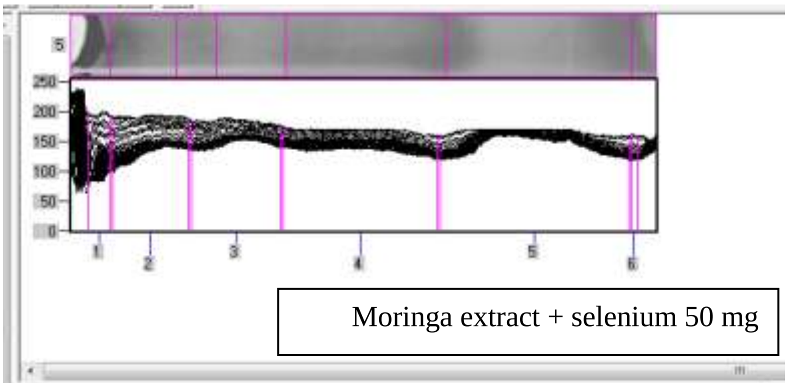
Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	173290	214	1378		204.539	4	699464	168	4505		48.257
2	1439698	198	8904		130.026	5	75034	154	530		38.344
3	729532	170	4558		78.430						

Figure 1(a): Specifications of the protein bands on the polyacrylamide gel of Moringa trees (part of the PhotoCapt program(

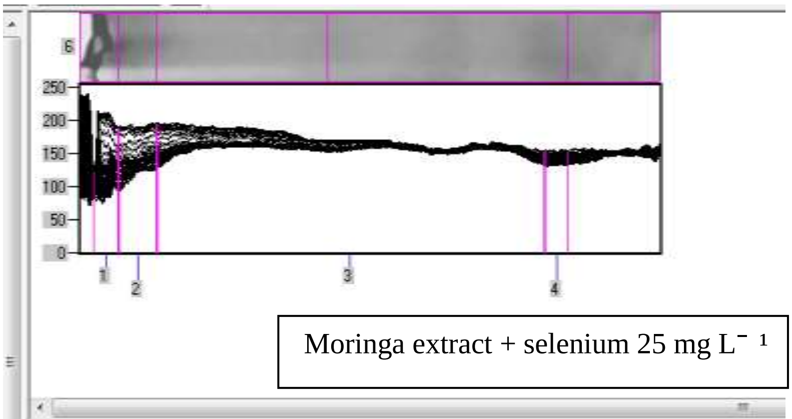
ISSN 2072-3857



Number	Volume	Height	Area	-----	M.W.	Number	Volume	Height	Area	-----	M.W.
1	143314	222	1166		207.436	4	67864	156	477		48.466
2	1421038	202	9169		130.474	5	1230032	170	7897		34.295
3	679734	170	4505		76.868						



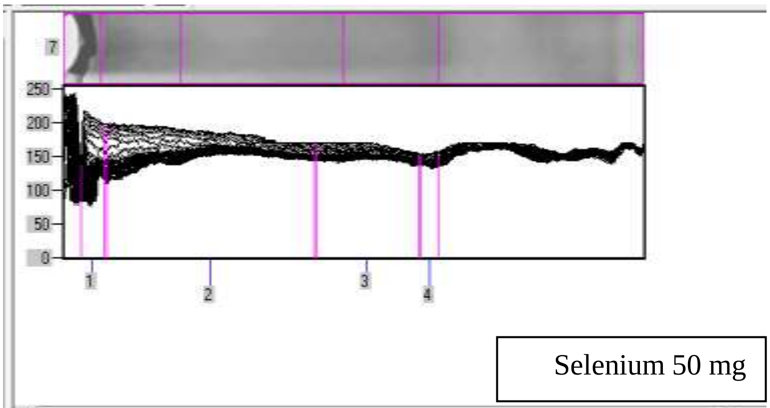
Number	Volume	Height	Area	-----	M.W.	Number	Volume	Height	Area	-----	M.W.
1	110640	202	1007		210.345	4	961176	174	6625		96.126
2	467484	194	3286		136.720	5	1206476	170	8109		48.678
3	595654	190	3869		122.360	6	40010	162	318		34.396



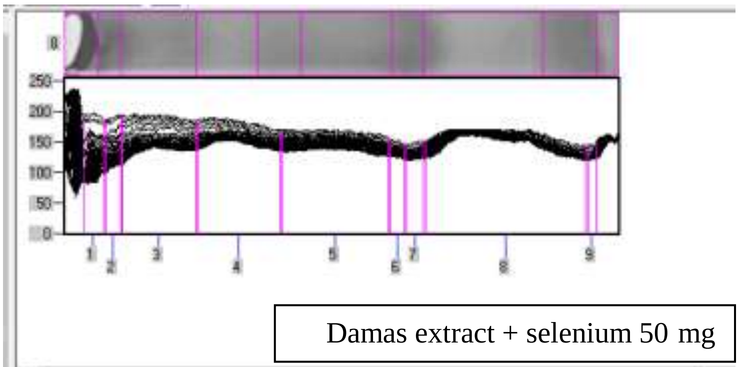
Number	Volume	Height	Area	-----	M.W.	Number	Volume	Height	Area	-----	M.W.
1	134844	214	1113		213.264	3	2665962	196	16642		82.899
2	234352	196	1643		146.908	4	142086	156	1007		37.152

Figure 1(b): Specifications of the protein bands on the polyacrylamide gel of Moringa trees (part of the PhotoCapt program).

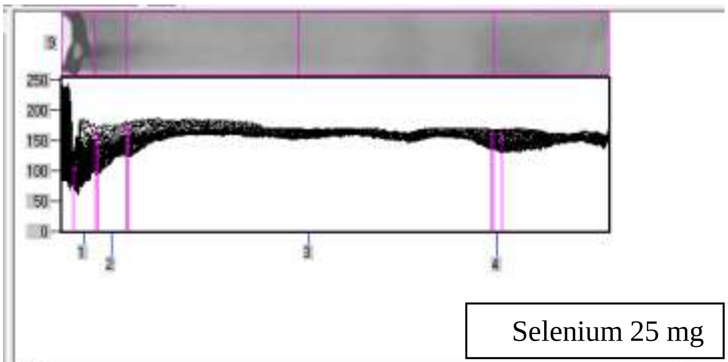
ISSN 2072-3857



Number	Volume	Height	Area	-----	M.W.	Number	Volume	Height	Area	-----	M.W.
1	125312	218	1060		216.191	3	668412	170	4452		71.721
2	1373948	200	8957		132.263	4	112898	158	795		48.257



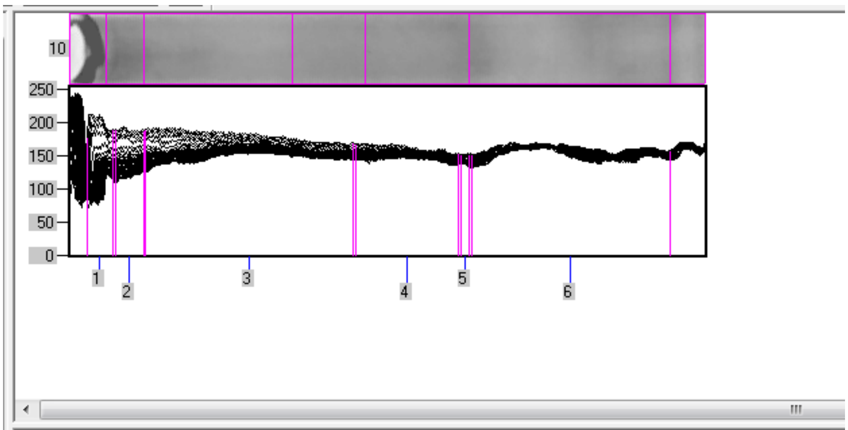
Number	Volume	Height	Area	-----	M.W.	Number	Volume	Height	Area	-----	M.W.
1	104284	198	954		219.124	6	91462	156	689		54.516
2	94198	194	742		166.354	7	102282	152	795		48.050
3	493264	196	3392		124.625	8	1088788	170	7261		36.565
4	578204	190	3763		100.480	9	53770	150	424		34.295
5	702922	170	4823		82.899						



Number	Volume	Height	Area	-----	M.W.	Number	Volume	Height	Area	-----	M.W.
1	118398	186	1060		222.061	3	2653236	188	16642		81.539
2	187680	182	1378		149.558	4	61228	168	424		38.819

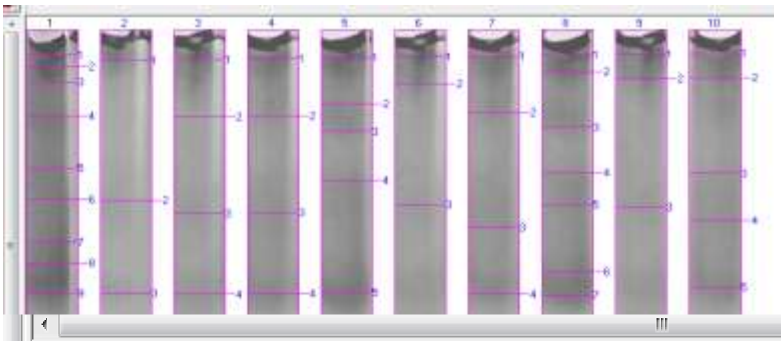
Figure 1(c): Specifications of the protein bands on the polyacrylamide gel of Moringa trees (part of the Photocapt program.(

ISSN 2072-3857



Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	128030	212	1080		225.000	4	621676	168	4158		75.000
2	163350	194	1188		150.000	5	53666	154	378		50.000
3	1298626	192	8370		100.000	6	1234908	170	7992		35.000

Figure 1(d): Specifications of the protein bands on the polyacrylamide gel of Moringa trees (part of the Photocapt program(



	M.W.									
	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10
1	219.124	201.656	204.539	207.436	210.345	213.264	216.191	219.124	222.061	225.000
2	182.032	85.197	130.026	130.474	136.720	146.908	132.263	166.354	149.558	150.000
3	147.791	48.466	78.430	78.868	122.360	82.899	71.721	124.625	81.539	100.000
4	130.474	35.335	48.257	48.466	96.126	37.152	48.257	100.480	38.819	75.000
5	102.869	33.389	38.344	34.295	48.678			82.899		50.000
6	85.661				34.396			54.516		35.000
7	65.272							48.050		
8	57.379							36.565		
9	48.466							34.295		
10	34.799									

Figure (2): Number and locations of protein bands and their molecular weights for mango trees (part of the PhotoCapt program.(

ISSN 2072-3857

Table 1. Number of protein bands and their molecular weights in Moringa trees (kDa(

Protein bands	1	2	3	4	5	6	7	8	9	10
Marker	219.12	182.03	147.79	130.47	102.8	85.66	65.27	57.37	48.46	34.79
Control	201.65	85.19	48.46	35.335	33.38	0	0	0	0	0
Moringa extract	204.53	130.02	78.43	48.25	38.34	0	0	0	0	0
Damas extract	207.43	130.47	78.86	48.46	34.29	0	0	0	0	0
Moringa extract + selenium 50 mg L^{-1}	210.34	136.72	122.36	96.12	48.67	34.39	0	0	0	0
Moringa extract + selenium 25 mg L^{-1}	213.26	146.90	82.89	37.15	0	0	0	0	0	0
Selenium 50 mg L^{-1}	216.19	132.26	71.72	48.25	0	0	0	0	0	0
Damas extract + selenium 50 mg L^{-1}	219.12	166.35	124.62	100.48	82.89	54.51	48.05	36.56	34.29	0
Selenium 25 mg L^{-1}	222.06	149.55	81.53	38.81	0	0	0	0	0	0
Damas extract + selenium 25 mg L^{-1}	225.00	150.00	100.00	75.00	50.00	35.00	0	0	0	0

Conclusion

Stress is a significant problem for plants, which have developed numerous mechanisms to resist challenges such as drought and high temperatures. These mechanisms are often genetically controlled and involve a reduction in the synthesis of natural proteins. Changes in transcription and translation processes can also occur, leading to the production of new proteins through gene expression according to the plant's needs and the type of stress encountered. This ensures the plant's resistance to adverse conditions, as many proteins produced in response to drought play a direct or indirect role in stress resistance. The unfavorable environmental factors were controlled by using plant extracts and nano fertilizers and their assistance in future research to control various stresses.. Proteins

ISSN 2072-3857

accumulate to mitigate stress conditions, and changes in the protein pattern due to stress positively impacts mango seedlings, enhancing their resistance. This, in turn, reduces water loss through mechanisms present in the leaves.

References

[1]Yadav, D and Singh, S. P. 2017. Mango: History origin and distribution, J. Pharmacog. Phytochem. 6(6):1257- 1262.

[2] Parvez, G. M. 2016. Pharmacological activities of mango (Mangifera indica): A review. Journal of Pharmacognosy and Phytochemistry, 5(3):1-7.

[3] Kumar, M.; Saurabh, V.; Tomar, M.; Hasan, M.; Changan, S.; Sasi, M.; Maheshwari, C.; Prajapati, U. and Singh, S. 2021. Mango (Mangifera indica L.) leaves: Nutritional composition, phytochemical profile, and health-promoting bioactivities. Antioxidants, 10(2): 299.

[4]Bally, I.S.E. 2006. Mangifera indica (Mango). Species Profiles for Pacific Island Agroforestry, 1-25.

[5]Maldonado-Celis, M. E.; Yahia, E. M.; Bedoya, R.; Landázuri, P.; Loango, N.; Aguillón, J.; Restrepo, B. and Guerrero Ospina, J.C. 2019. Chemical composition of mango Mangifera indica L. fruit: Nutritional and phytochemical compounds. Front. Plant Sci.,10, 1073 .

[6]Lebaka, V. R.; Wee, Y. J.; Ye, W and Korivi ,M. 2021. Nutritional Composition and Bioactive Compounds in Three Different Parts of Mango Fruit. Int. J. Environ Res Public Health. 16;18(2):741.

[7]Jignasa, H. R.; Varu, D. K.; Farheen, H. H. and Meera, B. S. 2018. Correlation of Climatic Parameters with Flowering Characters of Mango. Int J Pure App Biosci. 6 (3): 597-601.

[8]Khalifa, S. M. and Abobatta, W. F. 2023. Climate Changes and Mango Production Temperature. Science: Botany; Ecology; Evolutionary Biology. 1(1): 043-046 .

[9]Sharma, S. and Manjeet (2020). Heat Stress Effects in Fruit Crops: A Review. Agricultural Reviews. 41(1):73-78.

[10]Abobatta, W.F. 2021. Fruit orchards under climate change conditions: adaptation strategies and management. J. Appl Biotechnol Bioeng. 8(3):99-102. DOI: https://doi.org/10.47275/2692-5222-113 .

[11]Wach, D. and Skowron, P. 2022. An overview of plant responses to the drought stress at morphological, physiological and biochemical levels. Polish Journal of Agronomy. 50,50(wrz.2022),25–34. DOI: https://doi.org/10.26114/pja.iung.435.2022.04.

[12]Ihsan, M. Z.; Daur, I.; Alghabari, F., Alzamanan, S.; Rizwan, S.; Ahmad, M.; Waqas, M. and Shafqat, W. 2019. Heat stress and plant development: role of sulphur metabolites and management strategies. Acta Agriculturae Scandinavica Section B:Soil and Plant Science, 69(4), 332–342. https://doi.org/10.1080/09064710.2019.1569715.

[13]Gaballah, M.S.; Shaaban; S.M and Abdallah, E.F 2014. The use of antitranspirants and organic compost in sunflower grown under water stress and sandy soil. International Journal of Academic Research. 6(6):211-215.

[14]Zhang, Y.; Xu, J.; Li, R.; Ge, Y.; Li, Y. and Li, R. 2023. Plants' Response to Abiotic Stress: Mechanisms and Strategies. Int.J.Mol. Sci., 24, 10915. https://doi.org/10.3390/ijms241310915.

ISSN 2072-3857

[15]Alheidary, M. H. 2023. Spraying Technology and Foliar Application Result in a Smooth Layer of the Spray: a Literature Review. Basrah J. Agric. Sci., 36(2), 334-374.

[16]Bulgari, R.; Cocetta, G.; Trivellini, A.; Vernieri, P. and Ferrante, A. 2015. Biostimulants and Crop Responses: A. Review. Biol. Agric. Hortic. 31, 1–17.

[17]Hopkins, W. G. and Muner, N. P. 2008. Introduction to plant physio- logy. 4th Edition, J. Wiley and Sons, U. S. A. 526 pp.

[18]Piasecka, A.; Kachlicki, P., and Stobiecki, M. 2019. Analytical methods for detection of plant metabolomes changes in response to biotic and abiotic stresses. International Journal of Molecular Sciences, 20(2). https://doi.org/10.3390/ ijms20020379 .

[19]Al-Najjar, M. Abd. H.; Al-Ibrahimi, M. S. Abd. and Fadel, W. F. 2021. The Manual in the Laboratory, Guide to Laboratory Analysis for Postgraduate and Primary Students. Enheduana House for Printing, Publishing, and Distribution. Iraq, 223 p

[20]Bavei, V.; Shiran, B.; Khodambashi, M. and Ranjbar, A 2011. Protein electrophoretic profiles and physiochemical indicators of salinity tolerance in sorghum (Sorghum bicolor L.). African Journal of Biotechnology. 10(14):2683-2697.

[21]David M. O. and Nilsen, E. T. 2000. The Physiology of Plant Under Stress . John Wiley & Sons, Inc. New York.

[22]Abd, A.M.; El-Dahab, E and Taain, D.A. 2019. Molecular studies of cultivars of date palms of phoenix dactylifera L. Al-Hussein Bin Talal University Journal for Research;4(1.(

[23]Shareef, H. J. and Al-khayri, J. M. 2021. Salt and Drought Stress Exhibits Oxidative Stress andModulated Protein Patterns in Roots and Leaves of Date Palm (Phoenix dactylifera L.). Acta Agriculturae Slovenica, 117(1):1-10 .

[24]Taain, D. A.; Al-najjar, M. A. H. and El-qatrani, N. A. 2021. investigation the protein pattern of leaves and roots of barhi and khalas date palm (phoenix dactylifera L.) cultivars propagated by offshoots and tissue culture techniques. Plant Cell Biotechnology and Molecular Biology 22(1and 2):9-17.

[25]Rashad, S. E.; Heiba, S. A. A.; Emam, M. A., Osman, S. A and Eldemerdash, I. S. 2023. Effect of PEG induced drought stress on Genetic diversity using SDS-PAGE and ISSR markers for Egyptian barley varieties. Egypt. J. Chem. 66 (SI13): 647-658.

ISSN 2072-3857