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Proteomic and Molecular Responses of Date Palm Offshoots (cv. Al-Jabjab) under Water Stress and Treated Wastewater Treatments

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Abstract

This study aimed to evaluate the impact of different irrigation treatments, including drought stress and biologically treated wastewater irrigation, on protein pattern and gene expression in date palm offshoots (cv. Al-Jabjab). Polyacrylamide gel electrophoresis analysis revealed that all studied trees shared the first and second protein bands with closely similar molecular weights, confirming their genetic uniformity and common plant origin.

However, significant variations were observed in the number, position, and molecular weights of protein bands depending on the applied treatment. Drought stress treatments led to an increase in the number of protein bands, indicating activation of gene expression and stimulation of intracellular defense mechanisms. The newly synthesized proteins, particularly low-molecular-weight proteins, are likely osmoprotectant or stress shock proteins that play essential roles in maintaining cellular structure, regulating damaged proteins, and preserving metabolic balance under water deficit conditions.

In contrast, irrigation with biologically treated wastewater resulted in a clearer and more regular protein pattern, reflecting stable gene expression and improved metabolic efficiency. This improvement may be attributed to the balanced nutrient content and reduced osmotic and oxidative stress associated with treated wastewater. Overall, the findings suggest that irrigation treatments directly influence molecular reprogramming in date palm, and that the use of biologically treated wastewater represents a sustainable irrigation strategy capable of enhancing growth performance and drought tolerance under arid and semi-arid conditions.

I. Introduction

The date palm (Phoenix dactylifera L.) is regarded as one of the most important plant species within the Arecaceae family, which comprises more than 200 genera and over 2,500 species.

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This family ranks second in importance to humans after the Poaceae (Gramineae) family. The date palm belongs to the order Arecales, a major botanical order that includes numerous palm species. Taxonomically, it is classified under the genus Phoenix and the species dactylifera (El-Hadrami and El-Hadrami, 2009; Jain et al., 2011).

Plants growing under full sunlight are frequently subjected to intense irradiance and high temperatures, conditions that commonly result in drought stress (Allen and Ort, 2001). Drought is a critical environmental factor influencing plant growth and development, and prolonged exposure can cause substantial damage by inducing physiological imbalances that disrupt enzymatic functions and hormonal regulation (Ihsan et al., 2019). Water deficit stress adversely affects various plant processes and promotes oxidative stress through the overproduction of reactive oxygen species (ROS). The accumulation of these reactive molecules leads to cytotoxic damage, including lipid membrane peroxidation and disturbances in cellular transport and regulation (Hussain et al., 2019).

The application of biologically treated wastewater aims to enhance the physical and chemical characteristics, as well as the fertility status, of the soil's surface layer. Such improvements create favorable conditions for plant growth by increasing soil moisture availability and supplying essential nutrients. Additionally, treated wastewater has been shown to stimulate antioxidant defense systems by elevating both enzymatic and non-enzymatic antioxidant activities, while encouraging the accumulation of osmoprotectants such as soluble sugars and proline (Yamada et al., 2002).

In light of the declining water quality of the Shatt al-Arab River, caused by the discharge of metallic and organic contaminants from domestic sewage and industrial effluents containing petroleum-derived hydrocarbons—and given the limited research on irrigating date palm offshoots with biologically treated wastewater as a strategy to alleviate drought stress—this study was undertaken. With increasing global water scarcity, the reuse of treated wastewater represents a practical alternative irrigation resource that may contribute to mitigating drought stress and supporting sustainable agricultural production.

II. Materials and Methods

The study was conducted in the Al-Hartha region, located north of Basra Governorate, Iraq, within the Palm Research Unit of the Ministry of Agriculture during the 2024–2025 growing seasons. A total of 27 offshoots of the date palm cultivar Jabjab were selected, ensuring

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uniformity in vegetative growth vigor, height, and age. The palms were 5–6 years old and planted in a permanent field. The experiment was carried out using biologically treated wastewater under three irrigation treatments:

1. Control treatment: Irrigation with Shatt al-Arab River water.
2. Mixed treatment: Irrigation with a mixture of Shatt al-Arab River water and biologically treated wastewater at a ratio of 1:1.
3. Treated wastewater: Irrigation with biologically treated wastewater only.

In addition, three irrigation intervals were applied: every 3, 10, and 15 days. The following parameters were studied:

Samples were collected from palm fronds and dried at -26 °C using a freeze-dryer (lyophilization process). The samples' proteins were extracted using the procedure described by Al-Najjar et al., (2021). 1 gram of palm leaves was mixed with 3 milliliters of Tris-HCl-buffer (0.1M, pH 7.5) solution containing Phenyl Methane Sulfonyl Fluoride (PMSF) at 4 °C. Centrifugation was then performed at 4 °C for 30 minutes at a speed of 18,000 rpm, and 40 microliters of the supernatant was transferred to a polyacrylamide gel. Proteins were electrophoresed on a polyacrylamide gel using the Slab Electrophoresis technique with SDS present, as outlined by Bavei et al., (2011). Additionally, Promega's Broad Range Protein Molecular Weight Markers wereemployed. A specialized program called Photo Capt. Mw version was used to estimate and illustrate the molecular weights of the proteins.17. The treatments were numbered on the gel as

follows:

Column 1 =marker / Column 2= Irrigation for 3 days + Shatt al-Arab water treatment / Column3= Irrigation every 3 days + (Shatt Al-Arab water and biologically treated water)

Column 4= Irrigation every 5 days + Shatt Al-Arab water

Column 5= Irrigation every 5 days + biologically treated water

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Column 6= Irrigation every 5 days + (Shatt Al-Arab water and biologically treated water)

Column 7= Irrigation every 15 days + Shatt Al-Arab water

Column 8= Irrigation every 15 days + biologically treated water

Column 9= Irrigation every 15 days + (Shatt Al-Arab water and biologically treated water)

III. Results and discussion

The results of the protein pattern analysis of date palm offshoot leaves (cv. Al-Jabjab) under investigation (Figure 1) revealed that all trees shared the first and second protein bands on the polyacrylamide gel. The molecular weights of the proteins in the first band ranged between (241.463–257.927) kDa, whereas those in the second band ranged between (127.676–137.912) kDa. This similarity in molecular weights among all studied trees may indicate that these trees originated from a single plant source (cv. Al-Jabjab), as they exhibited closely related molecular weights.

The appearance of low-molecular-weight protein bands may be attributed to a mechanism adopted by the plant to cope with stress conditions. These proteins increase with prolonged stress duration and may be classified as osmotic proteins. This is consistent with findings reported by several researchers, who indicated that certain proteins increase in expression under stress conditions and are referred to as osmoprotectant proteins (Hare et al., 1998; Grossman and Rhodes, 2002).

Furthermore, the protein pattern results of date palm offshoot leaves (cv. Al-Jabjab) (Figure 2) demonstrated differences among trees in the number, position, and characteristics of protein bands on the polyacrylamide gel. The number of protein bands ranged from four to six depending on the type of treatment.

Four protein bands appeared in the interaction treatment (biologically treated wastewater combined with irrigation every three days). Five protein bands were observed in three treatments: irrigation every three days; the interaction treatment (Shatt Al-Arab water + treated wastewater) with irrigation every three days; and treated wastewater with irrigation every 15 days. Observation of these three treatments indicates that biologically treated wastewater, when combined with drought intervals, prevented alterations in the protein pattern of the trees, as the number of protein bands was equal to that of the control treatment (irrigation every three days), despite differences in the molecular weights of the protein bands.

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Eight protein bands appeared in only one treatment, namely the interaction between Shatt Al-Arab water and irrigation every 15 days. Seven protein bands were observed in two treatments: (irrigation every 5 days + Shatt Al-Arab water) and (irrigation every 15 days + Shatt Al-Arab water + treated wastewater).

The protein pattern results clearly indicate that the studied treatments—particularly drought treatments—significantly affected gene expression in the studied trees and resulted in the appearance of new protein bands. This suggests that the treatments stimulated the cells to synthesize new proteins that support growth and developmental indicators in plants subjected to drought stress. The treatments also caused changes in the position and molecular weights of protein bands, indicating activation of gene expression and synthesis of new proteins that may contribute to improved plant growth and drought tolerance. It is well established that each cellular activity is governed by one or more proteins, which are classified according to their function into transport proteins, storage proteins, structural proteins, and protective proteins (Lesk, 2010).

These findings suggest that the treatments led to protein synthesis and may also have induced alterations in transcription and translation processes, resulting in the production of new proteins through gene expression according to plant requirements and treatment type, thereby ensuring improved plant growth (David and Nilson, 2000; Khairallah, 2009). These synthesized proteins contribute to the structure of the nucleus and cytoplasm, and some function as enzymes with essential roles in various metabolic processes during plant growth, fruit development, and ripening (Dalali, 1986; Cooper and Hausman, 2007).

Drought stress induces the synthesis of new low-molecular-weight proteins that play an important role in repairing and regulating damaged proteins associated with various vital activities in plants. These newly synthesized proteins may represent drought-induced stress shock proteins, which play a fundamental role in plant responses to physiological changes caused by drought and are typically present at low levels under normal conditions (Polenta et al., 2020; Ghirardo et al., 2021).

The protein pattern results also showed greater clarity and regularity of protein bands in plants treated with biologically treated wastewater, which represents a positive indicator reflecting stable gene expression and improved metabolic status. This type of water is relatively balanced in salts and contains mineral nutrients and absorbable organic compounds, thereby providing a favorable growth environment with reduced osmotic and oxidative stress.

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The clarity and regularity of protein bands in this treatment indicate enhanced synthesis of proteins associated with growth, cellular construction, and essential metabolic activities, such as photosynthetic proteins, nitrogen metabolism proteins, and enzymatic proteins involved in intracellular biochemical transformations. This pattern also reflects membrane integrity stability and efficient ribosomal activity, allowing continuous protein synthesis under normal physiological conditions without activating stress-related defense pathways.

Therefore, the balanced protein pattern observed under biologically treated wastewater irrigation does not represent a stress response, but rather indicates improved functional efficiency and the plant's ability to utilize available resources to support growth and cellular differentiation. This confirms the positive role of treated wastewater as a sustainable water resource in enhancing plant physiological performance under agricultural conditions (Hasanuzzaman et al., 2022; Zulfiqar et al., 2024).

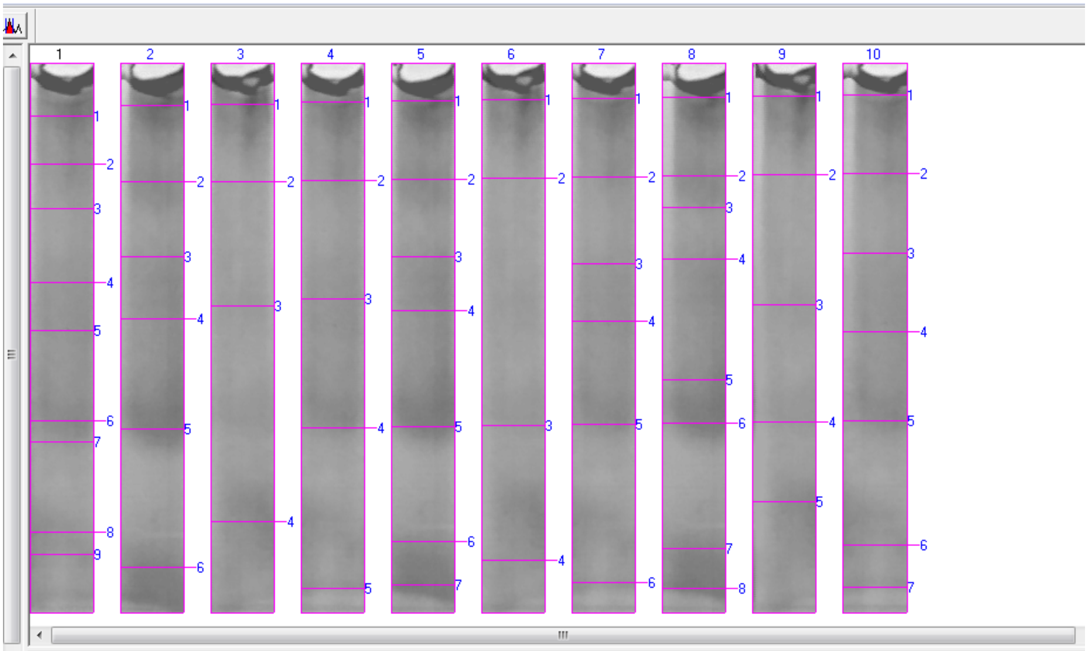

Figure (1): Number and sites of protein bands and their molecular weights (a part of the Photocopy program)

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Table (1): Some specifications of protein bands on polyacrylamide gel

Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	200440	220	1540		225.000	6	109540	166	770		35.000
2	387134	204	2585		150.000	7	744926	176	4730		25.000
3	696884	204	4345		100.000	8	24514	160	165		15.000
4	354594	194	2310		75.000	9	155458	162	1045		10.000
5	643900	180	4290		50.000						

Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	131550	218	1100		241.463	4	757664	170	5225		54.606
2	520772	200	3575		127.676	5	79434	156	605		30.789
3	868676	190	5665		85.843	6	1173142	170	7810		7.105

Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	147850	200	1265		243.293	3	1509584	170	9570		61.093
2	1467118	196	9240		127.676	4	95586	170	660		16.302

Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	129794	210	1100		246.951	4	164898	158	1155		31.378
2	1373006	190	8965		129.107	5	1172294	170	7535		2.368
3	733780	168	4895		65.074						

Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	119980	218	1045		248.780	5	107562	156	825		31.972
2	489302	200	3410		130.550	6	1096452	170	7260		13.159
3	829212	186	5445		85.843	7	13754	136	110		3.158
4	753158	170	5225		58.594						

Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	125876	196	1100		250.610	3	710694	170	4565		32.572
2	2325920	196	14630		132.003	4	672234	170	4455		8.684

Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	132066	214	1100		252.439	4	648188	170	4345		53.572
2	501352	196	3410		133.467	5	94314	158	660		33.175
3	1018676	190	6490		83.212	6	1162272	170	7480		3.684

Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	85220	224	715		254.268	5	189024	162	1375		46.657
2	542276	206	3685		134.941	6	87684	156	660		33.782
3	587212	190	3740		100.923	7	1130664	170	7480		11.484
4	840892	176	5610		84.990	8	27660	144	220		2.368

Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	132834	204	1100		256.098	4	711204	170	4510		34.390
2	1482696	198	9240		136.422	5	8224	170	55		17.228
3	784568	172	4895		61.739						

Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	156000	218	1232		257.927	5	88302	158	616		35.000
2	500514	198	3304		137.912	6	919844	170	5936		12.343
3	1071284	190	6720		87.082	7	306172	162	2016		2.632
4	658860	170	4368		49.969						

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	M.W.									
	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10
1	225.000	241.463	243.293	246.951	248.780	250.610	252.439	254.268	256.098	257.927
2	150.000	127.676	127.676	129.107	130.550	132.003	133.467	134.941	136.422	137.912
3	100.000	85.843	61.093	65.074	85.843	32.572	83.212	100.923	61.739	87.082
4	75.000	54.606	16.302	31.378	58.594	8.684	53.572	84.990	34.390	49.969
5	50.000	30.789		2.368	31.972		33.175	46.657	17.228	35.000
6	35.000	7.105			13.159		3.684	33.782		12.343
7	25.000				3.158			11.484		2.632
8	15.000							2.368		
9	10.000									

IV. Conclusion

The findings of the present study demonstrate that different irrigation treatments, particularly drought stress treatments, had a significant impact on gene expression and protein synthesis in date palm offshoots (cv. Al-Jabjab). The consistent presence of the first and second protein bands with closely similar molecular weights across all studied trees confirms their genetic uniformity and common plant origin. However, the appearance of additional protein bands and variations in their molecular weights reflect adaptive molecular responses triggered by the imposed treatments.

The increased number of protein bands under drought conditions indicates activation of stress-responsive pathways, including the synthesis of low-molecular-weight proteins that are likely osmoprotectant or stress shock proteins. These proteins play essential roles in maintaining cellular integrity, repairing damaged proteins, regulating metabolic balance, and enhancing plant tolerance to water deficit. Therefore, drought stress appears to induce molecular reprogramming involving transcriptional and translational modifications aimed at improving plant survival and stress adaptation.

In contrast, irrigation with biologically treated wastewater resulted in a clearer and more stable protein pattern, suggesting enhanced metabolic efficiency and balanced gene expression. This indicates that treated wastewater may provide a favorable growth environment by supplying essential nutrients and reducing osmotic and oxidative stress. Consequently, the use of biologically treated wastewater represents a sustainable irrigation strategy that supports physiological stability and promotes improved growth performance in date palm under semi-arid conditions.

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