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Effect of Superabsorbent Polymer and Irrigation Intervals on changes in the Protein pattern of tissue culture- derived Date Palm *Phoenix dactylifera* L. cv.Barhi grown under drought stress

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Abstract: A study was conducted on a 5-year-old tissue culture- derived date palm cv. Barhi will investigate the effect of superabsorbent polymer and irrigation intervals on changes in the protein patterns of leaves. The soil was treated with a super absorbent polymer (Green Back) (0, 250, 500) grams per tree added to the root zone with a depth of 50 cm and a width of 30 cm. Trees were irrigated every 5, 10, and 30 days. Leaves were dried using a freeze – dryer (a lyophilization technology). The Slab-Electrophoresis method was used in the presence of SDS to electrophorese the proteins on a polyacrylamide gel. Aspecialized program called Photo Capt Mw was used to estimate and illustrate the ' molecular weights of the ptoteins. The analysis of the protein pattern of the leaves revealed that all trees could identify the first and second protein bands on the polyacrylamide gel. The molecular weights in the first band ranged from 187.048 to 225.000 KDa, while the molecular weights of the proteins in the second band ranged from 137.137 to 150.000 KDa. That suggests they all originate from the same plant, Barhi cv. The protein pattern data demonstrated that the treatments affected the trees' gene expression, creating new protein bands, which reached seven bands at 10- and 30-day watering intervals. The treatments encourage the cells to produce new proteins that promote the growth markers. The treatments triggered the process of gene expression and the synthesis of new proteins, which improve plant growth and drought resistance.

Keywords: Drought stress, Molecular weight, Protein bundles, Photo Capt .

Introduction

Date palm *Phoenix dactylifera* L. of the family Arecaceae is spread across various regions around the world. Iraq is regarded as one of the nations with a large population of date palm trees, which has occupied great importance from both religious, and economic aspects, as well as for it, nutritional and environmental benefits for the Arab people since ancient times. (Johnson, 2011; Taain *et al.*,2023).

Most plants that grow under direct sunlight frequently face high light intensity, high temperatures, and drought (Allen & Ort, 2001; Sarmad & Al-Asadi 2024). Drought plays a fundamental role in plant growth and development and may cause drought stress that affects the efficiency of physiological processes, enzyme activity, and the level of internal hormones. Drought stress disrupts specific plant functions and induces oxidative stress leading to increased reactive oxygen species. This, in turn, affects cellular toxicity and causes deterioration of the lipid membranes in cell walls, thereby disrupting the regulation of cell-to-cell exchange (Ihsan *et al*., 2019; Al-Aradi,2020). Reduced availability of water resources in many arid countries, particularly in response to the indiscriminate harvesting of water reservoirs and climate change, has created real concerns (Razavi & Davary, 2014)

Increased temperatures in low - rainfall areas worldwide are widespread, encouraging efficient water conservation through soil amendments such as plant residues, zeolite, and synthetic superabsorbent polymers (SAP) (Dabhi *et al*., 2013; Najafinezhad *et al*., 2014). In dry areas, water scarcity is the main limiting factor for crop production. Thus, the use of SAP may effectively increase plant growth by improving nutrient use efficiency and soil water retention. These polymers are soil conditioners, developed to aid plant establishment and growth in drought condition (Eneji *et al*., 2013; Mazen *et al*.,2015).

For plants to grow more effectively and be healthier, natural improvers must be incorporated into the top layer of the soil in the root zone. In this manner, the soil's fertility is raised, and its structure is enhanced. By doing this, the soil's fertility and structure are enhanced, creating a favorable environment for plant growth by supplying nutrients and increasing soil moisture. The quantities of osmotic defenders, such as sugars and proline, as well as the activity of enzymatic and non-enzymatic antioxidants, are all improved when plants are treated with polymer compounds (Yamada *et al*., 2002).

Utilizing soil amendments represents a sound strategy for enhancing date palm yields under drought- stress conditions. This approach enables farmers to decrease the number of irrigations without causing significant water stress, thus saving irrigation water and increasing water use efficiency (Hassan *et al*.,2022)

Tissue - culture date palms are susceptible to environmental factors such as heat, drought, and salinity, as they are produced under laboratory conditions, and have thin leaves. Besides, the amount of protective wax on their leaves differs between trees produced by offshoots (Al-Busaidi, 2002; Al-Najjar *et al*.,2020; Taain *et al*.,2021).

Approximately half of the dry cell weight is composed of proteins, with large molecular weights that are impermeable to membranes. Depending on the requirements of the plant and how it reacts to the treatment, alterations may take place in the genetic transcription and translation processes, which could result in the production of new proteins through gene expression, enhancing plant growth and fruit development, several studies have shown characterized the proteins and metabolites involved in various biological processes, which affect the quality and ripening of the fruit and its response to various physiological processes (Alpresem *et al.*,2025; Al-Shewaily & Alpresem,2019; Al-Khayri & Naik, 2017;Hopkins & Muner,2008). The majority of plant stressors result in a significant reduction in protein and nucleic acid synthesis in plant tissues (Al-Mahmoudi *et al.*,2023;

Al-Najjar *et al.*,2020). Effective molecules that contribute to adaptive responses in developing plants under stress conditions are integral to determining stress resistance. Proteins and metabolites, such as the amino acid proline, are examples of these functional molecules. They work closely together to control essential plant functions in reaction to stressors, ensuring the plant's resilience to them (Al-Najjar *et al*., 2011), This study was conducted out to investigate the individual and interactive effects of superabsorbent polymer (SAP) application and varying irrigation intervals on the protein expression pattern of tissue – culture-derived date palm (*Phoenix dactylifera* L. cv. Barhi) under induced drought stress.

Materials & Methods

The 5-year-old tissue- culture- derived Barhi cultivar of date palm was used. A trench 50 cm deep and 30 cm wide was excavated around each tree, Barhi was treated with the superabsorbent polymer (Green Back)**.**

At three dosages (0, 250, and 500 grams per tree) the product was applied to the root zones of the trees. Every five, ten, or thirty days, 150 liters of water were used to irrigate the trees. 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 were employed. 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=control (irrigation every 5 days) / Column 3=irrigation every 10 days / Column 4= irrigation every 30 days / Column 5=polymer 250gm+ irrigation every 5 days / Column 6= polymer 250gm+ irrigation every 10 days / Column 7= polymer 250gm+ irrigation every 30 days / Column 8= polymer 500gm+ irrigation every 5 days / Column 9= polymer 500gm+ irrigation every 10 days / Column 10= polymer 500gm+ irrigation every 30 days.

Results &Discussion

The results of this study focused on drought stress-response mechanisms. The review examines the following aspects, antioxidants, late embryogenesis abundant and heat-shock proteins.

Molecular control mechanisms for abiotic stress tolerance involve the activation and regulation of specific stress-related genes. These genes are involved in the entire stress response cascade, including signaling, transcriptional control, protection of membranes and proteins, and scavenging of free radicals and toxic compounds. Recently, research into the molecular mechanisms of stress responses has started to bear fruit and, in parallel, genetic modification of stress tolerance has also shown promising results that may ultimately apply to agriculturally and ecologically important plants. The present study summarizes the recent advances in elucidating stress-response mechanisms and their biotechnological applications.

Because the size, area, and height of the protein bands varied with treatment, the protein pattern of the Barhi leaves (Table 1) differed across all research conditions. All trees in the study were able to identify the first and second protein bands on the polyacrylamide gel, as indicated by the protein pattern of the leaves (Figure 1). The molecular weights of the proteins in the first band ranged between 187.048 and 225.000 KDa, while those in the second band ranged between 137.137 and 150.000 KDa. The similar molecular weights of the protein bands in each tree may suggest that these palms originated from the same plant (the Barhi cv.) The appearance of protein bundles with low molecular weight may be due to a mechanism that the plant uses to respond to stress. These proteins increase with the length of the stress period, they can be considered a type of osmotic protein. Findings from some researchers indicate that there are proteins whose expression increases under stress conditions, called osmoprotectant proteins, and that growth and total protein content were increased at 40 days and 80 days post- treatment (Grossman & Rhodes, 2002; Salih,2024).

Studies have shown that the date palm employs a sophisticated two-pronged survival strategy under severe drought, achieved through active defense, mechanisms that upregulate proteins involved in detoxification, ROS scavenging, and energy production. The plant actively protects its cells from stress-induced damage, and the second strategy is the reallocation of resources. By downregulating proteins involved in growth-related processes like photosynthesis and protein synthesis, the plant conserves water and energy, essentially entering a "survival mode (El- Rabet *et al*.,2016).

Findings in Figure 1 also demonstrated that the quantity, locations, and characteristics of the protein bands on the polyacrylamide gel varied among Barhi trees. Depending on the kind of treatment, there were anywhere from four to seven protein bands.

Four protein bands appeared in the interaction treatment (500 g polymer with irrigation every five days). In comparison five protein bands were observed across three treatments (irrigation every five days, the interaction treatment between 250 g polymer and irrigation every five days and the interaction treatment between 500 g polymer and irrigation every ten days). It can be found by observing these three treatments, that this polymer when interacted with dry periods prevented changing the protein pattern of the trees, as the number of protein bands for polymer treatment was equal to that in the control treatment (irrigation every five days) despite the difference in the molecular weights of the protein bands.

Only the interaction treatment including 500 g of polymer and irrigation every 30 days showed six protein bands. Only two treatments—drought stress treatments (irrigation every 10 days and irrigation every 30 days)—showed seven protein bands.

The protein pattern's findings demonstrated that the investigated treatments particularly the drought ones had a noticeable impact on the trees' gene expression process and led to the creation of new protein bands, suggesting that they had encouraged the cells to produce new proteins that support the growth and development markers in plants facing drought stress. Additionally, it was evident that the treatments changed the locations of the protein bands and their molecular weights, suggesting that they activated the gene expression process and produced new proteins that might help plants grow and withstand drought., as it became clear that each cellular activity is responsible for one or more proteins that are divided according to their function into several types, including, for example, transport proteins, storage proteins, structural proteins, and protection proteins (Lesk, 2010; Saleh *et al*.,2023).

These findings showed that the treatments under study led to protein synthesis and might also alter transcription and translation processes, thereby enabling the production of new proteins through gene expression in response to the type of treatment and the plant's needs,thereby guaranteeing better plant growth. (David & Nilson, 2000; Alaa & Mohammed, 2022). synthesized proteins enter into the composition of the nucleus and cytoplasm, and some of them are enzymes that play important functional roles in various metabolic processes during the growth of plants and fruits and their ripening (Cooper & Hausman, 2007). Drought stress triggers the synthesis of low-molecular-weight proteins with low molecular weights that play an important role in repairing and regulating damaged proteins, thereby supporting various vital activities in plants. These new proteins may be drought stress shock proteins, which play a fundamental role in the plant's response to physiological changes caused by drought and are usually at low levels under normal conditions (Polenta *et al*., 2020; Ghirardo *et al*., 2021; Shaban & Alpresem,2025).

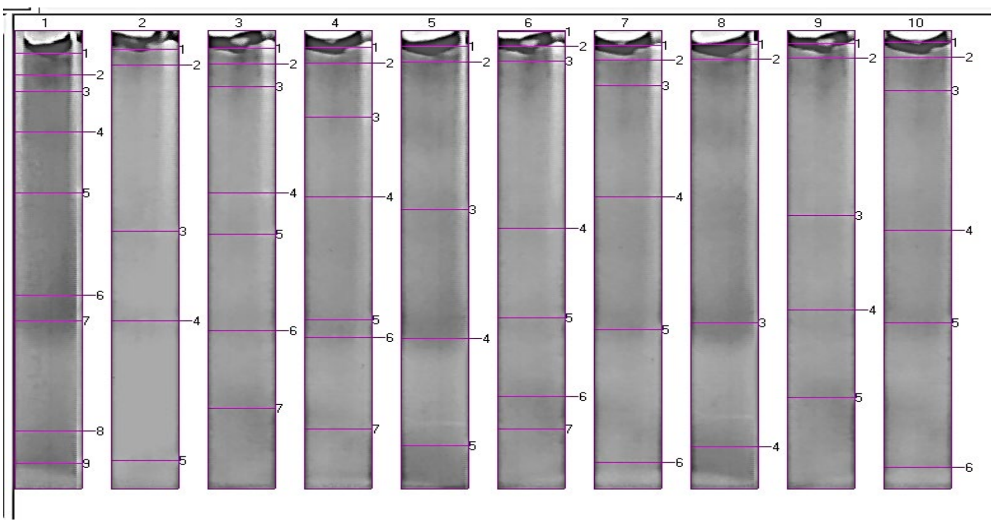

Figure (1): Number and sites of protein bands and their molecular weights (a part of the Photocopy program)

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

Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	31616	190	270		167.259	6	183692	176	1512		56.857
2	151166	190	1188		121.647	7	19174	172	162		50.440
3	122464	190	918		99.862	8	1075594	178	7614		36.776
4	342582	190	2430		94.068	9	19834	178	162		35.178
5	1149542	186	8316		83.529						

Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	68046	208	594		187.048	4	34750	168	216		50.440
2	126198	198	864		137.137	5	1487276	174	8856		35.311
3	2336518	198	13824		74.747						

Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	66406	212	594		192.283	5	760036	170	4752		73.978
2	115026	208	864		138.732	6	734618	166	4752		48.335
3	178956	196	1242		104.917	7	30530	156	216		38.238
4	1284606	198	7830		83.529						

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4	1284606	198	7830		83.529						

Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	38522	218	378		197.604	5	69174	154	486		50.663
2	112620	212	864		140.332	6	85232	160	594		46.995
3	438942	200	2970		96.239	7	1174982	170	7506		36.888
4	1689810	190	10908		82.723						

Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	5816	202	54		213.941	3	2082276	196	14148		50.000
2	117302	206	1026		146.769	4	1178798	170	7884		35.956

Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	5816	202	54		213.941	3	2082276	196	14148		50.000
2	117302	206	1026		146.769	4	1178798	170	7884		35.956

Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	5816	202	54		213.941	3	2082276	196	14148		50.000
2	117302	206	1026		146.769	4	1178798	170	7884		35.956

Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	32564	216	324		219.463	4	730082	170	4698		53.010
2	85832	210	702		148.384	5	61716	164	432		39.104
3	2172766	198	13662		78.610						

	M.W.									
	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10
1	167.259	187.048	192.283	197.604	202.997	280.000	208.447	213.941	219.463	225.000
2	121.647	137.137	138.732	140.332	141.936	202.997	145.156	146.769	148.384	150.000
3	99.862	74.747	104.917	96.239	79.961	143.545	106.205	50.000	78.610	100.000
4	94.068	50.440	83.529	82.723	46.812	75.501	82.723	35.956	53.010	75.000
5	83.529	35.311	73.978	50.663	36.004	51.116	48.535		39.104	50.000
6	56.857		48.335	46.995		39.190	35.222			35.000
7	50.440		38.238	36.888		36.888				
8	36.776									
9	35.178									

Conclusion

The appearance of low - molecular -weight proteins may result from the mechanisms the plant uses to cope with stress, and these proteins increase as the stress period lengthens. These proteins can be considered a type of osmotic protein, and several researchers have confirmed that appear increases under stress conditions and are called osmotic protection proteins. The study results, which involved the protein pattern analysis of date palm, indicated that drought tolerance genes were differentially expressed resulting in either increased or decreased protein abundance at the selected protein sites under drought stress. All trees could identify the first and second protein bands on the polyacrylamide gel, according to the study of the protein pattern of leaves. The molecular weights of the proteins in the first band ranged between 187.048 and 225.000 KDa, while those in the second band ranged between 137.137 and 150.000 KDa. Given their similar molecular weights, identifying the protein band molecular weights across all trees may suggest that they all originate from the exact plant, the Barhi cultivar. Results indicated that four protein bands appeared in the interaction treatment (500 g polymer with irrigation every five days) in comparison to five protein bands that appeared in three treatments (irrigation every five days, the interaction treatment between 250 g polymer and irrigation every five days and the interaction treatment between 500 g polymer and irrigation every ten days). It can be found by observing these three treatments, that this polymer when interacted with dry periods prevented changing the protein pattern of the trees, as the number of protein bands for polymer treatment was equal to that in the control treatment (irrigation every five days) despite the difference in the molecular weights of the protein bands.

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Contributions of Authors

N.J.M.: Collection of specimens, Laboratory techniques, wrote and revised the manuscript.

M.A.H.A.: Suggestion the proposal of the article, wrote and revised the manuscript, identification of the plant.

D.A.T.: Suggestion the proposal of the article, revised the manuscript.

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Conflicts of interest

The authors declare that they have no conflict of interests.

Ethical approval

All ethical guidelines related to plant and care issued by national and international organizations were implemented in this report.

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تأثير البوليمر فائق الامتصاص وفترات الري في تغيرات النمط البروتيني لفسائل نخيل التمر النسيجي *Phoenix dactylifera* L. صنف البرحي النامي تحت ظروف اجهاد الجفاف

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المستخلص: اجريت هذه الدراسة على فسائل نخيل التمر صنف البرحي الناتج من الزراعة النسيجية بعمر 5 سنوات عوملت بالبوليمر (Green Back) (0، 250غم , 500غم للفسيلة الواحدة) اضيفت الى منطقة الجذور بعمق 50 سم. كما تمت معاملة الفسائل بفترات ري مختلفة (رية واحدة كل 5 , 10, 30 يوم). أخذت عينات من الوريقات وجفدت العينات بتقنية التجفيف بوساطة التبريد Freeze-dryer (Lyophilization technique) واستخلص البروتين من العينات واجري الترحيل البروتيني على هلام Polyacrylamide باستعمال طريقة Slab-Electrophoresis بوجود العوامل الماسخة SDS وقدرت الأوزان الجزيئية للبروتينات ورسمت عبر برنامج حاسوبي خاص PhotoCapt Mw. بينت نتائج الدراسة للنمط البروتيني للأوراق ان جميع اشجار الدراسة قد اشتركت بالحزمة البروتينية الاولى والحزمة البروتينية الثانية على هلام Polyacrylamide, اذ تراوحت الاوزان الجزيئية لبروتينات الحزمة الاولى بين (187.048–225.000) كيلو دالتن. في حين تراوحت الاوزان الجزيئية لبروتينات الحزمة الثانية بين (137.137–150.000) كيلو دالتن. ان هذا التطابق لجميع اشجار الدراسة قد يدل على ان هذه الاشجار تعود لأصل نباتي واحد (وهو صنف البرحي). كما ان معاملات الدراسة قد اثرت بصورة واضحة في عملية التعبير الجيني للأشجار وسببت ظهور حزم بروتينية جديدة وصلت الى سبعة حزم (كما في معاملتي الري كل 10 ايام والري كل 30 يوم) وهذا يدل ان معاملات الدراسة قد حفزت الخلايا لتصنيع بروتينات جديدة تدعم مؤشرات نمو وتطور النباتات النامية تحت اجهاد الجفاف، كما كان للمعاملات تأثيرا واضحا في تغيير مواقع واوزان الحزم البروتينية مما يدل على ان المعاملات قد سببت تنشيط عملية التعبير الجيني وتصنيع بروتينات جديدة قد يكون لها دور في تحسين نمو النباتات ومقاومة الجفاف.

الكلمات المفتاحية: الاجهاد الجفافي، الوزن الاجزيئي، الحزم البروتينية، الفوتوكابت.