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Impact of Superabsorbent Polymer and Irrigation Intervals on Biochemical Characteristics of Tissue Culture- Derived Date Palm *Phoenix dactylifera* L. cv. Barhi Grown Under Drought Stress

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Abstract: This study was conducted on 5-year-old tissue culture-derived date palm cv. Barhi to investigate the impact of superabsorbent polymer with three dosages (0, 250, 500) grams per tree and irrigation intervals of (5, 10, 30) days on biochemical characteristics of the leaves. A randomized complete block design was used in a factorial experiment (3x3) with three replicates. Results showed that the 500 g tree^{-1} polymer dosage had a significant effect on total soluble carbohydrates, while the 250 g tree^{-1} polymer dosage was superior to the other dosages in enhancing superoxide dismutase (SOD) enzyme activity. Findings also showed that control treatment (0 g tree^{-1} polymer dosage) was the best in increasing total phenol, peroxidase (POD) activity, amino acids and proline. As for the effect of irrigation periods, the 30-day irrigation interval significantly affected total phenol, SOD and POD activities, amino acids and proline by recording the highest values compared to the rest irrigation periods, whereas the 5-day irrigation period outperformed the others in increasing of total soluble carbohydrates in leaves. The interaction between 0 g tree^{-1} polymer dosage irrigation every 30 days gave the highest rate of proline (10.123 µg g^{-1}), while the treatment of 500 g tree^{-1} polymer and the irrigation every 5 days gave the lowest rate (3.897) µg g^{-1}.

Keywords: Drought stress, Irrigation intervals, proline, Superoxide dismutase (SOD).

Introduction

The date palm (*Phoenix dactylifera* L.) belongs to the family Arecaceae. It is a monocotyledonous, dioecious plant and is considered to be one of the oldest cultivated tree fruits, a symbol of life in the desert because it tolerates high temperatures, drought, and salinity batter than many other fruit plants (Taain *et al*., 2021).

Iraq is one of the driest countries in the world, located within the Middle East and North Africa region, where climate projections predict a decrease in rainfall, an increase in temperatures, and a noticeable and frequent rise in drought cycles (Al-Ansari, 2013). Drought plays a fundamental role in plant growth and development and causes significant damage, affecting physiological processes, internal hormone levels, and enzyme activity (Aqeela*,et al*,2023 ;Ihsan *et al*., 2019). It disrupts certain plant functions, leading to oxidative stress, and an increase in reactive oxygen species levels, which, in turn, contribute to cellular toxicity and cause deterioration of the lipid membranes in cell walls, thereby affecting the regulation of exchange between cells (Hussain *et al*., 2011).

Ghazzawy *et al*., (2023) studied four irrigation levels (80, 100, 120, 140) % of the water consumption of date palm cv. Sukari and found an increase in leaf length and width at an irrigation level of 100% of the water consumption compared to the other treatments.

Polymers are hydrophilic materials with a high-water absorption capacity, so they are used as soil conditioners to reduce water loss and increase plant yield. At the same time, they can absorb and retain water solutions up to hundreds of times their weight. Therefore, they are added to soil to enhance its properties and provide water supplies near the root zone, thereby minimizing nutrient loss, reduce irrigation costs, improve root growth, prevent soil erosion, increase soil cation exchange capacity, and mitigate global warming (Sun *et al*., 2013).

In a study conducted by Oraee & Moghadam (2013) on Myrobalan cherry peach trees (*Prunus cerasifera*), four levels of polymer (0, 0.5, 1, 2, 3) % were applied, the results showed that the 3% polymer level resulted in the highest leaf area compared to the other treatments.

Khaleghi & Moallemi (2018) found that treating two olive cultivars, Baghmalek and Dezphol, with polymer at levels of (1, 2, 3, 4) g kg^{-1} of soil led to a significant improvment in all vegetative growth parameters, such as leaf area and number of leaves compared to the control treatment.

The present study was aimed to determine the impact of superabsorbent polymer and irrigation intervals on the biochemical parameters of date palm *Phoenix dactylifera* L.leaves cv. Barhi.

Materials & Methods

This study was conducted on 5-year-old tissue culture-derived date palm cv. Barhi treated with the superabsorbent polymer (Green Back) at three dosages (0, 250, and 500) grams per tree applied to the root zone by digging a trench around the tree with a depth of 50 cm and a width of 30 cm. The trees were irrigated with 150 liters water every 5, 10, or 30 days. Samples were taken from date palm leaves to determine the following parameters**:**

1. Total soluble carbohydrates determined by using the Phenol- Sulfuric acid Colorimetric Method Modification as described by (Belkhadi *et al.,* 2010).

2. Total phenols were determined by using Folin-Denis method mentioned in Al-Najjar *et al*., (2020).

3. Superoxide dismutase (SOD) activity was determined according to the method described by Beauchamp & Fridovich (1971), and the absorbance was read using a spectrophotometer at a wavelength of 760 nm with gallic acid as a standard solution.

4. Peroxidase (POD) activity was determined according to method of Whitaker. (1994) and the absorbance was read by spectrophotometer at a wavelength of 436 nm.

5.Amino acids were determined according to the method described by Mello *et al.* (2005), and the absorbance was read at a wavelength of 570 nm using a spectrophotometer.

6. Proline acid in leaves was determined according to the method described by Porra (2002) and the absorbance was read at a wavelength of 520 nm using a spectrophotometer.

Factorial Experiment was conducted including two factors, ground addition of superabsorbent polymer and irrigation periods, using Randomized Complete Block Design (RCBD) with three replicates. The results were statistically analyzed using the statistical program Genstat. The mean values differences were compared by using the least significant difference (LSD) test at the probability level of 0.05.

Results & Discussion

1.Total soluble carbohydrates (mg g^{-1})

The results of Table 1 show the effect of polymer and irrigation intervals on total soluble carbohydrates in leaves, as the 500 g $tree^{-1}$ polymer dosage had a significant effect on carbohydrates and recorded the highest value, which amounted to 13.878 mg g^{-1}, with a significant difference from the other treatments, while the control treatment (0 mg. $palm^{-1}$ polymer dosage) recorded the lowest value, which amounted to 10.835 mg g^{-1}, while the dosage of 250 g $tree^{-1}$ gave 13.025 mg g^{-1}. As for the effect of irrigation intervals, the 5-day irrigation period recorded the highest average of 14.81 mg g^{-1}, while the irrigation treatment of 30 days recorded the lowest value, which amounted to 9.636 mg g^{-1}, with a significant difference from the rest of the treatments.

As for the interaction between polymer dosages and irrigation periods, the interaction between 500 g $tree^{-1}$ polymer dosage with 5-day irrigation recorded the highest value of 16.358 mg g^{-1}, while the interaction between 0 g $palm^{-1}$ polymer dosage with the irrigation period every 30 days recorded the lowest value of 8.641 mg g^{-1}.

Table (1): Effect of polymer dosages and irrigation periods on leaf carbohydrate content mg. g^{-1}

Polymer concentration (gm palm-1)	Means of Polymer			Means of Polymer
	5	10	30	
0	12.321	11.542	8.641	10.835
250	15.751	13.981	9.342	13.025
500	16.358	14.351	10.925	13.878
Means of irrigation periods	14.810	13.291	9.636	

RLSD 0.05		
Polymer	**Irrigation periods**	**Polyme× irrigation periods**
0.104	0.104	0.181

Total phenols (mg g^{-1})

Table 2 showed the effect of the polymer and irrigation intervals on the total phenol of leaves. The control treatment recorded the highest value of 6.93 mg g^{-1}, while the 500 g $tree^{-1}$ treatment recorded the lowest value of 5.36 mg g^{-1}. Regarding irrigation intervals, the 30-day irrigation interval led to the highest phenol accumulation (8.09 mg g^{-1}). while the 5-day irrigation interval resulted the lowest value (4.78 mg g^{-1}).

For the interaction between polymer application and irrigation intervals, the combination of 0 g. tree $^{-1}$ polymer with 30-day irrigation interval recorded the highest phenol content (8.73 mg g^{-1}), whereas the combination of 500 gm tree $^{-1}$ polymer with the 5-day irrigation interval resulted in the lowest value (3.85 mg g^{-1}).

Table (2): Effect of polymer dosages and irrigation periods on leaf content of total phenols mg g^{-1}

Polymer concentration (gm palm^{-1})	Irrigation intervals (day)			Means of Polymer
	5	10	30	
0	5.76	6.32	8.73	6.93
250	4.73	5.12	8.21	6.02
500	3.85	4.90	7.33	5.36
Means of irrigation intervals	4.78	5.45	8.09	

RLSD 0.05		
Polymer	**Irrigation intervals**	**irrigation intervals Polyme**
0.642	0.642	1.111

Superoxide dismutase (SOD) activity (minute units g^{-1})

The results shown in Table 3 illustrate the effect of polymer application and irrigation intervals on the leaf content of Superoxide Dismutase (SOD) enzyme activity. The treatment with 250 g tree^{-1} polymer resulted in the highest SOD activity, with a value of 1.192-minute units g^{-1}, whereas the 500 g tree^{-1} polymer treatment recorded the lowest SOD activity, at0.927-minute units g^{-1}. Regarding irrigation intervals, the 30 days irrigation period led to the highest SOD enzyme activity, with a value of 1.763-minute units. g^{-1}, while the 5-day irrigation period resulted in the lowest activity, recorded at 0.503-minute units g^{-1}.

For the interaction between the polymer dosages and irrigation intervals, the combination of 0 gm tree^{-1} polymer and 30-day irrigation period yielded the highest SOD enzyme activity, at 1.989-minute units g^{-1}, while the combination of 500 g tree^{-1} polymer and the 5 day of irrigation period resulted in the lowest activity, at 0.363-minute units g^{-}.

Peroxidase (POD)Activity (minute units g^{-1})

Table 4 shows that the 0 g tree^{-1} polymer dosage resulted in the highest rate of 99.21-minute units g^{-1}, while the 500 g tree^{-1} polymer treatment recorded the lowest rate of 73.48-minute units g^{-1}. Additionally, the results showed that the 30-day irrigation period produced the highest rate of 114.76-minute units g^{-1}, while the 5-day irrigation period resulted in the lowest rate of 55.77minute units g^{-1}.

Regarding the interaction between polymer dosage and irrigation intervals, the combination of 0 g tree^{-1} polymer dosage and the 30-day irrigation period yielded the highest rate of 134.17-minute units g^{-1}, while the combination of the 500 g tree^{-1} polymer dosage and the 5-day irrigation period showed the lowest rate of 41.71-minute units g^{-1}. .

Table (3): Effect of polymer dosages and irrigation periods on superoxide dismutase (SOD) activity minute units g⁻¹.

Polymer concentration (gm palm⁻¹)	Irrigation intervals (day)			Means of Polymer
	5	10	30	
0	0.648	0.776	1.989	1.38
250	0.498	1.321	1.756	1.192
500	0.363	0.876	1.543	0.972
Means of irrigation intervals	0.503	0.991	1.763	
RLSD 0.05				
Polymer	**Irrigation intervals**	**irrigation intervals Polyme**		
0.2564	0.2564	0.4441		

Table (4): Effect of polymer dosages and irrigation periods on peroxidase (POD)Activity minute units g⁻¹

Polymer concentration (gm palm⁻¹)	Irrigation intervals (day)			Means of Polymer
	5	10	30	
0	73.26	90.21	71134.	199.2
250	52.34	13.79	112.79	4287.
500	41.71	81.41	2.397	73.48
Means of irrigation intervals	55.77	89.58	114.76	
RLSD 0.05				
Polymer	**Irrigation intervals**	**irrigation intervals Polyme**		
1.758	1.758	3.046		

Amino acids (mg g⁻¹)

Table (5) shows that the control treatment (0 g. tree⁻¹) resulted in the highest rate of 4.75 mg g⁻¹, while the 500 g tree⁻¹ polymer treatment recorded the lowest rate of 3.33 mg g⁻¹.

Regarding irrigation intervals, the 30-day irrigation period produced the highest rate of 5.87 mg g⁻¹, whereas the 5-day irrigation period resulted in the lowest rate of 2.67 mg g⁻¹.

Regarding the combination polymer dosage and irrigation intervals, the combination of 0 g tree⁻¹ polymer dosage and the 30-day irrigation period yielded the highest rate of 5.35 mg g⁻¹, while the combination of 500 g tree⁻¹ polymer dosage and the 5-day irrigation period showed the lowest rate of 1.98 mg g⁻¹.

Table (5): Effect of polymer dosages and irrigation periods on amino acids content of leaves mg g⁻¹

Polymer concentration (gm palm⁻¹)	Irrigation intervals (day)			Means of Polymer
	5	10	30	
0	08 3.	4.21	5.35	4.75
250	2.22	3.45	6.12	3.93
500	1.98	2.88	5.12	3.33
Means of irrigation intervals	2.67	3.51	5.87	

RLSD 0.05		
Polymer	**Irrigation intervals**	**irrigation intervals× Polyme**
0.853	0.853	1.478

Proline (μg g⁻¹)

Table 6 shows that the 0 g tree⁻¹ polymer dosage resulted in the highest content of proline, reaching 8.086 μg g⁻¹, while the 500 g. tree⁻¹ dosage gave the lowest value of 5.656 μg g⁻¹. The same table also reveals that the irrigation period every 30 days recorded the highest value of 9.077 μg g⁻¹, while the irrigation period every 5 days gave the lowest value of 4.872 μg g⁻¹.

Regarding the interaction between polymer dosages and irrigation intervals, the interaction between 0 g tree⁻¹ polymer dosage and 30-day irrigation period resulted in the highest rate of 10.123 μg g⁻¹, while the combination of 500 g tree⁻¹ polymer and the 5-day irrigation gave the lowest rate of 3.897 μg g⁻¹.

Table (6): Effect of polymer dosages and irrigation periods on leaf content of proline μg g-¹

Polymer concentration (gmpalm⁻¹)	Irrigation intervals (day)			Means of Polymer
	5	10	30	
0	6.321	7.815	10.123	8.086
250	4.398	5.255	8.892	6.182
500	3.897	4.857	8.215	5.656
Means of irrigation intervals	4.872	5.976	9.077	

RLSD 0.05		
Polymer	**Irrigation intervals**	**Polyme× irrigation intervals**
0.4848	0.4848	0.8398

Discussion

The results presented in Tables 1,2,3,4,5, and 6 clearly demonstrate that superabsorbent polymer and irrigation periods significantly affected the biochemical parameters of date palm leaves (cv.Barhi) including total soluble carbohydrates, phenols, superoxide dismutase and peroxidase activities, amino acids , proline content . Carbohydrates are essential molecules in plants as they are vital for life. They serve as stored energy, provide energy through the oxidation process, and form part of the structural composition of plant cells and tissues such as cellulose. Water stress can decrease in metabolic activities such as photosynthesis, ion absorption, transport, carbohydrate formation, nutrient metabolism and growth stimulants as well as inhibiting enzymatic activities (Mewis *et al*., 2012). Carbohydrates, the primary product of photosynthesis, play a crucial role the plant's defense mechanism against various stresses (Norwood *et al*., 2000). When exposed to environmental stress, carbohydrates accumulation helps modify osmosis within plant cells and nutrient metabolism, in addition to contributing to maintaining the integrity of plasma membranes (Hummel *et al*., 2009).These findings align with those of Zouari *et al*.,(2016) who observed an increase in total soluble sugars in date palm offshoots under stress, from 168.8 µmol g^{-1} in the control treatment to 248.89 µmol g^{-1} FW in the stressed treatment.

Water content reduction causes a decrease in the transfer of water molecules, leading to limited stomatal movement and decreased cell turgor pressure, which negatively affects the carbon fixation rate. Additionally, the movement of nutrients from the soil to the plant is hindered (Ragel *et al*., 2019), resulting in a decrease in water nutrients absorption (SAS, 2004; Alpresem, *et al*.,2023; Alpresem *et al*.,2025). Long-term water shortages lead to tissue dehydration,increased oxidation, and reduced chlorophyll concentration, ultimately decreasing photosynthesis activity. Adequate irrigation improves the efficiency of root absorption of nitrogen absorption, which is essential chlorophyll synthesis. Severe drought can trigger the production of free radicals such as superoxide and hydrogen peroxide, which oxidize chlorophyll pigments (Karimpour, 2019). Water stress often leads to a reduction in chlorophyll content and photosynthetic efficiency in many plants (Gurumurthy *et al*., 2019). This may be due to the activation of the hormone abscisic acid (ABA), which inhibits the chlorophyll synthesis (Saqr, 2010). The decrease in the content of chlorophyll pigment in leaves under water stress is typical and results from pigments= degradation (Rahdari *et al*., 2012).

Phenolic compounds are common in plant tissuesand act as antioxidants that scavenge free radicals (ROS), donate hydrogen, and have the ability to inhibit the enzyme Liopoxygenase, in addition to stimulating the enzymes such as catalase when the plant is exposed to stress (Vitor *et al*., 2004; Del Rio *et al*., 2010).

Amino acids, the basic building blocks of protein, play a key role in plant metabolism They contribute to the formation of nucleotides and protoplasm, enzymes, plant hormones, and vitamins. Tryptophan, for example, is essential for the synthesis of Indoleacetic acid (IAA) (Kang & Kini, 2009). Proline is a non-enzymatic antioxidant that helps mitigate oxidative damage during severe stress and regulates osmatic pressure (Abdullah *et al*.,2025; Al-Rubaie, 2021).

Exposure of date palm trees to prolonged drought negatively affects the growth and their condition. However, exposure to limited periods of drought may improve their drought tolerance through osmotic adjustment (Al-Khayri & Al-Bahrany, 2004; Al-Mahmoudi *et al*,2023). Increased proline accumulation, is key of osmotic adjustment within plant tissues (Al-Khayri, 2002). The severity of drought leads to increase amino acids, including proline, which decreases osmotic tension, reduces water stress and enhancing the plant ability to absorb water and nutrients from the growth medium, then increasing the vegetative growth of plants (Claussen, 2004; AL-Shewailly & Alpresem,2019).

Peroxidase enzymes, found throught the plant kingdom, participate in various physiological activities, including lignin biosynthesis, defense against pathogens and responses to environmental stresses. However, the specialized activities of peroxidase enzymes remain unclear (Passardi *et al*., 2007; Cosio & Dunand, 2009). Peroxidase play role in the plant defense against reactive oxygen species (ROS) by removing oxygen free radicals and protect cell membrane lipids from oxidation, also reduce hydrogen peroxidase to water and oxygen (He *et al*., 2011). Water stress elevates the production of ROS, leading to an imbalance between antioxidants and oxidizing agents, which can damage plant tissues (Al-Rubaie, 2021). As observed, the activity of antioxidant enzymes such as superoxide dismutase and peroxidase decreases under stress (Viera Santos *et al*., 2001; Alnajjar,*et al*,2020).

The results of this study are consistent with those of Kataa (2022) who investigated the effect of different irrigation intervals, on two date palm cultivars (Sayer and Halawi). The highest total amino acid content in the leaves (0.0915 mg g^{-1}) was observed in the treatment with weekly irrigation in winter and bi-weekly irrigation in summer. Conversely, the lowest total amino acid content was found in the treatment with irrigation every 30 day (0.0470 mg g^{-1}). Regarding the proline content, the highest value (1.074 µg g^{-1}) was recorded in the treatment every 30 day, while the lowest (0.460 µg g^{-1}) was found in the treatment with weekly irrigation in winter and bi-weekly irrigation in summer.

Conclusion

The results indicate that superabsorbent polymer and irrigation periods significantly influenced the studied biochemical characteristics, the 500 g $tree^{-1}$ polymer dosage treatment yielded the highest total soluble carbohydrates of leaves, while 250 g $tree^{-1}$ polymer dosage treatment enhanced the superoxide dismutase (SOD) enzyme activity. The 30-day irrigation period significantly increased the total phenol, SOD and POD activities, amino acids and proline levels. The interaction between 0 g $tree^{-1}$ polymer dosage and 30-day irrigation led to the highest accumulation of amino acids, particularly proline, while the treatment of 500 g tree⁻¹ polymer and 5-day irrigation resulted in the lowest amino acids content. These findings highlight the role of superabsorbent polymer at the dosage of 500 g per tree and frequent irrigation (every 5 day) in mitigation drought stress in date palm trees, as drought severity correlate with increased amino acids accumulation, including proline.

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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 الصفات الكيموحيوية لنخيل التمر صنف البرحي *Phoenix dactylifera* L. cv. Barhi الناتج من زراعة الأنسجة والنامي تحت ظروف الجفاف

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المستخلص: أجريت هذه الدراسة على نخيل التمر صنف البرحي الناتج من زراعة الأنسجة وبعمر 5 سنوات لمعرفة تأثير البوليمر فائق الامتصاص بثلاث مستويات (0، 250، 500) غم شجرة$^{-1}$ وفترات ري كل (5، 10، 30) يومًا في الصفات الكيموحيوية للأوراق. تم استخدام تصميم القطاعات العشوائية الكاملة في تجربة عاملية (3 × 3) بثلاث مكررات. أظهرت النتائج أن معاملة البوليمر 500 غرام. شجرة$^{-1}$ كان لها تأثير كبير على إجمالي الكربوهيدرات القابلة للذوبان، بينما كانت معاملة البوليمر 250 غرام. شجرة$^{-1}$ متفوقة على المعاملات الأخرى في تعزيز نشاط إنزيم سوبر أوكسيد ديسميوتاز (SOD). أظهرت نتائج الدراسة أيضًا أن معاملة المقارنة (0 غرام شجرة$^{-1}$ بوليمر) كانت الأفضل في زيادة إجمالي نشاط الفينول والبيروكسيديز (POD) والأحماض الأمينية والبرولين. أما بالنسبة لتأثير فترات الري، فقد أثرت فترة الري (30 يومًا) بشكل معنوي على إجمالي الفينول، ونشاطي SOD وPOD، والأحماض الأمينية، والبرولين، مسجلةً أعلى القيم مقارنةً بفترات الري المتبقية، بينما تفوقت فترة الري (5 أيام) على فترات الري الأخرى في زيادة إجمالي الكربوهيدرات الذائبة في الأوراق. وقد أعطى التداخل بين معاملة 0 غرام من بوليمر كل 30 يومًا أعلى معدل للبرولين (10.123 مايكروغرام غرام$^{-1}$)، بينما أعطت معاملة 500 غرام من بوليمر والري كل 5 أيام أقل معدل (3.897 مايكروغرام غرام$^{-1}$).

الكلمات المفتاحية: الإجهاد، الجفاف، فترات الري، البرولين، سوبر أكسيد ديسموتيز (SOD).