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Enhancing Physiological Performance of Tissue Culture-Derived Date Palm Plants under Salinity Stress with Kelpak Seaweed Extract

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Abstract

Date palms are a valuable crop that is often grown on saline soils, which limits their development and yield. In this study, the effects of Kelpak seaweed extract on nutrient levels, photosynthetic pigments, and biochemical parameters in date palm leaves under salt stress were investigated. We applied different concentrations of Kelpak (0, 2.5, 5, 7.5, and 10 ml L−1) to date palm plants exposed to salt stress. The results showed that when compared to the control treatment, the Kelpak treatment significantly increased nutritional levels in date palm leaves, including nitrogen (N), phosphorus (P), potassium (K), and calcium (Ca). In addition, treatment with Kelpak extract increased the levels of photosynthetic pigments in the leaves, including chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids, and this increase was associated with concentration increases. Moreover, biochemical analysis revealed that with increasing concentrations of Kelpak, total soluble protein and total soluble carbohydrates increased, whereas proline content decreased. According to the findings, Kelpak Seaweed extract boosted nutrient absorption, increased photosynthetic pigments, and altered biochemical markers related with plant development and stress responses. This study provides evidence that Kelpak may be a beneficial treatment for salt-stressed date palm plants. However, further studies are needed to fully understand the mechanisms of action and to optimize the application of Kelpak in date palm cultivation.

Keywords: carbohydrates, carotene, chlorophyll, proline, protein.

Introduction

Date palm (*Phoenix dactylifera* L.) is an economically important crop cultivated in arid and semi-arid regions worldwide. It serves as a significant source of income, food, and shelter for millions of people, particularly in regions where other crops struggle to thrive due to harsh environmental conditions. However, the productivity and quality of date palm cultivation are often hampered by various abiotic stresses, including salinity stress (Muhsen et al., 2020; Ahmad et al., 2023). Salinity stress, caused by the presence of high salt levels in the soil, severely affects the growth, development, and overall performance of date palm plants. The detrimental effects of salinity stress on plant physiology and biochemistry include reduced water uptake, ion imbalance, oxidative damage, and disruption of essential metabolic processes. (Almutawa, 2022). Developing strategies to mitigate the negative impacts of salinity stress and enhance the stress tolerance of date palm plants is of paramount importance for sustainable cultivation and increased yield (Acosta-Motos et al., 2020). In recent years, researchers have turned their attention towards natural biostimulants as potential tools for improving plant growth and stress tolerance (Ma et al., 2022). Seaweed extracts, derived from various species of marine macroalgae, have gained recognition as effective biostimulants due to their rich composition of bioactive compounds, including plant growth regulators, amino acids, vitamins, and minerals (Ali et al., 2021). Kelpak, a commercially available seaweed extract derived from the brown algae Ecklonia maxima, has shown promising results in promoting plant growth, enhancing nutrient uptake, and improving stress tolerance in a wide range of crops. (Kulkarni et al., 2019). While the benefits of Kelpak have been extensively studied in different plant species (Aremu et al., 2022). Therefore, the objective of this study was to investigate the effect of Kelpak seaweed extract on the physiological and biochemical responses of date palm offshoots derived from tissue culture under salinity stress. We hypothesized that Kelpak treatment would enhance the stress tolerance of date palm offshoots by improving water relations, maintaining ion homeostasis, and modulating key metabolic processes. To test this hypothesis, we evaluated various parameters, including plant growth, leaf water potential, electrolyte leakage, chlorophyll content, antioxidant enzyme activities, and osmolyte accumulation.

To the best of our knowledge, this is the first study to explore the potential of Kelpak seaweed extract in enhancing salinity stress tolerance in date palm offshoots. The findings of this study could have significant implications for date palm cultivation in salt-affected regions, offering a sustainable and environmentally friendly approach to improve crop productivity.

Materials and Methods

Study Location:

The study was conducted in a private orchard located in Basrah city, Iraq, during the 2021-2022 growing season. The field's soil properties, as presented in Table 1.

Experimental Design:

Fifteen five-year-old Barhi cultivar date palm trees, uniform in size and growth, were selected for the experiment. The trees were grown in soil affected by salinity.

Application of Kelpak Seaweed Extract:

Five concentrations of Kelpak seaweed extract (0, 2.5, 5, 7.5, and 10 ml L^{-1}) were applied as a foliar spray. The first application was conducted in early January, followed by a second application one month later. The application was carefully performed to ensure uniform coverage of the tree canopy.

Assessment of Chemical Characteristics:

Various chemical characteristics were estimated to evaluate the effects of Kelpak seaweed extract on the date palm trees. The following assessments were conducted:

1. Photosynthetic Pigments: The photosynthetic pigments, including chlorophyll a, chlorophyll b, and carotene, were determined using the procedures described by Kumar et al. (2019). Leaf samples were collected from each tree and analyzed spectrophotometrically to quantify the pigment content.
2. Leaf Nutrient Content:

The leaf content of nitrogen (N), phosphorus (P), potassium (K), sodium (Na), calcium (Ca), and chloride (Cl) was quantified. Leaf samples were collected and subjected to digestion using a mixture of H_2SO_4 and H_2O_2, following the protocol described by Parkinson and Allen (1975). The nutrient content was analyzed using specific colorimetric or titrimetric methods as outlined below:

A. Total nitrogen (N) was determined using the micro-Kjeldahl method, following the procedure described by Jackson (1962).
B. Total phosphorus (P) was measured photocolorimetrically utilizing the Bray No. 1 method, as outlined by Bray and Kurtz (1945).
C. Potassium (K) contents was determined using the flame photometric method, following the procedure established by Black (1965).

D. Calcium (Ca) content was determined using the ethylene diaminetetra-acetic acid (EDTA) method, as described by Al-lison (1973).

3. Total Soluble Protein: The total soluble protein content was analyzed using the Bradford assay (Bradford, 1976). Leaf samples were homogenized, and the protein content was determined based on the absorbance of the protein-dye complex formed.
4. Carbohydrate Content: The carbohydrate content was estimated following the anthrone method (Yemm and Willis, 1954). Leaf samples were homogenized, and the carbohydrates were hydrolyzed. The resulting sugars were reacted with anthrone reagent, and the absorbance was measured to determine the carbohydrate content.
5. Proline Content: The proline content was measured according to Bates et al. (1973). Leaf samples were homogenized, and proline was extracted using acid ninhydrin reagent. The absorbance of the developed color was measured to quantify the proline content.

Statistical Analysis:

The collected data were analyzed using a completely randomized block design (CRBD). Analysis of variance (ANOVA) was performed to determine the significant treatment effects. To identify specific differences between treatments, the least significant difference (LSD) test was applied at a probability level of 0.05 ($p<0.05$).

Table 1: Physical and Chemical Properties of the Study Field Soil

Property	Value	Unit
Ec	14.60	ds m^{-1}
pH	6.90	
Aviable N	230	mg km^{-1}
Avaible P	20.85	mg km^{-1}
Avaible K	157.00	mg km^{-1}
$CaCO_3$	390.50	mg km^{-1}
Na^{+2}	20.44	mM L^{-1}
Cl^{-1}	84.90	mM L^{-1}
Organic matter	10.22	g kg^{-1}
Clay	58.5	%
Silt	25.2	%
Sand	16.3	%

Results

Photosynthetic Pigments:

Table 2 presents the effects of different concentrations of Kelpak seaweed extract on the photosynthetic pigments (Chlorophyll A, Chlorophyll B, Total Chlorophyll, and carotene) in

Date Palm leaves, measured in mg $100g^{-1}$. The lowest values were observed at 0 ml L^{-1} of Kelpak, where Chlorophyll A was measured at 1.580 mg $100g^{-1}$, Chlorophyll B at 0.414 mg $100g^{-1}$, Total Chlorophyll at 1.994 mg $100g^{-1}$, and carotene at 0.003 mg $100g^{-1}$. Conversely, the highest values were recorded at 10 ml L^{-1} of Kelpak, with Chlorophyll A reaching 2.414 mg $100g^{-1}$, Chlorophyll B at 0.830 mg $100g^{-1}$, Total Chlorophyll at 3.244 mg $100g^{-1}$, and carotene at 0.009 mg $100g^{-1}$. These results clearly demonstrate that as the concentration of Kelpak seaweed extract increased, there was a corresponding increase in the levels of photosynthetic pigments in Date Palm leaves. This indicates a positive relationship between Kelpak concentration and the production of photosynthetic pigments, suggesting the potential benefits of using higher concentrations of Kelpak in Date Palm cultivation to enhance photosynthetic activity and overall plant health.

Table 2: Effects of Different Concentrations of Kelpak Seaweed Extract on Photosynthetic Pigments (mg $100g^{-1}$) in Date Palm Leaves.

Kelpak concentration (ml L^{-1})	Chlorophyll A	Chlorophyll B	Total Chlorophyll	carotene
0	1.580	0.414	1.994	0.003
2.5	1.896	0.552	2.438	0.005
5	2.120	0.789	2.909	0.008
7.5	2.663	0.975	3.638	0.010
10	2.414	0.830	3.244	0.009
LSD ($p<0.05$)	0.022	0.018	0.030	0.002

Nutrient Content:

Table 3 displays the nutrient content (N, P, K, Ca) in Date Palm leaves in response to different concentrations of Kelpak Seaweed Extract. At a concentration of 0 ml L^{-1}, the N content was measured at 1.379 mg g^{-1}, P at 0.503 mg g^{-1}, K at 1.860 mg g^{-1}, and Ca at 36.32 mg g^{-1}. As the concentration of Kelpak increased to 2.5 ml L^{-1}, the nutrient levels also increased, with N reaching 1.541 mg g^{-1}, P at 0.625 mg g^{-1}, K at 2.084 mg g^{-1}, and Ca at 40.40 mg g^{-1}. Subsequent increases in Kelpak concentration to 5 ml L^{-1}, 7.5 ml L^{-1}, and 10 ml L^{-1} resulted in further elevation of nutrient content in Date Palm leaves. The highest levels were recorded at 7.5 ml L^{-1}, with N measured at 1.990 mg g^{-1}, P at 1.065 mg g^{-1}, K at 3.127 mg g^{-1}, and Ca at 51.82 mg g^{-1}. These results indicate a positive correlation between the concentration of Kelpak Seaweed

Extract and the nutrient content in Date Palm leaves. These findings suggest that the application of Kelpak Seaweed Extract can enhance the nutrient content in Date Palm leaves, potentially leading to improved plant health and productivity.

Table 3: Nutrient Content (mg g^{-1})in Date Palm Leaves in Response to Different Concentrations of Kelpak Seaweed Extract.

Kelpak concentration (ml L^{-1})	N	P	K	Ca
0	1.379	0.503	1.860	36.32
2.5	1.541	0.625	2.084	40.40
5	1.726	0.878	2.650	43.67
7.5	1.990	1.065	3.127	51.82
10	1.828	0.910	2.724	48.90
LSD ($p<0.05$)	0.029	0.020	0.036	1.15

Biochemical Parameters:

Table 4 presents the biochemical parameters (Total Soluble Protein, Total Soluble Carbohydrates, and Proline) in Date Palm leaves treated with different concentrations of Kelpak Seaweed Extract. The lowest value for Total Soluble Protein was observed at 7.5 ml L^{-1} (1.036 mg g^{-1}), while the highest value was recorded at 10 ml L^{-1} (2.261 mg g^{-1}). For Total Soluble Carbohydrates, the lowest value was obtained at 0 ml L^{-1} (0.444 mg g^{-1}), and the highest value was measured at 10 ml L^{-1} (1.160 mg g^{-1}). Regarding Proline, the lowest value was observed at 10 ml L^{-1} (1.914 mg g^{-1}), and the highest value was recorded at 0 ml L^{-1} (3.020 mg g^{-1}).

Overall, the general trend observed for Total Soluble Protein was an initial increase from 0 ml L^{-1} to 2.5 ml L^{-1}, followed by a slight decrease at 7.5 ml L^{-1}, and a subsequent increase at 10 ml L^{-1}. Total Soluble Carbohydrates showed a similar trend, with an increasing pattern from 0 ml L^{-1} to 5 ml L^{-1}, followed by a relatively stable level from 5 ml L^{-1} to 7.5 ml L^{-1}, and another increase at 10 ml L^{-1}. In contrast, Proline exhibited a decreasing trend as the concentration of Kelpak Seaweed Extract increased, with the lowest value observed at 10 ml L^{-1} and the highest value at 0 ml L^{-1}.

These results suggest that Kelpak Seaweed Extract treatment has varying effects on different biochemical parameters in Date Palm leaves. While Total Soluble Protein and Total Soluble

Carbohydrates generally increased with increasing Kelpak concentration, Proline levels decreased.

Table 4: Biochemical Parameters (Total Soluble Protein, Total Soluble Carbohydrates, and Proline) in Date Palm Leaves Treated with Various Concentrations of Kelpak Seaweed Extract.

Kelpak concentration (ml L^{-1})	Total soluble protein (mg g^{-1})	Total soluble carbohydrates (mg g^{-1})	Proline (µg g^{-1})
0	1.455	0.444	3.020
2.5	1.590	0.658	2.846
5	1.882	0.873	2.318
7.5	1.036	0.902	2.135
10	2.261	1.160	1.914
LSD ($p<0.05$)	0.022	0.018	0.030

Discussion

Biostimulants obtained from extracts of seaweed (SWE) are renowned for their capacity to stimulate plant growth through diverse mechanisms. Research has demonstrated that SWE can boost the growth and maturation of root cells by activating them and encouraging the synthesis of cytokinins (Battacharyya et al., 2015; Baltazar et al., 2021). SWE is a chemical that affects plant growth and has been shown to have various beneficial effects on plant growth and development. These effects include improved leaf hydration, increased nutrient absorption, increased shoot development, and reinforced roots. In general, SWE is a promising plant growth regulator with the potential to improve plant health and performance. (Yao and colleagues, 2020). SWE has the ability to alter the level of plant hormones, particularly cytokinins and auxins, which are essential for growth (Muetasam Jafr et al., 2022). SWE can help protect plant cells from damage by oxidation and can activate enzymes that help plants cope with stress, which can improve plant tolerance (Elansary et al., 2017; Hamouda et al., 2022), according to Ali et al. (2021), SWE has the potential to enhance the functioning of antioxidant enzymes such as superoxide dismutase

(SOD), glutathione reductase (GR), and ascorbate peroxidase (ASP), thereby safeguarding plants against unfavorable environmental condition. The utilization of SWE may also help to protect plants from damage by increasing the synthesis of substances such as tocopherol, ascorbic acid, and carotenoids, which assist in preserving the photosynthetic system of PSII (Alebidi et al., 2021). SWE has the ability to improve plant nutritional status by lowering sodium chloride (NaCl) absorption, thereby reducing their negative effects (Bhupenchandra et al., 2022), while increasing the content of essential elements like potassium (K) and calcium (Ca) in leaves (Deolu-Ajayi et al., 2022). Furthermore, SWE may have other benefits such as increasing chlorophyll synthesis, controlling cell membrane components during a biotic stress, and reducing free radical activity to prevent chlorophyll degradation (Espinosa-Antón et al., 2023). Ali et al. (2021) demonstrated that SWE can enhance the levels of reducing sugars in plants, potentially bolstering their ability to withstand wilt and promote nucleic acid metabolism. Overall, the many effects of SWE, such as root activation, hormonal modulation, antioxidant activity, nutrient absorption increase, and stress tolerance, contribute to its usefulness as a biostimulant for boosting plant growth and development.

Conclusion

In conclusion, the results of this study highlight the positive effects of Kelpak Seaweed Extract on Date Palm leaves. The application of Kelpak led to increased nutrient content, enhanced photosynthetic pigment levels, and altered biochemical parameters. These findings indicate that Kelpak has the potential to improve nutrient uptake, promote photosynthesis, and influence important biochemical processes in Date Palm plants. These results suggest that Kelpak Seaweed Extract can be considered as an effective plant growth regulator for enhancing the growth, development, and overall health of Date Palm trees. Further studies are needed to investigate the underlying mechanisms and optimize the use of Kelpak in agricultural practices.

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تعزيز الأداء الفسيولوجي لنباتات نخيل التمر المشتقة من زراعة الأنسجة تحت ضغط الملوحة بمستخلص الأعشاب البحرية من كيلباك

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الخلاصة

يعتبر نخيل التمر .*Phoenix dactylifera* L محصولا قيما يزرع غالبا في التربة المالحة، مما يحد من نموها ومحصولها. في هذه الدراسة، تم التحقيق في آثار مستخلص الأعشاب البحرية كيلباك على مستويات المغذيات, صبغات البناء الضوئي, وبعض الصفات الكيموحيوية في اوراق نخيل التمر تحت اجهاد الملوحة. طبقنا تركيزات مختلفة من كيلباك (0 ، 2.5 ، 5 ، 7.5 ، و 10 مل لتر −1) لنباتات النخيل المعرضة لاجهاد الملح. وأظهرت النتائج أنه بالمقارنة مع العلاج السيطرة ، ادى الرش بمستخلص كيلباك الى زيادة معنوية في مستوى العناصر الغذائية في أوراق النخيل، بما في ذلك النيتروجين (N) والفوسفور (P) والبوتاسيوم (K) والكالسيوم (Ca). بالإضافة إلى ذلك، أدى الرش بمستخلص كيلباك إلى زيادة مستويات صبغات البناء الضوئي في الأوراق ، بما في ذلك الكلوروفيل a، والكلوروفيل b، والكلوروفيل الكلي، والكاروتينات، وارتبطت هذه الزيادة بزيادة التركيز. علاوة على ذلك.، كشف التحليل الكيميائي الحيوي أنه مع زيادة تركيز الكيلباك، زاد البروتين القابل للذوبان الكلي والكربوهيدرات القابلة للذوبان الكلية ، بينما انخفض محتوى البرولين. وفقا للنتائج، عزز مستخلص الأعشاب البحرية كيلباك امتصاص العناصر الغذائية، وزيادة صبغات البناء الضوئي، وتغيير الصفات الكيموحيوية المتعلقة بتطور النبات واستجابات الإجهاد. تقدم هذه الدراسة دليلا على أن كيلباك قد يكون علاجا مفيدا لنباتات نخيل التمر المجهدة بالملح. ومع ذلك ، هناك حاجة إلى مزيد من الدراسات لفهم آليات العمل بشكل كامل ولتحسين تطبيق الكلباك في زراعة نخيل التمر.

الكلمات المفتاحية: الكربوهيدرات، الكاروتين، الكلوروفيل، البرولين، البروتين.