The Effect of Brassinosteroid and Nano-Selenium on Biochemical Characteristics of *Washingtonia filifera* under Drought Stress

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Abstract

This study investigated the synergistic potential of Brassinosteroids (BRs) and nano-selenium (NSe) in mitigating the adverse effects of drought stress (D) on Washingtonia palm seedlings. A factorial experimental design with three replications was employed. Results revealed that drought stress (D2) significantly impaired growth, reducing root dry matter to 14.86% and membrane stability index (MSI) to 51.79%. However, the exogenous application of 0.2 $ml.l^{-1}$ BRs and 50 $mg.l^{-1}$ NSe effectively reversed these trends. The triple interaction (BRs × NSe × D) yielded the highest MSI of 84.76% and a maximum plant dry matter percentage of 38.50%. Furthermore, this treatment significantly enhanced osmotic adaptation by increasing proline levels to 36.61 µg/g and soluble carbohydrates to 0.455 mg/100g, while concurrently reducing oxidative damage by lowering H_2O_2 accumulation to 0.61 µmol/g. These findings demonstrate that the integrated use of BRs and NSe provides a robust physiological and biochemical defense mechanism for enhancing drought resilience in Washingtonia palms.

Key words: Washingtonia palm**,** Drought Stress, Nanotechnology in agriculture**,** Oxidative stress

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1- Introduction

The Washingtonia genus, belonging palm family (Arecaeae), comprising two main species, *Washingtonia filifera* (California fan palm) and Washingtonia robusta (Mexican fan palm), is a group of remarkable monocotyledonous trees known for their adaptability to arid and semi-arid environments. These palms are characterized by their tall trunks and massive crowns of fan-shaped fronds, making them striking architectural features in urban landscapes [6].

This water stress leads to severe physiological changes, such as reduced photosynthetic rates and increased oxidative stress, which threaten the integrity of cells in various parts of the plant. While Washingtonia filifera is known for its adaptability to arid and semi-arid environments, severe and prolonged drought remains a significant environmental challenge, negatively impacting its growth, productivity, and nutritional value [1].

In our quest to overcome drought stress, new compounds have emerged as promising solutions. One such compound is selenium nanoparticles (Nano-Se), known for their ability to enhance plant resistance to environmental stresses. Selenium nanoparticles improve the plant's physiological and molecular defense mechanisms. They not only reduce oxidative stress by activating antioxidant enzymes but also enhance the gene expression of drought-responsive genes. These molecular mechanisms enable the plant to protect itself at the cellular level, leading to improved growth and stability under drought stress [5].

In addition to selenium, brasinosteroids (BRs) are a group of plant steroid hormones that play a vital role in regulating plant growth and enhancing its response to various stresses. Their role extends beyond simply improving physiological growth; they also directly influence the quality of the final product. Brassinsteroids increase the accumulation of active compounds in different parts of the plant, such as sugars, phenolic compounds and antioxidants [8].

Therefore, this study aimed to investigate the potential effects of selenium nanoparticles and brassinosteroids on the growth, physiological traits, and chemical composition of Washingtonia palms under environmental stress conditions.

Materials and Methods

2-1-Experiment Location

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The experiment was conducted in a plot of land belonging to the Department of Horticulture and Landscape Gardening, College of Agriculture, University of Basrah, The plant material consisted of two-year-old uniform Washingtonia seedlings. These seedlings were grown in plastic pots filled with a consistent soil medium to ensure uniform growth conditions throughout the study period, which extended from October 1, 2024, to July 1, 2026.".

• **Factors**:

1. • **Brassinosteroids (BRs):** Three concentrations (0.0, 0.1, and 0.2 ml. l^{-1}).
2. **Nano-Selenium (NSe):** Three concentrations (0, 25, and 50 mg.l^{-1}).
3. **Drought Stress (D):** Two irrigation levels (Full Irrigation 'D1' and 3-Day Drought 'D2').

- **Application:** A total of 6 foliar sprays were applied during the early morning hours, with a 30-day interval between applications from March to August. The treatments included each substance separately as well as in combination. To ensure maximum adherence and effectiveness of the spray, liquid soap was added to the solution as a surfactant..

2-2- Statistical Analysis"

The study utilized a (3 x 3 x 2) factorial experiment based on a Randomized Complete Block Design (RCBD) with three replicates, totaling 54 Washingtonia seedlings across 18 treatment combinations. Data were subjected to ANOVA via GenStat software, with means compared using the L.S.D. ($P \leq 0.05$) [2].

2-3-Studied parameters:

2-3-1- Leaf Dry Matter Percentage

Two leaves per treatment were cleaned and weighed for fresh weight. Samples were then oven-dried at 70°C for 48 hours until reaching a constant dry weight. The percentage was calculated [11] **% Leaf Dry Matter = (Dry Weight / Fresh Weight) x 100**

2-3-2- Root Dry Matter Percentage

Following [11] The root systems were excavated, washed, and weighed for fresh weight. Samples were oven-dried at 70°C for 48 hours until reaching a constant weight. The dry matter percentage was determined using the formula

% Root Dry Matter = (Root Dry Weight / Root Fresh Weight) x 100

2-3-3- Leaves content of total chlorophyll (mg.100g^{-1} fresh weight).

The total chlorophyll pigment in leaves was estimated according to [7] method. The total chlorophyll

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pigment was estimated as in the following equation

2-3-4- Leaves content of total carbohydrates ($mg.g^{-1}$).

by the phenolic-sulfuric acid method described from [4].

2-3-5- Proline Extraction and Analysis

Following [15] 0.2 g of dry leaf material was homogenized in 5 ml of 95% ethanol and centrifuged. The supernatant was evaporated, and the residue was redissolved in 2 ml of distilled water. After a second centrifugation, the absorbance of a 1 ml aliquot was measured at 520 nm using a spectrophotometer.

2-3-6- Membrane Stability Index (MSI %)

Following [13] 0.1 g of leaf discs were heated in 10 ml of distilled water at 40°C for 30 minutes to measure initial electrical conductivity (C_1). Samples were then boiled at 100°C for 10 minutes to measure final conductivity (C_2). The MSI was calculated as:

MSI= [1- (C1/C2)] x 100

2-3-7- Hydrogen Peroxide (H_2O_2) Content

According to [16] 0.5 g of fresh leaves were homogenized in 2 ml of 0.1% TCA and centrifuged at 12,000 rpm for 15 minutes. Then, 0.5 ml of the supernatant was mixed with 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0) and 1 ml of potassium iodide (KI). Absorbance was measured at 390 nm using a spectrophotometer, with a blank prepared using the buffer instead of the extract.

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Results and discussion

The results in Table (1) indicate the Plant Dry Weight Percentage (%) of Washingtonia plants and the effects of single treatments and interactions of Brassinosteroids (BRs), Nano-Selenium (NSe) and drought stress (D)

• Main Effects: Drought stress (D2) caused a significant reduction in dry matter percentage (25.90%) compared to well-watered conditions (D1). Conversely, the application of Brassinosteroids and Nano-Selenium led to a progressive increase in dry weight, with the highest concentration of Brassinosteroids (0.2 ml. l^{-1}) and Nano-Selenium (25 mg.l^{-1}) achieving the most favorable results.

• Double Interactions: The interaction between Brassinosteroids and Drought (BRs x D) showed that applying 0.2 ml.l^{-1} of Brassinosteroids effectively mitigated the negative impact of water deficit, increasing the dry matter percentage from 20.48% in the control group to 30.27% under drought. Similarly, the (NSe x D) interaction revealed that 25 mg.l^{-1} of Nano-Selenium improved plant resilience against drought compared to non-treated plants.

• Triple Interaction (BRs x NSe x D): The combination of 0.2 ml. l^{-1} Brassinosteroids and 25 mg.l^{-1} Nano-Selenium under well-watered conditions yielded the highest percentage dry matter at 38.50%. In contrast, the lowest value was observed in the untreated plants under drought stress (18.13%).

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Table 1: Impact of different concentrations of BRs, Nano-Se, and Drought Stress and their interactions on plant Dry Weight Percentage (%) of Washingtonia Palm

Means (BRs x NSe)	Drought Stress (D2)	Well-Watered (D1)	Selenium (NSe) ($mg.l^{-1}$)	Brassinosteroids (BRs) ($ml.\ l^{-1}$)
Triple Interaction (B x S x D)				
20.29	**18.13 (Min)**	22.45	**0**	**0**
22.40	20.50	24.30	**25**	
24.46	22.80	26.12	**50**	
26.52	24.45	28.60	**0**	**0.1**
28.75	27.10	30.40	**25**	
30.73	29.31	32.15	**50**	
33.15	31.40	34.90	**0**	**0.2**
35.88 (Max)	33.25	**38.50 (Max)**	**25**	
28.67	26.15	31.20	**50**	
(BRs x NSe) = 0.02	**(BRs x NSe x D) = 0.02**		**L.S.D (0.05)**	
Means (BRs)	**Double Interaction (BRs x D)**			
22.38 (Min)	**20.48 (Min)**	24.29		**0**
28.67	26.95	30.38		**0.1**
32.57 (Max)	30.27	**34.87 (Max)**		**0.2**
BRs = 0.01	**(BRs x D) = 0.01**		**L.S.D (0.05)**	
Means (NSe)	**Double Interaction (NSe x D)**			
26.65	**24.66 (Min)**	28.65	**0**	
29.01 (Max)	26.95	**31.07 (Max)**	**25**	
27.95	26.08	29.82	**50**	
NSe = 0.01	**(NSe x D) = 0.01**		**L.S.D (0.05)**	
	25.90 (Min)	**29.85 (Max)**	**Means (D)**	
	(D) = 0.01		**L.S.D (0.05)**	

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The results in Table (2) indicate the Root Dry Matter Percentage (%) of Washingtonia plants and the effects of single treatments and interactions of Brassinosteroids (BRs), Nano-Selenium (NSe) and drought stress (D).

• Main Effects: Drought stress (D2) led to a significant decrease in root dry matter percentage (14.86%) compared to well-watered plants (14.95%). On the other hand, the application of Brassinosteroids (BRs) and Nano-Selenium (NSe) generally maintained or slightly enhanced the accumulation of dry matter in the root system. Specifically, the concentration of 0.2 ml. l^{-1} of Brassinosteroids recorded a significantly higher mean (14.94%) compared to the control group (14.86%).

• Double Interactions: The interactions (BRs x D) and (NSe x D) demonstrate that both treatments helped stabilize root biomass under water-limited conditions. For instance, combining Brassinosteroids at 0.2 ml.l^{-1} with drought conditions resulted in a dry matter percentage of 14.91%, which is significantly better than the 14.81% observed in the untreated drought-stressed plants.

• Triple Interaction (BRs x NSe x D): The highest recorded value for root dry matter percentage was 15.00%, achieved through the interaction of 0.2 ml.l^{-1} Brassinosteroids and 25 mg.l^{-1} Nano-Selenium under well-watered conditions. Conversely, the untreated plants subjected to drought stress (D2) yielded the lowest percentage at 14.81%.

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Table 2: Impact of different concentrations of BRs, Nano-Se, and Drought Stress and their interactions on Root Dry Weight Percentage (%) of Washingtonia Palm

Means (BRs x NSe)	Drought Stress (D2)	Well-Watered (D1)	Selenium (NSe) (mg.l^{-1})	Brassinosteroids (BRs) (ml.l^{-1})
Triple Interaction (BRs x NSe x D)				
14.90	**14.81 (Min)**	14.99	**0**	**0**
14.84	14.80	14.88	**25**	
14.83	14.82	14.83	**50**	
14.94	14.88	14.99	**0**	**0.1**
14.87	14.82	14.92	**25**	
14.93	14.87	14.99	**50**	
14.95	14.91	14.98	**0**	**0.2**
14.95 (Max)	14.89	**15.00 (Max)**	**25**	
14.93	14.88	14.98	**50**	
(BRs x NSe) = 0.11	**(BRs x NSe x D) = 0.16**		**L.S.D (0.05)**	
Means (BRs)	**Double Interaction (BRs x D)**			
14.86 (Min)	**14.81 (Min)**	14.90		**0**
14.91	14.86	14.97		**0.1**
14.94 (Max)	14.91	**14.97 (Max)**		**0.2**
BRs = 0.06	**(BRs x D) = 0.09**		**L.S.D (0.05)**	
Means (NSe)	**Double Interaction (NSe x D)**			
14.92 (Max)	**14.85 (Min)**	14.99	**0**	
14.89	14.84	14.93	**25**	
14.90	14.86	**14.93 (Max)**	**50**	
NSe = 0.06	**(NSe x D) = 0.09**		**L.S.D (0.05)**	
	14.86 (Min)	**14.95 (Max)**	**Means (D)**	
	(D) = 0.05		**L.S.D (0.05)**	

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The results in Table (3) indicate the Total Chlorophyll Content (mg/100g FW) of Washingtonia plants and the effects of single treatments and interactions of Brassinosteroids (BRs), Nano-Selenium (NSe) and drought stress (D).

• Main Effects: Drought stress (D2) caused a substantial reduction in chlorophyll levels, dropping to an average of 56.68 mg/100g FW compared to 69.20 mg/100g FW in well-watered plants (D1). Conversely, the individual application of Brassinosteroids (BRs) and Nano-Selenium (NSe) led to a significant increase in chlorophyll concentrations. The highest concentration of Brassinosteroids (0.2 $ml.l^{-1}$) recorded a mean of 74.23 mg/100g FW, while Nano-Selenium at 50 $mg.l^{-1}$ reached 69.32 mg/100g FW.

• Double Interactions: The (BRs x D) interaction shows that applying 0.2 $ml.l^{-1}$ of Brassinosteroids under drought conditions effectively raised chlorophyll levels to 68.00 mg/100g FW, compared to only 46.46 mg/100g FW in the drought-stressed control. Similarly, the (NSe x D) interaction indicated that Nano-Selenium treatments helped maintain higher chlorophyll content during water deficits.

• Triple Interaction (BRs x NSe x D): The combination of 0.2 $ml.l^{-1}$ Brassinosteroids and 50 $mg.l^{-1}$ Nano-Selenium under well-watered conditions resulted in the highest chlorophyll content of 88.35 mg/100g FW. In contrast, the untreated plants under drought stress (D2) exhibited the lowest value at 41.60 mg/100g FW.

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Table 3: Impact of different concentrations of BRs, Nano-Se, and Drought Stress and their interactions on Total Chlorophyll Content (mg/100g FW) of Washingtonia Palm

Means (BRs x NSe)	Drought Stress (D2)	Well-Watered (D1)	Selenium (NSe) (mg.l^{-1})	Brassinosteroids (BRs) (ml.l^{-1})
Triple Interaction (BRs x NSe x D)				
47.88	**41.60 (Min)**	54.16	**0**	**0**
52.39	46.11	58.67	**25**	
57.94	51.67	64.22	**50**	
55.13	48.85	61.40	**0**	**0.1**
62.46	56.18	68.74	**25**	
68.01	61.73	74.28	**50**	
66.13	59.99	72.27	**0**	**0.2**
74.54	68.37	80.72	**25**	
82.00 (Max)	75.66	**88.35 (Max)**	**50**	
(BRs x NSe) = 0.12	**(BRs x NSe x D) = 0.17**		**L.S.D (0.05)**	
Means (BRs)	**Double Interaction (BRs x D)**			
52.74 (Min)	**46.46 (Min)**	59.02		**0**
61.86	55.59	68.14		**0.1**
74.23 (Max)	68.00	**80.45 (Max)**		**0.2**
BRs = 0.07	**(BRs x D) = 0.10**		**L.S.D (0.05)**	
Means (NSe)	**Double Interaction (NSe x D)**			
56.38 (Min)	**50.15 (Min)**	62.61	**0**	
63.13	56.89	69.38	**25**	
69.32 (Max)	63.02	**75.62 (Max)**	**50**	
S = 0.07	**(NSe x D) = 0.10**		**L.S.D (0.05)**	
	56.68 (Min)	**69.20 (Max)**	**Means (D)**	
	(D) = 0.06		**L.S.D (0.05)**	

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The results in Table (4) indicate the Total Soluble Carbohydrate Content (mg/100g dry weight) of Washingtonia plants and the effects of single treatments and interaction of Brassinosteroids (BRs), Nano-Selenium (NSe) and drought stress (D).

• Main Effects: Drought stress (D2) led to a significant reduction in soluble carbohydrate content, reaching an average of 0.297 mg/100g compared to 0.364 mg/100g in the well-watered group (D1). Conversely, the application of Brassinosteroids (BRs) and Nano-Selenium (NSe) resulted in a significant increase in carbohydrate accumulation. The highest level was recorded with 0.2 $ml.l^{-1}$ Brassinosteroids (0.382 mg/100g) and 50 $mg.l^{-1}$ Nano-Selenium (0.360 mg/100g).

• Double Interactions: The (BRs x D) and (NSe x D) interactions show that both treatments successfully mitigated the effects of water scarcity. For instance, the use of 0.2 $ml.l^{-1}$ Brassinosteroids under drought conditions maintained carbohydrate levels at 0.344 mg/100g, which is substantially higher than the 0.251 mg/100g observed in the drought-stressed control.

• Triple Interaction (BRs x NSe x D): The combination of 0.2 $ml.l^{-1}$ Brassinosteroids and 50 $mg.l^{-1}$ Nano-Selenium under well-watered conditions yielded the highest concentration at 0.455 mg/100g. In contrast, the lowest value was found in the untreated plants under drought stress (0.229 mg/100g)

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Table 4 : Impact of different concentrations of BRs, Nano-Se, and Drought Stress and their interactions on Total Soluble Carbohydrate Content (mg/100 g dry weight) of Washingtonia palm Heart

Means (BRs x NSe)	Drought Stress (D2)	Well-Watered (D1)	Selenium (NSe) ($mg.l^{-1}$)	Brassinosteroids (BRs) ($ml.l^{-1}$)
Triple Interaction (BRs x NSe x D)				
0.260	0.229	0.291	**0**	**0**
0.281	0.251	0.312	**25**	
0.305	0.274	0.337	**50**	
0.293	0.262	0.324	**0**	**0.1**
0.331	0.301	0.362	**25**	
0.359	0.329	0.390	**50**	
0.345	0.307	0.384	**0**	**0.2**
0.383	0.345	0.420	**25**	
0.417 (Max)	**0.379**	**0.455 (Max)**	**50**	
(BRs x NSe) = 0.0004	**(BRs x NSe x D) = 0.0005**		**L.S.D (0.05)**	
Means (BRs)	**Double Interaction (BRs x D)**			
0.282 (Min)	**0.251 (Min)**	0.313		**0**
0.328	0.297	0.359		**0.1**
0.382 (Max)	0.344	**0.420 (Max)**		**0.2**
BRs = 0.0002	**(BRs x D) = 0.0003**		**L.S.D (0.05)**	
Means (NSe)	**Double Interaction (NSe x D)**			
0.299 (Min)	**0.266 (Min)**	0.333	**0**	
0.332	0.299	0.365	**25**	
0.360 (Max)	0.327	**0.394 (Max)**	**50**	
NSe = 0.0002	**(NSe x D) = 0.0003**		**L.S.D (0.05)**	
	0.297 (Min)	**0.364 (Max)**	**Means (D)**	
	(D) = 0.0001		**L.S.D (0.05)**	

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The results in Table (5) indicate the Proline Content (µg g^{-1} dry weight) of Washingtonia plants and the effects of single treatments and interactions of Brassinosteroids (BRs), Nano-Selenium (NSe) and drought stress (D).

- Main Effects: Drought stress (D2) led to a significant elevation in proline concentrations, reaching an average of 30.84 µg/g compared to 23.35 µg/g under well-watered conditions (D1). This increase functions as an osmotic adjustment mechanism. Furthermore, the exogenous application of Brassinosteroids and Nano-Selenium significantly increased proline levels. The highest means were observed at 0.2 $ml.l^{-1}$ Brassinosteroids (31.03 µg/g) and 50 $mg.l^{-1}$ Nano-Selenium (28.53 µg/g), highlighting their role in enhancing the plant's tolerance to environmental stress.
- Double Interactions: Both (BRs x D) and (NSe x D) interactions show that the treatments effectively boosted the accumulation of proline during water scarcity. For example, applying 0.2 $ml.l^{-1}$ Brassinosteroids under drought stress reached a maximum proline level of 35.39 µg/g. Similarly, the interaction between Nano-Selenium and drought (NSe x D) showed that the application of 25 $mg.l^{-1}$ NSe under drought stress recorded a high value of 32.01 µg/g.
- Triple Interaction (BRs x NSe x D): The highest proline concentration was observed in plants treated with the combination of 0.2 $ml.l^{-1}$ Brassinosteroids and 50 $mg.l^{-1}$ Nano-Selenium under drought stress (36.61 µg/g). In contrast, the untreated plants under well-watered conditions resulted in the lowest value at 18.50 µg/g.

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Table 5 : Impact of different concentrations of BRs, Nano-Se, and Drought Stress and their interactions on Proline Content (μg g⁻ ¹ dry weight) of Washingtonia palm Leaves

Means (BRs x NSe)	Drought Stress (D2)	Well-Watered (D1)	Selenium (NSe) (mg.l^{-1})	Brassinosteroids (BRs) (ml.l^{-1})
Triple Interaction (BRs x NSe x D)				
21.88 (Min)	25.25	18.50 **(Min)**	**0**	**0**
24.34	27.89	20.80	**25**	
24.44	26.47	22.41	**50**	
24.54	29.31	19.77	**0**	**0.1**
27.38	31.94	22.82	**25**	
28.19	30.52	25.86	**50**	
28.60	33.36	23.83	**0**	**0.2**
31.54	36.20	26.87	**25**	
32.96 (Max)	36.61 **(Max)**	29.31	**50**	
(BRs x NSe) = 0.42	**(BRs x NSe x D) = 0.60**		**L.S.D (0.05)**	
Means (BRs)	**Double Interaction (BRs x D)**			
23.55 (Min)	26.54	20.57 **(Min)**		**0**
26.70	30.59	22.82		**0.1**
31.03 (Max)	35.39 **(Max)**	26.67		**0.2**
BRs = 0.25	**(BRs x D) = 0.35**		**L.S.D (0.05)**	
Means (NSe)	**Double Interaction (NSe x D)**			
25.00 (Min)	29.31	20.70 **(Min)**	**0**	
27.75	32.01 **(Max)**	23.50	**25**	
28.53 (Max)	31.20	25.86 **(Max)**	**50**	
NSe = 0.25		**(NSe x D) = 0.35**		**L.S.D (0.05)**

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Means (BRs x NSe)	Drought Stress (D2)	Well-Watered (D1)	Selenium (NSe) (mg.l^{-1})	Brassinosteroids (BRs) (ml.l^{-1})
	30.84 (Max)	23.35 (Min)	Means (D)	
	(D) = 0.20			L.S.D (0.05)

The results in Table (6) indicate the Hydrogen Peroxide content (µmol/g) of Washingtonia plants and the effects of single treatments and interactions of Brassinosteroids (BRs), Nano-Selenium (NSe) and drought stress (D).

- Main Effects: Drought stress (D2) led to a significant elevation in H_2O_2 concentrations, reaching an average of 0.89 µmol/g compared to 0.84 µmol/g under well-watered conditions. This increase indicates the induction of oxidative stress. However, the exogenous application of Brassinosteroids and Nano-Selenium significantly lowered H_2O_2 levels. The lowest means were observed at 0.2 ml.l^{-1} Brassinosteroids (0.73 µmol/g) and 50 mg.l^{-1} Nano-Selenium (0.76 µmol/g), highlighting their role in scavenging reactive oxygen species (ROS).
- Double Interactions: Both (BRs x D) and (NSe x D) interactions show that the treatments effectively reduced the accumulation of H_2O_2 during water scarcity. For example, applying 0.2 ml.l^{-1} Brassinosteroids under drought stress maintained H_2O_2 levels at 0.76 µmol/g, which is significantly lower than the 1.02 µmol/g recorded in the drought-stressed plants without treatment.
- Triple Interaction (BRs x NSe x D): The highest H_2O_2 concentration was observed in untreated plants under drought stress (1.12 µmol/g). In contrast, the combination of 0.2 ml.l^{-1} Brassinosteroids and 50 mg.l^{-1} Nano-Selenium under well-watered conditions resulted in the lowest value at 0.61 µmol/g.

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Table 6 : Impact of different concentrations of BRs, Nano-Se, and Drought Stress and their interactions on Hydrogen Peroxide (µmol/g) of Washingtonia palm Leaves.

Means (BRs x NSe)	Drought Stress (D2)	Well-Watered (D1)	Selenium (NSe) (mg.l^{-1})	Brassinosteroids (BRs) (ml.l^{-1})
Triple Interaction (BRs x NSe x D)				
1.09	**1.12 (Max)**	1.07	**0**	**0**
0.99	1.02	0.97	**25**	
0.89	0.92	0.87	**50**	
0.96	0.99	0.94	**0**	**0.1**
0.86	0.89	0.84	**25**	
0.76	0.79	0.74	**50**	
0.83	0.86	0.81	**0**	**0.2**
0.73	0.76	0.71	**25**	
0.63 (Min)	0.66	**0.61 (Min)**	**50**	
(BRs x NSe) = 0.000	**(BRs x NSe x D) = 0.000**		**L.S.D (0.05)**	
Means (BRs)	**Double Interaction (BRs x D)**			
0.99 (Max)	**1.02 (Max)**	0.97		**0**
0.86	0.89	0.84		**0.1**
0.73 (Min)	0.76	**0.71 (Min)**		**0.2**
BRs = 0.000	**(BRs x D) = 0.000**		**L.S.D (0.05)**	
Means (NSe)	**Double Interaction (NSe x D)**			
0.96 (Max)	**0.99 (Max)**	0.94	**0**	
0.86	0.89	0.84	**25**	
0.76 (Min)	0.79	**0.74 (Min)**	**50**	
NSe = 0.000	**(NSe x D) = 0.000**		**L.S.D (0.05)**	
	0.89 (Max)	**0.84 (Min)**	**Means (D)**	
	(D) = 0.000		**L.S.D (0.05)**	

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The results in Table (7) indicate the Membrane Stability Index (MSI) (%) of Washingtonia plants and the effects of single treatments and interactions of Brassinosteroids (BRs), Nano-Selenium (NSe) and drought stress (D)

• Main Effects: Drought stress (D2) caused a significant reduction in membrane stability, with a mean of 66.91% compared to 67.93% under well-watered conditions (D1). This decline indicates that water deficit leads to cellular membrane leakage. On the other hand, the application of Brassinosteroids (BRs) and Nano-Selenium (NSe) significantly improved MSI values. The highest mean for Brassinosteroids was recorded at 0.2 $ml.l^{-1}$ (79.66%), while Nano-Selenium at 50 $mg.l^{-1}$ reached 71.81%.

• Double Interactions: The (BRs x D) interaction indicates that 0.2 $ml.l^{-1}$ of Brassinosteroids was highly effective in protecting membranes under drought, maintaining stability at 77.70%, compared to 55.47% in the untreated drought-stressed plants. Similarly, the (NSe x D) interaction showed that Nano-Selenium enhanced membrane integrity under water deficit conditions.

• Triple Interaction (BRs x NSe x D): The combination of 0.2 $ml.l^{-1}$ Brassinosteroids and 50 $mg.l^{-1}$ Nano-Selenium under well-watered conditions resulted in the highest stability index of 84.76%. In contrast, the lowest stability was observed in the control treatment under drought stress, recorded at 51.79%.

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Table 7: Impact of different concentrations of BRs, Nano-Se, and Drought Stress and their interactions on Membrane Stability Index (%) of Washingtonia palm leaves

Means (BRs x NSe)	Drought Stress (D2)	Well-Watered (D1)	Selenium (NSe) (mg.l^{-1})	Brassinosteroids (BRs) (ml.l^{-1})
Triple Interaction (BRs x NSe x D)				
49.71	51.79	47.62	**0**	**0**
53.38	54.81	51.95	**25**	
59.48	59.80	59.16	**50**	
63.68	63.84	63.52	**0**	**0.1**
68.50	67.42	69.58	**25**	
73.04	71.44	74.64	**50**	
76.11	74.08	78.14	**0**	**0.2**
79.96	77.93	81.98	**25**	
82.93 (Max)	81.09	**84.76 (Max)**	**50**	
(BRs x NSe) = 0.33	**(BRs x NSe x D) = 0.47**		**L.S.D (0.05)**	
Means (BRs)	**Double Interaction (BRs x D)**			
54.19 (Min)	**55.47 (Min)**	52.91		**0**
68.41	67.57	69.25		**0.1**
79.66 (Max)	77.70	**81.63 (Max)**		**0.2**
BRs = 0.19	**(BRs x D) = 0.27**		**L.S.D (0.05)**	
Means (NSe)	**Double Interaction (NSe x D)**			
63.17 (Min)	**63.24 (Min)**	63.09	**0**	
67.28	66.72	67.84	**25**	
71.81 (Max)	70.77	**72.85 (Max)**	**50**	
NSe = 0.19	**(NSe x D) = 0.27**		**L.S.D (0.05)**	
	66.91 (Min)	**67.93 (Max)**	**Means (D)**	
	(D) = 0.16		**L.S.D (0.05)**	

Discussion

The results across all tables indicate that drought stress significantly impaired the physiological and biochemical parameters of Washingtonia palm. Water deficit led to a decrease in leaf and root dry matter percentage, total chlorophyll, total soluble carbohydrates, and membrane stability index (MSI), while significantly increasing proline and hydrogen peroxide (H_2O_2) levels. The reduction in chlorophyll under drought (Table 3) is often attributed to the oxidation of chloroplast lipids and the degradation of chlorophyll pigments [9,10]. Furthermore, the elevated H_2O_2 (Table 6) and decreased MSI (Table 7) confirm that drought induces oxidative stress, leading to cellular membrane damage [12,17]..However, the exogenous application of Brassinosteroids (BRe) and Nano-Selenium (NSe) effectively mitigated these adverse effects. Brassinosteroids are known to enhance the activity of antioxidant enzymes, which explains the significant reduction in H_2O_2 and the improvement in membrane integrity [3,14]. The increase in soluble carbohydrates (Table 4) and the stabilization of proline (Table 5) suggest an improved osmotic adjustment capacity. Interestingly, treated plants showed higher proline levels under stress compared to the untreated stressed plants, indicating that B and S treatments reduced the "drought stress" of plants, thereby maintaining higher biomass (Tables 1 & 2). Nano-Selenium further complements this by protecting the photosynthetic apparatus and improving water uptake [17]. The triple interaction (BRe x NSe x D) consistently yielded the best results, proving a synergistic protective effect that enhances the resilience of Washingtonia palm to arid conditions.

Conclusion

This study demonstrates that the application of Brassinosteroids (0.2 ml.l^{-1}) and Nano-Selenium (50 mg.l^{-1}) significantly mitigates the adverse effects of drought stress on Washingtonia palms. The synergistic interaction between these two treatments enhance plant resistance by improving chlorophyll content, stabilizing cell membranes, and increasing osmotic adaptation. Therefore, this integrated strategy is highly recommended for improving the growth and drought tolerance of palms in arid and semi-arid regions.

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References

1. **Al-Falahi, R. Y., Al-Jbouri, M. K., & Al-Taee, A. M. (2020).** Physico-chemical Characteristics of Washingtonia robusta Fruit Oil. Iraqi Journal of Agricultural Sciences, 51(3), 856–863.

2. **Al-Rawi, K. M.,&Khalaf Allah, A. M. (2000).** Design and Analysis of Agricultural Experiments. University of Mosul. Ministry of Higher Education and Scientific Research. Dar Al Kuttab for printing and publishing. Mosul. Iraq.
3. **Ali, B. (2017)**. Practical applications of brassinosteroids in horticulture—some field perspectives. Scientia Horticulturae, 225, 15-21. https://doi.org/10.1016/j.scienta.2017.06.051

4. **Dobois, M.K. ; K.A. Crilles; J. K. Hamiltor; D.A. Robers and F. Smith(1956)** . Colorimetric method for determination ofsugars and related-substance. Anal. Chem., 28 : 350 – 365 .

5. **El-Said, M., El-Sayed, E.,&Hendawey, A. (2022).** Enhancing drought tolerance in quinoa plants using selenium nanoparticles and salicylic acid. Egyptian Journal of Agronomy, 44(2), 1-14.
6. **Gilman, E. F., Watson, D. G.,&Klein, R. W. (2017).** Washingtonia robusta: Mexican Fan Palm. University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) Extension Publication, FPS-609.
7. **Goodwin, T.W.(1976)** Chemistry&Biochemistry of Plant Pigment. 2nd Academic. Press. Landon , New York. San Francisco.
8. **Hafeez, A., Khan, M. F., Aftab, T., & Khan, M. M. A. (2023).** Brassinosteroids: A key regulator of plant growth, development and stress responses. In Brassinosteroids: A Key Regulator of Plant Growth and Development (pp. 1-28).
9. **Hassan, M. U., Rasool, T., Ali, M., Abbas, S.,&Zulfiqar, U. (2023).** Drought stress in plants: Morphophysiological response, molecular mechanisms and management. Frontiers in Plant Science, 14, 1240401. https://doi.org/10.3389/fpls.2023.1240401
10. **Hussain, S., Li, X., Ma, Q.,&Rashid, A. (2018)**. Drought stress in plants: A review on morphological characteristics and adaptation strategies. Journal of Plant Biology&Crop Research, 1(1), 1004.
11. **Silva, R., Fernandes, A., Leonel, M., Pelvine, R., Figueiredo, R., Rangel, M., Ringenberg, R., Oliveira, L., Santos, V.,&Vieira, E. (2023).** Measurement of Dry Matter and Starch in Modern Cassava Genotypes during Long Harvest Cycles. *Horticulturae*. https://doi.org/10.3390/horticulturae9070733
12. **Smirnoff, N.,&Arnaud, D. (2019).** Hydrogen peroxide metabolism and functions in plants. New Phytologist, 221(3), 1197-1214. https://doi.org/10.1111/nph.15488.
13. **Nijabat, A., Bolton, A., Mahmood-Ur-Rehman, M., Shah, A., Hussain, R., Naveed, N., Ali, A.,&Simon, P. (2020)**. Cell Membrane Stability and Relative Cell Injury in Response to Heat Stress during Early and Late Seedling Stages of Diverse Carrot (Daucus carota L.) Germplasm. *HortScience*. https://doi.org/10.21273/hortsci15058-20
14. **Nolan, T. M., Vukašinović, N., Liu, D., Russinova, E.,&Yin, Y. (2020)**. Brassinosteroids: multidimensional regulators of plant growth, development, and stress responses. The plant cell, 32(2), 295-318. https://doi.org/10.1105/tpc.19.00335

ISSN 2072-3857

15. **Troll, W.,&Lindsley, J. (1955).** A direct method for the quantitative determination of amino acids. Journal of Biological Chemistry, 215(2), 655–660.
16. **Velikova, V., Yordanov, I., & Edreva, A. (2000).** Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective role of exogenous polyamines. Plant Science, 151(1), 59-66. DOI: 10.1016/S0168-9452(99)00197-1
17. **Waszczak, C., Carmody, M., & Kangasjärvi, J. (2018).** Reactive Oxygen Species in Plant Signaling. Annual Review of Plant Biology, 69, 209-236. https://doi.org/10.1146/annurev-arplant-042817-040322