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**Adaptive response of *Balanites aegyptiaca*
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Adaptive response of *Balanites aegyptiaca* (L.) Delile to severe aridity in the Western Desert of Egypt

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Desert date or *Balanites aegyptiaca* (L.) Del is one of the most prevalent, wild plant species in the arid regions of Africa and the Middle East. The desert date tolerates a broad range of soil types and can be found in a variety of habitat types. It has a wide ecological distribution but is mainly found on level alluvial sites with deep sandy loam and free access to water. *B. aegyptiaca* is used in African and Indian traditional medicine. Many underutilized tree species are good sources of food, fodder and possible therapeutic agents. *B. aegyptiaca* tree is considered drought tolerant and a potential source of many secondary metabolites. This study investigated the adaptive and physiological response to severe aridity and drought stress on the growth of *B. aegyptiaca* in El-Kharga Oasis, which is the driest region in the Western Desert. Clay soil was found to be dominant in mechanical soil analysis. Weakly to moderately calcareous soil carbonate was found, and the estimated pH values of the soil solution appeared to be slightly alkaline and exhibited a comparatively high electric conductivity. Total free amino acid concentration was greater than that of soluble protein and sugar. Results indicated that *B. aegyptiaca* is a drought-tolerant plant in severely arid areas that are subject to drought stress.

Keywords: *Balanites aegyptiaca*, Drought resistance, Plant-environment relations, Chlorophyll, Carbon and Nitrogen metabolism

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INTRODUCTION

Balanites aegyptiaca (L.) Delile, a member of the Zygophyllaceae (Balanitaceae) family, is also known as the “desert date” (Heiglige in Arabia) (Hall & Walker, 1991). It is a prickly, multibranched tree or shrub that grows to almost ten meters in height. According to Yadav & Panghal (2010) and Chothani & Vaghasiya (2011), it is a tropical evergreen perennial tree that tolerates drying. The short trunk of *B. aegyptiaca* frequently branches off close to the base. This tree has a wide ecological distribution. It is native to the Middle East, South Asia, Africa, and the Arabian Peninsula, as well as other arid and semi-arid locations (Arbonnier, 2004).

The well-known medicinal wild tree *B. aegyptiaca* is indigenous to South Asia and Africa, according to Hall (1992). It has a wide biological range throughout almost all of Africa, depending on the soil, climate, rainfall, and geographic conditions. Except for the areas surrounding the Mediterranean and Red Sea, it naturally grows across the Egyptian desert (Alshamy, 2016; Milto et al., 2019; Morsy et al., 2010). Worldwide, traditional medicine has made use of *B. aegyptiaca*'s roots, stems, leaves, bark, and seeds, among other parts. The fruit and its water extract are widely used as herbal diabetes treatments in Egypt (Abdelaziz et al., 2020). Asian and African countries use other parts of the plant, such as the seeds, leaves, bark, and root, to treat an array of illnesses. According

to Chothani & Vaghasiya (2011) and Yadav & Panghal (2010), they include haemorrhoids, intestinal worms, constipation, dysentery, stomach aches, asthma, epilepsy, jaundice, malaria, fever, and wounds. Over two thirds of Egypt's entire land area—roughly 681,000 km²— is made up of the Western Desert. Sand that shifts causes problems in an oasis because it not only fills in houses, farms, and wells, but also settles where there is stability, under vegetation, boosting its level. One of the most important geographic areas in Egypt is the New Valley Governorate which occupies one-third of the country's entire area. The El-Kharga Oasis, the last oasis on the ring before the Nile Valley, is a vast depression that reaches 220 km from the north to the southwest of the Nile and encompasses all of southern Egypt, excluding the territory adjacent to the Red Sea. There is little information in the literature about the botanical characteristics of *B. aegyptiaca*. Therefore, it is imperative to welcome any new botanical information regarding this medicinal plant.

According to previous studies, *B. aegyptiaca* maintains its metabolic activity for several years. This behavior illustrates the plant's resistance to water stress. The aim of the current study is to determine the physiological processes and metabolic activities that *B. aegyptiaca* uses as adaptation mechanisms to withstand drought and aridity.

MATERIAL AND METHODS

Study area

One of the principal Oases of Egypt's Nubian Desert (El-Hadidi, 2000), El-Kharga Oasis is situated in a depression in the southern section of the Western Desert, covering an area of 7200 km. Assiut City is located 224 km to the west, whereas Dakhla Oasis is located 104 km east. The desert lands extend between latitudes 24° 30' and 25° 40' N and longitudes 30° and 31° E. The depression has a long and narrow shape, extending 185 km from north to south and 15 to 30 km from east to west. Its total surface area is more than 3000 km², of which just 1% is cultivated. Rainfall is erratic; almost zero (mm year⁻¹) whereas the mean annual relative humidity is lower in summer (26-32%) than in winter (53-60%). The temperature is moderate in winter, but summer temperatures are rather high.

Soil analysis

Soil samples from certain sites were collected and plenty of plants were observed. During the wet (winter) season, samples were collected at seven different sites. Every site had three duplicates, which were removed and transported in plastic bags to the laboratory. The same locations were used to gather leaf samples of *B. aegyptiaca* plants during the summer and winter. The fresh soil sample was weighed, dried for 24 hours at 105 °C in an oven, and its dry weight was calculated to measure the water content of the soil samples. According to Kapur & Govil (2000), the water content of the sample was determined as a percentage of its dry weight. Water extracts with a ratio of 1:5 (soil: water) were made in accordance with the US Salinity Laboratory staff's protocol (Richards, 1954). To prepare soil extracts (1:5) for this proposal, twenty gm of soil were shaken with 100 milliliters of distilled water for 60 minutes. A clear filtrate was then obtained by filtering the mixture. The dichromate oxidation method, as described by Walkley & Black (1934), was used to assess the amount of organic matter in the soil samples. While sulphate was measured turbid metrically as BaSO₄ using methods provided by Bardsley & Lancaster (1965), chloride was determined volumetrically in accordance with Jackson (1967). Williams & Twine (1960) reported that flame photometry was used to detect sodium and potassium. The Versene method was used to calculate the volumetric values of calcium and magnesium (Johnson & Ulrich, 1959). Jackson (1967) reported that phosphorus was measured calorimetrically as

phospho-molybdate. Accordingly, a conductivity meter (model 4310, JEN WAY) was used to measure the soil filtrate's total soluble salts (TSS) and electric conductivity (EC). To ascertain the soil reactivity of the samples that were obtained, an electric pH meter (model AD-8000) was utilized. Jackson (1967) was followed in determining the soil's total carbonates.

Plant examination

Balanites aegyptiaca tree is 4-10 m tall, with grey bark and branches pubescent with axillary spreading spines to 4 cm (Boulos, 2000). Its trunk is short and is often branching near the base. The bark is dark brown to grey, deeply fissured. The branches are armed with stout yellow or green thorns up to 8 cm long. The leaves have two separate leaflets: leaflets obovate, asymmetric, 2.5 to 6 cm long, bright green, leathery, with fine hairs when young.

In test tubes, 10 milliliters of 80% aqueous ethanol were used to extract chlorophyll from a specific weight of fresh, healthy leaves. After that, the tubes were heated for 12 to 15 minutes at 70°C in a water bath, with glass marbles placed on top of them, until the leaf tissue turned colorless (Lichtenthaler & Buschmann, 2001). After that, the samples were quickly placed in ice to chill and were then filled to a capacity of 10 milliliters. The extract's optical density was determined at 663, 645 and 470 nm, the maximal absorption wavelengths of chlorophyll a, chlorophyll b and carotenoids respectively, using a spectrophotometer. The chlorophyll content was expressed as (mg. g⁻¹ f. wt.). A certain amount of healthy, fresh leaves was immersed in 10 mL of distilled water and heated for 30 minutes in a water bath at 56 ± 1°C to determine chlorophyll stability.

For either chlorophyll a or b, the chlorophyll stability index (CSI) was given as a percentage, comparing the amount of chlorophyll in the heated sample with the fresh sample. Ultimately, mg. g⁻¹ fresh weight (FW) was obtained for each of these pigment components. Furthermore, the ratio of chlorophyll a to b was estimated. Fresh leaves were dried for 24 hours at 70 °C in an oven before being milled into a fine powder for plant extraction. El-Sharkawi & Michel (1977) adopted the extraction procedure. To investigate soluble osmotically active metabolites, soluble sugars (carbon metabolites), total free amino acids, and soluble proteins (nitrogen metabolites) were all estimated. The methods given by Lee & Takahashi (1966), Lowry et al., (1951), and Dubois et al., (1956) were utilized to determine them. The contents of various anions and cations in plant extracts were

determined, as was previously mentioned in the soil extract part. The ions and soluble osmotically active metabolites were measured in milligrams per dry weight (mg DW).

Statistical analysis

To assess the impact of individual components and their interactions on the parameters under examination, statistical inferences were required, including determining the coefficient of determination (η^2) and calculating the analysis of variance (F value). The SPSS software was used to statistically analyze the data. In order to evaluate the respective contributions of every single element and interaction to the whole response, the coefficient of determination (η^2) has been developed.

RESULTS

Physical properties of soil

Soil water content and total soluble salt

Water contents in the winter soil sample (Figure 2) varied from 0.41% to 5.49%. Site 1 recorded the highest values of water content (5.49%), while the lowest value of water content (0.41 %) was recorded at site 6. TSS values in the winter ranged from 0.03% to 2.09% in most of the sites under study that were inhabited by *B. aegyptiaca* (Figure 2). The site with the highest TSS value (2.09%) was site 3. At site 2, the lowest figure (0.03%) was recorded.

Organic matter, pH value and Electric conductivity

Organic matter content (%) of soil samples was illustrated in (Table 1). The maximum value of the organic matter content (1.14%) was at site 3, whereas the minimum value (0.41%) occurred at site 5 during winter. Table 1 showed the PH values of soil solutions. In winter, values varied from 8.07 to 8.68. In these habitats, the soil solution was slightly alkaline. Site 3 reported the highest pH value (8.68), while site 7 recorded the lowest pH value (8.07). The electric conductivity (EC) values of the soil solutions are listed in table 1. During the winter, its values varied from 0.09 to 6.53 mS cm⁻¹. At site 3, the greatest EC value of 6.53 mS cm⁻¹ was noted. At site 2, the lowest value (0.09 mS cm⁻¹) was recorded. In general, the carbonate concentrations (at sites 1 and 6, respectively) varied from 5.05% to 6.77% (Table 1).

Major ion concentration in the soil

Concentrations of the main soluble cations and anions in soil samples taken from the locations where *B. aegyptiaca* plants were discovered are presented in Table 2. The soil's sodium contents varied from 0.16

to 15.66 mg g⁻¹. In contrast, the potassium concentration varied from 0.06 to 2.49 mg g⁻¹. The calcium contents of soil samples ranged from 0.18 to 1.87 mg g⁻¹. The magnesium contents of soil samples, however, fluctuated from 0.09 to 1.43 mg g⁻¹. The concentrations of chlorides found in soil samples varied from 0.68 to 6.11 mg g⁻¹. Site 5 had the highest concentration of chlorides detected. Small amounts of phosphates and sulphates were found in the investigated sites.

Plant examination

Water content

Figure 3 illustrates the water content of leaves in winter, which varied from 33.07% to 62.41%. It fluctuated between 47.33% and 69.91% during the summer. Two single elements and the combination between them had highly significant effects (Table 4).

Chlorophyll content and chlorophyll stability index

The carotenoid, chlorophyll a, and chlorophyll b concentrations are shown in Figure 4 and Table 3, respectively. Except for sites 1, 3, and 4, the summer had a higher chlorophyll a content than the winter. In winter, the content of chlorophyll a ranged from 0.03 to 0.44 mg g⁻¹f.wt., whereas in summer, it varied from 0.13 to 0.30 mg g⁻¹f. wt. Except for sites 5, 6, and 7, the concentration of chlorophyll b was higher in the winter than it was in the summer. Site 1 had the highest concentration of chlorophyll b, 0.21 mg g⁻¹f. wt.

Both seasons had a relatively slight carotenoid content, ranging from 0.03 to 0.15 mg g⁻¹ f. wt. In winter, total chlorophyll a+b was higher than in summer, except for sites 5, 6, and 7. Chlorophyll a/b ratio was between 0.70 and 2.64 in winter, but in summer, the ratio was between 1.48 and 2.19. According to the statistical analysis shown in Table 4, seasonality's effect on chlorophyll a was statistically non-significant, whereas regionality and the interaction of the two components were extremely significant. The regionality had dominant effect ($\eta^2 = 0.82$) followed by interaction ($\eta^2 = 0.18$). The minor ones were the seasonality factor ($\eta^2 = 0.003$). The effects of regionality and the interaction between the two components were extremely significant in chlorophyll b, while the seasonality factor had no effect. The main influence was regionality ($\eta^2 = 0.86$) followed by interaction ($\eta^2 = 0.14$). The seasonality factor was the minor one ($\eta^2 = 0.004$). The two signal components had a highly significant influence in carotenoid, but their interaction was not significant.

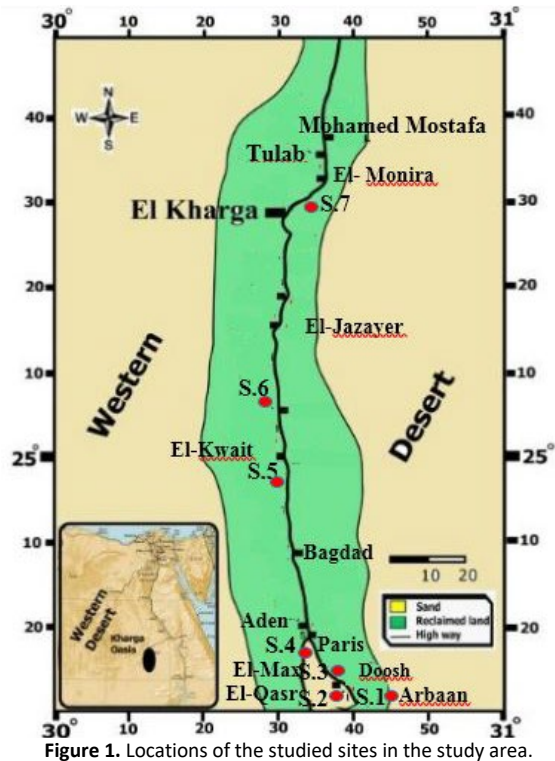


Figure 1. Locations of the studied sites in the study area.

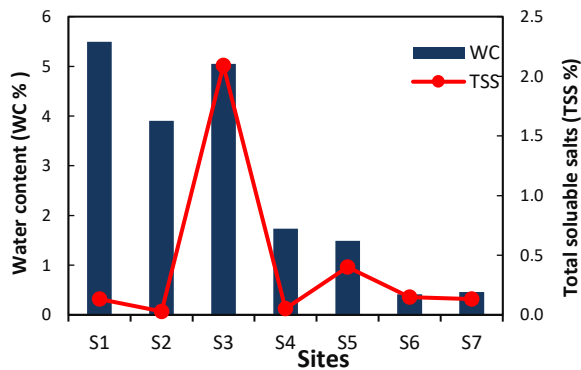


Figure 2. Water content (WC%) and total soluble salts (TSS %) in soil samples of the studied sites inhabited by *Balanites aegyptiaca* plants in El-Kharga Oasis during winter season.

The most significant influence was regionality ($\eta^2 = 0.54$) followed by seasonality ($\eta^2 = 0.43$). The minor factors ($\eta^2 = 0.03$) were exhibited by interaction between the two components.

The summer season had a greater chlorophyll stability index (CSI) than winter at all sites. In winter, the chlorophyll-a stability index varied from 51.54% to 96.44%, and in summer, it was between 86.22% and 98.23%. All sites except sites 5, 6, and 7 exhibited the opposite pattern, with chlorophyll stability b being higher in the summer than in the winter (Table 3).

Table 1. pH values, organic matter content (OM %), electric conductivity (EC mS cm⁻¹) and CO₃⁻² expressed in (%) of soil samples taken from various study sites in El-Kharga Oasis that are inhabited by *Balanites aegyptiaca* plants in the winter.

Sites	pH	EC mS cm ⁻¹	OM %	CO ₃ ⁻² %
S1	8.22	0.41	0.78	5.05
S2	8.61	0.09	0.52	5.87
S3	8.68	6.53	1.14	5.98
S4	8.61	0.16	0.55	6.04
S5	8.23	1.25	0.41	6.24
S6	8.60	0.47	0.88	6.77
S7	8.07	0.41	0.83	6.17

Table 2. Major soluble ions, cations (Ca²⁺, Mg²⁺, Na⁺, K⁺), expressed in mg g⁻¹ soil, SO₄⁻² and PO₄⁻³ (expressed in µg g⁻¹ soil) Cl (expressed in mg g⁻¹ soil) at several studied sites in El-Kharga Oasis inhabited by *Balanites aegyptiaca*.

Sites	Cations				Anions		
	Ca ²⁺	Mg ²⁺	Na ⁺	K ⁺	Cl ⁻	SO ₄ ⁻²	PO ₄ ⁻³
S1	1.87	0.09	2.48	0.46	3.52	1.62	0.46
S2	0.37	0.11	0.16	0.11	0.68	4.88	0.84
S3	1.65	1.43	15.66	2.49	1.69	6.05	0.72
S4	0.18	0.20	0.97	0.06	0.83	0.94	0.84
S5	0.85	0.54	5.23	0.36	6.11	3.83	0.74
S6	0.35	0.38	1.38	0.21	2.00	0.76	0.20
S7	0.83	0.12	1.33	0.19	0.68	3.61	0.31

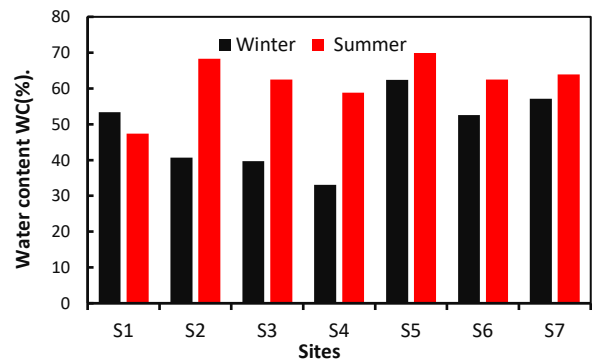


Figure 3. Water content (%) in the leaves of *Balanites aegyptiaca* plants at different studied sites in El-Kharga Oasis during both seasons winter and summer.

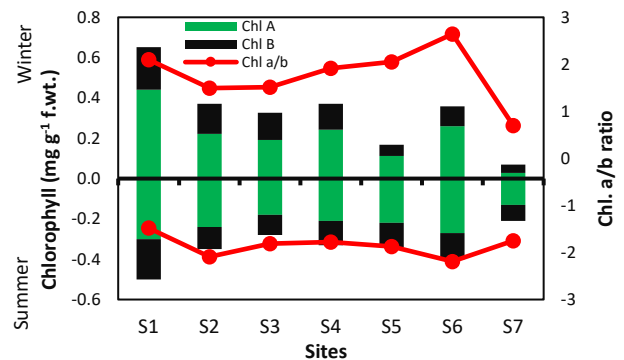


Figure 4. Chlorophyll (Chl a, Chl b) concentrations (mg g⁻¹ f.wt.) and Chl. a/b ratio in *Balanites aegyptiaca* plants at different studied sites in El-Kharga Oasis during winter and summer seasons.

Table 3. Chlorophyll concentrations (Chl a, Chl b, Chl a+b, Chl a/b and Carotenoid) in mg g⁻¹ f. wt., chlorophyll a/b ratio and chlorophyll stability index (as percentage %) in *Balanites aegyptiaca* plants at different studied sites in El-Kharga Oasis during winter and summer seasons.

St	Chl. a		Chl. b		Carotenoid		Chl a+b		Chl a/b		CSI a		CSI b	
	W	S	W	S	W	S	W	S	W	S	W	S	W	S
1	0.44	0.30	0.21	0.20	0.08	0.15	0.65	0.51	2.10	1.48	54.73	91.67	66.75	92.66
2	0.22	0.24	0.15	0.11	0.05	0.09	0.37	0.35	1.49	2.09	71.59	86.22	74.90	83.97
3	0.19	0.18	0.13	0.10	0.03	0.06	0.33	0.28	1.51	1.81	96.44	98.23	92.87	96.74
4	0.24	0.21	0.13	0.12	0.06	0.08	0.37	0.33	1.92	1.78	51.54	98.21	67.54	96.46
5	0.11	0.22	0.05	0.12	0.06	0.09	0.17	0.34	2.05	1.87	89.05	97.74	95.43	91.94
6	0.26	0.27	0.10	0.13	0.06	0.09	0.36	0.40	2.64	2.19	52.39	96.89	90.82	90.75
7	0.03	0.13	0.04	0.08	0.03	0.07	0.07	0.21	0.70	1.75	75.45	90.64	88.45	86.94

According to the statistical analysis shown in Table 4, regionality and the interaction between the two components were highly significant in the chlorophyll a/b ratio, but the influence of the seasonality factor was not statistically significant. Seasonality showed a slight effect ($\eta^2=0.01$), whereas regionality had the main effect ($\eta^2=0.58$), followed by interaction ($\eta^2=0.48$). Regarding CSI a, seasonality had the largest effect ($\eta^2 = 0.49$) followed by regionality ($\eta^2 = 0.27$), with the interaction role playing a subdominant role. For CSI b, the main influence was the interaction ($\eta^2=0.40$), with regionality following next ($\eta^2=0.39$).

Ionic composition of the plant tissue

The major ion concentrations in *B. aegyptiaca* leaves that were collected from various sites are shown in Figures 5 and 6, respectively. Except for sites 2 and 5, the concentrations of sodium in *B. aegyptiaca* leaves were higher in the winter than in the summer. The sites with the highest concentrations were site 2 (16.71 mg g⁻¹d.wt.) in the summer and site 3 (10.28 mg g⁻¹d.wt.) in the winter. Statistical analysis (Table 5) indicated that the impact of regionality and the interaction between the two parameters were found to be highly significant. Seasonality exhibited a slight effect ($\eta^2 = 0.01$) whereas interaction had the main effect ($\eta^2 = 0.68$). Except for sites 3 and 5, potassium concentrations in *B. aegyptiaca* leaves were greater in the summer than in the winter. They ranged from 8.60 to 23.07 mg g⁻¹d.wt. in the winter and from 14.07 to 22.87 mg g⁻¹d.wt. in the summer. Both the single-factor effect and the two-factor interaction were highly significant (Table 5). While seasonality had a minor role ($\eta^2 = 0.06$), regionality had the main effect ($\eta^2 = 0.74$).

The content of calcium in the leaves of *B. aegyptiaca* plants ranged between 4.00 mg g⁻¹d.wt. at site 4 and 9.33 mg g⁻¹d.wt. at site 6 in winter and between 4.40 mg g⁻¹d.wt. at site 6 and 8.00 mg g⁻¹d.wt. at site 2 in summer. Winter plant samples taken from site 6 had the highest calcium content (9.33 mg g⁻¹d.wt.).

Table 4. Statistical analysis of *Balanites aegyptiaca* water content, chlorophyll and organic components, showing analysis of variance (F-value) and determination coefficient (η^2).

Parameters	S.O.V	F	η^2
Water content	Seasonality	2003.945**	0.38
	Regionality	295.972**	0.34
	Interaction	238.991**	0.28
Chlorophyll a	Seasonality	0.931ns	0.003
	Regionality	72.332**	0.82
	Interaction	15.683**	0.18
Chlorophyll b	Seasonality	0.947ns	0.004
	Regionality	36.677**	0.86
	Interaction	5.812**	0.14
Carotenoid	Seasonality	128.763**	0.43
	Regionality	26.442**	0.54
	Interaction	1.962ns	0.03
Chl. a+b	Seasonality	1.291ns	0.003
	Regionality	75.038**	0.85
	Interaction	12.589**	0.14
Chl. a/b ratio	Seasonality	1.2021ns	0.01
	Regionality	11.644**	0.58
	Interaction	8.151**	0.48
CSI a	Seasonality	89.533**	0.49
	Regionality	8.219**	0.27
	Interaction	7.346**	0.24
CSI b	Seasonality	11.935**	0.21
	Regionality	3.626**	0.39
	Interaction	3.751**	0.40
Soluble sugars	Seasonality	56.049**	0.26
	Regionality	8.009**	0.22
	Interaction	18.731**	0.52
Soluble proteins	Seasonality	133.015**	0.45
	Regionality	21.274**	0.43
	Interaction	6.521**	0.12
Total Amino acids	Seasonality	233.39**	0.85
	Regionality	3.957**	0.09
	Interaction	2.793*	0.06

**Significant at 0.01 confidence level. *Significant at 0.05 confidence level.

Results of the statistical study (Table 5) showed that while seasonality was not significant, the effects of regionality and interaction variables were significant. All sites, except for sites 6 and 7, had higher concentrations of magnesium in the summer than in the winter. Sites 2 and 5 recorded the lowest concentration (3.09 mg g⁻¹d.wt.) during the winter, whereas site 3 recorded the maximum concentration

(11.87 mg g⁻¹d.wt.) during the summer. The effects of both the individual components and their interactions were found to be highly significant according to statistical analysis (Table 5). Regionality had the largest influence ($\eta^2=0.66$). Except for sites 4 and 5, the concentrations of chlorides in the leaves of *B. aegyptiaca* plants were higher in the summer than in the winter. Statistical analysis revealed that the effect of single factors was highly significant but the interaction between both factors had a non-significant effect. The main factor was regionality ($\eta^2=0.80$), followed by seasonality ($\eta^2=0.11$), and their combination ($\eta^2 = 0.09$). In comparison to chlorides, phosphates and sulphates had lower values in the summer and winter. In winter, sulphates varied between 7.74 and 27.74 $\mu\text{g g}^{-1}$ d.wt., and in summer, between 29.17 and 51.67 $\mu\text{g g}^{-1}$ d.wt. Site 3 had the highest number of sulphates (51.67 $\mu\text{g g}^{-1}$ d.wt.) in plants during the summer. Based on the statistical analysis, it was found that there was significant effect from both individual elements and their interactions. The values of phosphates varied from 95.55 to 157.64 $\mu\text{g g}^{-1}$ d.wt. in the winter to 96.52 to 139.39 $\mu\text{g g}^{-1}$ d.wt. in the summer. The results of the statistical analysis showed that the interactions and regionality had major effects. The largest effect was that of regionality ($\eta^2=0.84$), with the subdominant role of interaction ($\eta^2=0.16$) and a minor effect of seasonality ($\eta^2=0.0007$).

Table 5. The inorganic components (anions and cations) in leaves of *Balanites aegyptiaca* statistical analyzed demonstrating analysis of variance (F-value) and determination coefficient (η^2).

Parameters	S.O.V	F	η^2
Na ⁺	Seasonality	4.931*	0.01
	Regionality	34.56**	0.31
	Interaction	76.296**	0.68
K ⁺	Seasonality	57.614**	0.06
	Regionality	113.656**	0.74
	Interaction	30.026**	0.20
Ca ⁺²	Seasonality	3.635ns	0.04
	Regionality	6.905**	0.48
	Interaction	7.003**	0.48
Mg ⁺²	Seasonality	38.21**	0.08
	Regionality	52.664**	0.66
	Interaction	20.883**	0.26
Cl ⁻	Seasonality	9.667**	0.11
	Regionality	11.444**	0.80
	Interaction	1.299ns	0.09
SO ₄ ⁻²	Seasonality	358.591**	0.62
	Regionality	15.094**	0.16
	Interaction	21.564**	0.22
PO ₄ ⁻³	Seasonality	0.241ns	0.0007
	Regionality	45.61**	0.84
	Interaction	8.832**	0.16

**Significant at 0.01 confidence level. *Significant at 0.05 confidence level.

Metabolic components

In comparison to the other metabolites examined, the TAA level was the greatest, and the SP concentration was the lowest metabolic component of the cell. As shown in Figure 7, except for sites 3 and 4, winter values of soluble sugar levels were greater than summer values. Site 1 recorded the greatest concentration of soluble sugars in the plant samples, which was 0.42 mg g⁻¹d. wt. in the winter. In site 2, the lowest value that was recorded over the summer was 0.18 mg g⁻¹d. wt. The result of statistical analysis (Table 4) revealed that there was a highly significant effect from both the individual components and their interaction. The main effect was the interaction ($\eta^2 = 0.52$), with seasonality coming in second ($\eta^2 = 0.26$). Soluble protein concentrations are displayed in Figure 7. All sites had higher levels of soluble proteins in the winter than in the summer. In winter, the levels of soluble proteins varied from 0.11 mg g⁻¹d. wt to 0.19 mg g⁻¹d. wt. In contrast, they varied between 0.08 mg g⁻¹d. wt and 0.13 mg g⁻¹d. wt in summer. A statistical analysis showed that the effects of both the individual components and their interactions were extremely significant (Table 4). As Figure 7 illustrates, in all sites, the summer had a larger total free amino acid level than the winter. In the winter, the values fluctuated from 0.10 to 0.43 mg g⁻¹d.wt., while in the summer, they ranged from 0.79 to 1.30 mg g⁻¹d.wt. Site 1 recorded the highest values of amino acid content in the summer. The results of the statistical analysis (Table 4) showed that the effect of both the individual components and their interactions were highly significant. The main influence was that of seasonality ($\eta^2 = 0.85$).

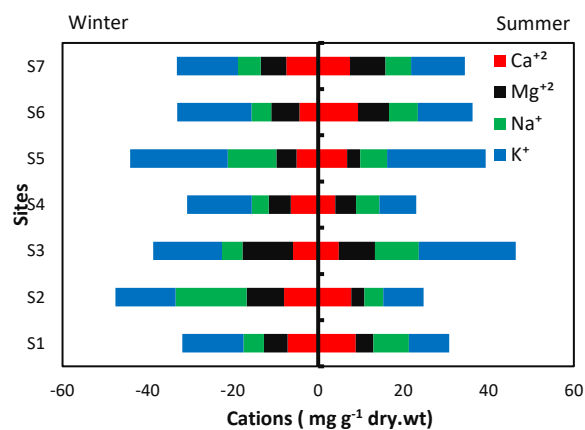


Figure 5. Major soluble cations Concentrations (Ca⁺², Mg⁺² and Na⁺, K⁺) expressed in mg g⁻¹d.w.t in leaves of *Balanites aegyptiaca* plants in both seasons winter and summer at different sites under investigation in El-Kharga Oasis.

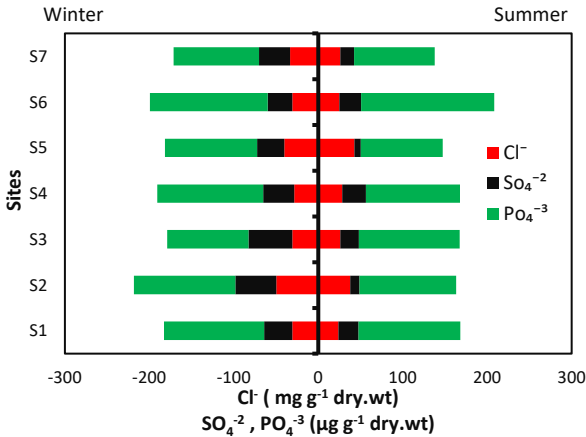


Figure 6. Concentrations of major soluble anions SO_4^{2-} and PO_4^{3-} in $\mu\text{g g}^{-1}$ d. wt. and Cl^- in mg g^{-1} d. wt. in leaves of *Balanites aegyptiaca* plants at studied sites in El-Kharga Oasis during winter and summer seasons.

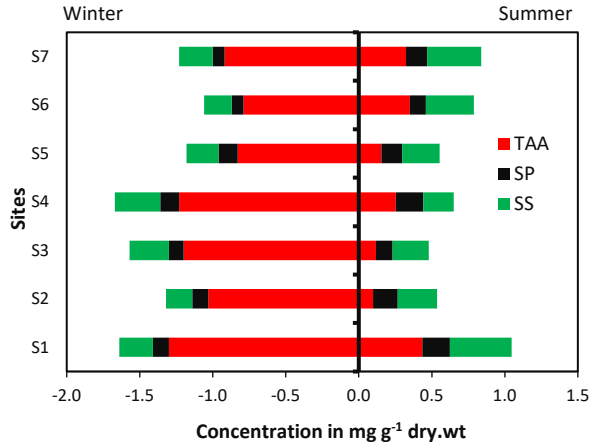


Figure 7. Soluble proteins (SP), total free amino acids (TAA) and soluble sugars (SS) Concentrations expressed as mg g^{-1} d.wt. in leaves *Balanites aegyptiaca* plants at studied sites in El-Kharga Oasis during winter and summer seasons.

DISCUSSION

The aim of recent data was to investigate the adaptive response of *Balanites aegyptiaca* (L.) Del to severe aridity in the Western Desert of Egypt. Thus, seven sites that were inhabited by the plant under study were selected to clarify the ecological features of these sites and how this species endured such extreme aridity. One of the world's driest regions is the Western Desert, which accounts for the low soil water content. Its remote location from the oceans and the absence of high altitudes, which may attract orographic rain, contribute to its extreme aridity. Even though it does not rain much, a rise in the groundwater table could increase the potential of perennials serving perennials.

In desert environments, the geographical distribution of soil moisture was found to be significantly influenced by the texture of the soil (Jafari et al., 2004; Li et al., 2018). Among other soil particle components, clay soil was found to be dominant in mechanical soil analysis. The results obtained showed that the OM% of the soil correlated with species richness, while high summer temperatures and dispersed vegetation had a negative effect on it (Salama et al., 2015). Weakly to moderately calcareous soil carbonate was found, and the estimated pH values of soil solution appeared to be slightly alkaline. Alkaline pH values were generally found in arid regions where soluble sodium salts (like Na_2CO_3) may build up. According to many authors, the results presented aligned with the general characteristics of soils in arid regions and how they related to vegetation and climate (Zahran & Willis, 2009).

As reported by El-Khatib & Abd-Elaah (1998), who reported low OM levels as well as alkaline soil surface reaction (pH) and poor biological activity, this is typical of the general characteristics of arid-region soil and its relationship with climate and vegetation, a result of the study area's extreme aridity and low moisture content. In general, there were a lot of total soluble salts. The total soluble salt content of the soil was influenced by the rates of evaporation and precipitation. High rates of evaporation resulted in a build-up of salt in the unsaturated zone, which can be dissolved by water seeping through (Tizro & Voudouris, 2007). The soil solutions in the study area therefore exhibited a comparatively high electric conductivity. This demonstrates the abundance of these habitats with soluble salts. Consequently, in semi-arid regions, the evaporation process causes groundwater to become more ionically enriched, which increases salinity. Na^{+2} and Cl^- constituted most of the estimated soluble salts in the soil. An accelerated rate of evaporation that concentrated the soil solution could be a result of the elevated concentration of salt. Salama et al., (2012, 2016) and Badri et al., (1996) reported results that are in line with the recent data. Phosphates and sulfates were generally the lowest measured anion in the soil at all sites.

One of the main environmental factors limiting plant growth and development is water scarcity (Harb et al., 2010; Song et al., 2012). In response to a scarcity of water, plants modify many physiological and metabolic processes through multiple regulatory pathways (Morison et al., 2008; Ahuja et al., 2010; Park et al., 2012; Khamis & Papenbrock, 2014). Plants

have developed two adaptation techniques to resist external stress. To reduce water loss, plant cells often reset their osmotic potential. This is accomplished by taking up inorganic ions from the environment and synthesizing suitable solutes such as soluble proteins, amino acids, and soluble carbohydrates that function as osmolytes (Du Jinyou et al., 2004; Sayed et al., 2013). The water content of *Balanites aegyptiaca* (L.) Del increased considerably in the summer than in the winter due to the combination of high summer temperatures and few water resources. When stomata closed due to high temperatures, plant transpiration continued through the cuticle (Schreiber, 2001).

According to the revealed data, chlorophyll a and chlorophyll b in the plant under study were significantly higher in the summer than in the winter. Desert plants accumulated higher chlorophyll concentrations because of their adaptive mechanisms to dry conditions (Morsy et al., 2008). The ratio of Chl a/b ranged between 0.70 and 2.64. According to Quarmby & Allen (1989), the two main pigments, essential (Chl a) and accessory (Chl b), are normally found in a 3:1 ratio. There could be two reasons for the decreasing ratio of chlorophyll a/b in the leaves: either there is more chlorophyll b than chlorophyll a, or chlorophyll a is degrading. It has been recently shown that Chl b is transformed to Chl a in higher plants as part of the Chl a/b inter-conversion cycle, allowing plants to adjust to light changes (Ito et al., 1996). Drought stress considerably reduces chlorophyll a and b contents as well as the chlorophyll a/b ratio, which has an adverse effect on photosynthetic efficiency (Sharifi & Mohammadkhani, 2016). Within chloroplasts, carotenoids are one of the most prevalent groups of lipid-soluble antioxidants (Della-Penna & Pogson, 2006). The ability of plant pigments to withstand extremely high temperatures is demonstrated by the chlorophyll stability index. It is therefore an effective indication for arid plants. In general, summer values of the chlorophyll a and b stability index are considerably higher than winter estimates. According to Al-Tantawy (1983) and Abd El-Maksoud (1983, 1987), the greater levels of chlorophyll and carotenoids found in desert plants are a result of their adaptation mechanisms to the dry climate.

There are two strategies for dealing with environmental stress. The first is based on inorganic solutes and is rapid. In stressed plants, osmotic gradient re-adjustment can be effectively achieved by the absorption, exclusion, or removal of inorganic

osmoregulatory ions such as K^+ , Na^+ , Ca^{+2} , Mg^{+2} , and Cl^- (Salama et al., 2015). The simplest way to manage external biotic and/or abiotic challenges brought on by an accumulation of inorganic solutes is by osmoregulation (Salama et al., 2018). The second approach is the accumulation of organically appropriate solutes such as amino acids, soluble proteins, and carbohydrates. The synthesis of the various organic solutes takes an extended time in this process (Wyn Jones & Pritchard, 1989).

An assessment of the role of ions primarily present in the plant solution is required to account for methods of osmoregulation through the ionic fraction of the plant osmotic material. Whether it is summer or winter, the molar quantity of accumulated cations is greater than the molar number of accumulated anions. Summer is when plants become less active biologically. They build up the quantities of organic solutes required to keep the living branches' cells turgid. However, biological activity will resume in the winter and the accumulated organic solutes will be directed to use. Consequently, plants tend to accumulate inorganic solutes rather than the organic solutes required for biological processes. The plant under study employed a unique approach to accumulate various inorganic solutes. Tolerance appears to be largely dependent on the regulation of Ca^{+2} and Cl^- uptake from soils and the partitioning of these ions within plants. The investigated species therefore relied more on K^+ and Ca^{+2} . These findings corroborated those made by Kamel (2008). Generally, the vacuole accumulates Na^+ and Cl^- , which raises the vacuole osmotic pressure, according to Wyn Jones & Storey (1981).

The results of this study show that the studied plant had significant calcium and magnesium accumulation. According to Kamel (2008), under drought stress, succulent species accumulated significant levels of Ca^{+2} and Mg^{+2} . The investigated plants enhanced uptake of Cl^- to guarantee a high osmotic pressure and raise the specific heat of cell sap to combat the high temperatures of the desert. According to Harvy (1985), chloride is primarily collected in the vacuole, where it raises the vacuole's osmotic pressure. This indicates how Cl^- plays a part in osmoregulation. It is necessary for the plants to store sulfate to prevent chloride poisoning. Summer saw a greater build-up of sulphates in *Balanites aegyptiaca* than winter. To maintain their succulence, it is well known that in arid conditions or during dry seasons, plants tend to accumulate more sulfates. Furthermore, the synthesis of amino acids containing thiol (-SH) groups requires

sulphate. In the plants under investigation, phosphorus was present in trace levels. This could be due to either the absence of sufficient phosphates in the soil or because phosphates are incorporated into plant metabolism quickly. Complementary solutes provided by the plants themselves are known as organic solutes. Biological processes require organic solutes. Plants gather under stress to withstand external stresses. Plants under heat or water stress have been shown by Irigoyen et al., (1992) and Mohammadkhani & Heidari (2008) to accumulate soluble sugars to a level that can provide significant osmotic potential.

According to Prado et al., (2000), soluble sugars are essential for maintaining water balance in plants placed under drought stress. The plant under study had a slightly higher accumulation of soluble sugars. Increased drought stress lowers the amount of chlorophyll in leaves and the activities of photosynthetic enzymes, which decreases the efficiency of photosynthesis (Anjum et al., 2011; Al-Falahi et al., 2022). The decrease in photosynthetic activity might also be attributed to a reduction in stomatal mobility (Abid et al., 2018; Salama et al., 2021).

In response to environmental stress, plants may increase their water binding molecules to prevent amino acids from being incorporated into proteins. During the winter, the soluble protein level of the plants under study increased significantly. The surface exposed to binding water rises with protein content, and drought resistance is associated with bound water (Du Jinyou et al., 2004). Nitrogen metabolites can assist certain xerophytic organisms osmotically respond to stress (Rayan & Farghali, 2007). Generally, under stressful conditions, soluble sugars act through hydrogen bonding to interact with proteins and cell membranes to avoid protein denaturation. To preserve the hydrophilic contacts in the cell membrane and the structure and function of proteins, the free hydroxyl groups of soluble sugars balance those of water. Because of this, the soluble sugar levels of the research plant dropped during the summer. These findings corroborate the study conducted by (Poonam et al., 2016). The level of total free amino acids in *Balanites aegyptiaca* (L.) Del was generally much higher in the summer than it was in the winter. These results align with Migahid's (2003).

According to Ali et al., (2019), amino acids behave similarly through three different mechanisms: serving as suitable osmolytes, controlling pH, or serving as a

store for carbon or nitrogen. Total free amino acid concentration is greater than that of soluble protein and sugar. Several authors (Singh et al., 1973; Miller & Leschine, 1984; Reddy & Vora, 1985) who used different economic plants also had similar outcomes. One could consider this rise in amino acid content to be a plant's adaptation strategy to salinization. It is evident from the data in the current study that *Balanites aegyptiaca* (L.) Del is more suited to the drought-prone circumstances prevalent in the study area. Tesfay et al., (2014) stated that *Balanites aegyptiaca* (L.) Del is a multipurpose tree that is prized for its ability to offer fuel wood, food, and medicinal goods for subsistence living in dry and semi-arid regions with limited other options. As stated by Chothani & Vaghasiya (2011), creating a picture of variation within the natural range and developing the ability to produce plants with desirable traits are priorities because the potential of *Balanites aegyptiaca* (L.) Del under control is still unexplored. According to Orwa et al., (2009), the plant is resistant to extreme heat, intense light, high wind, and drought.

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