

Turkish Journal of Botany

http://journals.tubitak.gov.tr/botany/

Research Article

Ecophysiological responses of *Calligonum polygonoides* and *Artemisia judaica* plants to severe desert aridity

Fawzy SALAMA*, Suzan SAYED, Ayat ABD EL-GELIL Botany Department, Faculty of Science, Assiut University, Assiut, Egypt

Received: 03.04.2014 • Accepted: 30.07.2014 • Published Online: 16.03.2015	•	Printed: 10.04.2015
---	---	---------------------

Abstract: The present study, conducted in 2011 and 2012, deals with the response of *Calligonum polygonoides* and *Artemisia judaica* to the drought conditions in Wadi El-Assiuty and its tributary Wadi Habib in the middle part of the Eastern Desert of Egypt. Soil and plant samples were collected from 50 stands in the wet (winter) and dry (summer) seasons. In the selected plants chlorophyll (Chl a and b) contents as well as the Chl stability index tended to increase significantly during the summer season. Calcium and magnesium were accumulated in considerable amounts in the studied plants. Amounts of calcium accumulated were higher than those of magnesium. Phosphates appeared in the plants in low amounts and correlated positively with those found in the soil. The studied plants showed an increase in soluble sugars accumulation. Soluble protein content increased significantly during the winter season with a corresponding decrease in free amino acids. It is quite clear that *Calligonum polygonoides* plants were better adapted to drought conditions prevailing in the area under study than *Artemisia judaica*. This was judged by the average metabolic potentiality as soluble metabolites (soluble sugars and soluble proteins) are much higher in *Calligonum than* in *Artemisia*.

Key words: Chlorophyll stability, Egypt, osmoregulation, soluble sugars, soluble proteins, xerophytes

1. Introduction

Plants are continuously exposed to environmental stimuli that influence development and growth and determine productivity. High and low temperatures, mineral imbalance, excess or insufficient light, and lack of water are stressors that compromise productivity (Lawlor, 2002).

Water availability is not the only restrictive factor for plant growth in the arid and semiarid zones; nutrients are also usually scarce, and the excess of solar radiation is often an important additional source of stress for plants in these ecosystems (Valladares, 2003). Results reported by Radwan (2007) showed that a combination of drought, high temperature, and irradiation imposed a complex of stresses on seed germination, seedling establishment, and plant survival in arid habitats.

Drought is a meteorological and environmental event, defined as the absence of rainfall for a period of time long enough to cause depletion of soil moisture (water stress) and damage to plants, or as an increase in the rate of transpiration accompanied by high temperature (heat stress), hastening the occurrence of injurious dehydration (Wang et al., 2005). Drought may be permanent, as in a desert area, or seasonal, as in areas with well-defined wet and dry seasons; it can also be unpredictable, as in many humid climates (Akhondi et al., 2006). Rain, though scanty, is the main source of water supply to the Egyptian deserts and semideserts. In the southern and western rainless deserts of Egypt there is no available moisture in the soil that would support natural vegetation. The desert surface soil is air dried all year except for a few days in winter and spring. There is a permanently wet layer in the deep soil strata. This layer, with low water content of about 2–4%, is within reach of the deeply penetrating roots of perennials, and these plants can make use of such low moisture (Salama et al., 2012).

Plants grown in drying soil regulate their water status via numerous tactics, i.e. osmotic adjustment, stomatal pores, turgor maintenance, root distribution, and leaf canopy properties (Davies and Meizner, 1990). An increase in the concentration of organic solutes will also increase the amount of bound water inside cells. This is useful for desert plants that suffer from high temperature and water deficit (Sayed et al., 2013). Compartmentalization of ions into vacuoles, especially for plants that have large vacuoles, is helpful in decreasing the water potential inside the plant tissues and helps prevent cytoplasm destruction (Cheesman, 1988). Osmotic adjustment provides the means to avoid cellular dehydration, which is essential for maintaining cellular activity (Bartels and Ramanjulu,

^{*} Correspondence: fawzy_salama2010@yahoo.com

2005). Initially, it was thought that osmotic adjustment occurred only in plants subjected to high salinities; however, later studies in plants grown on dry soils showed that this response is also common in conditions of water stress (Cushman, 2001; Sayed et al., 2013). Xerophytic plant species adapt to drought due to their ability to maintain turgidity and water uptake (Martínez et al., 2005). According to Mile et al. (2002), the ability of plants to accumulate the inorganic ions in high quantities inside their tissues is the most important mechanism to maintain the plant water potential more negative than the external medium to maintain the water uptake.

Drought resistance is a complex trait involving several interacting properties. Accordingly, the present study aims to study the physiological behavior of 2 desert plant species to identify and understand drought resistance mechanisms.

2. Materials and methods

2.1. Soil analysis

The soil of certain stands was sampled where a great abundance of plants was observed. Samples were collected from 50 stands in the wet (winter) and dry (summer) seasons. Three replicates were taken from each stand and carried to the laboratory in plastic bags.

2.1.1. Organic matter determination

Organic matter was determined in the soil samples by the dichromate oxidation method, according to Walkley and Black (1934).

2.1.2. Determination of soil water content and soil extract Water content of the soil samples was determined by weighing the fresh soil sample and drying it in an oven at 105 °C for 24 h. Then the dry weight was determined. The water content of the sample is calculated as percent of the dry weight. Soil extracts (1:5) were prepared by shaking 20 g of soil with 100 mL of distilled water for 1 h, followed by filtration to obtain a clear filtrate.

2.1.3. Determination of water soluble ions

Chloride was determined volumetrically according to Jackson (1967), and sulfate was determined turbid metrically as $BaSO_4$, according to procedures described by Bardsley and Lancaster (1965). Sodium and potassium were measured by flame photometry, according to Williams and Twine (1960). Calcium and magnesium were determined volumetrically by the versene method, as described by Johnson and Ulrich (1959). Phosphorus was determined colorimetrically as phosphomolybdate, according to Jackson (1967). Carbonates and bicarbonates were estimated according to the method described by Piper (1947).

2.1.4. Electric conductivity, total soluble salts, and pH value

Electric conductivity (EC) and total soluble salts (TSS) of the soil filtrate were determined using a conductivity meter (model 4310, JEN WAY), according to Jackson (1967). An electric pH meter (model pH-206, Lutron) was used to determine the soil reaction of the collected samples.

2.2. Plant analysis

2.2.1. The selected species

Calligonum polygonoides and *Artemisia judaica* plants were assessed for their ecophysiological behavior. *Calligonum polygonoides* from the family Polygonaceae is a shrub, stems many-branched; main branches woody, rigid; young branches herbaceous, thin, green; leaves minute. *Artemisia Judaica* from the family Asteraceae is an annual, perennial, or shrub; leaves alternate strongly aromatic, densely grayish, tomentose low shrub, 30–80 cm; stems manybranched from the base.

2.2.2. Determination of chlorophyll parameters

Chlorophyll was extracted in test tubes from a definite weight of fresh healthy leaves in 10 mL of 80% aqueous ethanol (Welfare et al., 1996). To estimate chlorophyll stability a definite weight of fresh healthy leaves was placed in 10 mL of distilled water and heated in a water bath at 56 \pm 1 °C for 30 min. Chlorophyll stability index (CSI) was expressed as percentage between the chlorophyll content in the heated sample and the fresh sample for chlorophyll a or b. Finally, these pigment fractions were calculated as mg g⁻¹ fresh weight (FW). Chlorophyll a/b ratio was also calculated.

2.2.3. Preparation of plant extraction

The fresh shoots were dried in an oven at 70 $^{\circ}$ C for 24 h, and then the dry shoots were ground into fine powder. The extraction technique was adopted from El-Sharkawi and Michel (1977).

2.2.4. Estimation of soluble solutes in plant extract

In the plant extract several analyses were run including the following.

2.2.4.1. Determination of soluble osmotically active metabolites

The analysis of soluble osmotically active metabolites included the determination of soluble sugars (as carbon metabolites), total free amino acids, and soluble proteins (as nitrogen metabolites). They were determined according to procedures described by Dubois et al. (1956), Lee and Takahashi (1966), and Lowry et al. (1951), respectively.

2.2.4.2. Determination of different constituents of the ionic fraction

The contents of different anions and cations in plant extracts were determined as previously mentioned for soil extract.

2.3. Statistical analysis

Statistical inferences necessary to evaluate the effects and relative roles (shares) of single factors and their interactions on the parameters tested included analysis of variance (F value), coefficient of determination (η^2), and simple linear correlation coefficient (r). Data were statistically analyzed using SAS and SPSS programs. The coefficient of determination (η^2) has been devised to evaluate the relative effect of each single factor and interaction in contributing to the total response. The simple linear correlation coefficient (r) is used to elucidate the relationship between the internal mineral element contents in the plant tissues and those in the soil.

3. Results

3.1. Soil analysis

3.1.1. Soil water content and total soluble salts

Generally, soil water content (Table 1) varied with collection stand differences. It ranged between 0.11% and 0.89% in summer, while in winter its range was between 0.12% and 0.81%. Stand 11 recorded the highest values of water content during summer. The lowest value of water content was recorded at stand 45.

The total soluble salts (TSS%) of soil extracts during summer were higher than in winter (Table 1). The highest

Table 1. pH values, electric conductivity (EC mS cm⁻¹), organic matter content (OM %), water content (WC%), and total soluble salts (TSS%) in the collected soil samples from different studied stands inhabited by *Calligonum polygonoides* and *Artemisia judaica* plants in Wadi El-Assiuty in summer and winter seasons.

Ct l	pH		EC	EC		OM %		WC%		TSS%	
Stand	Sum	Win									
05	8.15	6.45	0.18	0.17	0.14	0.11	0.33	0.18	0.06	0.05	
08	8.04	6.73	0.25	0.21	0.09	0.10	0.20	0.20	0.08	0.07	
09	7.99	6.62	0.36	0.29	0.07	0.07	0.44	0.69	0.11	0.09	
10	8.14	6.91	0.45	0.41	0.12	0.11	0.71	0.81	0.14	0.13	
11	8.14	6.60	0.27	0.30	0.45	0.23	0.89	0.73	0.09	0.10	
12	8.22	6.43	0.44	0.58	0.18	0.16	0.22	0.44	0.14	0.19	
13	8.42	6.60	0.18	0.16	0.23	0.13	0.24	0.20	0.06	0.05	
14	8.23	6.77	0.29	0.14	0.23	0.10	0.21	0.21	0.09	0.05	
15	7.86	6.63	0.45	0.19	0.24	0.15	0.20	0.20	0.14	0.06	
16	8.04	6.76	0.15	0.24	0.10	0.09	0.31	0.51	0.05	0.08	
17	8.07	6.60	0.21	0.20	0.15	0.08	0.14	0.31	0.07	0.07	
18	8.11	6.63	0.14	0.19	0.11	0.05	0.47	0.14	0.05	0.06	
19	7.89	6.67	0.20	0.19	0.18	0.04	0.18	0.21		0.06	
27	8.08	6.63	0.95	0.20	0.06	0.05	0.14	0.16	0.09	0.06	
28	8.03	6.68	0.30	0.24	0.07	0.08	0.44	0.29	0.10	0.08	
29	8.02	6.62	0.13	0.20	0.05	0.03	0.20	0.29	0.04	0.06	
30	7.92	6.75	0.35	0.19	0.10	0.07	0.24	0.29	0.11	0.06	
31	7.99	7.26	0.41	0.20	0.05	0.07	0.29	0.32	0.13	0.06	
32	8.05	7.15	0.26	0.19	0.14	0.14	0.13	0.16	0.08	0.06	
33	8.14	7.11	0.19	0.18	0.11	0.11	0.18	0.18	0.06	0.06	
34	8.00	6.68	0.18	0.18	0.09	0.10	0.27	0.30	0.06	0.06	
35	8.04	7.21	0.29	0.16	0.09	0.05	0.19	0.19	0.09	0.05	
36	7.92	6.74	0.14	0.18	0.18	0.11	0.17	0.18	0.05	0.06	
37	8.11	6.83	0.17	0.20	0.18	0.07	0.19	0.30	0.06	0.06	
38	8.14	6.69	0.18	0.21	0.10	0.07	0.19	0.22	0.06	0.07	
39	8.03	6.75	0.15	0.20	0.09	0.12	0.23	0.26	0.05	0.07	
40	7.88	6.99	0.20	0.18	0.07	0.08	0.22	0.26	0.06	0.06	
41	7.93	7.18	0.25	0.17	0.11	0.10	0.29	0.18	0.08	0.05	
42	7.85	6.74	0.20	0.19	0.08	0.11	0.26	0.18	0.06	0.06	
43	7.84	7.13	0.23	0.24	0.13	0.09	0.12	0.16	0.07	0.08	
44	7.92	7.29	0.17	0.19	0.16	0.11	0.12	0.24	0.05	0.06	
45	7.87	7.64	0.21	0.21	0.12	0.07	0.11	0.27	0.07	0.07	
46	7.88	7.52	0.20	0.17	0.10	0.12	0.17	0.12	0.06	0.05	
47	7.86	7.24	0.17	0.20	0.09	0.09	0.21	0.21	0.05	0.07	
48	7.84	7.21	0.23	0.25	0.07	0.09	0.11	0.17	0.07	0.08	
49	7.81	6.71	0.24	0.20	0.16	0.16	0.20	0.20	0.08	0.06	
50	7.63	6.91	0.19	0.19	0.09	0.05	0.17	0.31	0.06	0.06	

value (0.14%) of TSS was recorded during summer in stands 14 and 15. The lowest value (0.04%) was reported at stand 29. In winter the highest value was in stands 11 and 12, and the lowest concentration was recorded at stand 31.

3.1.2. Organic matter

Generally, organic matter content (%) of soil samples was higher in summer than in winter, except in 9 stands where the opposite trend was noted (Table 1). Maximum value of organic matter content (0.45%) was recorded at stand 11 during summer, whereas the minimum value (0.03%) was at stand 29 during winter. In summer, organic matter content fluctuated between 0.05% and 0.45%.

3.1.3. pH value

The soil solution in these habitats is in the neutral or slightly alkaline range. The pH values of soil solutions are illustrated in Table 1. They ranged between 7.63 and 8.42 in the summer season and between 6.43 and 7.64 in winter. The highest pH value (8.42) was recorded at stand 13 during summer, and the lowest value (6.43) was at stand 12 during winter.

3.1.4. Electric conductivity

As shown in Table 1, the values of electric conductivity (EC) of soil solutions were higher in summer than in

winter in most stands. Its values ranged between 0.13 and 0.95 mS cm⁻¹ during summer, while in winter it ranged between 0.14 and 0.58 mS cm⁻¹. The highest (0.95 mS cm⁻¹) and lowest (0.13 mS cm⁻¹) values of EC were recorded at stand 27 and stand 29, respectively.

3.1.5. Concentration of major ions in the soil

Generally, concentrations of sodium were higher in summer than in winter (Figure 1). In summer, sodium concentrations ranged between 0.02 and 0.16 mg g⁻¹ soil, while in winter they ranged between 0.01 and 0.07 mg g⁻¹ soil. The highest value recorded during summer was at stand 15.

Potassium was higher in summer than in winter at all stands (Figure 1). Its contents ranged between 0.018 mg g⁻¹ soil and 0.071 mg g⁻¹ soil in summer, while in winter the values ranged from 0.01 to 0.041 mg g⁻¹ soil. The highest concentration was recorded at stand 15.

Calcium and magnesium show the same behavior in most stands (Figure 1). During summer calcium concentrations of the soil samples ranged between 0.10 and 0.37 mg g⁻¹ soil. In winter, concentrations fluctuated between 0.13 and 0.43 mg g⁻¹ soil. Stand 12 had the highest concentration of calcium during winter. Concentrations

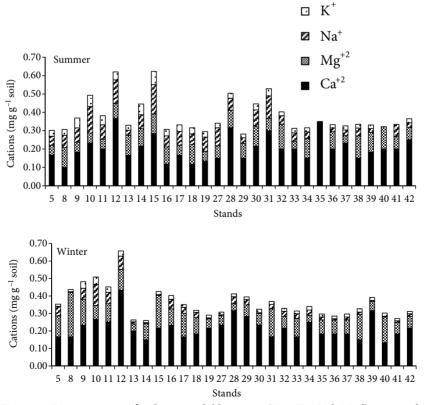


Figure 1. Concentrations of soil major soluble cations (Na⁺, K⁺, Ca⁺², Mg⁺²) expressed in mg g⁻¹ soil of the different studied stands inhabited by the investigated plants in Wadi El-Assiuty in summer and winter seasons.

of magnesium in soil samples during summer ranged between 0.04 and 0.18 mg g⁻¹ soil, while in winter they varied from 0.04 to 0.25 mg g⁻¹ soil. The highest value of magnesium was recorded in stand 8.

Chlorides concentrations in soil samples showed higher values during summer in most stands in comparison with winter (Figure 2). Concentrations ranged between 0.06 and 0.16 mg g^{-1} soil in summer. In winter, the concentrations varied from 0.04 to 0.12 mg g^{-1} . The highest concentration of chlorides was recorded at stand 15.

Sulfates were higher during summer months than winter months (Figure 2). The highest recorded concentration was during the summer season at stand 10 (4.86 μ g g⁻¹ soil). Sulfates concentrations ranged between 0.15 and 4.86 μ g g⁻¹ soil in summer, while during winter concentrations were between 0.28 and 3.45 μ g g⁻¹ soil.

Phosphates in the soil samples of studied stands appeared in small quantities in both summer and winter seasons (Figure 1). In general, phosphates concentrations during winter were higher than during summer.

Generally, bicarbonates contents were higher in summer than in winter months (Figure 1). They did not exceed 1.83% (stand 37) in summer and 0.15% (stands 11, 30, 31, and 40) during winter.

3.2. Plant analysis

3.2.1. Chlorophyll content and chlorophyll stability index The concentrations of chlorophyll a and chlorophyll b in *Calligonum polygonoides* are illustrated in Figures 3a and b. Chlorophyll a content was higher in summer than in winter in most studied stands. Concentrations in summer ranged between 0.09 and 0.27 mg g⁻¹ FW, while in winter it ranged between 0.05 and 0.27 mg g⁻¹ FW. Chlorophyll b content was higher in summer than in winter with few exceptions. In winter, chlorophyll b concentrations ranged between 0.050 and 0.186 mg g⁻¹ FW, while in summer they ranged between 0.047 and 0.191 mg g⁻¹ FW The chlorophyll a/b ratio ranged between 0.74 and 2.92 in winter, and 1.27 to 2.93 in summer.

The stability index (CSI) of chlorophyll was higher in summer than in winter at most studied stands. Chl a stability ranged between 20.86% and 97.17% in summer and 39.89% and 96.56% in winter. Chl b stability fluctuated between 25.59% and 94.38% in summer, but in winter it ranged between 33.68% and 88.38%.

The statistical analysis in Table 2 shows that the effect of single factors and their interactions were significant for chlorophyll parameters tested. Seasonality had a dominant effect (Chl a) followed by interaction. For chlorophyll b the

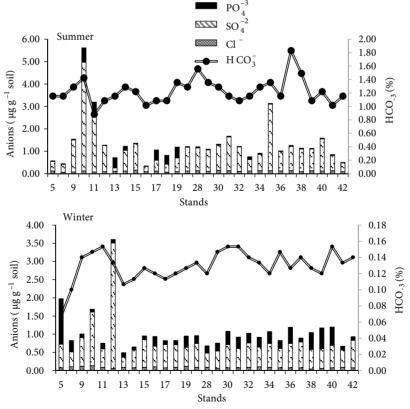


Figure 2. Concentrations of Cl⁻ (mg g⁻¹ soil), SO₄⁻², PO₄⁻³ (μ g g⁻¹ soil), and HCO₃⁻ (%) in the different studied stands inhabited by investigated plants in Wadi El-Assiuty in summer and winter seasons.

SALAMA et al. / Turk J Bot

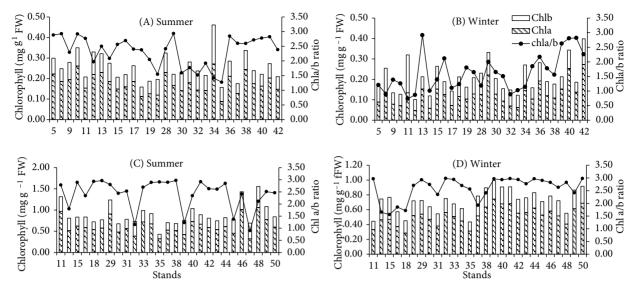


Figure 3. Chlorophyll (Chl a, Chl b) concentrations (mg g^{-1} FW) and Chl a/b ratio at different stands inhabited by *Calligonum polygonoides* (A and B) and *Artemisia judaica* (C and D) in summer and winter, respectively.

e i					
Parameters	S.O.V.	C. polygonoid	es	A. judaica	
	5.0. v.	F	η^2	F	η^2
	Seasonality	1169.2**	0.59	73.96**	0.08
Chlorophyll a	Regionality	13.05**	0.18	13.61**	0.43
	Interaction	16.49**	0.23	15.52**	0.49
	Seasonality	6.3*	0.02	147.5**	0.17
Chlorophyll b	Regionality	12.03**	0.47	12.46**	0.37
	Interaction	13.06**	0.51	15.46**	0.46
	Seasonality	740.1**	0.63	03.70*	0.02
Chl _{a/b} ratio	Regionality	10.34**	0.25	5.04**	0.47
4,0	Interaction	5.23**	0.12	5.40**	0.51
	Seasonality	9.6*	0.02	74.30**	0.13
CSI	Regionality	15.4**	0.46	12.30**	0.47
	Interaction	17.3**	0.52	10.40**	0.40
	Seasonality	95.20**	0.08	24.00**	0.04
CSI _b	Regionality	22.94**	0.55	11.94**	0.56
	Interaction	15.38**	0.37	08.55**	0.40
	Seasonality	9.6**	0.03	20.40**	0.09
Soluble sugars	Regionality	9.81**	0.68	05.77**	0.54
	Interaction	4.14**	0.29	03.99**	0.37
	Seasonality	84.4**	0.10	59.40**	0.10
Soluble proteins	Regionality	19.43**	0.60	12.31**	0.51
	Interaction	9.55**	0.30	09.32**	0.39
	Seasonality	101.4**	0.14	53.40**	0.02
Total amino acids	Regionality	9.9**	0.38	79.00**	0.81
	Interaction	12.58**	0.48	16.16**	0.17

Table 2. Statistical analysis for chlorophyll and organic components of *Calligonum polygonoides* and *Artemisia judaica* showing analysis of variance (F-value) and determination coefficient (η^2).

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

role of interaction was dominant, followed by regionality, and the role of seasonality was minor. Seasonality had the dominant effect on the Chl a/b ratio followed by regionality, while the interaction role was minor. In the case of CSIa interaction had the dominant effect followed by regionality. The reverse held true for CSIb where regionality had the dominant effect, and interaction was subdominant.

The concentrations of chlorophyll a and chlorophyll b in *Artemisia judaica* are illustrated in Figures 3C and 3D. Chlorophyll a content was higher in summer than in winter at most studied stands. In summer it ranged between 0.32 and 1.05 mg g⁻¹ FW, while in winter it ranged between 0.74 and 0.28 mg g⁻¹ FW. Chlorophyll b content was higher in summer than in winter with few exceptions. In winter these concentrations ranged between 0.11 and 0.30 mg g⁻¹ FW, while in summer concentrations varied from 0.11 to 0.50 mg g⁻¹ FW. The chlorophyll a/b ratio ranged between 1.56 and 2.98 in winter, while in summer it varied from 0.90 to 2.

The stability index of chlorophyll was higher in winter than in summer in most studied stands. CSIa ranged between 26.39% and 83.58% in summer and 19.55% and 92.53% in winter; CSIb fluctuated between 28.15% and 87.76% in summer, but in winter it ranged between 22.22% and 94.97%. The effects of single factors and their interactions were significant for chlorophyll parameters (Table 2). Interaction had a dominant effect on Chl a content, followed by seasonality. For chlorophyll b, the role of interaction was dominant followed by regionality, and the role of seasonality was minor. Interaction had the dominant effect for Chl a/b ratio followed by regionality. In the case of CSI a and b, regionality had the dominant effect followed by interaction.

3.2.2. Ionic contents of the plant tissue

In general, cations were found in higher concentrations inside the plant tissues than in soil samples. Concentrations of sodium were higher in winter than in summer in the 2 studied plants. In *Calligonum polygonoides* (Figure 4) they ranged between 0.83 and 10.85 mg g⁻¹ DW in summer and between 0.67 to 17.75 mg g⁻¹ DW in winter. The highest concentrations were recorded in stand 17. The effects of single factors and their interactions on Na⁺ content were highly significant (Table 3). Regionality had the dominant role followed by interaction.

Potassium concentrations were higher in summer than in winter at all stands except stand 8, where concentrations showed the reverse trend. Concentrations of potassium ranged between 3.15 and 13.23 mg g⁻¹ DW in summer and 1.51 and 6.00 mg g⁻¹ DW in winter. The highest concentration was recorded in stand 10, while the lowest

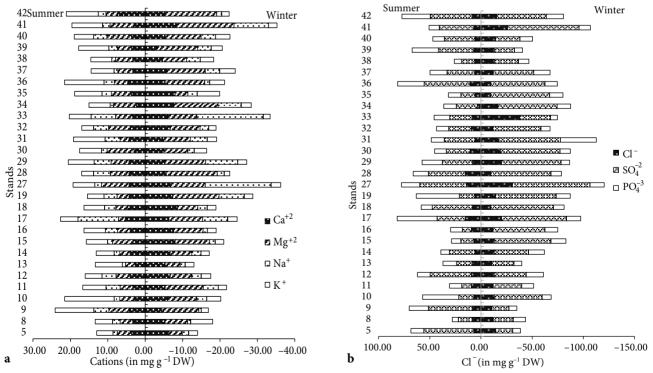


Figure 4. Concentrations of *Calligonum polygonoides* major soluble anions (Cl⁻) in mg g⁻¹ DW and (SO₄⁻² and PO₄⁻³) μ g g⁻¹ DW, and major soluble cations (Na⁺, K⁺, Ca⁺², Mg⁺²) in mg g⁻¹ DW at different studied stands in summer and winter seasons.

Demonsterne	S.O.V.	C. polygon	oides	A. judaica		
Parameters		F	η^2	F	η^2	
	Seasonality	154.5**	0.05	01.36	0.01	
Na ⁺	Regionality	81.28**	0.58	17.79**	0.49	
	Interaction	51.37**	0.37	18.23**	0.50	
	Seasonality	372.8**	0.50	527.5**	0.35	
K+	Regionality	7.23**	0.29	25.80**	0.45	
	Interaction	5.57**	0.21	10.90**	0.19	
	Seasonality	77.06**	0.50	334.0**	0.44	
Ca+2	Regionality	1.30ns	0.23	10.10**	0.35	
	Interaction	1.52ns	0.27	05.94**	0.21	
	Seasonality	421.7**	0.45	28.25**	0.14	
Mg ⁺²	Regionality	10.54**	0.32	03.47**	0.38	
	Interaction	7.51**	0.23	04.40**	0.48	
	Seasonality	384.8**	0.33	76.05**	0.22	
Cl-	Regionality	15.95**	0.38	06.99**	0.50	
	Interaction	12.48**	0.29	03.80**	0.28	
	Seasonality	124.2**	0.26	351.1**	0.28	
SO_4^{-2}	Regionality	6.9**	0.39	19.32**	0.41	
	Interaction	6.14**	0.35	14.60**	0.31	
	Seasonality	6.21	0.13	35.64**	0.13	
PO_4^{-3}	Regionality	1.28	0.42	05.60**	0.51	
	Interaction	1.37	0.45	03.97**	0.36	

Table 3. Statistical analysis of the inorganic components (anions and cations) of *Calligonum polygonoides* and *Artemisia judaica* plants showing analysis of variance (F-value) and determination coefficient (η^2).

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

concentration was recorded during winter. Statistical analysis (Table 3) revealed that the single factor effect was highly significant. Seasonality had the dominant effect followed by regionality.

Calcium and magnesium were more concentrated in winter than in summer (Figure 4). Concentrations of calcium ranged between 2.33 and 7.33 mg g⁻¹ DW in summer, while in winter they ranged between 3.00 and 8.67 mg g-1 DW The highest value of calcium concentrations was recorded in plants collected from stand 18 during winter. The effects of single factors were highly significant. Seasonality had the dominant effect (Table 3). Concentrations of magnesium ranged between 1.80 and 7.40 mg g $^{\scriptscriptstyle -1}$ DW in summer and 3.40 and 15.40 mg g-1 DW in winter. The highest concentration was detected at stand 41 during winter. Statistical analysis for magnesium concentrations (Table 3) revealed that the effect of single factors and their interactions were highly significant. Seasonality had the dominant effect followed by regionality and interaction.

Chlorides concentrations were higher in winter than in summer at all studied stands (Figure 4). The concentrations of Cl⁻ varied from 3.55 to 15.38 mg g^{-1}

DW during summer, and ranged between 9.47 and 37.87 mg g⁻¹ DW during winter. Data in Table 3 revealed that the single factors as well as their interactions were highly significant in affecting Cl⁻. Regionality had the dominant role, followed by seasonality, and then interaction.

Sulfates and phosphates had low values in summer and winter compared with Cl⁻ (Figure 4). Sulfates contents fluctuated between 0.08 and 0.02 mg g⁻¹ DW in winter and 0.05 and 0.01 mg g⁻¹ DW in summer. The highest value of sulfates concentration was recorded at stand 27. The effect of single factors was highly significant. Regionality had the dominant role followed by interaction.

Phosphates concentrations ranged between 0.01 and 0.035 mg g⁻¹ DW in winter, while in summer they ranged between 0.01 and 0.042 mg g⁻¹ DW Statistical treatment revealed that the single factor effects were nonsignificant. The dominant role was occupied by the interaction ($\eta^2 = 0.45$).

Concentrations of sodium in *Artemisia juadica* were higher in summer than in winter (Figure 5). They ranged between 0.67 and 9.80 mg g⁻¹ DW in summer and 1.50 and 7.05 mg g⁻¹ DW in winter. The highest concentrations were recorded at stands 29 and 34. The effects of single

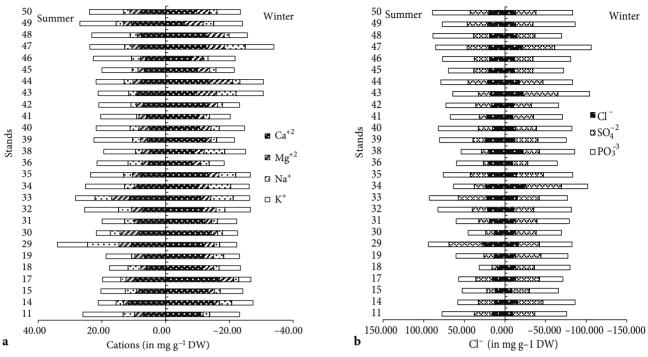


Figure 5. Concentrations of *Artemisia judaica* major soluble anions (Cl⁻) in mg g⁻¹ DW and (SO₄⁻² and PO₄⁻³) μ gm g⁻¹ DW, and major soluble cations (Na⁺, K⁺, Ca⁺², Mg⁺²) in mg g⁻¹ DW at different studied stands in summer and winter seasons.

factors and bifactorial interactions on Na⁺ content were highly significant (Table 3). Interaction and seasonality had equally dominant roles ($\eta^2 = 0.50$ and $\eta^2 = 0.49$, respectively).

Potassium concentrations in *Artemisia juadica* were higher in summer than in winter except at stands 14, 18, and 30, where concentrations showed the reverse trend (Figure 5). Concentrations of potassium ranged between 4.41 and 12.65 mg g⁻¹ DW in summer and 3.89 and 9.43 mg g⁻¹ DW in winter. The highest concentration was recorded at stand 11 during summer, while the lowest concentration was reported at stand 17. Statistical analysis (Table 3) revealed that the single factors effect was highly significant. Regionality had the dominant role followed by seasonality.

Calcium and magnesium (Figure 5) were more concentrated in winter than in summer. Concentrations of calcium ranged between 5 and 11.67 mg g⁻¹ DW in summer, while in winter they ranged between 6 and 17 mg g⁻¹ DW The highest value of calcium concentrations was detected in plants collected during winter. Single factors significantly affected calcium and magnesium concentrations. Seasonality had the dominant effect.

Concentrations of magnesium ranged between 0.40 and 6 mg g⁻¹ DW in summer and 0.80 and 7.40 mg g⁻¹ DW in winter. The highest concentration was detected at stand 44. The effect of single factors and their interactions were

highly significant (Table 3). Interaction had the dominant effect followed by regionality.

In most studied stands chlorides concentrations were higher in summer than in winter (Figure 5). Concentrations of Cl⁻ varied from 9.47 to 28.43 mg g⁻¹ DW during summer and 8.28 and 26.03 mg g⁻¹ DW during winter. Data in Table 3 revealed that the single factors as well as their interactions were highly significant in affecting Cl⁻. Regionality had the dominant effect followed by interaction.

Sulfates and phosphates (Figure 5) had low values in summer and winter as compared with Cl⁻; sulfates contents fluctuated between 18.5 and 43.7 μ g g⁻¹ DW in winter and 6.3 and 40.5 μ g g⁻¹ DW in summer. The highest value was recorded at stand 34. The effects of single factors and their interactions were highly significant. Regionality had the dominant role followed by interaction.

Phosphates concentrations (Figure 5) ranged between 28.9 and 52.1 μ g g⁻¹ DW in winter, while in summer its range was between 15.7 and 49.9 μ g g⁻¹ DW Statistical treatment revealed that the effects of single factors and their interactions were significant. The dominant role was regionality.

3.2.3. Correlation analysis

The correlation analysis was carried out between the concentrations of major soluble ions in *Calligonum polygonoides* shoots and soil samples (Table 4).

Concentrations of SO_4^{-2} in plant shoots correlated negatively with those in soil in both winter and summer. Cl^- was correlated negatively in summer and positively in winter. Both correlations were statistically nonsignificant. PO_4^{-3} had a positive significant correlation in summer and negative nonsignificant correlation in winter. Ca^{+2} , Mg^{+2} , Na⁺, and K⁺ were correlated positively (nonsignificant) in summer and negatively during winter.

Table 4 shows that concentrations of Na⁺ and PO₄⁻³ in *Artemisia judaica* shoots correlated negatively (nonsignificant) with those in soil in both winter and summer. Mg was correlated negatively in summer and positively in winter. Both correlations were statistically nonsignificant. Ca⁺² had positive nonsignificant correlation in summer and negative nonsignificant correlation in winter. Cl⁻ and SO₄⁻² were correlated positively (nonsignificant) in summer and winter seasons. K⁺ had a negative significant correlation during summer and positive (nonsignificant) correlation during winter.

3.2.4. Metabolic components

As shown in Figure 6 the concentrations of soluble sugars in *Calligonum polygonoides* measured in summer were higher than those measured in winter at most studied stands. The highest value was measured in plant samples collected from stand 37. The lowest value was recorded at stand 9. In summer, concentrations of soluble sugars ranged between 22.75 and 157.08 mg g⁻¹ DW, while in winter concentrations ranged between 18.43 and 104.88 mg g⁻¹ DW The effects of single factors as well as their interactions were statistically significant (Table 2). Regionality had the dominant effect followed by interaction.

In winter, concentrations of soluble proteins (Figure 6) were higher than in summer except at 8 stands. Concentrations ranged between 8.48 and 37.88 mg g^{-1}

DW during summer and 12.12 and 49.88 mg g^{-1} DW in winter. The highest value was recorded at stand 35, while the lowest value was detected at stand 5. Regionality had the dominant effect (Table 2) followed by interaction.

In comparison with the other metabolites, the content of total free amino acids in *Calligonum* shoots (Figure 6) showed low values and ranged between 1.11 and 3.26 mg g^{-1} DW in winter and 1.33 and 9.06 mg g^{-1} DW in summer. The highest values were recorded at stands 10 and 37. Bifactorial interaction had the dominant effect followed by regionality (Table 2).

Concentrations of soluble sugars in *Artemisia judaica* (Figure 6) measured in winter were higher than those in summer at most studied stands. The highest value was measured in the plant samples collected in summer from stand 33. The lowest value was recorded in summer at stand 11. The effects of single factors were statistically significant (Table 2). Regionality had the dominant role followed by interaction.

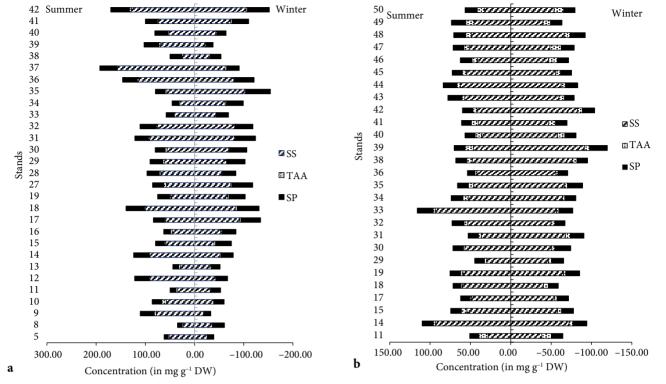
In winter, concentrations of soluble proteins (Figure 6) were higher than in summer, except in 9 stands. Soluble proteins concentrations ranged between 9.27 and 19.63 mg g⁻¹ DW during summer and 11.90 and 21.95 mg g⁻¹ DW during winter. The highest value was recorded at stand 39 while the lowest value was at stand 36. The effects of single factors and their interactions were highly significant (Table 2). Regionality had the dominant role followed by interaction.

The content of total free amino acids in *Artemisia* shoot showed low values (Figure 6). Content ranged between 2.83 and 14.13 mg g⁻¹ DW in winter and 1.46 and 11.66 mg g⁻¹ DW in summer. The highest content was at stand 47 during winter. Regionality had the dominant effect followed by interaction (Table 2).

	Summer		Winter		
	C. polygonoides	A. judaica	C. polygonoides	A. judaica	
Parameters					
Ca ²⁺	0.088	0.112	-0.098	-0.025	
Mg^{2+}	0.209	-0.213	-0.323	0.195	
Na ⁺	0.034	-0.099	-0.169	-0.073	
K ⁺	0.119	-0.401*	-0.014	0.273	
Cl⁻	-0.034	0.174	0.060	0.010	
SO_4^{-2}	-0.269	0.023	-0.102	0.170	
PO_4^{-3}	0.521**	-0.065	-0.175	-0.242	

Table 4. Correlation coefficient values (r) between internal mineral elements in *Calligonum polygonoides* and *Atremisia judaica* plants and the content in soil samples in both summer and winter seasons.

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



SALAMA et al. / Turk J Bot

Figure 6. Concentrations of soluble sugars (SS), soluble proteins (SP), and total free amino acids (TAA) expressed as mg g^{-1} DW at different studied stands inhabited by *Calligonum polygonoides* and *Artemisia judaica* plants in Wadi El-Assiuty during summer and winter seasons.

4. Discussion

The obtained data showed that soil water content of Wadi El-Assiuty was very low over the year. It suffers from severe aridity. Although rain falls rarely, a rise in the ground water table may serve perennials. Unless rainfall occurs, the underground water is the main resource for surviving plants in the main channel of the wadi. The situation is different in the deltaic plain where there are 2 other ways for water to be supplied. Firstly, the Nile water seeps into porous deposits; and secondly, the ground water pumped up in newly reclaimed lands plays the main role in increasing the water content of the deltaic soil.

The soil organic matter is correlated to the vegetation. The very scattered vegetation and high temperatures in summer affected the soil organic matter content, which was low. The estimated pH values in the soil solution tended to be slightly alkaline. In arid regions where soluble salts of sodium (such as Na_2CO_3) may accumulate, an alkaline pH is usually attained. These results were consistent with the general characteristics of soils of arid regions and their relationship with climate and vegetation as described by many authors (Zahran and Willis, 2009).

Total soluble salts were generally higher during summer. This may be due to the severe aridity and low moisture content. High rates of evaporation in semiarid areas lead to salt accumulation in the unsaturated zone; this can be dissolved by infiltrating water (Tizro and Voudouris, 2007). Consequently, electric conductivity of the soil solutions of the studied area was also relatively high. This reflects the richness of these habitats in soluble salts.

Concentrations of Ca^{+2} and Cl^- were high in summer. The high rate of evaporation concentrated the soil solution. The present data agreed with results obtained by Salama et al. (2012).

Data revealed that chlorophyll a and chlorophyll b in the studied plants were significantly higher in summer than in winter. These results agreed with Morsy et al. (2008).

The Chl a/b ratio ranged between 1.5 and 2.8. According to Quarmby and Allen (1989), the 2 main essential (Chl a) and accessory (Chl b) pigments are normally present in the ratio of about 3:1. The decreased ratio of Chl a/b in the leaves may be due to an increase in Chl b relative to Chl a or due to degradation of Chl a. Recently, it has been demonstrated that in higher plants Chl b is converted to Chl a as part of the Chl a/b inter-conversion cycle, which permits plants to adapt to changing light conditions (Ito et al., 1996). Generally, the chlorophyll a and b stability index was significantly higher in summer than as estimated in winter. The present data also indicated that chlorophyll b showed more stability than chlorophyll a in summer in response to both higher temperature levels and gradual soil moisture depletion; this coincides with findings in Radwan (2007).

Osmoregulation is the easier way to overcome the external stress. There are 2 ways to face the environmental stress. The first is quick and depends on the inorganic solutes. The absorption, excluding or extraction of the osmo-regulator inorganic ions such as Na⁺, K⁺, Ca²⁺, Mg²⁺, and Cl⁻ is very helpful for readjusting the osmotic gradient in stressed plants (Du Jinyou et al., 2004; Kamel, 2008; Sunkar, 2010; Sayed et al., 2013). This will occur immediately. The second method depends on the accumulation of organic compatible solutes as soluble sugars, soluble proteins, and amino acids. This process requires a long time in order to synthesize the different organic solutes (Wyn Jones and Pritchard, 1989).

To account for mechanisms of osmoregulation through the ionic fraction of the plant osmotic material, an evaluation of the role of ions predominantly found in the plant solution is necessary. Generally, the molar summation of accumulated cations was greater than that of accumulated anions, whether in winter or summer. The plants in summer decrease their biological activity. They accumulate the necessary amounts of organic solutes to maintain the cell turgidity in the live branches. On the other hand, in winter the biological activity will start again and the accumulated organic solutes will be used. As a result, the plants tend to accumulate inorganic solutes instead of organic solutes, which are needed in biological processes. Each of the 2 studied plants had its own strategy to accumulate the different inorganic solutes.

Apparently, the control of Ca^{+2} and Cl^- uptake from soils and the partitioning of these ions within plants is an essential component of tolerance. The studied plants depended more on Ca^{+2} and K^+ . These results agreed with Kamel (2008). Due to the limited available amounts in the soil, sodium accumulated in lower amounts than potassium. *Leptadenia pyrotechnica* had the lowest K^+ concentration. K^+ is preferred in the cytoplasm due to its beneficial effect and lower toxicity on metabolism, and K^+ is required for enzyme activity and protein synthesis (Wyn Jones et al., 1979).

The amounts of calcium accumulated were higher than accumulated magnesium in the studied plants. Kamel (2008) reported that succulent species accumulated considerable amounts of Ca^{2+} and Mg^{2+} under drought stress.

To ensure a high osmotic pressure to increase the specific heat of cell sap to overcome desert high temperatures, the studied plants increased uptake of Cl⁻. Chloride is mostly accumulated in the vacuole, as described by Harvy (1985), and this accumulation increases the osmotic pressure in the vacuole. This reflects the role of Cl⁻ in osmoregulation.

Both *Calligonum polygonoides* and *Artemisia judaica* accumulated more SO_4^{-2} in winter than in summer. Plants tend to accumulate more sulfates in dry seasons or habitats to maintain their succulence. In addition, sulfate is needed for biosynthesis of amino acids that contain a thiol (–SH) group.

Phosphates appeared in low amounts in the investigated plants. This may be due to the rapid incorporation of phosphates into plant metabolism, or poverty of phosphates in the soil.

Content of some inorganic solutes inside plants was correlated significantly with their content in the soil solution. In summer K⁺ correlated negatively in *Artemisia judaica*, while PO₄⁻³ correlated positively in *Calligonum polygonoides*. Organic solutes are compatible solutes furnished by the plants themselves. The organic solutes are needed for biological processes. Under stress plants accumulate them to overcome external stresses. The studied species are frequently adapted for drought conditions prevailing in their habitats during the summer season by accumulating more considerable soluble sugars, soluble proteins, and amino acids than in winter when the prevailing ecological conditions may be more favorable for such plants. These results agreed with the study by Salama et al. (2012) in *Ochradenus baccatus* in Wadi Qena.

The studied plants showed slight increases in soluble sugars accumulation. *Calligonum polygonoides* plants tend to increase their soluble sugars, especially in summer. *Artemisia judaica* increased their content of soluble sugars in winter. This may be due to an increase in photosynthesis while temperature was low (Prat and Fathi-Ettai, 1990). Mohammadkhani and Heidari (2008) found that plants under heat or water stress tend to accumulate soluble sugars at a level high enough to generate considerable osmotic potential. Prado et al. (2000) stated that soluble sugars play an important role in keeping water balance in plants under drought stress.

Plants respond to their environmental stress in 2 ways, either by increasing their water binding molecules or by preventing the incorporation of amino acids into proteins. Soluble protein content in the studied plants increased significantly during winter. The increase in protein content increases the surface exposed to binding water, and bound water is correlated to drought resistance (Du Jinyou et al., 2004). Some xerophytic species may adjust osmotically to stress through the contribution of nitrogen metabolites (Rayan and Farghali, 2007).

Generally, total free amino acids content in the 2 species was significantly higher in summer than in winter. These results are in accordance with Migahid (2003).

Thus, from the previous observations about soil elements and metabolic constituents in the studied plants, we assume that there are close relationships between the presence of organic matter and soluble K⁺, Na⁺, and Cl⁻ in high concentrations in the plants during the summer season on one hand and the presence of soluble sugars and soluble proteins on the other. The presence of such soil constituents may stimulate the synthesis of greater amounts of metabolic constituents, e.g., carbohydrates, proteins, and amino acids, that help osmoregulation

References

- Akhondi M, Safarnejad A, Lahouti M (2006). Effect of drought stress on proline accumulation and mineral nutrients changes in alfalfa (*Medicago sativa* L.). J Sci Technol Agr Nat Resour 10: 175–175.
- Bardsley CE, Lancaster JD (1965). Sulfur. In: Black CA, Evans DD, White JL, Ensminger LE, Clark FE, editors. Methods of Soil Analysis. Part 2. Agronomy. Series No. 9. Madison, Wisconsin, USA: American Society of Agronomy, Inc., pp. 1102–1116.
- Bartels D, Ramanjulu S (2005). Drought and salt tolerance in plants. Plant Sci 24: 23–58.
- Cheesman JM (1988). Mechanisms of salinity tolerance in plants. Plant Physiol 87: 547–550.
- Cushman JC (2001). Osmoregulation in plants: implications for agriculture. Am Zool 41: 758–769.
- Davies WJ, Meizner FC (1990). Stomatal responses of plants in drying soil. Biochem Physiol Pfl 186: 357–366.
- Du Jinyou, Xiaoyang C, Wei L, Qiong G (2004). Osmoregulation mechanism of drought stress and genetic engineering strategies for improving drought resistance in plants. For Stud Chin 6: 56–62.
- Dubois M, Gilles KA, Hamilton JK, Rabers PA, Smith F (1956). Colorimetric method for the determination of sugars and related substances. Anal Chem 28: 350–356.
- El-Sharkawi HM (1977). Osmo-metabolic adjustments in linum, cotton and wheat under salinity stress. Bull Fac Sci Assiut Univ 6: 205–223.
- El-Sharkawi HM, Michel BE (1977). Effects of soil water matric potential and air humidity on CO_2 and water vapour exchange in two grasses. Photosynthetica 11: 176–182.
- Harvy DMR (1985). The effect of salinity on ion concentrations within the root cells of *Zea mays* L. Planta 165: 242–248.
- Ito HT, Ohtsuka T, Tanaka A (1996). Conversion of chlorophyll b to chlorophyll a 7-hy droxymethyl chlorophyll. J Biol Chem 271: 475–479.
- Jackson ML (1967). Soil Chemical Analysis-Advanced Course. Washington Department of Soil Sciences.
- Johnson CM, Ulrich A (1959). Analytical Methods for Use in Plant Analysis. US Dept Agric Calif Univ Agric Inform Bull.

under drought conditions. Such metabolites might serve as energetic materials for plants to persist (i.e. respiratory material) or as raw material for metabolic processes that enable plants to maintain a fair rate of growth during the dry season (El-Sharkawi, 1977).

Based on data presented in this study, *Calligonum polygonoides* plants are better adapted to drought conditions prevailing in the area under study, more tolerant to drought, and more favorable to the conditions of the arid desert than *Artemisia judaica*.

- Kamel M (2008). Osmotic adjustment in three succulent species of Zygophyllaceae. Afr J Ecol 46: 96–104.
- Lawlor DW (2002). Limitation to photosynthesis in water-stressed leaves: stomata *vs.* metabolism and the role of ATP. Ann Bot-London, Oxford 89: 871–885.
- Lee YP, Takahashi T (1966). An improved colorimetric determination of amino acids with use of ninhydrin. Anal Biochem 14: 71–77.
- Lowry CH, Rosebrough NJ, Farr AL, Bandall HJ (1951). Protein measurement with folin phenol reagent. J Biol Chem 193: 256–275.
- Martínez J, Kinet J, Bajji M, Lutts S (2005). NaCl alleviates polyethylene glycol-induced water stress in the halophyte species *Atriplex halimus* L. J Exp Bot 56: 2421–2431.
- Migahid MM (2003). Effect of salinity shock on some desert species native to the northern part of Egypt. J Arid Environ 53: 155– 167.
- Mile O, Meszaros I, Verses S, Lakatos G (2002). Ecophysiological study on the salt tolerance of a pannonian endemism (*Lepidium crassifolium* (W. *et* K.)) in inland saline area. Acta Biol Szeg 46: 249–250.
- Mohammadkhani N, Heidari R (2008). Drought-induced accumulation of soluble sugars and proline in two maize varieties. World Appl Sci J 3: 448–453.
- Morsy AA, Youssef AM, Mosallam HAM, Hashem AM (2008). Assessment of selected species along Al-Alamein-Alexandria International Desert Road, Egypt. J Appl Sci Res 4: 1276–1284.
- Piper CS (1947). Soil and Plant Analysis. New York, NY, USA: Interscience.
- Prado FE, Boero C, Gallarodo M, Gonzalez JA (2000). Effect of NaCl on germination, growth and soluble sugar content in *Chenopodium quinoa* willd seeds. Bot Bull Acad Sinica 41: 27–34.
- Prat D, Fathi-Ettai RA (1990). Variation in organic and mineral components in young *Eucalyptus* seedlings under saline stress. Physiol Plantarum 79: 479–486.
- Quarmby C, Allen SE (1989). Organic constituents. In: Allen SE, editor. Chemical Analysis of Ecological Materials, 2nd ed. London, UK: Black Well Scientific Publications, pp. 160–200.

- Radwan AAU (2007). Plant water relations, stomatal behavior, photosynthetic pigments and anatomical characteristics of *Solenostemma arghel* (Del.) Hayne under hyper-arid environmental conditions. Amer-Eurasian J Sci Res 2: 80–92.
- Rayan AM, Farghali KA (2007). Some physiological adaptations to drought in xerohalophytic plants inhabiting two oases in Western Desert of Egypt. Saudi J Biol Sci 14: 271–281.
- Salama FM, Ahmed MK, El-Tayeh NA, Hammad SA (2012). Ecophysiological studies on *Ochradenus baccatus* Delile in Wadi Qena, Eastern Desert, Egypt. Assiut Univ J Bot 41: 203–223.
- Sayed A, Mohamed AA, Salama FM (2013). Ecophysiological studies on three desert plants growing in Wadi Natash, Eastern Desert, Egypt. J Biol Earth Sci 3: 35–43.
- Sunkar R (2010). Plant Stress Tolerance, Methods and Protocols. Dordrecht, Heidelberg, London, New York: Springer, pp. 401.
- Tizro AT, Voudouris KS (2007). Groundwater quality in the semiarid region of the Chahardouly basin, West Iran. Int J Hydrol Proc 22: 3066–3078.
- Valladares F (2003). Light heterogeneity and plants: from ecophysiology to species coexistence and biodiversity. In: Esser K, Lüttge U, Beyschlag W, Hellwig F, editors. Prog Bot 64: 439–471.
- Walkley A, Black IA (1934). An examination of the Degtjareff method for determining organic carbon in soils: effect of variations in digestion conditions and of inorganic soil constituents. Soil Sci 63: 251–263.

- Wang FZ, Wang QB, Kwon SY, Kwak SS, Su WA (2005). Enhanced drought tolerance of transgenic rice plants expressing a pea manganese superoxide dismutase. J Plant Physiol 162: 465– 472.
- Welfare K, Flower TJ, Taylor G, Yeo AR (1996). Additive and antagonistic effects of ozone and salinity on the growth, ion contents and gas exchange of five varieties of rice (*Oryza sativa* L.). Environ Pollut 92: 257–266.
- Williams CH, Twine ME (1960). Flame photometric method for sodium, potassium and calcium. In: Paech K, Tracey MV, editors. Modern Methods of Plants Analysis. Berlin: Springer-Verlag, pp. 535.
- Wyn Jones RG, Brady CJ, Speirs J (1979). Ionic and osmotic relations in plant cells. In: Laidman DL, Wyn-Jones RG, editors. Recent Advances in the Biochemistry of Cereals. London: Academic Press, p. 63.
- Wyn Jones RG, Pritchard J (1989). Stresses, membranes and cell walls. In: Jones HG, Flowers TJ, Jones MB, editors. Plants Under Stress: Biochemistry, Physiology and Ecology and Their Application to Plant Improvement. Cambridge University Press, pp. 95–114.
- Zahran MA, Willis AJ (2009). The Vegetation of Egypt. Vol. 2. Plant and Vegetation. London, UK: Springer.