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Ecophysiological studies on *Achillea fragrantissima* and *Artemisia judaica* in two wadis of Southern Sinai, Egypt

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Key words: *Achillea fragrantissima*, amino acids, *Artemisia judaica*, metabolites, DNA, seasons, Southern Sinai.

Abstract

The physiological and molecular responses of *Achillea fragrantissima* (Forssk.) Sch. Bip. and *Artemisia judaica* L. growing in Wadi El-Sheikh (WSH) and Wadi El-Arbaean (WAR) in Saint Catharine, Southern Sinai, Egypt during the wet and dry seasons were investigated. The results revealed that.in both wadies, soil had higher electrical conductivity (EC) and - higher water content . in WSH possessed higher Na$^+$, K$^+$ and Cl$^-$ during wet season and higher Ca^{+2} and Mg^{+2} during dry season. . In wet season, *A. fragrantissima* showed higher value of total carbohydrates in the downstream and higher value of total nitrogen and protein in the upstream of WSH. . . A comparable increase in selenium content was noticed in *A. fragrantissima* in downstream of WSH during dry season while the same was the case during wet season of WAR. Both species in the two wadis had higher values of total phenols, total alkaloids and total glycosides during the dry season. Values of the amino acids in *A. fragrantissima* of WAR were higher in dry season while in WSH, they were detected in wet season. *A. fragrantissima* was considerably rich in four amino acids: asparagine, glutamine, proline and tyrosine and relatively poor in isoleucine and phenylalanine. Higher values of aspartic acid and proline were recorded in *A. judaica* in the downstream of the WAR during dry season. *A. fragrantissima* from both wadies was characterized by the presence of bands of molecular sizes ~~of~~ 1290, 1193, 586 and 481 bp. However, bands of molecular sizes of 1693, 1647, 1189, 1010, 896, 759, 730, 719, 616, 549, 413, 380 and 155, bp were detected only in *A. fragrantissima* from WSH, while they were absent in specimens from WAR. . *A. judaica* from the two wadis was characterized with bands of molecular sizes of 1861, 1402, 1342, 1161, 1032, 973,854, 824, 777, 734,724, 629, 574, 326 and 206 bp. There were three common bands in both plants from both wadis with molecular sizes of 908, 246 and 179 bp.

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Introduction

Achillea fragrantissima (Forssk.) Sch.Bip. and *Artemisia judaica* L. (Compositae) are shrubby herbs growing naturally in Egypt and distributed in the desert regions (Tächholm, 1974; Boulos, 2005). Drought tolerance is achieved by cell- and tissue-specific physiological, biochemical, and molecular mechanisms (Reddy *et al.*, 2004; Mossallam *et al.* 2000) reported that one of the most adaptive responses of *Artemisia judaica* growing on rocky habitat in the winter season is the increase in the values of DNA, RNA, free amino acids, total nitrogen and total carbohydrates which together with organic acids serve as compatible solutes to maintain the osmotic balance under conditions of water stress. The total inorganic ion concentration increased rapidly in stressed plants and seemed to be the major component of its osmotic adjustment (Patakas *et al.*, 2002**)**. Plants exposed to drought or salt stress are known to produce higher amounts of secondary metabolites such as alkaloids which have a role in the defense of plants against active oxygen species (Selmar, 2008). The phenolic compounds comprise a large family of therapeutic agents possessing anticarcinogenic action (Patil and Pike, 1995), antioxidant activity (Torel *et al.*, 1986) and also have important functions as antibiotics and natural pesticides (Heldt, 1997). Flavonoids act as protective pigments to shield plants from the damaging effect of ultraviolet light (Heldt, 1997). Drought stress may also induce changes in gene expression and induction of repair systems and chaperones which influence plant distribution, survival, and crop yields worldwide (Ingram and Bartels, 1996).

The present study aimed to investigate the physiological responses of the two medicinally valuable plants *A. fragrantissima* and *A. judaica* under the drought stress conditions prevailing in Wadi [valley] El-Sheikh (WSH) and Wadi El-Arbaeen (WAR) in S. Sinai, Egypt. These two wadis [vallies] are markedly different both environmentally and edaphically (El-Absy, 2011). The results might be of a highly predictive value in determining the best season and habitat from which certain medicinally active components might be obtained from these two species.

Material and methods

Soil analysis

Electrical conductivity of soil (EC) was carried out using soil-water paste, according to Jackson (1962) and expressed in mmohs/cm. The soil moisture content was determined according to the method described by Rowell (1994). Sodium and potassium were determined photometrically using the flame photometer, according to Johnson and Ulrich (1959) while calcium and magnesium were determined by the titration method according to Jackson and Thomas (1960). Chlorides were determined in the soil extract by the $AgNO_3$ method according to Jackson (1967). Cation and anion values were expressed as meq./L.

Plant analysis

1. Succulence ratio: Shoot samples of *A. fragrantissima* and *A. judaica* were collected from their natural populations in the up and down streams of Wadi Al-Arbaeen (WAR) and Wadi Al-Sheikh (WSH) in Saint Catherine area, South Sinai in March and August, representing the peaks of the wet and dry seasons. The succulence ratio (SR) was calculated as the initial fresh weight / dry weight ratio (Fr.wt / Dry wt.) according to Dehan and Tal (1978).

2. Minerals: The mixed- acid digestion method of Chapman and Pratt (1978) was applied in preparing the sample solution for the estimation of chlorides, sulfates, sodium and potassium. Sodium and potassium contents were measured using Flame Photometer. Values were expressed as meq /100g dry wt. of plant material. Calcium, magnesium and iron were determined by using ICP emission spectroscopy (Jones, 1977). Chlorides were estimated according to Jackson and Thomas (1960**)**. Sulfate content was estimated by the turbidimetric method according to the Standard Methods (1989). Chlorides and sulfate values were expressed as meq/100 g dry

wt. From the same plant digest some micro-elements were estimated. Iron, zinc and selenium were determined using Atomic Absorption Spectrophotometer (Sp1900) as described by Allen *et al.* (1974).

3. Total nitrogen: The total nitrogen content of shoot system was determined using the modified micro Kjeldahl method of Peach and Tracey (1956) and the results were expressed as mg/ 100g dry wt.

4.Crude fiber: Crude fiber content was determined in ashed-plant material of the shoot system according to Askar and Treptow, (1993).

5. Total available carbohydrate: The total available carbohydrate content was estimated according to Chaplin and Kennedy (1994) and calculated as mg/100 g dry wt.

6. Amino acids: Protein-bound and free amino acids were determined using the amino acid analyzer according to the method of Pellet and Young (1980).

7. Total alkaloids, total glycosides and total phenols: Total alkaloids were determined quantitatively according to the method described by Harbone (1973). Total glycosides were determined in air-dried material according to Harper (1975). Total phenols were estimated by the method of Malik and Singh (1980).

8. PCR-analysis of DNA: Extraction of DNA from *A. fragrantissima* and *A. judaica* was conducted according to the method described by Dellaporta *et al.* (1983). Fifteen 10-mer random DNA oligonucleotide primers were independently used in the PCR reactions according to Williams *et al.* (1990). Only five primers generated reproducible polymorphism in the DNA profiles. These five primers had the following initials and sequences:

OPA-10 (5'-GTGATCGCAG-3'),
OPE-04 (5'-GTGACATGCC-3'),
OPM-09(5'-GTCTTGCGGA-3'),
OPB-10 (5'-CTGCTGGGAC-3'),
and OPG-19 (5'-GTCAGGGCAA-3').
Each experiment was repeated twice and only the stable products were scored.

Results

1. Soil analysis

The highest value of soil EC during the dry season (256 millimohs/cm) was recorded in the upstream of WSH. The maximum value of soil water content was recorded in downstream of both wadis during wet season, (Table 1). It appeared from Table 1 that Na^+ content was higher in the up- and downstream of the two wadis in the wet season compared to the dry one, where the maximum value (19.90 meq/100g dry wt.) was recorded in the upstream of WSH. K^+ content tended to increase in each of the two wadis during the wet season in the up- and down-streams except for WSH, which recorded a slight increase in K^+ content (0.50 meq^{-1}100g dry wt.) during the dry season for the downstream (Table 1). In both wadis, Ca^{2+} content in the soil associated with the two species was higher during the wet season than in the dry one. However, the upstream of WSH showed a marked decrease in its Ca^{2+} content (Table 1). Table 1 showed also that Mg^{2+} content decreased in each of WSH and WAR during the wet season, except in the downstream of WSH where it displayed an increase (183.50 meq /100g dry wt.).

Table 1. The values of EC (mmohs/cm), water content (%) and mineral analysis of the soil from up and down streams of Wadi El-Sheikh (WSH) and Wadi El- Arbaeen (WAR) during the wet and dry seasons (as meq/100g dry wt.).

Sites	EC	WC	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-
WSH							
Wet season							
Upstream	2.05	5.80	19.90	3.20	5.50	193.50	36.80
Downstream	0.52	8.96	4.10	0.40	5.60	183.50	6.75
Dry season							
Upstream	2.56	1.69	1.97	1.37	19.32	481.87	17.38
Downstream	0.72	5.01	2.54	0.50	4.44	153.06	5.83
WAR							
Wet season							
Upstream	1.71	2.90	12.10	0.99	6.60	140.40	21.75
Downstream	1.01	3.13	5.30	0.57	4.40	122.61	1.13
Dry season							
Upstream	0.93	1.20	1.99	0.05	5.72	190.98	8.73
Downstream	0.87	1.96	1.88	0.02	3.70	167.09	4.72
Statistical analysis							
Season	-	**	**	**	**	**	**
Wadis	**	**	**	**	**	**	**
Season x wadis	**	**	*	-	**	**	**
Up x down	**	**	**	**	**	**	**
Seasons x wadis x (Up x down)	**	**	**	**	**	**	-

* = Significant at ** = Significant at $P \leq 0.0$ - = Not significan

Plant analysis

1. Succulence: The water content and succulence ratios of the shoot systems of the two species increased significantly in WSH during the wet season than in the dry one, and the maximum value was recorded for *A. judaica* at the downstream, while in WAR the increase in water content and succulence ratio were recorded in the upstream for the two species and in the downstream for *A. fragrantissima* only during the wet season (Table 2).

2. Crud fiber: There was a significant increase in the crude fiber content of the shoot during the dry season in both species in upstream of WSH, while it decreased significantly in *A. fragrantissima* at the downstream (Table 2). In WAR there was an increase in the crude fiber content for the two species during the wet season, except for *A. judaica* which has the lowest value (12.33%) at the downstream.

3. Total available carbohydrate: The total available carbohydrate content of *A. fragrantissima* shoot was significantly increased during the wet season than in the dry one in the two wadis, except in the upstream of WAR. In case of *A. judaica*, the increase was recorded during the wet season in the up- and down-streams of WSH and only in the upstream of WAR (Table 2).

4. Total nitrogen: In the wet season, a wide variation in the total nitrogen and crude protein contents occurred in the two wadis and the highest value was determined for *A. fragrantissima* in the upstream of WSH. Meanwhile, in the dry season, the highest values of the total nitrogen and crude protein contents were recorded for the same species in the upstream of WAR (Table 2).

Table 2. Changes in % water content (WC), succulence ratio (SR), % crude fiber (CF), total available carbohydrates (TAC) and total nitrogen (TN) in g 100 g^{-1} dry weight for each species in each site of Wadi El-Sheikh (WSH) and Wadi El-Arbaeen (WAR) during the wet and dry seasons.

Sites	**Species**	**WC (%)**	**SR**	**CF (%)**	**TAC**	**TN**
		WSH				
		Wet Season				
Up Stream	*A. fragrantissima*	35.0	1.5	12.7	12.5	14.9
	A.judaica	50.0	2.0	11.0	16.7	4.6
Down Stream	*A. fragrantissima*	34.5	1.5	25.0	19.4	2.4
	A.judaica	56.9	2.4	9.7	14.0	5.2
		Dry season				
Up Stream	*A. fragrantissima*	30.1	1.4	15.7	10.7	6.2
	A.judaica	42.4	1.8	18.7	12.0	7.9
Down Stream	*A. fragrantissima*	32.5	1.5	13.7	9.9	6.3
	A.judaica	48.0	2.0	23.7	10.5	2.6
		WAR				
		Wet Season				
Up Stream	*A. fragrantissima*	22.4	1.3	14.7	14.6	3.7
	A.judaica	28.8	1.4	24.3	14.1	4.6
Down Stream	*A. fragrantissima*	33.4	1.5	12.3	16.6	4.9
	A.judaica	29.9	1.4	19.3	14.3	4.9
		Dry season				
Up Stream	*A. fragrantissima*	18.2	1.2	14.0	15.7	8.3
	A.judaica	25.4	1.3	13.0	9.0	6.5
Down Stream	*A. fragrantissima*	10.8	1.1	18.0	13.4	4.1
	A.judaica	37.5	1.7	10.7	15.0	4.6
	Statistical analysis					
	Species	**	**	ns	**	**
	Wadis	**	**	**	**	**
	seasons	**	**	**	**	*
	sites	**	**	**	**	**
	Species x wadis	**	**	ns	**	**
	Species x seasons	ns	ns	**	**	**
	Wadis x seasons	ns	**	**	**	**
	seasons x species x wadis x sites	*	*	**	**	**

**= Significant at P<0.01, *=Significant at P<0.05 and ns=not significant

5. mineral contents: In *A. fragrantissima*, sodium content decreased in the wet season than in the dry one in WSH, while in *A. judaica*, it increased in the up- and down- streams of WSH and in the downstream of WAR (Table 3). Potassium content showed a significant decrease in both wadis during the wet season for each species and the lowest value was recorded in the downstream of WSH for *A. fragrantissima* (0.40 mg/100g dry wt.). During the wet season, calcium content attained the highest value in both species and in both wadis (Table 3), except in the downstream of WSH for *A. judaica* and the mid- stream of WAR for the two species where it decreased. Magnesium content increased in the two species during the wet than in dry season in the upstream of WSH, while it declined at the downstream. Regarding WAR, a significant accumulation of magnesium was recorded during the wet season for *A. fragrantissima* in both up- and down-streams and in *A. judaica* in the downstream only. Chlorides decreased in *A. judaica* in the upstream of WAR during the wet season. Sulfur content significantly decreased in *A. fragrantissima* in the up- and down-streams of WSH and the opposite was true for *A. judaica* as a maximum value of 2210 (mg /100 dry wt.) was recorded during the wet season. It was evident that in both species, sulfur content always increased significantly in WAR. Iron content decreased in *A. fragrantissima* during the wet season in WSH., while, it was significantly increased in *A. judaica* during the wet season in WSH and the downstream of WAR, where it was decreased slightly in the upstream (Table 3). Zinc content was significantly decreased during the wet season in both species in the up- and down-streams of both wadis, except for *A. judaica* in the downstream of WAR

during wet season where it showed a significant increase (1.72 mg/100g dry wt.). Selenium content increased significantly during the dry season in both species and in the two sites of both wadis, except in the downstream of WAR where it decreased in *A. judaica* (0.08 mg/100g dry wt.) during the dry season.

Table 3. Changes in mineral elements (In mg100 g^{-1} dry weight) of *A. fragrantissima* and *A. judaica* in each site of Wadi El-Sheikh (WSH) and Wadi El-Arbaeen (WAR) during the wet and dry seasons*.

Sites	**Species**	**Na^+**	**K^+**	**Ca^{2+}**	**Mg^{2+}**	**Cl^-**	**SO_4**	**Fe**	**Zn**
WSH									
Wet season									
Up Stream	*A. fragrantissima*	5.4	0.9	19.0	12.0	63.3	105.0	2.39	0.09
	A.judaica	35.7	2.3	93.8	19.2	125.8	2110	1.05	0.11
Down Stream	*A. fragrantissima*	2.2	0.4	12.3	8.8	61.8	92.7	0.97	0.07
	A.judaica	20.7	1.6	13.3	10.4	186.7	198.7	0.97	0.10
Dry season									
Up Stream	*A. fragrantissima*	21.3	15.9	17.1	11.2	62.3	190.3	2.48	0.13
	A.judaica	20.8	4.8	9.9	7.2	51.7	141.3	0.09	1.06
Down Stream	*A. fragrantissima*	29.9	19.3	11.6	19.2	57.3	240.0	4.41	0.12
	A.judaica	6.1	8.6	391.6	12.8	38.3	81.0	0.11	0.48
WAR									
Wet season									
Up Stream	*A. fragrantissima*	6.4	0.7	80.2	12.7	111.7	107.0	0.66	0.11
	A.judaica	3.1	1.9	33.8	16.1	51.7	149.0	1.19	0.08
Down Stream	*A. fragrantissima*	3.7	1.4	14.7	8.9	50.8	147.0	0.99	0.12
	A.judaica	3.1	2.6	37.1	40.0	125.8	459.7	3.45	1.72
Dry season									
Up Stream	*A. fragrantissima*	2.8	1.7	15.3	7.9	50.0	91.7	1.55	0.12
	A.judaica	20.8	17.4	20.6	16.7	53.3	121.3	1.56	0.18
Down Stream	*A. fragrantissima*	3.7	2.1	12.1	8.7	49.8	70.0	1.30	0.13
	A.judaica	2.9	22.1	10.6	10.6	55.8	131.0	0.48	0.08

* statistical analysis indicated that the effect of species, Wadis and sites besides the interactions between species x Wadis, species x seasons, wadis x seasons and the interaction between the four factors were highly significant for each element ($P<0.01$).

6. Total alkaloids, total glycosides and total phenols: In both species and in both wadis, the total alkaloids increased during the dry than in wet season (Fig.1a). The highest value was recorded during the dry season in WAR where it reached 0.97 mg/100g dry wt. for *A. fragrantissima* in the upstream. A similar trend was revealed also for the total phenolic compounds (Fig.1b) where it increased during the dry season in the two wadis for both species. The highest value was recorded during the dry season in *A. judaica* (10.27 mg/100g dry wt.) in the downstream of WSH. Total glycosides increased in *A. fragrantissima* during the dry season in the upstream of WSH and downstream of WAR (Fig.1c). In *A. judaica*, total glycosides increased during the dry season in the up- and down-stream of WAR and only in the upstream of WSH.

7. Amino acids: The protein amino acids of *A. fragrantissima* in the upstream of WAR were higher during the wet season than in dry one (Table 4), and the opposite was true in WSH. However, in the downstream, where the values of the amino acids in *A. fragrantissima* of WAR were higher in the dry season, in WSH the higher values were recorded in the wet season. Protein of *A. fragrantissima* was considerably rich in four amino acids (aspartic, glutamic, proline and tyrosine) and relatively poor in isoleucine and phenylalanine. During the dry season in the upstream of both wadis, all protein amino acids of *A. judaica* showed higher values than their counterparts in the wet season (Table 4). However, in the downstream of WAR, the same trend was recorded while the opposite was true in the downstream of WSH.

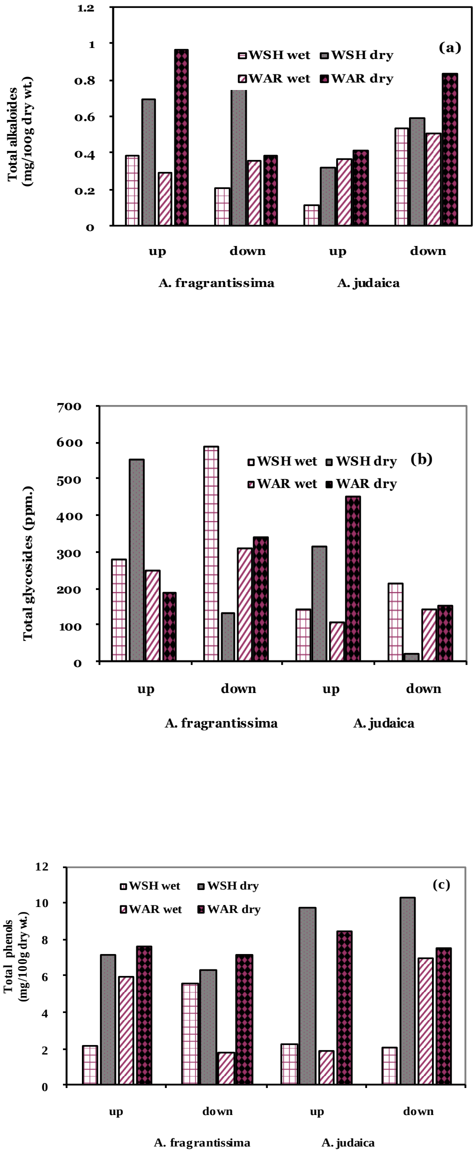

Fig. 1. Changes in total alkaloids (a), total glycosides (b) and total phenols (c) of *A. fragrantissima* and *A. judaica* from the upstream and downstream localities of Wadi El-Sheikh (WSH) and Wadi El-Arbaeen (WAR) during the wet and dry seasons.

All free amino acids of *A. fragrantissima* in the upstream of both Wadies were higher in the dry season than in the wet one while the opposite was recorded in the downstream of WSH for all amino acids and only for proline in WAR (Table 5). As regards *A. judaica*, the accumulation of the free amino acids, aspartic, threonine, serine, glutamic and proline was achieved in the plants from up- and down-streams of the two wadis during the dry season, in addition to alanine, valine and tyrosine for up- and down-streams in WAR only (Table 5). In the up- and down-streams of WAR, accumulation of all free amino acids in *A. judaica* was greater during the dry season than in the wet one. The same behavior was recorded in the upstream of WSH, while in the downstream accumulation of only proline and serine was obvious.

8. PCR- analysis of DNA: The molecular sizes of base pairs of amplified DNA fragments produced by five primers with *A. fragrantissima* and *A Judaic* revealed that several bands were detected in each species in both wadis. Some others were recorded as characteristic bands for each wadi and not found in the same plant in the other wadi which has been summarized in Table 6. In *A. fragrantissima*, bands of molecular size of 1693, 1647, 1505, 1189, 1010, 896, 759, 730, 719, 616 and 549 bps were characteristic for WSH and not expressed in the same plant in WAR, while a band of molecular size 1276 bps was present exclusively in WAR and can be considered as a unique band for *A. fragrantissima* characteristic for WAR. In case of *A. judaica,* bands of molecular size of 2019, 1647, 1010, 672, 549, 533, 506, 416 and 323 bps were characteristic for WSH and not expressed in the same plant in WAR, while bands of molecular size of 463 and 385 bps were characteristic for WAR and not expressed in the same plant in WSH.

In *A. fragrantissima*, bands of molecular size of 586, 1193, 841 and 1290 bp were expressed as characteristic bands only for *A. fragrantissima* in both wadis, while in *A. judaica*, bands with molecular size of 1861, 1402, 1342, 1161, 1032, 973, 854, 824, 777, 734, 724, 629, 574, 326, and 206 bps were recorded in both wadis which can be considered as characteristic for *A. judaica* in the two wadis.

Table 7 showed that DNA concentration in *A. judaica* was almost twice the corresponding value in *A. fragrantissima* in the two wadis and reached its highest value (465 µg/ml) in WAR. It was also noticeable that DNA concentration of each species in WAR was nearly twice that in WSH.

Table 4. Seasonal changes in some protein amino acid for *A. fragrantissima* and *A. judaica* from upstream (US) and downstream (DS) of wadi Al-Sheikh (WSH) and wadi El-Arbaeen (WAR) during the wet and dry seasons. (as mg/100g dry wt.).

A. fragrantissima					***A. judaica***			
	WSH		**WAR**		**WSH**		**WAR**	
	U S	**DS**	**U S**	**DS**	**US**	**DS**	**U S**	**DS**
	Wet season							
Aspartic	323	394	662	168	233	337	245	255
Threonine	119	138	166	64	110	153	119	106
Serine	119	142	204	67	103	140	116	107
Glutamic	302	396	441	175	256	434	259	258
Proline	239	280	1407	176	135	245	171	331
Glycine	113	170	324	81	127	183	132	141
Alanine	92	135	425	80	124	168	135	150
Valine	131	178	369	86	146	199.	160	141
Isoleucine	3.7	1.7	0.4	0.5	5.8	3.4	0.4	1.9
Leucine	185	97	221	60	59	94	76	109
Tyrosine	402	175	466	97	97	169	130	207
Ph.alanin	52	12	85	3.5	6.8	12.7	8.2	12.2
Histidine	234	59	104	51.3	20.3	44	35	109
Lysine	69	65	64	36	64.	77	72	44
Argenine	90	95	22	20	72	110	95	55
	Dry season							
Aspartic	109	90	469	256	333	247	648	893
Threonin	49	40	179	69	155	100	329	238
Serine	51	47	216	90	143	100	314	225
Glutamic	125	101	580	325	315	253	678	604
Proline	121	91	320	293	405	313	592	605
Glycine	57	42	468	88	176	126	344	344
Alanine	54	45	277	69	170	112	383	474
Valine	99	56	268	87	226	106	426	408
Isoleucin	2.4	1.8	1.8	3.8	16	9.3	4	1
Leucine	69	45	197	47	253	69	323	228
Tyrosine	134	86	318	150	480	166	513	625
Ph.alanin	19.4	6.9	37.7	3.9	64.8	7.7	127	17.5
Histidine	92	68	135	110	390	108	294	93
Lysine	29.7	39.5	109	23.6	102	36	195	21
Argenine	37	24	196	50	136	44	404	76

Table 5. Seasonal changes in some free amino acids for *A. fragrantissima* and *A. judaica* from upstream (US) and downstream (DS) of Wadi El-Sheikh (WSH) and Wadi El-Arbaeen (WAR) during the wet and dry seasons. (as mg/100g dry wt.).

A. fragrantissima					***A. judaica***			
	WSH		**WAR**		**WSH**		**WAR**	
	U S	**DS**	**U S**	**DS**	**U S**	**DS**	**U S**	**DS**
	Wet season							
Aspartic	8.4	11.6	4	7.6	4.6	6.7	5.8	6.5
Threonine	9.3	1.8	2.3	3.7	2.4	5.9	2.5	5.8
Serine	22.9	31.7	10.9	21.2	10.9	13.8	11.3	22.5
Glutamic	13.2	0.2	2.3	9.8	2.4	3.9	4.7	10.4
Proline	412	248	108	141	111	170	124	177
Glycine	35.9	22.6	7.2	12.8	18.9	35.7	18	18.5
Alanine	31.3	17.4	7.9	7.8	20.2	31.9	14.2	18.8
Valine	18.5	24	7	8.3	12.8	18.6	13.1	13
Isoleucine	0.21	0.04	0.32	0.23	0.22	0.65	0.013	0.53
Leucine	10.6	3.9	1.1	0.55	2.3	9.9	4.3	8.6
Tyrosine	17.4	7.3	3.9	3.1	17.5	13.7	8.1	15.3
Ph.alanin	6.3	0.8	0.3	0.3	2.9	1.1	0.4	6.1
Histidine	5.8	0.8	1	0.4	2.2	0.3	0.2	4.1
Lysine	35	9	10	13	17.1	33.8	20.6	24.6
Argenine	6	1.9	7.6	10.8	1	2.9	11.4	3
	Dry season							
Aspartic	5.5	64.6	5.	10.8	8.6	9.8	10.1	30.7
Threonine	2.8	12.7	2.5	4.7	5	6.2	13.1	13
Serine	18.7	189	13.3	48.4	30.1	41.7	14.1	214
Glutamic	3	18.6	3	11.7	12	13	15.5	21.6
Proline	195	388	75	166	243	266	127	442
Glycine	10	22.5	17.2	24.8	22.2	26.2	19.7	37.6
Alanine	9.2	17.4	15.1	14.8	11.8	19.3	19.7	30.4
Valine	8.4	64.7	7.7	11.8	12.1	13.7	20.9	56.8
Isoleucin	0.00	0.10	0.10	0.31	0.05	0.14	23.7	0.12
Leucine	2	20.1	1.1	0.65	4.4	5.5	24.7	13.1
Tyrosine	4.5	13	5.1	0.96	10	11.2	25.9	16.9
Ph.alani.	0.2	4.2	1	1.5	2.1	2.6	34.2	7.1
Histidin.	1.4	14.3	2.1	1.1	1.6	1.8	36.2	23.5
Lysine	13.5	15.4	17.6	3.6	16.1	17.9	50	33.8
Argenin.	15.2	10	4.3	11.1	5.8	6.2	54.4	11.4

Table 6. The characterstic molecular sizes of amplified DNA fragments with *Achillea fragrantissima* and *Artemisia judaica* from Wadi El-Sheikh (WSH) and Wadi El- Arbaeen (WAR) based on the results produced by five primers.

Size (bp)	WSH		WAR	
	A. fragrantissima	*A. judaica*	*A. fragrantissima*	*A. judaica*
2019	0	1	0	0
1693	1	0	0	0
1647	1	1	0	0
1505	1	0	0	0
1276	0	0	1	0
1189	1	0	0	0
1010	1	1	0	0
896	1	0	0	0
759	1	0	0	0
730	1	0	0	0
719	1	0	0	0
672	0	1	0	0
616	1	0	0	0
549	1	1	0	0
533	0	1	0	0
506	0	1	0	0
463	0	0	0	1
416	0	1	0	0
413	1	1	0	1
385	1	0	1	1
380	1	0	0	0
323	0	1	0	0

Table 7. The concentration of DNA in *Achillea fragrantissima* and *Artemisia judaica* from Wadi El-Sheikh (WSH) and Wadi El- Arbaeen (WAR).

DNA concentration µg/ml	**WSH**		**WAR**	
	A.fragrantissima	***A. judaica***	***A.fragrantissima***	***A. judaica***
	120	215	210	465

Discussion

Sinai lies in the arid belt of North Africa and belongs to the Saharan Mediterranean area with a true desert climate (Danin, 1983). The bed of WAR is moderately vegetated with a rocky substrate and the plant richness is relatively high with high vegetation coverage and it has a granitic geology, while the bed of WSH is flat with a high proportion of small rock component and high vegetation cover (Guenther, 2005).

The recorded increase in the succulence ratio of *A. judaica* in the downstream of WSH may be related to the increase in soil moisture and chloride content (Abu- Taha, 2010; Abd El- Maboud, 2011). Abd El-Fattah *et al.* (1993) reported a positive correlation between the degree of succulence of the plant with each of soil moisture and the Cl^- content of the plant. In WSH, however, the decrease of succulence ratio during the dry season in *A. judaica* can be related to the decrease of soil water content. It is known that succulence is one of the most common features of many halophytes as well as xerophytes which can be increased by univalent more than by divalent or trivalent ions (Greenway and Munns, 1980). Kasim *et al.* (2008) showed that in Southern Sinai *Arthrocnemum macrostachyum* possessed higher succulence ratio in both summer and spring compared to *Nitraria retusa*, while the opposite was true in cell-membrane electrical conductivity and in each species the cell-sap osmotic potential was decreased during the summer which was appreciably lower in *A. macrostachyum*.

The high values of some mineral ions such as K in *A. fragrantissima* during the dry season In WSH can be referred to its role in osmotic adjustment to survive water deficit during the dry season (Miller *et al.*, 1995). In addition, Salisbury and Ross (2000) reported that K^+ acts as an activator of many enzymes which are important for photosynthesis and respiration, and it is also involved in carbohydrate metabolism and protein synthesis (Jain, 1997). The present results revealed that crude fiber content experienced fluctuations in the two different seasons and species where the highest value was recorded for *A. fragrantissima* at downstream of WSH during wet season while in WAR the highest value was recorded for *A. judaica* in upstream during wet season. The increase in crude fiber during the wet season can be related either to the decrease in soil EC, potassium and chloride or to the increase in water content. Kamel (2011) reported that during the autumn in upstream of wadi El-Bagha, the highest value of crude fiber percentage was obtained from *Deverra tortuosa,* while the lowest value was from *Acacia raddiana*.

The highest value of total available carbohydrates was observed in the two species during the wet season, while the lowest value was recorded in the dry one. These results may be related to more favorable conditions necessary for carrying out metabolic processes beside the greater mass of photosynthetic tissue during the wet season (Abd El-Rahman *et al.*, 1994). However, the decline in total carbohydrate content in the dry season can be due to increased water stress resulting in decreased photosynthetic activity associated with increased respiration (El-Lamey, 1999). It has been reported that under water stress conditions the total available carbohydrates are converted to soluble sugars leading to a decrease in osmotic potential, thus increasing drought tolerance (Pelah *et al.*, 1997).

Total nitrogen and crude proteins showed their highest values in *A. fragrantissima* at the upstream of WSH during the wet season, associated with a decrease in soil moisture content. However, total nitrogen increased significantly during the dry season for upstream of WAR, contributing partially in the building up of the osmotic potential of plants under xeric conditions as reported by Youssef (1988). The present results are in agreement with those of Abd El-Rahman (1973) who reported that decreasing soil moisture can be associated with an increase of nitrogen content of the plant tissue. For *A. fragrantissima* in WSH, the increase in total protein content during the wet season may be attributed to the effect of increased EC, potassium and chlorides in the soil due to decreased soil water content.

Calcium was the dominant cation in the downstream of WSH during the dry season, followed by magnesium, sodium and potassium and the variations in mineral concentrations in response to the effect of seasons and wadis as well as their interactions were found to be significant in both plants. These findings were similar to those recorded by Abd El-Maboud (2006). The increase in Ca^{2+}concentration can influence tissue elasticity; it is generally assumed that calcium increases the rigidity of the cell wall (Marigo and Peltier, 1996). Atkinson (1991) reported that the increase in Ca^{2+} concentration in stressed plants may be responsible for the drastic reduction in stomatal conductance.

The accumulation of S, which was much greater than that of Cl^- for *A. fragrantissima* in WAR especially in the dry season, may be due to the prevailing water stress associated with the decrease in soil moisture as reported by Abo Sitta *et al.* (1995). They also reported that sulfate ion exerts dehydrating action on proteins and the lack of succulence can be due to specific effects of sulfate.

In each of the two species the total alkaloids ~~were~~ increased during the dry season in the two wadis. Virk and Singh (1990) reported that moisture stress during the dry season favored the synthesis of alkaloids in the root and their translocation to cell vacuoles in shoot, which might contribute to the decrease in symplastic osmotic potential in *Catharanthus roseus* because moisture stress induced osmotic adjustment. Alkaloids may have a role in the defense of the plants against singlet oxygen ($^1O_2$), which is damaging to all living organisms, and is produced in plant tissues in the presence of light (Evans, 1999).

During the dry season in the downstream of WSH, there was high accumulation of total phenols in *A. judaica*. This accumulation might be considered as one of the chemical adaptations to the environmental stresses as explained by Mann (1978). Total phenols are known to be accumulated in different xerophytic plants and attained their highest levels during summer (Morsy, 1996). Similarly, in the downstream of WSH, there was an increase in glycoside content in *A. fragrantissima* during the wet season.

In *A. fragrantissima* the increase in the free amino acids aspartic, threonine, serine, glutamic, alanine, phenylealanine and histidine during the dry season in WSH at the downstream only and in WAR at both up- and down-streams may be due to the correlation between the capacity of plants to accumulate specific amino acids and their ability to resist or tolerate a particular environmental stress as reported by Stewart and Larcher (1980). The accumulation of the free amino acids under drought stress may be considered as cytoplasmic osmotica, for which there has been evidence in certain species, including proline, glutamic acid, aspartic acid and a number of other amino acids (Wang *et al.*, 2004). The highest value of proline recorded in *A. judaica* in WSH and WAR at the up- and down-streams during the dry season can be considered as one of the characteristic metabolic changes often observed under stress and also a metabolic device to maintain osmotic balance under stressful conditions (Mohammed and Sen, 1987). Proline plays an important role in the stimulation of root elongation at low water potentials and acts as osmoprotectant in plants subjected to drought (Yamada *et al.*, 2005). It acts also as a free radical scavenger and can be more important in overcoming stress than in acting as a simple osmolyte as reported by Lawlor and Cornic, (2002), who added that accumulation of other amino acids like glycine, serine and glutamate has been known to regulate and integrate the metabolism in stressed photosynthetic tissues. Kasim *et al.* (2008) reported that in Southern Sinai, *Netraria retusa* has experienced higher proline level in spring and attributed this accumulation to its role as an osmoprotectant for enzymes and membranes against stresses rather than a compatible solute and they added that each of *Nitraria retusa and Arthrocnemum macrostachyum* possessed higher nitrogen content during summer. It was noticeable that values of free amino acids in the up- and down-streams of WSH were close to each other due to the fact that water content of these two sites were also comparable and their elevation is 1421 m, while in WAR, with an elevation of 1718 m, amino acid values in the upstream were higher than those in the downstream, corresponding to the difference in elevation between up- and down-stream as well as the difference in water content. It appears that, in both wadis the two species tend to incorporate amino acids into proteins under relatively favorable conditions of the wet season but dissociate them to free amino acids under the stressful conditions of the dry season.

The RAPD results showed the appearance of definite bands specific for each species which may be expressed as characteristic positive markers for drought tolerance. One of these bands appeared with the primer OPE-04 (molecular size 908 bp.) and two bands appeared with the primer OPM-09 (molecular sizes of 246 and 179 bp.) which distinguished the two ~~plants~~ species in the two wadis. Furthermore, four positive markers (with molecular size 1647, 1010, 549 and 413 bp.) appeared with the primer OPB-10 as four bands in both species only in WSH and these bands may be considered as a genetic finger print or positive genetic markers for these plants under the studied environmental conditions (Khalaf, 2005).

In *A. fragrantissima*, specific bands appeared only in WSH with molecular sizes of 1693, 1505, 1189, 896, 759 (OPA-10), 719 (OPE-04), 616 (OPM-09), 1647, 1010, 730, 549, 413 and 380 bp. (OPB-10) which were absent in WAR. However, there was a fragment of 1276 bp. (OPA-10 and OPG-19) present exclusively in WAR and can be considered as a unique band for *A. fragrantissima* .

A. judaica showed an increase in DNA concentration in WAR than in WSH. This was in agreement with the results of Mossallam *et al.* (2000) who reported that the most adaptive response of *A. judaica* growing on rocky habitat in the winter season is to increase the levels of auxins and cytokinins and to decrease the level of growth inhibitors which was accompanied with an increase in the levels of DNA, RNA, free amino acids, total -N and total carbohydrates. Similarly, *A. fragrantissima* attained the highest content of DNA concentration in WAR. Key (1989) suggested that hormonal imbalance may occur in some plants where cytokinin is thought to increase the synthesis of DNA; besides, that mRNA and auxin increase the ribosomal RNA component of cell division. Reddy *et al.* (2004) revealed that drought tolerance is achieved by cell-and tissue-specific physiological, biochemical, and molecular mechanisms, which include specific gene expression and accumulation of specific proteins.

The observed differences in the physiological responses of *Achillea fragrantissima* and *Artemisia judaica* growing in the two different wadis may throw some light on the differences in their osmoregulatory mechanisms under abiotic stresses. In addition, the increase in DNA concentration of the two species in WAR compared to WSH suggests that high variations which have been observed among samples of the same species collected from different sites could be due to differences in environmental and edaphic factors.

References

Abd El-Fattah RI , Baraka DM, El-Hady AF. 1993. Anions and cations accumulation in some plants from south Sinai under different habitat conditions. Desert Institute Bullitin, Egypt **43**(2), 281 – 293.

Abd El-Maboud MM. 2006. Ecophysiological responses of some xerophytes from Wadi El-Gafra, the Eastern Desert of Egypt. M. Sc. Thesis, Faculty of Science, Al-Azhar University, Cairo, Egypt.

Abd El-Maboud MM. 2011. Ecophysiological responses of *Salsola tetrandra* Forssk. and *Deverra tortuosa* Desf. under different habitat conditions of the North Western Coast of Egypt. Ph. D. Thesis, Faculty of Science, Al-Azhar University, Cairo, Egypt.

Abd El-Rahman AA. 1973. Effect of moisture stress on plants. Phyton (Austria) **15**(1 – 2), *67 – 86*.

Abd El-Rahman KM, Eissa, AGM. 1994. Nutritional studies on two most common range land species (*Stipagrostis scoparia* and *Hammada elegans*) in Al-Qassim region, Saudi Arabia. 1- Chemical analysis, feeding value and productivity. World Review of Animal Production **29**(2), 48 – 52.

Abo Sitta MY, Al Taisan AW. 1995. Variations in mineral ion composition of some succulent halophytes in the Estern Coastal Province of Saudi Arabia. Desert Institute Bullitin, Egypt **45**(1), 99 – 100.

Abu-Taha MM. 2010. Habitat and species diversity in some wadis in Sinai Peninsula. M. Sc. Thesis, Botany Deptment, Faculty of Science, Ain Shams University, Egypt.

Allen S, Grimshaw HM, Parkinson JA Quarmby C. 1974. Chemical Analysis of Ecological Materials. Blackwell Scientific Publications, Oxford. London, pp. 565.

Askar A Treptow H. 1993. "Quality assurance in tropical fruit processing" Springer-Verlag, Berlin, pp. 238.

Atkinson CJ. 1991. The flux and distribution of xylem sap calcium to adaxial and abaxial epidermal tissue in relation to stomatal behaviour. Journal of Experimental Botany, **42**, 987 – 993.

Boulos L. 2005. Flora of Egypt, Vol. 4: Monocotyledons (Alismataceae-Orchidaceae (Al Hadara Buplishing), 617 p.

Chaplin MF, Kennedy JF. 1994. "Carbohydrate Analysis" A practical approach. 2nd Ed. Oxford Univ. Press, Oxford, New York, Tokyo, 324 pp.

Chapman VD, Pratt EP. 1978. Methods of Analysis of Soils, Plants and Waters. Univ. of California, Division of Agricultural Science.

Danin A. 1983. Desert vegetation of Israel and Sinai. Jerusalem Publishing House. pp 148.

Dehan K, Tal M. 1978. Salt tolerance of the wild relatives of the cultivated tomato. Response of *Solanum pennclli* L. to high salinity. Irrigation Science. **1**, 71.

Dellaporta SL, Wood J, Hicks JB 1983. A plant DNA mini preparation. Version III. Plant Molecular Bioliology, Rep. **1**, 19 – 21.

El-Absy KM. 2011. Ecophysiological studies on some economic plants from wadi El-Arbaeen and wadi El-Sheikh, South Sinai, Egypt. Ph.D. Thesis, Botany Department, Faculty of Science, Tanta University, Egypt.

El-Lamey TM. 1999. Ecological and phytochemical studies on *Schouwia thebaica* Webb. in South Sinai. M.Sc. Thesis, Environmental Studies and Research Institute, Ain Shams University, Egypt.

Evans WC. 1999. Trease and Evans Pharmacognosy, 4th ed. WB Saunders Ltd". pp. 224-239

Greenway H, Munns R. 1980. Mechanisms of salt tolerance in non-halophytes. Annual Review of Plant Physiology **31**, 149 – 190.

Guenther R. 2005. Vegetation and grazing in the St. Katherine Protectorate, South Sinai, Egypt. Opera Wall Plant Report 1 – 44.

Harper HA. 1975. Review of Physiological Chemistry. 15th ed., Long Medicinal Publications, Los Angles, California P.570.

Harbone JB. 1973. Phytochemical methods, a guide to modern techniques of plant analysis. Chapman and Hall. London, 185-186.

Heldt HW. 1997. Plant Biochemistry and Molecular Biology. Oxford Univ. Press pp. 352 – 414, 501.

Ingram J, Bartels D. 1996. The molecular basis of dehydration tolerance in plants. Annual Review of Plant Physiology and Plant Molecular Biology **47**, 377- 403.

Jackson ML. 1962. Soil Chemical Analysis. Constable and Co. Ltd. London.

Jackson ML. 1967. Soil Chemical Analysis. Prentice-Hall of India, New Delhi., India.

Jackson WA, Thomas GW. 1960. Effect of KCl and Dolometic Limestone on growth and on uptake of sweat potato. Soil Science **89**, 347 – 352.

Jain VK. 1997. In "Fundamentals of Plant Physiology". Published by S.Chand and Company Ltd., Ram Nagar, New Delhi, pp 444.

Johnson CM, Ulrich A. 1959. Analytical Methods for Use in Plant Analysis. U.S. Dep. Agric., Calif. Univ., Agricultural Information Bullitin pp. 766.

Jones JB. 1977. Elemental analysis of soil extracts and plant tissue ash by plasma emission spectroscopy. Soil Science and Plant Analysis **8**, 349 *– 365.*

Kamel AM. 2011. Ecological survey and domestication of two range plants from wadi el-bagha at South Sinai. Ph. D. Thesis, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt.

Kasim WA, El-Shourbagy MN, Ahmed AM, El-Absy KM. 2008. Physiological adjustment of *Arthrocnemum macrostachyum* and *Nitraria retusa* to saline habitats in Sinai, Egypt. Austuralian Journal of Basic and Applied Sci*ence* **2**(3), 418-428.

Key JL. 1989. Modulation of gene expression by auxin. Biological Assays **11**, 52 – 58.

Khalaf MA. 2005. Biotechnological and physiological studies on *Artemisia* species in Sinai Peninsula. M. Sc. Thesis, Botany Department, Faculty of Science (Girls), Al-Azhar University, Cairo, Egypt.

Lawlor DW, Cornic G. 2002. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. Plant Cell Envionment **25**, 275 – 294.

Malik CP, Singh MB. 1980. Plant Enzymology and Histo-enzymology. A last manual Kalyani Publishers, New Delhi – Ludhiana.

Mann J. 1978. Secondary Metabolism. Oxford Univ. Press, Oxford.

Marigo G, Peltier JP. 1996. Analysis of the diurnal change in osmotic potential in leaves of *Fraxinus excelsior* L. Journal of Experimental Botany **47**, 763 – 769.

Miller RW, Donahue RL, Miller JU. 1995. Soils in our Environment. 7th ed. Prentice Hall, Englewood Cliffs, New Jersey, USA.

Mohammed S, Sen DN. 1987. Proline accumulation in arid zone plants. Journal of Arid Environment **13**, 231 – 236.

Morsy AA. 1996. Physiological studies on certain medicinal plants. M. Sc. Thesis, Faculty of Science, Ain Shams University, Egypt.

Mossallam HA, Abd Allah MM, Hassanein RA, Morsy AA. 2000. Eco-physiological studies on some lithophytes growing in Sinai, Egypt. Desert Institute Bullitin, A.R.E., Egypt **50**(1), 147 – 174.

Patakas A, Nikolaou N, Zioziou E, Redoglou K, Niotsakis B. 2002. The role of organic solute and ion accumulation in osmotic adjustment in drought stressed grapevines. Plant Science **163**, 361 – 367.

Patil BS, Pike LM. 1995. Distribution of quercetin content in different rings of various coloured onion (*Allium cepa* L.) cultivars. Journal of Horticultural Science **70**(44), 643 – 650.

Peach K, Tracey MV. 1956. Modern Methods of Plant Analysis. vol. 1. Springer-Verlag, Berlin, **4**, p 643.

Pelah D, Wang W, Altman A, Shoseyov O, Bartels D. 1997. Differential accumulation of water stress related proteins, sucrose synthase and soluble sugars in *Populus* species that differ in their water stress response. Physiologica Plantarum **99**, 153 – 159.

Pellet PL, Young VR. 1980. Nutritional Evaluation of Protein Foods. Published by the United Nation University.

Reddy AR, Chaitanya KV, Juture PP, Sumithra K. 2004. Differential antioxidative responses to water stress among five mulberry (*Morus alba*, L.) cultivars. Environmental and Experimental Botany **52**, 33 – 42.

Rowell DL. 1994. Soil Science: Methods and Application. Longman Publishers, Singapore. 350 pp.

Salisbury FB, Ross CW. 2000. Plant Physiology, 5th ed. Wadsworth Publishing Company, Inc. Belmont. California.

Selmar D. 2008. Potential of salt and drought stress to increase pharmaceutically significant secondary compounds in plants. Agriculture and Forestry Research **85**, 139 – 144.

Standard Methods for the examination of water and waste water 1989. APHA, AWWA, WPCE, Washington DC. U.S.E.P.A.

Stewart CR, Larcher F. 1980. Accumulation of amino acids and aelated compounds in relation to environmental stress. In "The Biochemistry of Plants". Vol. 5, B.J. Miflin (ed.). Academic Press, New York, pp, 609- 630.

Täckholm V. 1974. Students' Flora of Egypt, (ed. 2). Cairo Univ., Beirut, pp 888.

Torel J, Cillard J, Cillard P. 1986. Antioxidant activity of flavonoids and reactivity with redoxy radical. Phytochemistry **25**(2), 383 – 385.

Virk SS, Singh OS. 1990. Osmotic properties of drought stressed periwinkle (*Catharanthus roseus*) genotypes. Annals of Botany **66**, 23 – 30.

Wang S, Wan C, Wang Y, Chen H, Ztou Z, Fu H, Sosabookee ER. 2004. The characteristic Na^+, K^+ and free proline distribution in several drought-resistant plants of the Alaxa Desert, China Journal of Arid Environment **56**(3), 525 – 539.

Williams JGK, Kubelik AR, Livak KJ, Rafalski A, Tingey SV. 1990. DNA polymorphism amplified by arbitrary primers is useful as genetic markers. Nuclic Acid Research **18**, 6531 – 6535.

Yamada M, Morishita H, Urano K, Shiozaki N, Kazuko Y, Shinozaki K, Yoshiba Y. 2005. Effects of free proline accumulation in petunias under drought stress. Journal of Experimental Botany **56**(417), 1975 – 1981.

Youssef AM. 1988. Ecological studies on the plant communities of the desert area, South Ismailia. M.Sc. Thesis, Botany Department, Faculty of Science, Ain Shams University, Cairo, Egypt.