

Mechanisms of Stress Tolerance in Some Important Wild Plants Grown in the Arabian Desert

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Abstract: This work aimed to investigate mechanisms of stress tolerance for various wild plants widely distributed in the desert of central Saudi Arabia. The study included nine permanent herbaceous or sub shrubby species of different families and various morphological characteristics. The studied species were *Anthemis pseudocotula*, *Citrullus colocynthis*, *Heliotropium bacciferum*, *Heliotropium supinum*, *Plantago coronopus*, *Rhazya stricta*, *Rumex vesicarius*, *Scorzonera intricata* and *Zygophyllum coccineum*. Accumulation of organic and inorganic solutes was analyzed in both herbs and roots. Organic solutes comprised the total soluble sugars (TSS) and free amino acids (FAA), while the inorganic ones included potassium (K⁺), sodium (Na⁺), calcium (Ca²⁺) and magnesium (Mg²⁺). Accumulation of organic solutes specially soluble sugars was the main mechanism to increase osmotic pressure inside plant cells. Free amino acids were very low in all species except *Citrullus colocynthis*, *Scorzonera intricata* and *Zygophyllum coccineum*, which may follow such mechanism to prevent dehydration. TSS concentrations were higher in roots but that of FAA were higher in herbs. Most species maintained high percentages of potassium with very low levels of sodium and subsequently high K/Na ratio. Sodium was low in both roots and herbs however K, Ca and Mg were higher in herbs. Calcium and magnesium seemed to have role in stress tolerance for some species as *Rumex vesicarius* and *Zygophyllum coccineum*. Variability was found among the studied species in relation to their morphological characteristics. The location was an important factor affecting concentration of solutes in plant tissues. Results could be beneficial to understand stress tolerance and improve plants by the transfer of genes coding for the accumulation of solutes.

Key words: Desert plants • Abiotic stress • Osmotic potential • Adaptation • Tolerance

INTRODUCTION

Desert includes different types of abiotic stress including drought, salinity and temperature. Desert plants follow different mechanisms to avoid dehydration and adapt with these stresses. These mechanisms comprise escape, avoidance and tolerance. Herbaceous plants usually adapt with stress throughout escape or avoidance [1]. The escaping plants accelerate development and complete their life during rainy season to escape drought. The avoidance species prevent dehydration through low water consumption and low water loss. Tolerant plants include various woody and herbaceous plants possessing different mechanisms to adapt with stress [2]. These plants have the ability to withstand dehydration via osmotic adjustment and stabilize proteins throughout the production of large molecules [3]. Accumulation of

organic and inorganic solutes is one of the most important mechanism allowing the adjustment of osmotic pressure and subsequently maintaining water inside plant cells [4, 5].

Saudi Arabia covers a major part of Arabian Peninsula which is a vast desert including diverse ecosystems and various tolerant plants with interesting physiological aspects. The desert of the central region of Kingdom is characterized by drought sandy soils, low precipitation, high evaporation and dry climate of an average annual rains of less than 100 mm per day [6]. This region comprises about 450 wild and cultivated species of 62 different families [7]. Qassim is the most rich region in the central Saudi Arabia providing various wild species of multiple uses [8, 9]. The grown plants of Qassim region were divided into xerophytes, halophytes and mesophytes [10].

Very little information is available on the stress tolerance mechanisms of the different species successfully grown in the desert of such important region. The study of adaptation mechanisms in three different species including *Suaeda fruticosa* (halophyte), *Artemisia judaica* (xerophyte) and *Rumex vesicarius* (mesophyte) showed different responses regarding osmoregulation [11]. The ecophysiological study of three desert plants grown in the Eastern desert of Egypt showed that osmotic adjustment was the main adaptation way to adapt with drought [12]. No information is available on the tolerance mechanisms of other important plants grown in different locations of this region and the distribution of solutes in plant tissues is still unknown. The present work aimed to study the mechanisms of stress tolerance in nine herbaceous and sub shrubby wild species widely distributed as tolerant plants in the Arabian desert of the central region of Saudi Arabia. The distribution of organic and inorganic solutes within different plant organs and the effect of location were also investigated to understand the tolerance phenomenon.

MATERIALS AND METHODS

Area of Study and Studied Species: This study was carried out on wild plants grown in the desert of the central region of Saudi Arabia (Qassim). This region includes diverse ecosystems with various tolerant plants providing interesting physiological aspects. Nine wild species belonging to different families were studied to investigate their mechanisms of tolerance to abiotic stress (Table 1 and Figure 1). The selection of these species was based on their nature as permanent herbaceous or sub shrubby plants widely distributed in various locations of the region. They also had different growth nature with

various morphological characteristics (Figure 1). The survey of these tolerant species included 4 locations of about 1000 km². Six species were collected from one location (L₁) and four species were collected from another location (L₂) sited at 50 km to investigate the location effect (Table 1). The survey was carried out throughout two successive seasons of September 2016 and March 2017 where plant materials were collected. Plant materials included the herbs and roots of five species of similar location (L₁), the herbs of three species collected from the other location (L₂) and the herbs of one species collected from both locations (Table 1). The identification of the studied species was based on the previous published reports [8, 9].

Collection and Preparation of Plant Materials: Herbs (vegetative part including shoots and leaves) and roots of studied species were collected at early morning and put in plastic bags then transferred to the laboratory. The collected materials were washed twice in distilled water then dried at 70°C for 3 days. After full drying, samples were ground and stored in paper bags till analysis.

Analysis of Organic Solutes: Total soluble sugars (TSS) was determined colorimetrically in dry herb and root after extraction in ethanol 80% using sulphuric acid-phenol method and the absorbance was measured at wavelength of 490 nm [13]. The determination of free amino acids was achieved colorimetrically using ninhydrin 5% and the absorbance was recorded at 570 nm wavelength according to Moore and Sten [14]. Both organic solutes were measured using UV-120-02 spectrophotometer, Shimadzu, Kyoto, Japan. Sugars were recorded as percentage in dry materials while, amino acids were measured as ppm or mg kg⁻¹.

Table 1: The studied wild species, their families, location and collected organs

	Species	Family	Location	Organ
1	<i>Anthemis pseudocotula</i>	Compositae / Asteraceae	L ₁	Herb
2	<i>Heliotropium bacciferum</i>	Boraginaceae	L ₁	Herb
3	<i>Plantago coronopus</i>	Plantaginaceae	L ₁	Herb
4	<i>Rumex vesicarius</i>	Polygonaceae	L ₁	Herb
5	<i>Scorzonera intricata</i>	Compositae / Asteraceae	L ₁	Herb
1	<i>Anthemis pseudocotula</i>	Compositae / Asteraceae	L ₁	Root
2	<i>Heliotropium bacciferum</i>	Boraginaceae	L ₁	Root
3	<i>Plantago coronopus</i>	Plantaginaceae	L ₁	Root
4	<i>Rumex vesicarius</i>	Polygonaceae	L ₁	Root
5	<i>Scorzonera intricata</i>	Compositae / Asteraceae	L ₁	Root
6	<i>Citrullus colocynthis</i>	Cucurbitaceae	L ₂	Herb
7	<i>Heliotropium supinum</i>	Boraginaceae	L ₂	Herb
8	<i>Rhazya stricta</i>	Apocyanaceae	L ₂	Herb
9	<i>Zygophyllum coccineum</i>	Zygophyllaceae	L ₁	Herb
9	<i>Zygophyllum coccineum</i>	Zygophyllaceae	L ₂	Herb

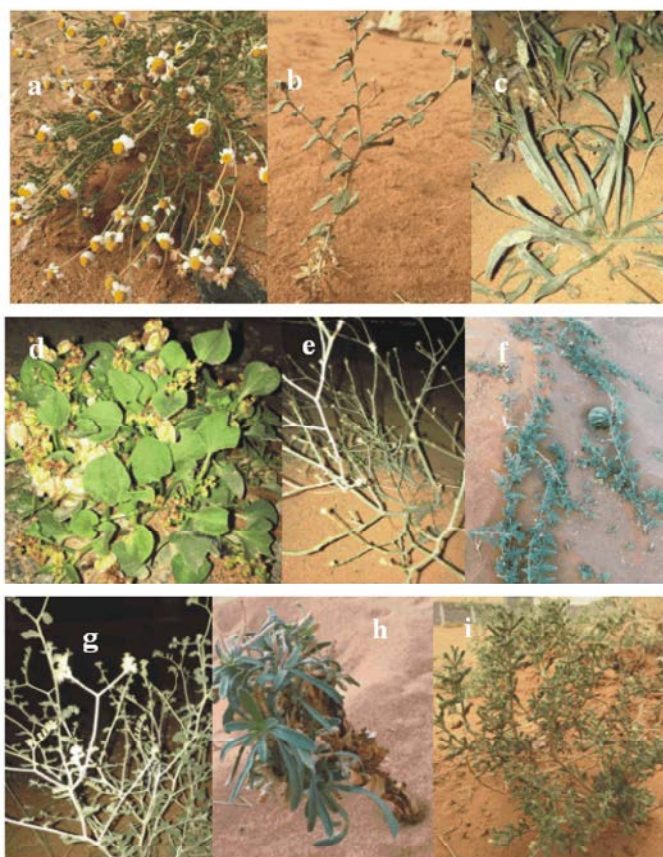


Fig. 1: The studied species including *Anthemis pseudocotula* (a), *Heliotropium bacciferum* (b), *Plantago coronopus* (c), *Rumex vesicarius* (d), *Scorzonera intricata* (e), *Citrullus colocynthis* (f), *Heliotropium supinum* (g), *Rhazya stricta* (h) and *Zygophyllum coccineum* (i)

Analysis of Inorganic Solutes: Dry samples of herbs and roots were digested in a mixture of H_2SO_4 and HNO_3 then the concentrations of potassium (K^+) and sodium (Na^+) were determined using Flame Photometer following to Jones [15]. Both elements were recorded as percentages and K/Na ratio was calculated for all treatments. Calcium (Ca^{+2}) and magnesium (Mg^{+2}) were measured in samples using ICP (Inductively Coupled Plasma (Emission Spectrometry Thermo 7000, according to Jones [15]. Both elements were recorded as percentages in dry weights (%).

Statistical Analysis: Analysis of variance (ANOVA) was applied to all the collected data followed by the comparison of means at $\alpha = 0.05$ using Duncan's multiple range tests (SAS 9.1.3 service pack 4). Percentages were transformed before analysis using the equation ($x + 0.5$ then square root) to normalize the distribution of data [16].

RESULTS

Analysis of Organic Solutes: Contents of total soluble sugars and free amino acids in the herbs and roots of studied species are presented in Table (2). Results showed highly significant differences among species for both sugars and amino acids.

Total Soluble Sugars: High percentages of sugars were found in all the studied species whatever plant organ or location (Table 2). Generally, average of sugar percentage in roots was higher than that in herbs for similar species grown in location 1; 4.7% versus 3.2% respectively. These five species also showed significantly different sugar percentages regardless plant organ where it mainly varied from 1.7% in *Rumex vesicarius* to 9.5% in *Scorzonera intricata*. Sugars percentage in herb and root varied according to species where some species (*Scorzonera intricata* and *Plantago coronopus*) showed significantly

Table 2: Organic solutes in the herbs and roots of various wild species in different locations

Species	Location	Organ	Sugars %	Amino acids ppm
<i>Anthemis pseudocotula</i>	L ₁	Herb	3.00 d	22.50 g
<i>Heliotropium bacciferum</i>			2.50 e	270.00 def
<i>Plantago coronopus</i>			3.10 d	22.50 g
<i>Rumex vesicarius</i>			1.70 f	112.50 efg
<i>Scorzonera intricata</i>			5.90 b	787.50 b
<i>Anthemis pseudocotula</i>	L ₁	Root	1.40 f	9.00 g
<i>Heliotropium bacciferum</i>			2.40 e	45.00 fg
<i>Plantago coronopus</i>			4.86 c	12.17 g
<i>Rumex vesicarius</i>			1.63 f	5.63 g
<i>Scorzonera intricate</i>			13.00 a	45.00 fg
<i>Citrullus colocynthis</i>	L ₂	Herb	4.40 c	4950.00 a
<i>Heliotropium supinum</i>			3.50 d	112.50 efg
<i>Rhazya stricta</i>			1.80 f	450.00 cd
<i>Zygophyllum coccineum</i>	L ₁	Herb	3.10 d	585.00 bc
<i>Zygophyllum coccineum</i>	L ₂	Herb	2.30 e	292.50 de

Means with the same letter are not significantly different at $\alpha = 0.05$

Table 3: Inorganic solutes in the herbs and roots of various wild species in different locations

Species	Location	Organ	K %	Na %	K/Na ratio	Ca %	Mg %
<i>Anthemis pseudocotula</i>	L ₁	Herb	2.95 a	0.28 e	10.73 b	1.71 c	0.29 e
<i>Heliotropium bacciferum</i>			1.00 f	0.30 e	3.33 g	2.17 a	0.38 cd
<i>Plantago coronopus</i>			1.90 c	0.30 e	6.33 d	2.04 b	0.41 cd
<i>Rumex vesicarius</i>			1.00 f	2.60 a	0.38 j	2.06 b	1.10 b
<i>Scorzonera intricate</i>			1.80 c	0.38 e	4.80 e	1.45 d	0.35 de
<i>Anthemis pseudocotula</i>	L ₁	Root	1.25 de	0.28 e	4.55 ef	0.73 h	0.08 g
<i>Heliotropium bacciferum</i>			0.60 g	0.28 e	2.18 h	0.85 g	0.13 fg
<i>Plantago coronopus</i>			1.38 d	0.35 e	3.93 fg	1.02 f	0.18 f
<i>Rumex vesicarius</i>			0.90 f	1.53 b	0.59 j	1.19 e	0.40 cd
<i>Scorzonera intricata</i>			1.18 e	0.28 e	4.27 ef	0.72 h	0.15 fg
<i>Citrullus colocynthis</i>	L ₂	Herb	3.05 a	0.53 d	5.81 d	1.17 e	0.45 c
<i>Heliotropium supinum</i>			2.73 b	0.15 f	18.17 a	1.79 c	0.34 de
<i>Rhazya stricta</i>			2.63 b	0.35 e	7.50 c	1.21 e	0.36 de
<i>Zygophyllum coccineum</i>	L ₁	Herb	1.35 d	0.88 c	1.54 hi	2.03 b	1.30 a
<i>Zygophyllum coccineum</i>	L ₂	Herb	1.00 f	0.93 c	1.08 ij	2.25 a	1.31 a

Means with the same letter are not significantly different at $\alpha = 0.05$

higher percentage in roots, while others (*Anthemis pseudocotula*) had significantly higher percentage in herbs and others (*Heliotropium bacciferum* and *Rumex vesicarius*) were similar in sugars concentration in herb and root. Similar trend was also found in sugars concentration in the species of second location (L₂) where they had significantly different sugar percentages (1.8 – 4.4%). Comparing sugars percentage in the herb of *Zygophyllum coccineum* plants from different locations showed significant differences between these locations.

Free Amino Acids: The variability in amino acids content was lower than that in sugars where most species had low concentrations of free amino acids whatever plant organ or location (Table 2). Generally, amino acids concentration in herbs was higher than that in roots for all studied species; 243.0 ppm versus 23.4 ppm, respectively. Most

species were similar in their contents of amino acids except *Heliotropium* which showed significantly higher concentration of amino acids compared to other species. The herbs of this genus had the maximum content of amino acids compared to its roots and the roots and herbs of other species which showed similar low concentrations of amino acids. However, a variability was found in the concentrations of amino acid in the herbs of species grown in location 2 where it varied from 112.5 ppm to 4950.0 ppm. Significant difference was also found between amino acid concentrations in *Zygophyllum coccineum* grown under two different locations.

Analysis of Inorganic Solutes: Data recorded in Table (3) showed percentages of inorganic solutes including potassium (K), sodium (Na), calcium (Ca) and magnesium (Mg) in the herbs and roots of studied species.

Potassium: Potassium percentage was generally higher in herbs (1.7%) in comparison with roots (1.1%) of the same species (Table 3). All species contained high level of potassium but they varied in its concentration from 0.8% to 2.1% as average. Potassium percentage in herb was higher than that in root for all species. The maximum potassium percentage was found in the herb and root of *Anthemis pseudocotula* and the minimum level was found in *Rumex vesicarius* and *Heliotropium bacciferum*. Species of second location (L_2) showed higher level of potassium compared to those of first location (L_1) except *Zygophyllum coccineum* showing higher potassium percentage in location 1. This species also showed the minimum concentration of potassium compared to other species of the same location.

Sodium: Most species showed low percentages of sodium except *Rumex vesicarius* which contained the highest sodium content in its herb and root (Table 3). No significant differences were found between sodium percentage in the herb and root of all species except in *Rumex vesicarius* which showed significantly higher sodium level in its herb compared to the root. Sodium percentage was also low in the species of location 2 and *Zygophyllum coccineum* showed similar sodium percentages in both locations.

Potassium/Sodium Ratio: Potassium/sodium ratio in the herb (5.1) was higher than that in root (3.1), (Table 3). All species had mean ratio higher than 1 except *Rumex vesicarius* which show a ratio of 0.5. This ratio varied among species where it ranged from 2.8 to 7.6. The maximum K/Na ratios were obtained from *Anthemis pseudocotula* and *Heliotropium supinum* and the minimum ones were found in *Rumex vesicarius* and *Zygophyllum coccineum*. The superior species (those of high K/Na ratio) maintained the maximum potassium content with the minimum sodium level in their tissues, in the contrary of inferior ones (species of low K/Na ratio) which had low level of potassium and high level of sodium. Species of location 2 also differed significantly in K/Na ratio and *Zygophyllum coccineum* showed the minimum ratio in both locations.

Calcium and Magnesium: Calcium percentage was higher than Mg percentage in all species whatever organ or location (Table 3). Calcium and magnesium percentages in the herbs of studied species were generally higher than those found in their roots. They varied from 1.8% and 0.5% in the herb to 0.9% and 0.2% in the root. Species

also differed in their concentrations of both elements specially Ca under both regions. The maximum percentage of both Ca and Mg was found in *Rumex vesicarius* and *Zygophyllum coccineum*. The last one showed different Ca percentages and similar Mg levels under the two studied regions.

DISCUSSION

The present study investigated the accumulation of organic and inorganic solutes in different organs of various herbaceous wild plants to identify the most important mechanisms of stress tolerance in these species. This mechanism is usually followed to increase osmotic pressure and subsequently maintaining water inside plant cells [5]. Results showed that the studied species followed different mechanisms regarding this phenomenon. Accumulation of sugars seemed to be the most important mechanism for all species where a large amount of soluble sugars, from 1.4% to 13 %, was found in their tissues compared to low amount of free amino acids. Sugars are well known as osmotic agents capable of increasing osmotic pressure relating to their large molecules. They were found to be the most important solutes in various plant species [17]. The transfer of genes coding to sugars accumulation was widely applied as the most effective way to improve stress tolerance [18]. The presence of amino acids in the form of proteins could explain its low level. The low concentration of free amino acids may be considered a tolerance indicator where they usually resulted from the degradation of protein under stress. Proline, as the most important amino acid, was considered an indicator of stress severity or sensitivity in many plant species [19]. Furthermore, amino acids are usually concentrated in cytosol to maintain osmotic balance between cytoplasm and vacuole [20]. However, the relatively high concentration of amino acids in some species including *Citrullus colocynthis*, *Scorzonera intricata* and *Zygophyllum coccineum* (0.1 - 0.5%) could be a specific mechanism of tolerance for these species. Results showed higher concentration of sugars in roots and higher concentration of amino acids in herbs. The low sugars percentage in herbs could be explained by their consumption in growth, development and other physiological activities achieved by vegetative system. However, the high concentration of free amino acids in herbs could be resulted from the degradation of proteins in vegetative system as the part more exposed to ambient temperature.

The inorganic solutes seemed to be very effective factors in the stress tolerance of the studied species. It was reported that uptake and accumulation of ions needs less energy compared to the synthesis of organic solutes [21]. Most species showed high percentage of potassium and very low level of sodium which resulted in high K/Na ratio. It seemed to be good mechanism of tolerance where tolerant plants accumulate potassium (K^+) as beneficial nutritional element to compete sodium (Na^+) and avoid its harmful effect [22]. The high selectivity of these species could be another factor leading to the maximum absorption of potassium and the minimum absorption of sodium. Potassium is well known as an essential macronutrient and a primary osmoticum maintaining low water potential in plant tissues [4]. Sodium was very low in both studied organs but potassium was higher in herb compared to root. Similar results were found in other wild species [11]. The low K/Na ratio in some species as *Rumex vesicarius* and *Zygophyllum coccineum* resulted from the relatively low potassium content and the high sodium absorption. These species probably had different mechanism of tolerance throughout accumulation of solutes where they showed the highest percentages of calcium and magnesium. The other species had a moderate percentages of both elements especially in vegetative parts. The role of Ca as important compounds of cell wall and the importance of Mg as main component in chlorophyll may explain this result. Similar results were reported in ecophysiological study effectuated on three desert plants grown in Egypt [12].

Results showed a variability in the contents of solutes in the studied species which could be related to their nature and genetic diversity. The morphological characteristics of different species could be also considered another factor affecting this result (Figure 1). It was reported that species differed in the regulation of osmotic potential through the accumulation of organic or inorganic solutes [11]. The variation found between studied locations may be due to the different soil structure, climatic changes and environmental conditions of each location.

CONCLUSION

The analysis of organic and inorganic solutes in the studied wild species showed that plants followed different mechanisms to adapt with abiotic stress under environmental conditions of desert. Accumulation of

organic solutes, specially soluble sugars, seemed to be the main mechanism to increase osmotic pressure and subsequently maintain water inside plant cells. Free amino acids were found in low quantity in most species which may indicate their tolerance to the degradation of proteins. Some species as *Citrullus colocynthis*, *Scorzonera intricata* and *Zygophyllum coccineum* showed a relatively high amino acids content which could be considered a specific mechanism of tolerance for these species. The analysis of organic solutes in herbs and roots showed that soluble sugars were higher in roots while amino acids were higher in herbs. The studied species also showed some mechanisms of tolerance regarding inorganic solutes. Most species maintained high concentrations of potassium with very low levels of sodium which resulted in high K/Na ratio. Sodium was very low in both roots and herbs but potassium was higher in herbs. Calcium and magnesium were also higher in vegetative parts. These elements seemed to have a tolerance role in some species as *Rumex vesicarius* and *Zygophyllum coccineum* which showed higher levels of both elements compared to other species. A variability was found among the studied species in relation to their morphological and genetic characteristics. The location was also an important factor affecting concentration of solutes in the studied species. The results showed different mechanisms for the most important wild species distributed in the central region of Saudi Arabia. They also provided valuable information about the transfer of organic and inorganic solutes between herb and root. Such results could be beneficial on both fundamental and applied levels to understand stress tolerance in desert plants and improve economic species by the transfer of genes coding for the accumulation of organic and/or inorganic solutes.

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