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Contribution of solutes to the osmotic adjustment of *Deverra tortuosa* (Desf.) DC

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Abstract

Deverra tortuosa is a xerophytic and salt tolerant plant and able to grow on very different soils. It was collected from Wadi Sudr, Wadi EL-Natrun EL-Alamein road and Wadi Um Ashtan during dry and wet seasons. This study aimed to investigate physiological responses and metabolic changes in *Deverra tortuosa* during stress conditions to identify metabolite compounds that are responsible for stress tolerance. The results of organic acids analysis in *Deverra* demonstrated that the concentration of the majority of detecting organic acids increased significantly with stress. The adaptation of *Deverra* to the arid environment in term of osmotic adjustment was documented in this study, it tended to accumulate certain compatible solutes to reduce its internal osmotic potentials. These osmotically active metabolites include inorganic ions solutes (Ca^{2+}, and SO_4^{2-}), carbohydrates, soluble sugars, reducing sugars, and organic acids, which may act in osmotic adjustment, assist in turgor maintenance and help to enhance drought tolerance. Osmotic adjustment through the synthesis of soluble sugars, has been postulated to have a significant role in drought tolerance in *Deverra*, it seems to be the main active compounds in the osmotic potential (Ψs). Since the estimated contributions of total soluble sugars to osmotic potential (Ψs) under stress conditions were 24.7% at Wadi Sudr and 30.4% at Wadi Um Ashtan and reached its maximum value of 35.9% at El-Alamein road. In conclusion, *Deverra tortuosa* depends on the accumulation of minerals, especially Ca^{2+} together with the organic solutes in its cytoplasmic osmoregulation to adapt to arid environments.

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Introduction

Under natural conditions plant can exposed to a variety of environmental stresses, such as drought, low or high temperature, excessive salinity. These abiotic stress factors generate secondary stress, i.e. osmotic and oxidative stress, which have negative influence on the plant, causing changes in its proper growth, development and metabolism (Bohnert *et al.*,1995) Environmental stresses cause significant loss of plant productivity. Abiotic stresses, e.g. light, UV, temperature, drought, soil salinity, air pollution, and mechanical damage, directly affect crop production (Vickers *et al.*, 2009). For example, the US drought of 2012, which was the most severe in at least 25 years, had a serious impact on production and yields of corn and soybean, leading to economic damage (Gilbert, 2012). This observation strongly suggests that a better understanding of the stress response is critically important for agricultural and economic performance to improve the tolerance of crops against such environmental stresses using phytochemical genomics and crop breeding (Mir *et al.*, 2012).

Plants exhibit a range of specific responses when exposed to mild or severe drought conditions, aimed to reduce water loss and/or to optimise water uptake. Among the earliest responses, reduction in vegetative growth, stomatal closure and a decrease in the rate of photosynthesis are observed (Lawlor and Tezara, 2009; Chaves *et al.*, 2009). Upon exposure to osmotic stress as a result of high temperature, drought and high salinity, plants accumulate a range of osmolytes with the primary function of turgor maintenance. Osmotic adjustment is considered as an important mechanism for drought adaptation. It assists in the maintenance of cell turgor through the accumulation of solutes and may reduce the effects of water stress both in vegetative and reproductive phases of crop growth (Subbarao *et al.,* 2000).

The types of osmoprotectant metabolites and their relative contribution in lowering the osmotic potential differ greatly among plant species (Silvente *et al.*, 2012).

Among xerophytic shrubs, *Deverra tortuosa* (Desf.)DC. (Qozaah) is a xerophytic and salt tolerant plant which grows in almost all the phytogeographical regions of Egypt especially desert wadis and sandy and stony plains. The plant is widely distributed in Tunisia, Libya, Egypt, Palestine and Saudi Arabia (Boulos, 2000). *Deverra* is a scleromorphic plant, which characterised by the presence of tissue (Sclerenchyma) composed of thick-walled cells that give their mechanical strength (Batanouny, 2001). Its roots may reach to several meters. Since, the root of *Deverra* was followed down to a depth of 5 meters, to the wetted soil layers in a small wadi at the Cairo to Suez road (Walter and Breckle, 2013).

The main goal of this study was to investigate metabolic changes in *Deverra tortuosa* during stress responses to identify metabolites compounds that are responsible for stress tolerance.

Materials and methods

Plant species

Deverra tortuosa (Desf.)DC. Synonym: *Pituranthos tortuosus* (Desf.) Asch. and Schweinf. Strongly aromatic glabrous shrub, 30-80 cm; stems dichotomously branched, striate; leaves caducous; basal leaves 3-8 cm, 2-pinnatisect into linear-subulate, acute lobes; petiole sheathing, with broad scarious margin; lower cauline leaves with sheaths to 1.5 cm; blade 1-2.5 cm, ternatisect, the lobes linear-subulate; upper leaves reduced to sheaths with filiform apices; umbels mostly terminal; peduncle 1.5-4 cm, stout; umbel-rays 6-10, 1-2 cm, subequal; bracts 2-3 x 1-1.5 mm, triangular, the margin scarious, the apex mucronate; bracteoles minute; bracts and bracteoles persistent; pedicel 0-1.5 mm; flowers hardly opening; petals almost glabrous; styles longer than the depressed stylopodium; fruit 1-1.5 mm, globose, hirsute Täckholm (1974) and Boluos (1999 and 2002).

Ecological studies

Study area

The study was carried out in three Areas; the first area was at Wadi Sudr in Ras Sudr (29° 35´30″ N, 32° 42´20″ E), South Sinai, and the second area was at 74 Km from the beginning of Wadi EL-Natrun EL-Alamein Road(30° 29´74″ - 30´ 43´65″ N, 30° 08´71″ -29° 01´14″E).While the third area lies in Wadi Um Ashtan, 18 km west of Mersa Matruh, Mersa Matruh Government between (31° 17´ 20″-31° 22´ 20″N latitude , 27° 00´ 00″-27° 02´ 30″E longitude), they represent the coastal area of western desert in Egypt.

Wadi Sudr is one of the most developed wadis of the northern group of south-west Sinai. The wadi is bounded by Gabel El-Raha (c-600m) from the North and Sinn Bishr (618m) from the South. The main trunk of the wadi extends roughly in NE-SW direction for a distance of about 55 Km and pours at Ras Sudr. Meteorological data of Ras Sudr indicated South Sinai is characterized by an arid to extremely arid climate and irregularity in rainfall. The climate is influenced by the orographic impact of the high mountains (Migahid *et al.*, 1959; Issar and Gilad, 1982). During the study period (from 2007 to 2008), mean of annual temperature was 23.2°C and total annual precipitation was 2.79 mm in 2007 and 11.42mm in 2008. Maximum annual temperature was 29.3°C in 2007 and 29.4°C in 2008, while minimum annual temperature was 17.4°C in 2007 and 17.6°C in 2008. Dry period extended from April to December in 2007 and 2008 at the two localities. The average of annual wind speed was 12.1 Km/h. These data indicate that the studied habitat have an arid type climate with high temperature especially during the dry period.

During the past century, mean of annual temperature has increased by 0.75°C and precipitation has shown marked variation throughout the Mediterranean basin (Osborne *et al.*, 2000). This change affected the wild vegetation of South Sinai in general and resulted in rarity of trees and change in vegetation composition (Moustafa *et al.*, 2001).

The second area; Wadi EL-Natrun EL-Alamein Road is located in the Libyan portion of the Sahara, which is called now the western desert of Egypt (Abd El Ghani and Marei, 2005), it is at 74 Km from the beginning of Wadi EL-Natrun EL-Alamein Road in Matruh governorate. The climate data (1998-2002) of Wadi El-Natrun indicated that mean maximum temperature was 36.2°C in July and mean minimum temperature was 8.2°C in January. The dry period extended for five years from May to September, means of annual rainfall (1998-2002) were 10.2,10, 24, 9.4 and 40.6mm, respectively. Another climate data was collected from El-Dabaa station, indicated that August was the hottest summer month and its mean temperature was 26.25°C, while the coldest month was January and its mean temperature was about 12.5°C.The dry period extended from June to August.While means of annual rainfall (1998- 2002) were 99.9, 47.5, 229.1, 70.3 and 123.5mm, respectively. The average of annual humidity was 65.88% in Wadi El-Natrun and 69.2 % in El-Dabaa.

The third area of the present study was at Wadi Um Asthan, 18 km west of Mersa Matruh, situated within a region of dry climate. This area is classified as arid with mild winter and warm summer (UNESCO, 1977). Means of annual rainfall was 263.13mm and 137.16mm during 2007 and 2008, respectively (increasing from West to East and from South to North). Mean of annual temperature was 20.3°C, and maximum annual temperature was 24.5°C in 2007 and 25.2 °C in 2008, while minimum annual temperature was 15.8°C in 2007 and 16.1°C in 2008.The average of annual humidity was 67.5% in 2007 and 65.7% in 2008.

Soil analysis

Samples from the soil supporting *Deverra tortuosa* were taken at two depths; (0 - 20 cm) and (20-40) in Wadi Sudr and one depth (0-40cm) in Wadi EL-Natrun EL-Alamein Road and Wadi Um Ashtan.

a) Soil physical properties

Soil texture (granulometric analysis) was determined through mechanical analysis by the sieve method (Jackson, 1967).

b) Soil chemical analysis

The preparation of samples was carried out according to Page, (1987).The hydrogen ion concentration (pH) and Electrical Conductivity (EC) measurements. The pH of soil extract was measured using pH meter instrument and the EC was measured by electrical conductivity meter.

Cations determination

The concentration of potassium and sodium was determined by a flame photometer (Jenway PFP7) and calcium and magnesium contents were determined by titration with ethylene diamine tetra-acetic acid (EDTA) according to the method of Rowell (1994). While Phosphorous was calorimetrically determined using ammonium molybdate and ascorbic acid (Rowell, 1994) and absorbance was read at 880 nm using spectrophotometer (Unicom UV-300).

Anions determination

Sulphate ions were precipitated as barium sulphates according to the turbidimetric method (Rowell, 1994**)** and absorbance was read at 480 nm using a spectrophotometer (Unicom UV-300.While the concentration of chloride was determined by titrating a known volume of the soil extract against silver nitrate (0.5N) using 1% potassium chromate as an indicator as described by Jackson (1967).

Physiological studies

Plant water status

Plant water content

Plant water content, the difference between fresh weight (FW) and dry weight (DW), was calculated on a dry basis using the following formula:

Plant water content (ml /100gDW) = $\frac{FW-DW}{DW} \times 100$.

Determination of plants osmotic potential

Sample preparation was done according to Simmelsgaard (1976). The fresh plants were collected in liquid nitrogen, and then stored at-20˚C until the time of measurement. Plant samples were thawed at room temperature and pressed to free the cell sap. The osmotic potential of the expressed sap was measured with freezing point osmometer (osmomat 030 - Gonotec - Berlin - Germany) which was calibrated with KCl. The osmotic potential of a given solute was calculated as: $\Psi_s = nRT/V$,where n is the number of solute molecules ;R. the universal gas constant ;T, temperature in K; and V, volum in liter(Baker,1984).

Plant chemical analysis

Mineral analysis

The aerial parts of *Deverra tortuosa* were collected from three natural field population and dried in the oven at 70˚C to a constant weight and ground to fine powder. Nitrogen (N) content of samples were estimated by the method described by Kjeldahl (1983) and crude protein was calculated as N×6.25 (James, 1995).

The dried sample (0.50) were taken and digested with 10 ml concentrated nitric acid. After adding (2-4ml) of perchloric acid, the contents were heated gently on a hot plate and used for mineral analysis (Baker and Smith, 1974).The concentrations of calcium, magnesium, Potassium and sodium were determined according to the method of Rowell (1994). Plant dry matter was ashed , part of ashed powder was dissolved in HCl to measure sulphur and phosphorus contents in plant by using turbidimetic and phosphomolybdat methods (Rowell 1994), respectively, while the other part was dissolved in diluted nitric acid (0.01N) to extract chloride from ashed powder samples and titrated the extracted chloride with standard silver nitrate (Jackson and Thomas, 1960).The concentrations of manganese, copper, zinc and iron were determined by using ICP emission spectroscopy (Jones, 1977).

Determination of total carbohydrates

The total carbohydrates in the arial parts of *Deverra tortuosa* were extracted by dissolving 0.3gm of plant powder in 10ml of 3% HCl. The tube was sealed and heated at 100˚C for 2-5 hours. The extracted sugars were estimated using the phenol–sulfuric acid method (Buysse and Merck, 1993).

Determination of total soluble sugar, reducing sugars

The plant sample was extracted twice in 40 ml of boiling water and twice in 40 ml of aqueous boiling ethanol (80%v/v) and clarified using saturated neutral lead acetate solution (AOAC method 2000). The total soluble sugar were estimated using the general phenol–sulfuric acid method (Buysse and Merck, 1993). while reducing sugar was estimated according to Nelson-Somogi method (Chaplin and Kenney, 1994).

Determination of organic acids

Organic acids in *Deverra tortuosa* were determined by HPLC according to the method of Zbigniew *et al*., (1991). 1ml of each sample was diluted by 10 ml water and take 35 µl for injection into HPLC Hewllet Packard (series 1050) equipped with auto sampling injector, solvent degasser, ultraviolet (UV) detector set at 210 nm and quaternary HP pump (series 1100). Packed column Hypesil BDS- C18, 4.0 × 250 mm was used to separate organic acid. The column temperature was maintained at 55°C, at flow rate 1ml/min. Organic acid standard from Sigma Co. were dissolved in a mobile phase (phosphoric acid) and injected into HPLC. Retention time and peak area were used to calculation of organic acids concentration by data analysis of Hewllet Packard software.

Determination total amino acids

The total amino acids (protein amino acids and free amino acids) in *Deverra tortuosa* were determined by using Amino Acid Analyzer apparatus model (LC 3000 Eppendorf, Central Lab. of Desert Research Center) according to the method of Pellet and Young (1980).

Statistical analysis

The data were statically analysed using the statistical program (CoStat Version 6.4).The significant differences between means were calculated by a split plot analysis of variance (ANOVA) using Student Newman–Keul's (SNK) test . Differences with $P < 0.05$ were considered as significant (Glantz, 1992).

Results and discussion

Soil analysis

Soil Physical and chemical properties at the studied habitats were represented in Tables 1and 2. Soil texture was similar in all the studied habitats and sandy, but with differences in the percentages of silt and clay, their percentages were 1.22 and 2.08% at first (0-20cm) and second (20-40cm) depths in Wadi Sudr soil, respectively. While in El-Alamein and Wadi Um Ashtan soils, their percentages increased to 2.31 and 7.1%, respectively.

Table.1 Physical properties of *Deverra tortuosa* associated soils at different habitats.

Habitats	Soil (cm)	Depth CaCO$_3$ %	Soil particles distribution (%)						Soil Texture Class	Soil Moisture Content	
			Very Coarse Sand 2.0-1.0mm	Coarse Sand 1.0-0.50 mm	Medim Sand 0.50-0.25mm	Fine Sand 0.25-0.125mm	Very Fine Sand 0.125-0.063mm	Clay& Silt <0.063mm		Winter	Summer
WadiSudr	0-20	39.5	31.44	30.24	16.95	12.50	7.65	1.22	Sand	1.12	0.13
	20-40	40.1	11.55	6.22	25.7	43.89	10.56	2.08	Sand	3..94	0.25
ElAlamein	0-40	24.5	2.40	12.38	25.02	28.41	29.48	2.31	Sand	4.12	0.33
UmAshtan	0-40	26.54	18.27	22.34	17.53	8.92	25.84	7.1	Sand	8.14	4.30

The pH values fluctuated in the basic range. Generally no significant differences in soil pH due to location changes were noticed. The lowest pH value of 7.28 was recorded at El-Alamein and the highest value of 7.88 at the first depth (0-20cm) of Wadi Sudr. The percentage of CaCO$_3$ was 39.5 % at first depth and

40.1% at second depth in Wadi Sudr soil. While at El-Alamein and Wadi Um Ashtan, its percentage was 24.5 and 26.5%, respectively. Soil moisture content at Wadi Sudr was 1.12 and 3.94% at first and second depths in winter. But, it decreased significantly in summer to 0.13 and 0.25%, respectively. While at El-Alamein and Wadi Um Ashtan, the soil moisture content was decreased from 4.12 and 8.14% in winter to 0.33 and 4.30% in summer, respectively. The presence of high soil moisture content in Wadi Um Ashtan soil may due to the high value of annual total rainfall (263.13mm) in winter 2007 and high water-holding capacities as a result of high percentage of silt and clay contents(7.15%) in comparing with the soils of Wadi Sudr (1.22, 2.08%) and El-Alamein(2.31%).

Table 2. Chemical properties of *Deverra tortuosa* associated soils at different habitats.

Habitats	Soil Depth (cm)	Ph at 1:2.5	EC dS/m	Cation Milliequivalent/Liter				Anion Milliequivalent/Liter			P ppm
				Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	Cl^{-}	SO_4^{2-}	HCO_3^{-}	
Wadi Sudr	0-20	7.88	4.17	1.4	0.60	11.3	1.28	13	1.55	8	0.1
	20-40	7.81	3.3	2.8	2.7	13	3.5	9.1	4.9	8	0.5
El-Alamein road	0-40	7.28	0.71	10.87	6.37	5.12	1.12	18.2	2.41	1.24	1.63
W.Um Ashtan	0-40	7.67	6.60	18.65	18.60	29.71	1.31	39.20	25.77	3.30	3.20

As shown in Table 2 Electric conductivity values differ between the studied habitats and ranged between 3.30 and 4.17dS/m at Wadi Sudr. While at El-Alamein and Wadi Um Ashtan, It was 0.71 and 6.60 dS/m, respectively. The results of soil chemical properties demonstrated that sodium (Na^+), was the dominant cation at Wadi Sudr and Wadi Um Ashtan soils, while calcium (Ca^{2+}) was the dominant at El-Alamein. Meanwhile (Cl^-) was the dominant anion in all the studied soils. In Wadi Sudr soil The mineral elements were arranged in the following order according to their concentrations; Cl^-> Na^+> SO_4^{2-}> Ca^{2+} > K^+> Mg^{2+} at first depth (0-20mm) and Na^+> Cl^-> SO_4^{2-} > K^+> Ca^{2+}> Mg^{2+} at the second depth (20-40cm).While in El-Alamein and Wadi Um Ashtan soils they arranged in the following order; Cl^- > Ca^{2+}> Mg^{2+}> Na^+> SO_4^{2-}> K^+ and Cl^-> Na^+> SO_4^{2-}> Ca^{2+} > Mg^{2+}> K^+, respectively. Generally, the maximum value of Ca^{2+} (18.65 $meql^{-1}$) was recorded in Wadi Um Ashtan soil and the minimum value (1.4 $meql^{-1}$) at the first depth in Wadi Sudr soil. The contents of Na^+, K^+ and Cl^- were 11.3, 1.28 and 13 $meql^{-1}$ in the soil of Wadi Sudr at first depth, while at the second depth, their concentrations were 13, 3.5 and 9.1 $meql^{-1}$, respectively. Whereas in the soil of El-Alamein, their concentrations were 5.12, 1.12 and 18.2 $meql^{-1}$, respectively. The maximum value of Mg^{2+} (18.60$meql^{-1}$) was recorded in Wadi Um Ashtan soil, while its minimum value (0.60$meql^{-1}$) was detected in Wadi Sudr soil. Increased NaCl concentration has been reported to cause nutrient deficiencies or imbalances, due to the competition of Na^+ and Cl^- with nutrients such as $Ca^{2+}$and K^+. Once sodium gets into the cytoplasm, it inhibits the activities of many enzymes (Jouyban, 2012).

Table 3. Analysis of variance showing the effect of different locations and two seasons on the minerals contents.

S.O.V	DF	Mean Sqaure											
		Na^+	K^+	Ca^{2+}	Mg^{2+}	SO_4^{2-}	P	Cl^-	N	Fe	Mn	Zn	Cu
Locations	2	0.016ns	0.110^{*}	0.057^{*}	0.042^{***}	0.056^{*}	0.019^{***}	4.87^{**}	0.018ns	41.9^{***}	2.99^{***}	5.13^{***}	52.8^{***}
Error	4	0.002	0.010	0.005	5.46	0.003	2.47	1.016	0.009	0.318	0.002	0.046	0.025
Seasons	1	0.085^{**}	0.015ns	1.23^{***}	0.069^{***}	0.024^{**}	0.001ns	1.8ns	1.525^{***}	1202^{***}	3.01^{***}	0.929^{**}	11.2^{***}
Location*Season	2	0.167^{***}	0.054ns	0.015ns	0.005^{*}	0.046^{***}	0.026^{***}	8.95^{***}	1.473^{***}	40.25^{***}	0.408^{***}	0.542^{**}	5.09^{***}
Error	6	0.003	0.015	0.004	8.908	8.444	2.263	2.166	0.0191	0.660	0.0019	0.0311	00.014

SOV: Source of Variance.

Significant effects are indicated for locations, seasons and their interactions ($^{*}P<0.05$,$^{**}p<0.01$,$^{***}P<0.001$ and ns, non-significant.

Mineral composition

As shown in Table 3 and Table 4, analysis of variance indicated that the variations of the contents of *Deverra tortuosa* from the majority of minerals (Mg^{2+}, P, Fe, Mn, Zn, and Cu) were highly significant between habitats and between seasons. Also, their concentration tented to increase under stress conditions in dry season.

Table 4. Mineral composition of *Deverra tortuosa* at different habitats.

Minerals	Habitats					
	Wadi Sudr		El-Alamein		Wadi Um Ashtan	
	Winter	Summer	Winter	Summer	Winter	Summer
Na^+ (g/100gDW)	0.65±0.17[c]	1.05±0.42[a]	1.06±0.12[a]	0.82±0.2[b]	0.82±0.14[b]	1.08±0.20[a]
K^+ (g/100gDW)	1.46±0.26[ab]	1.49±0.30[ab]	1.20±0.08[b]	1.46±0.22[ab]	1.66±0.36[a]	1.54±0.17[ab]
Ca^{2+} (g/100gDW)	2.12±0.20[b]	2.53±0.48[a]	1.83±0.43[c]	2.43±0.26[a]	1.92±0.18[c]	2.48±0.14[a]
Mg^{2+} (g/100gDW)	0.27±0.06[b]	0.35±0.14[a]	0.14±0.10[c]	0.24±0.17[b]	0.04±0.02[d]	0.24±0.12[b]
SO_4^{2-} (g/100gDW)	0.19±0.04[c]	0.32±0.04[b]	0.34±0.17[b]	0.53±0.16[a]	0.21±0.07[c]	0.37±0.06[b]
P (g/100gDW)	0.32±0.04[a]	0.19±0.04[b]	0.19±0.05[b]	0.14±0.10[c]	0.21±0.02[b]	0.34±0.06[a]
Cl^- (g/100gDW)	0.025±0.01[c]	0.04±0.01[b]	0.02±0.01[c]	0.03±0.02[bc]	0.06±0.02[a]	0.03±0.01[bc]
N (g/100gDW)	1.86±0.08[a]	1.30±0.09[b]	2.09±0.45[a]	0.88±0.22[c]	2.03±0.19[a]	0.94±0.12[c]
Fe (mg/100gDW)	31.04±1.6[c]	45.2±0.50[a]	22.2±0.33[e]	44.5±0.24[a]	27.3±0.38[d]	40.0±0.30[b]
Mn (mg/100gDW)	9.36±0.18[e]	10.5±0.24[c]	10.3±0.36[d]	10.5±0.19[c]	10.7±0.28[b]	11.8±0.40[a]
Zn (mg/100gDW)	3.10±1.08[a]	3.22±0.24[a]	3.44±0.17[a]	3.04±0.26[a]	2.14±0.10[b]	1.06±0.06[c]
Cu (mg/100gDW)	13.5±0.08[b]	14.3±0.20[a]	8.30±0.36[d]	11.98±0.84[c]	7.98±0.46[e]	8.22±0.28[de]

The values are the mean ±SD (n=3). Means in a single row followed by the same letters are not significantly different ($P < 0.05$).

The concentrations of Ca^{2+} and Mg^{2+} increased significantly with stress from 2.12 and 0.27g% in winter to 2.53 and 0.35g% in summer at Wadi Sudr and from 1.83 and 0.14g % to 2.43 and 0.24g% at El-Alamein, and from 1.92 and 0.04g% to 2.48 and 0.24g% at Wadi Um Ashtan, respectively. 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), and may be responsible for the drastic reduction in stomatal conductance in stressed plants (Atkinson, 1991). Bannister (1976) reported that the accumulation of high concentration of Ca^{2+} and Mg^{2+} can be considered as a protective adaptive response; which can counteract the harmful effects of Na^+ and Cl^-. The content of SO_4^{2-} increased significantly with stress from 0.19 to 0.32g% at Wadi Sudr and from 0.34 to 0.53% at El-Alamein. While at Wadi Um Ashtan, its value increased from 0.21g% in winter to 0.37g% in summer. The results revealed that the content of Fe, Mn and Cu in *Deverra* was significantly difference between seasons at all the studied habitats, since the concentrations of Fe and Mn increased significantly in dry season from 31.04 and 9.36 mg/100g in winter to 45.2 and 10.5 mg/100g in summer at Wadi Sudr and from 22.2 and 10.3 mg/100g to 44.5 and 10.5 mg/100g at El-Alamein, respectively. Whereas, at Wadi Um Ashtan, their values increased from 27.3 and 10.7 mg/100g in winter to 40 and11.8 mg/100g in summer, respectively. The rapidly increase in concentration of total inorganic ion in stressed plant, seemed to be the major component of its osmotic adjustment (Patakas *et al.*, 2002), since the accumulation of ions in the vacuole provides "cheap" solutes for osmotic from an energy consuming point of view (Flower *et al.*, 1977).

Carbohydrates and soluble Sugars

Data of statistical analysis (Table 5) revealed the existence of high significant difference between habitats and between seasons in the content of total carbohydrates and soluble sugars; particularly reducing sugars. The results in Table 6 show that the values of total carbohydrates were significantly increased from 4.73, 4.03 and 4.17 g% in winter to 8.34, 7.78 and 8.31 g% in summer at Wadi Sudr, El-Alamein and Wadi Um Ashtan, respectively. While the values of total soluble sugar increased under stress conditions to 1.95, 1.83 and 2 .22g%, at the same habitats, respectively. Such an increase in soluble sugars under drought conditions is considered as an adaptive mechanism to drought stress since drought tolerance can be partly attributed to soluble sugars accumulation (Pelah *et al.,* 1997) as they are able to protect the structural integrity of membranes during dehydration (Crowe *et al.,* 1988; Crowe and Crowe, 1992) and has a protective role for chloroplast from damage under water deficit conditions. (Santarius, 1973). Moreover, the value of reducing sugar increased significantly with stress from 0.25 to 0.95% and from 0.23 to 0.84g% at Wadi Sudr and El-Alamein, respectively. Also, there was a significant increase in the content of reducing sugar at Wadi Um Ashtan under stress conditions; its concentration increased from 0.63g% in winter to1.27g% in summer.

Table 5. Analysis of variance showing the effect of different locations and two seasons on protein, total carbohydrates, total soluble sugars, water content, osmotic content and osmotic potential in *Deverra tortuosa.*

S.O.V		Mean Sqaure							
	DF	Protein	T.Carb.	T. soluble sugars	Reducing sugars	Water content	Total phenols	Osmotic content	Osmotic potential
Locations	2	0.736ns	0.325**	3.059***	0.306**	4659**	34.95***	514267***	3.059***
Error	4	0.367	0.0140	0.009	0.0136	107.4	0.0340	1624.72	0.0096
Seasons	1	59.51***	70.29***	0.826***	1.875***	27149***	155.93***	138864***	0.8260***
Location*Season	2	57.53***	0.056ns	0.123**	0.0027ns	1412***	15.593***	2078.16**	0.1236**
Error	6	0.753	0.0455	0.0055	0.0094	15.886	0.0979	936.944	0.0055

SOV:Source of Variance.

Significant effects are indicated for locations, seasons and their interactions (*P<0.05 ,**p<0.01,***P<0.001 and ns, non-significant.

The results showed that *Deverra* under stress conditions tended to accumulate certain compound termed compatible solutes, these compatible solutes include carbohydrates, total soluble sugars; partially reducing sugars .Their major functions have been reported to be osmotic adjustment, carbon storage, and radical scavenging (Omami *et al.,* 2006).

Table 6. Total carbohydrates, t.soluble sugars, reducing in *Deverra tortuosa*.

Parameters	Habitats					
	Wadi Sudr		El-Alamein		Wadi Um Ashtan	
	Winter	Summer	Winter	Summer	Winter	Summer
T.Carbohydrates(g/100gDW)	4.37±0.55c	8.34±0.17a	4.03±0.34c	7.78±0.32b	4.17±0.20c	8.31±0.39a
T.Solu.Sugars (g/100gDW)	1.60±0.04e	1.95±0.26c	1.61±0.18e	1.83±0.15d	2.12±0.08b	2.22±0.10a
ReducingSugars (g/100gDW)	0.25±0.04d	0.94±0.15b	0.23±0.06d	0.84±0.24b	0.63±0.06c	1.27±0.42a

The values are the mean ±SD (n=3). Means in a single row followed by the same letters are not significantly different (P <0.05).

The most important adaptation mechanism in desert plants to maintain the plant water potential more negative than the external medium to insure the water uptake, is the ability of plants to accumulate the inorganic solutes in high quantities inside their tissues (Kan *et al.*, 2000; Kamel, 2007).

Table 7. Organic acids in *Deverra tortuosa* at different habitats.

Organic acids (mg/gDW)	Habitats					
	Wadi Sudr		El-Alamein road		Wadi Um Ashtan	
	Winter	Summer	Winter	Summer	Winter	Summer
Oxalic acid	0.61	0.89	0.50	0.46	1.86	0.74
Citric acid	7.55	20.5	0.91	32.2	6	5.36
Ascorbic acid	6.12	15.4	3.57	18.4	4.93	10.2
Formic acid	-	-	0.11	-	-	-
Succinic acid	20.9	45.7	18.9	64.5	22.2	41.7
Malic acid	0.01	0.05	0.01	0.02	-	-

Organic Acid

As shown in Table 7, the concentration of the majority of detected organic acids in *Deverra*, differed significantly between seasons and increased progressively with stress. Succinic acid was the most abundant organic acids in *Deverra* in dry season at all studied habitats, its concentration reached its maximum value (64.5mg/g) at El-Alamein. While at Wadi Sudr and Wadi Um Ashtan its values were 45.7 and 41.7 mg/g, respectively. Moreover, the content of *Deverra* from citric acid was increased 2.7-fold and 35.4-fold in summer at Wadi Sudr and El-Alamein, respectively. Also, the results indicated the increase of ascorbic acid 2.5- fold under stress conditions at Wadi Sudr and 2-fold at Wadi Um Ashtan. While at El-Alamein, its value increased 5-fold in summer. Ascorbic acid and glutathione are the most abundant soluble antioxidants in plants, which play a key role in plant defence against oxidative stress (Foyer and Noctor, 2011).

Table 8. Total amino acids and crude protein in *Deverra tortuosa*.

Parameters	Habitats					
	Wadi Sudr		El-Alamein road		Wadi Um Ashtan	
T.Amino acids (mg/gDW)	Winter	Summer	Winter	Summer	Winter	Summer
Aspartic acid	4.08	4.08	10.2	4.49	5.99	0.25
Threonine	1.37	1.43	3.86	1.77	2.01	0.77
Serine	2.05	2.11	5.37	2.06	2.26	1.32
Glutamic acid	7.15	6.32	13.9	7.85	9.00	3.64
Glycine	0.94	1.13	2.32	1.08	1.68	0.68
Alanine	3.61	3.20	7.04	3.79	7.51	2.13
Valine	2.56	2.16	5.08	1.90	5.37	1.56
Methionine	0.83	1.16	0.95	5.47	0.60	0.17
Isoleucine	2.11	1.73	4.12	1.23	2.36	1.14
Leucine	3.94	3.01	8.86	2.38	3.74	2.73
Tyrosine	2.77	2.46	5.70	1.59	1.79	1.14
Phenylalanine	2.78	2.46	8.37	0.26	2.83	1.66
Histidine	1.83	0.81	3.13	1.36	1.13	1.14
Lysine	0.28	3.79	5.84	3.20	2.47	1.35
Arginine	-	5.93	4.81	4.08	3.17	0.18
Total	36.3	41.78	89.55	42.51	51.91	15.99
Crude Protein(g/100gDW)	11.6 ± 0.50^{a}	8.14 ± 0.59^{b}	13.1 ± 2.82^{a}	5.50 ± 1.38^{c}	12.7 ± 1.19^{a}	5.87 ± 0.75^{c}

Timpa *et al.* (1986) reported that organic acids are involved in various roles in the metabolic and physiological responses of plants to water stress. However, Morgan (1984) reported that solutes involved in osmotic adjustment are typically sugars, amino acids, inorganic ions and organic acids.

Total amino acids

As shown in Table 8, the concentration of crude protein in *Deverra* was decreased in dry season at all habitats, its values decreased from 11.6 in winter to 8.14 g% in summer at Wadi Sudr. While at the other habitats, its value decreased significantly with stress from 13.1 to 5.50g% at El-Alamein and from 12.7 to 5.87g% at Wadi Um Ashtan. These results are supported by other reports; Vyas *et al.* (1996) reported that water stress causes both reductions in the rate of protein synthesis as well as changes in the type of proteins produced. Also, Al-Jebory (2012) reported that the content of protein in *Pisum sativum* decreased with increasing drought stress. The alternation in protein synthesis or degradation is one of the fundamental metabolic processes that may influence water stress tolerance (Jiang and Huang, 2002). The analysis of amino acids in *Deverra* indicated the increase in the concentration of total amino acids in winter at all habitats. This increase of total amino acids in winter samples is due to decomposed of proteins during preparation of sample to measure total amino acids (protein amino acids and free amino acids), since when proteins decompose through hydrolysis they give out amino acids. In spite of the increase in protein content in winter. The results revealed the increased in the concentration of total amino acid at Wadi Sudr in summer, which may be attributed to significant increase in the content of free amino acid in summer. Under stress conditions, the accumulation of amino acids may be actually a part of an adaptive process contributing to osmotic adjustment and has been taken as an index for drought tolerant potential of many plants (Duby, 1994; Gadallah, 1995).

Table 9. Plant water content and Osmotic potential (Ψs)of cell sap in *Deverra tortuosa*.

Parameters	Habitats					
	Wadi Sudr		El-Alamein		Wadi Um Ashtan	
	Winter	Summer	Winter	Summer	Winter	Summer
Plant water content (ml/100gDW)	79.7±4.66^{c}	35.5±5.61^{f}	131±14.2^{b}	47.1±6.05^{e}	165±26.1^{a}	60.9±13.2^{d}
Osmotic content(mOsmol l^{-1})	942±36.0^{b}	1254±126^{a}	499±60.8^{e}	614±37.0^{d}	584±35.3^{d}	685±35.0^{c}
Osmotic potential of cell sap (Ψs) MPa	-2.29±0.08^{b}	-3.06±0.30^{a}	-1.22±0.14^{e}	-1.49±0.09^{d}	-1.42±0.08^{d}	-1.66±0.08^{c}

The values are the mean ±SD (n=3). Means in a single row followed by the same letters are not significantly different (P <0.05).

Seasonal changes in the osmotic potential and osmotica contents

It was obvious from Table 9 that there was a significance decrease in water content in *Deverra* from winter to summer. Its values decreased from 79.7 to 35.5% and from 131to 47.1 % at Wadi Sudr and El-Alamein, respectively. The highest value of water content (165%) was recorded in winter at Wadi Um Ashtan and decreased to 60.9% in summer, which may be attributed to recent rains in winter and subject the investigated plants to extreme water deficit during the dry season. The ability of *Deverra* to maintain moderate water content in its tissue under sever water deficit; indicated that it tented to readjust its internal osmotic pressure, to insure the water uptake and may reduce the effect of water stress (Kamel, 2007).

The measured osmotic content per liter in cell sap of *Deverra* was illustrated in Table 9 and Fig 1a. The level of osmotic content attained its maximum value of (1254 mOsmol l^{-1}) in cell sap of *Deverra* in summer at Wadi Sudr, while in winter sample, its value decreased to 942 mOsmol l^{-1}. Similar behavior was observed at El-Alamein and Wadi Um Ashtan , since the values of osmotic content increased under stress from 499 and 584 mOsmol l^{-1} in winter to 614 and 685 mOsmol l^{-1}in summer, respectively.

Osmolyte accumulation in plant cell results in a decrease of cell osmotic potential and thus in maintenance of water absorption and cell turgor pressure, contributing to sustaining physiological processes, such as stomatal opening, photosynthesis and expansion growth (Subbarao *et al.*, 2000).

Table 10. Concentrations of soluble sugars, reducing sugar, succinic acids, Ca^{2+} and SO_4^{2-} (mmol l^{-1}) in *Deverra tortuosa* in dry season at different habitats.

Habitats	Parameters				
	Soluble Sugars	Red. Sugars	Succinic Acids	Ca^{2+}	SO_4^{2-}
Wadi Sudr	305.3±49.6[a]	146.6±23.8[a]	10.73±1.74[a]	178.0±28.8[a]	9.26±1.33[b]
Wadi El-Alamein road	215.6±28.7[b]	98.66±12.8[b]	11.0±2.55[a]	128.6±18.1[b]	11.5±1.66[a]
Wadi Um Ashtan	203.3±44.9[b]	116.3±26.6b	5.78±1.23[b]	101.6±22.4[c]	6.18±1.23[c]

The values are the mean ±SD (n=3). Means in a single column followed by the same letters are not significantly different (P <0.05).

The seasonal trend in osmotic potential in *Deverra* showed the capacity for osmotic adjustment under stress conditions (Table 9; Fig.1b).The difference in osmotic potential between seasons was highly significant in *Deverra* at Wadi Sudr, its value was -2.29Mpa in winter and decreased significantly (more negative) to -3.06 Mpa in dry season. While at El-Alamein and Wadi Um Ashtan, the values of osmotic potential decreased with stress from -1.22 and -1.42Mpa in winter to 1.49 and 1.66Mpa, respectively.

Table 11. Contribution of soluble sugars, reducing sugars, succinic acids, Ca^{2+} and SO_4^{2-} to osmotic potential (Ψs) in *Deverra tortuosa* in dry season at different habitats.

Habitats	Parameters				
	Soluble Sugars (%)	Red. Sugars (%)	Succinic Acids (%)	Ca^{2+} (%)	SO_4^{2-} (%)
Wadi Sudr	24.7±4.01[c]	11.9±1.92[b]	0.87±0.13[b]	14.4±2.35[b]	0.75±0.10[b]
Wadi El-Alamein road	35.9±4.79[a]	16.4±2.15[a]	1.85±047[a]	21.4±2.86[a]	1.92±0.27[a]
Wadi Um Ashtan	30.4±6.75[b]	17.4±3.87[a]	0.86±0.18[b]	15.2±3.37[b]	0.92±0.18[b]

The values are the mean ±SD (n=3). Means in a single column followed by the same letters are not significantly different (P <0.05).

The decrease in osmotic potential in response to water stress is a well-known mechanism by which many plants are adjusted to drought conditions (Patakas and Noitsakis, 1999; Morgan, 1984). Osmotic adjustment is considered as an important mechanism for drought adaptation. It assists in the maintenance of cell turgor through the accumulation of solutes and may reduce the effects of water stress both in vegetative and reproductive phases of crop growth (Subbarao *et al.,* 2000).

The concentrations of total soluble sugars, reducing sugars, succinic acid, Ca^{2+} and SO_4^{2-} were calculated on a tissue water basis (Tables 10). The accumulation of soluble sugars in high concentration in dry season, compared with Ca^{2+}, SO_4^{2-} and succinic acid at all habitats, revealed that soluble sugars seem to be the main active compounds in the osmotic potential (Ψs) in *Deverra* This result has been supported by a number of researchers (Kameli and Losel, 1993; Al Hakimi *et al.*, 1995; Kerepesi and Galiba, 2000; El-

Lamey, 2005). The estimated contribution of total soluble sugars to osmotic potential (Ψs) under stress conditions was about 24.7% at Wadi Sudr and 30.4% at Wadi Um Ashtan and reached to its maximum value (35.9%) at El-Alamein (Table11). While the percentage of contribution of reducing sugars reached to its maximum value (17.4 %) at Wadi Um Ashtan and decreased to 16.5% at El-Alamein and 11.9% at Wadi Sudr. Meanwhile the estimated contributions of Ca^{2+} and SO_4^{2-} reached to its maximum values (21.4 and 1.9%) at El-Alamein and minimum values (14.4 and 0.75%) at Wadi Sudr, respectively. While at Wadi Um Ashtan, their estimated contributions were 15.2 and 0.92%, respectively.

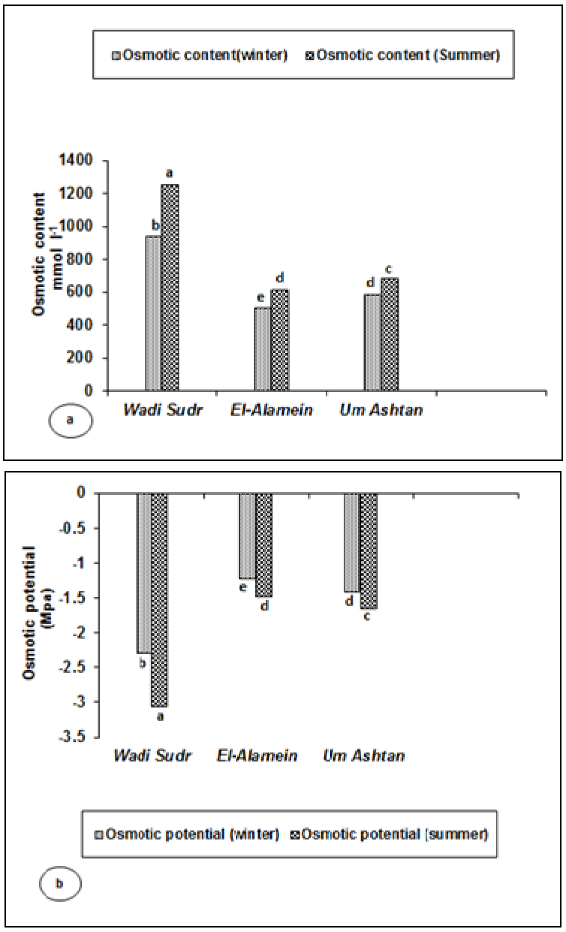

Fig. 1. Osmotic content (a) and osmotic potential (b) of shoot sap solution expressed from *Deverra tottousa*.

The adaptation of *Deverra* to the arid environment in term of osmotic adjustment was documented in this study, it tented to reduce its internal osmotic potentials through accumulation of osmotically active metabolites (e.g. soluble sugars), inorganic solutes($Ca^{2+}$and SO_4^{2-}).

Boscalu *et al.*, 2009 reported that the amount of bound water depends on the availability of organic solutes, especially soluble sugars, which played an important role in drought adaptation in the xerophytes. While Al Hakimi *et al.* (1995) reported that soluble sugar content proved to be a better marker for selecting improvement of drought tolerance in durum wheat (*Triticum durum* Desf.). Thus, the present research confirms the fact that soluble sugars seems to be a very sensitive marker for water tolerance improvement and they could be useful in selecting tolerant varieties against water stress.

Conclusion

Deverra tortuosa is a xerophytic and salt tolerant plant and able to grow on very different soils and in almost all the phytogeographical regions of Egypt, especially desert wadis and sandy and stony plains. The adaptation of *Deverra* to the arid environment in term of osmotic adjustment was documented in this study. The results demonstrated that *Deverra* can be used as a donor to transfer stress tolerance gene to other economical plants to increase their tolerance ability to drought. Since, it has a wide capacity to tolerate the period of water stress through increase in solute levels and decrease in osmotic potential.

The present research confirms the fact that soluble sugars seems to be a very sensitive and related marker for water tolerance improvement and they could be useful in selecting tolerant varieties against water stress. The increase of total ions content under drought stress, suggests that *Deverra tortuosa* depends on the accumulation of minerals together with the organic solutes in its cytoplasmic osmoregulation and to adapt to stress conditions. Thus, Attempts to improve drought tolerance in cultivated plants using transgenic approaches may be most effective if focused on metabolic pathway that produced commonly osmotically active solutes.

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