World Applied Sciences Journal 27 (7): 811-825, 2013
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2013.27.07.13669

Ecophysiological and Genetic Studies on Some Species of the Genus *Suaeda* Forssk ex Scop. In the Mediterranean Sea Coast

[1]*Mohamed M. Abd El-Maboud and* [2]*Rasha M.A. Khalil*

[1]Department of Ecology and Range Management, Desert Research Center, Cairo, Egypt
[2]Department of Plant Genetic Resources, Desert Research Center, Cairo, Egypt

Abstract: *Suaeda* is one of the most important native flora in Egypt deserts, have a great polymorphic characters between and within their species. Due to the lack of information on ecophysiological and genetic behaviors of *Suaeda* genus, this study aimed to clarify ecophysiological and genetic behaviors of three species of *Suaeda*, *S. vera*, *S. pruinosa* and *S. vermiculata*. Samples had been collected from different habitats along the Mediterranean Coast of Egypt. The results showed that $CaCO_3$ was higher in soil supporting both *S. vera* and *S. pruinosa* and lower in soil supporting *S. vermiculata*. Ca decreased under saline conditions in the three studied species and total soluble carbohydrates increased. Crude protein decreased under saline conditions in both *S. vera* and *S. vermiculata*. Ascorbic acid increased in *S. vera* and *S. pruinosa* under saline conditions and decreased in *S. vermiculata* under saline conditions. Proline increased and betaine decreased in *S. pruinosa* while betaine increased and proline decreased in *S. vermiculata* under saline conditions. SDS-PAGE protein of the studied species produced 50% of polymorphism. Eight isozyme systems including acid phosphatase, alcohol dehydrogenase, α and β-esterases, aldehyde oxidase, malic acid, malate dehydrogenase and peroxidase produced 76% of polymorphism. Five RAPD and five ISSR primers produced 73% and 99% of polymorphism. AFLP produced 48% of polymorphism. ISSR relatively produced higher polymorphism than RAPD and AFLP. The PCR product using specific primer of CMO and BADH genes generated bands with fragment size 500 bp and 1000 bp, respectively proofing that glycine betaine mechanisms for salinity tolerance is used by all *Suaeda* genotypes in different habitats.

Key words: Suaeda · Ionic composition · Proline · Ascorbic Acid · Betaine · Isozymes · RAPD · ISSR · AFLP

INTRODUCTION

The Mediterranean Coastal belt of Egypt extends around 1000 km long and 30 km inland. Its major characteristics as an arid Mediterranean climate are limited rainfall that varies between 80 and 250 mm/year. The North Mediterranean Coastal belt of Egypt is composed of two major sub-zones: the Northwestern Coast (NWC) and the Northeastern Coast (North Sinai) [1]. Khan *et al*. [2] reported that, water content in *Suaeda fruticosa* (L.) Forssk plants growing in saline conditions increased at low salinity but decreased with a further increase in salinity. Leaf Ca^{++}, Mg^{++} and K^+ concentration decreased with increasing salinity. Flowers [3] reported that, Na^+ replaced K^+ in regulating stomatal mechanism in the halophyte S*uaeda maritima*. Na^+ is not toxic in the vacuole and can undertake osmotic functions, reducing the total K^+ requirements for plants [4]. *Suaeda maritima* and *Suaeda asparagoides* contained high inorganic ions under saline conditions to maintain low water potential, but low water soluble carbohydrate contents [5]. Environmental stresses including salinity induce the production of reactive oxygen species (ROS) in plant cells [6, 7]. ROS are highly active, toxic to plants and can lead to cell death by causing damage to proteins, DNA and carbohydrates [8]. Proline and glycine betaine accumulation in plant cells enhance stress tolerance [9] and antioxidant enzyme activity [10]. Glycine betaine content in *Suaeda fruticosa* increased significantly ($p \leq 0.05$) from 0- 200 mM NaCl and remained unchanged in saline treatment up to 800mM NaCl. However, glycine betaine decreased significantly ($p \leq 0.05$) at 1000 mM

Corresponding Author: Mohamed M. Abd El-Maboud, Ecology and Range Management Department, Desert Research Center, Cairo, Egypt.

NaCl to a level similar to that of non-saline control [11]. Choline monooxygenase (CMO) and betaine aldehyde dehydrogenase (BADH) work together, CMO converts choline to betaine aldehyde and BADH converts betaine aldehyde to glycine betaine. These enzymes are compartmentalized within the chloroplast in plants such as *Suaeda sp* [12].

Biochemical markers such as proteins and isozymes were the cheapest and simplest methods that offer sufficient information and serve as a starting point for DNA-based studies. As a result of the high heterozygosity and high level of polymorphism that exist in *Suaeda sp*, biochemical techniques have been used to study genetic variations between and within different species of plants [13]. Randomized Amplified Polymorphic DNA (RAPD) have become widely used in studying genetic variation, plant taxonomy and conservation of wild plant species in natural populations because they are simple, quick, relatively not expensive and little amount of DNA quantities are required to detect genetic variation and there are no requirements for radioactive chemical [14]. Inter simple sequence repeats (ISSR) technique is considered simple and fast like RAPD, but has more stringency and specificity than RAPD. Also, ISSR markers are highly polymorphic, which makes them useful and more suitable for studies on genetic diversity, phylogeny, genetic coding, genomic mapping and evolutionary biology [15]. AFLP technique was introduced as a reliable and reproducible marker [16]. AFLPs have proven to be extremely proficient in revealing even the slightly polymorphism among and within species and provide an effective means of covering a wide area of the genome in a single assay with high multiplex ratio and no requirement of prior sequence information [17].

The present study aims studying ecophysiological and genetic behavior of *Suaeda vera*, *S. pruinosa* and *S. vermiculata* growing naturally in the Mediterranean coast of Egypt.

MATERIALS AND METHODS

Soil and shoot system of plant samples were collected from seven stands along the Mediterranean coast of Egypt during March 2012, three of them for *S. vera*, two stands for *S. pruinosa* and two stands for *S. vermiculata*. Stands of both *S. vera* and *S. pruinosa* were recorded at the Northwestern coast (west Matruh), while stands of *S. vermiculata* were recorded at the Northeastern coast of Egypt as shown in Table 1. The soil samples supporting plants were collected from the studied habitats at two successive depths; upper depth (0-20 cm) and lower depth (20-40 cm). These soil samples were dried and then powdered gently with wooden wallet and passed through 2mm sieve. Electrical conductivity (EC), Na^+, K^+, Ca^{++}, Mg^{++}, Cl^-, SO_4^{--}, HCO_3^- and CO_3^{--} were estimated and determined in soil water extract (1:1). EC, Na^+, K^+, Ca^{++} and Mg^{++} were determined following the methods described by Rowell [18]**.** Chlorides were determined as described by Jackson [19]. Sulphates were determined by the turbidity method according to Rainwater and Thatcher [20]**.** Bicarbonate and carbonate were determined according to Reitemeier [21]. CaCO3 was estimated using Collin's Calcimeter.

Plant Analyses: Total ash as described by A.O.A.C [22]. Sodium and potassium were measured by flame photometer as described by Yoshida *et al*. [23]. Calcium and magnesium by atomic absorption spectrophotometer using the method of A.O.A.C [22]. The chloride contents of the samples were determined according to Jackson and Thomas [24]. Sulphate contents of the samples were determined by the turbidimetric method according to Rowell [18]. Tissue water content percentage of plant shoots was determined as TWC (%) = 100 x (FW-DW)/FW. Total carbohydrates were estimated colorimetrically applying the phenol-sulphuric acid method as adopted by Chaplin and Kennedy [25]. Total soluble carbohydrates were estimated following the method of Dubois *et al*. [26]. Crude protein was estimated by Kjeldahl method as described by A.O.A.C [22]. Ascorbic acid was determined spectrophotometrically as described by Hussain *et al*. [27]. Free proline was quantified by the method of Bates *et al*. [28]. Glycine betaine was estimated colorimetrically as described by Grieve and Grattan [29].

Table 1: Habitats and geographical position of the studied *Suaeda sp.*

Stand	Species	Location along the Mediterranean coast	Habitats	The Geographical position system reading (GPS)
1	*S. vera*	Western coast	Coastal plain	31°32' 13.3" N and 26°12' 40.7" E.
2	*S. vera*	Western coast	Sand dunes	31°29' 19.6" N and 26°37' 43.6" E.
3	*S. vera*	Western coast	Salt marshes	31°23' 04.6" N and 27 °03' 51.7 " E.
4	*S. pruinosa*	Western coast	sand dunes	31°29' 19.6" N and 26°37' 43.6" E.
5	*S. pruinosa*	Western coast	Salt marshes	31°23' 04.6" N and 27 °03' 51.7 " E.
6	*S. vermiculata*	Eastern coast	Dry upper zones of salt marshes	31°02' 28.1" N and 33 °16' 43.6 " E
7	*S. vermiculata*	Eastern coast	Salt marshes	31°01' 54.5" N and 32°35' 16.3 " E

Statistical Analysis: Data obtained from the experiment of plant analysis were subjected to the proper statistical analysis of variance of the complete randomized design according to the procedure obtained by Snedecor and Cochran [30]. Mean values of treatments were differentiated by using Duncan at 5% level as mentioned by Duncan [31].

Extraction of Total Protein: Bulked leaf sample (0. 5g) of each sample was ground with liquid nitrogen and mixed with extraction buffer pH7.5 (50 mM Tris-HCl, 5% glycerol and 14 mM B- mercaptoethanol) in a mortar with pestle, left overnight then vortexed for 15 sec and centrifuged at 12.000 rpm at 4°C for 10 min. The supernatants were transferred to new eppendorf tubes and kept at -20°C until use for electrophoresis analysis according to Laemmli [32].

Isozyme Analysis: Eight isozymes were used such as: Acid phosphatase (Acph), alcohol dehydrogenase (Adh), α-esteras and β-esteras (Est), aldehyde oxidase (Ao), malic acid (Ma), malate dehydrogenase (Mdh) and peroxidase (Px). Isozymes were separated according to Stegemann *et al*. [33]. In gels staining, protocols of Wendel and Weeden [34] was used for Acph and Adh and Ao, Scandalios [35] was used for α and β-Est, Jonathan and Wendell [36] was used for Mdh and Ma, Heldt [37] was used for Px. Gels were washed two or three times with tap water, fixed in ethanol: 20% glacial acetic acid (9:11 v/v) for 24 hours and photographed.

DNA Extraction: Genomic DNA was extracted from fresh young leaves of ten plants which were chosen randomly for each species by CTAB method of Doyle and Doyle [38]. Leaves of ten plants from a single species were bulked prior to extraction. DNA was quantitated by spectrophotometer before gel electrophoresis.

RAPD for DNA Amplification: PCR reactions were performed according to Williams *et al*. [39] using six *10-mer* primers (Operon Technology, USA) such as: OPA4, OPA15, OP B10, OP B5 and OPZ10 with the sequences as shown in Table 3. The reaction conditions were optimized and mixtures were prepared (25µl total volumes) consisted of the following: 1.0µl dNTPs (8 mM), 1.0µl Taq DNA polymerase (1U/1µl), 2.5 µl10 X buffer, 3µl $MgCl_2$ (15 mM), 1.0µl Primer (10mM), 1.0µl Template DNA (10-50 ng/µl) and 15.5µl H_2O up to 25µl. Amplification were carried out in a Strategene Robocycler Greadient 96 Robocycler device programmed for 45 cycles as follows: Denaturation, (one cycle) 94°C for 4 minutes, followed by 35 cycle as follow: 94°C for 1minute, 36°C for 1 minute and 30 seconds and 72°C for 2 minutes and 30 second, extension, (one cycle) 72°C for 7 minutes. Agarose gel electrophoresis (1.2%) was used for resolving the PCR products according to Sambrook *et al*. [40]. The run was performed for one hour at 100 volt in Biometra submarine (40x20 cm). Fragments were detected on a UV- transilluminator and photographed by using Biometra Bio Doc Analyze 2005.

ISSR for DNA Amplification: ISSR-PCR reactions were conducted according to Sharma *et al*. [41] using specific primers which were synthesized by metabion GmbH Germany with the sequences shown in Table 9. The reaction conditions were optimized and mixtures were prepared (25µl total volumes) consisted of the following: 1.0µl dNTPs, 1µl Taq DNA polymerase 2.5µl 10 X buffer, 3µl $MgCl_2$, 1.0µl Primer, 1.0µl Template DNA and 15.5µl H_2O up to 25µl. Amplification were carried out in Stratgene Robocycler Gradient 96 which was programmed for 30 cycles as follows: Denaturation (one cycle) 94°C for 2 minutes, followed by 30 cycles: as follows 94°C for 40 second, 44°C for 45 sec, 72°C for 2 minute and 30 sec and finally one cycle extension at 72°C for 20 minutes and 4°C (infinitive). Agarose Gel electrophoresis (1.2%) was used for resolving the PCR amplification products. The run was performed for one hour at 120 volt in Biometra submarine (40x20 cm). Fragments were detected on UV- transilluminator and photographed by using Biometra Bio Doc Analyze 2005.

Primer Design: Specific degenerate primers were constructed according to its accession number, to recover partial-length of interesting genes. The primers were synthesized at Metabion, Germany. Primer sequences were checked for accuracy using the oligonucleotide software Oligo 4.1 (National Biosciences Inc., Plymouth, MN, USA).

Polymerase Chain Reaction (PCR): PCR reaction was accomplished by adding 5µl 10X buffer, 1µl dNTPs (10 mM each), 2.5 units Taq DNA polymerase, 10pmol of each primer (forward and reverse), 2µl DNA and RNase free water up to 50µl. All PCR reactions were performed in a thermal cycler (Gene Amp PCR System 2400, Perkin Elmer), by preheating at 94°C for 5 min followed by 30 cycles of 1 min at 94°C, 1 min at annealing

temperature and 2 min for extension at 72°C. Finally, the amplified DNA was incubated at 72°C for 7 min to accomplish a final extension. 20µl of PCR reaction were subjected to electrophoresis in 1.5% agarose gel containing ethidium bromide (0.01%), subjected to 100 volts for 1hr and then photographed using UVP gel documentation system, UVP corporation-UK.

AFLP Analysis: AFLP method was carried out following the standard procedure described according to Vos *et al*. [16]. High–quality genomic DNA (0.5g) was digested with a pair of restriction enzymes *(Pst*1/*Mse*1) then ligated to double stranded *Pst*1 and *Mse*1 adaptores. The ligated fragments were preamplified with nonselective primers and selective amplification was carried out using pairs of 2 bp and 3bp selective primers (Table 8). The products were separated on polyacrylamide gels using an M13 sequencing ladder as a standard size. Gel images were resolved and analyzed by Egy Gene Gel Analyzer Version One software to determine relative mobility (RF), molecular size (MS) of fragments in base pairs.

RESULTS

Regarding the parameters of soil profiles associated with the selected species at the seven studied stands (Table 2), electrical conductivity (EC) values started from 1.74 dS m^{-1} at the first layer and 4.67dS m^{-1} at the second layer of soil extract at coastal plain inhabiting *S. vera* only, then increased at sand dunes supporting *S. vera* and *S. pruinosa* and recorded the highest value at soil extracts of salt marshes supporting *S. vera* and *S. pruinosa* ranged from 9.4 to 10.52 dS m^{-1} at the first layer and 15.6 to 16.27 dS m^{-1} at the second layer respectively. EC value of soil supporting *S. vermiculata* was higher in salt marshes than dry upper zones of salt marshes as shown in Table 2. Concerning the analyzed ions of the soil samples representing the studied habitats, Table 2 clearly indicated that Na^+ and Cl^- were the dominant ions, while K^+ and HCO_3^- were the lowest. Generally, analyzed ions Na^+, K^+, Ca^{++}, Mg^{++}, Cl^-, $SO4^{--}$ and HCO_3^- tend to increase from first layer to second layer associated with the increase in EC value. $CaCO_3$ was high in soil supporting *S. vera* and *S. pruinosa* ranged from (28.6- 30%) of coastal plain to (66.8- 48.6%) of sand dunes from first to second layer, the salt marshes was intermediate between them. In case of soil supporting *S. vermiculata*, $CaCO_3$ recorded low values varied from 2.7% at the second layer of dry upper zones of salt marshes to 4.8% at the second layer of salt marshes.

Data presented in Table 3 indicated that all the studied; ash, ionic composition, water content and organic compounds had a significant effect. The highest value of ash and Na were observed in *S. vera* inhabiting sand dunes (30.96% and 13.53%), respectively, where in K content, *S. vera* at coastal plain recorded the highest value (2.37%) while that inhabiting sand dunes recorded the lowest value (1.43%). With regard to Ca^{++}, the highest value recorded in plants at sand dunes, while the lowest value at salt marshes (0.35%). Mg^{++} recorded the maximum value in plants at coastal plain (0.23%) and lower values in both inhabiting sand dunes and salt marshes equal (0.18%). Cl^- and water content recorded the highest values

Table 2: Chemical characters of soil supporting *Suaeda vera*, *Suaeda pruinosa* and *Suaeda vermiculata* at the studied habitats.

			Soluble cations and anions (meq/l)									
Species	Habitat	Depth (cm)	EC dS m^{-1}	Na^+	K^+	Ca^{++}	Mg^{++}	Cl^-	SO_4^{--}	HCO_3^-	CO_3^{--}	$CaCO_3$%
S. vera	Coastal plain	0-20	1.74	11.96	0.41	4.0	1.0	10	5.1	2.1	-	28.6
		20-40	4.67	33.91	1.03	7.0	7.0	40	5.6	2.1	-	30.0
	Sand dunes	0-20	3.31	24.60	1.61	3.8	3.1	27	4.1	1.5	-	66.8
		20-40	7.70	80.20	1.40	3.9	4.2	79	10.0	1.7	-	48.6
	Salt marshes	0-20	9.80	81.85	3.20	13.6	17.6	92	22.3	1.5	-	41.6
		20-40	15.60	152.00	5.10	15.0	24.0	161	34.8	1.5	-	45.1
S. pruinosa	*Sand dunes*	0-20	3.70	29.56	1.51	4.0	2.0	32	3.5	1.7	-	60.1
		20-40	8.20	91.00	1.97	5.0	3.3	89	10.5	1.9	-	45.5
	Salt marshes	0-20	10.52	91.16	1.92	17.0	20.0	105	24.8	1.4	-	38.2
		20-40	16.27	156.00	4.61	18.0	26.0	173	30.0	1.7	-	42.5
S. vermiculata	D.U. salt marshes	0-20	4.85	24.23	0.82	20.0	6.0	36	12.6	1.3	-	2.9
		20-40	5.29	30.00	0.95	23.0	7.0	40	19.8	1.5	-	2.7
	Salt marshes	0-20	11.92	103.80	1.38	25.0	18.0	77	68.3	1.7	-	3.8
		20-40	20.10	207.00	1.69	24.0	28.0	165	91.6	1.7	-	4.8

D.U. salt marshes = dry upper zones of salt marshes

Table 3: Ash, ionic composition and water content of *Suaeda vera*, *Suaeda pruinosa* and *S. vermiculata* growing at different habitats along the Mediterranean coast

Habitat	Ash%	Na^{+}%	K^{+}%	Ca^{++}%	Mg^{++}%	Cl^{-}%	S^{-}%	Water content%
(a) : *Suaeda vera*								
Coastal plain	20.55 b	11.13 b	2.37 a	0.51 b	0.23 a	1.85 c	0.52 a	64.82 c
Sand dunes	30.96 a	13.53 a	1.43 b	0.68 a	0.18 b	6.50 a	0.31 c	76.39 a
Salt marshes	22.21 b	10.73 b	1.70 c	0.35 c	0.18 b	4.09 b	0.47 b	66.3 b
(b) *Suaeda pruinosa*								
Sand dunes	25.40 b	12.23 a	1.60 b	2.16 a	0.70 a	7.47 b	0.47 b	78.14 a
Salt marshes	26.91 a	12.40 a	2.37 a	1.19 b	0.58 b	8.10 a	0.93 a	78.83 a
(c) : *Suaeda vermiculata*								
D. salt marshes	16.65 a	7.13 a	1.45 a	0.63 a	0.15 b	3.27 b	0.18 b	69.36 a
Salt marshes	14.43 b	7.34 a	1.23 b	0.53 b	0.38 a	4.02 a	0.26 a	64.91 b

D. salt marshes = dry upper zones of salt marshes

Table 4: Organic compounds of *Suaeda vera*, *Suaeda pruinosa* and *S. vermiculata* growing at different habitats along the Mediterranean coast

Habitat	Total carbohydrates%	Soluble carbohydrates%	Protein%	Ascorbic mg/100g FW	Proline µmol/g FW	Betaine µmol/g DW
(a) : *Suaeda vera*						
Coastal plain	30.31 a	1.87 b	13.94 ab	2.9 b	0.750 b	323.2 a
Sand dunes	18.54 c	2.11 a	15.05 a	3.0 b	0.883 a	310.4 a
Salt marshes	23.03 b	2.19 a	12.72 b	7.1 a	0.783 b	331.9 a
(b) : *Suaeda pruinosa*						
Sand dunes	26.52 a	3.01 b	6.18 a	1.9 b	0.987 b	273.2 a
Salt marshes	23.76 a	3.30 a	6.67 a	3.9 a	1.299 a	216.3 b
(c) : *Suaeda vermiculata*						
D. salt marshes	26.77 a	1.92 b	12.19 a	29.0 a	1.443 a	85.4 b
Salt marshes	28.75 a	2.30 a	8.98 b	27.3 b	0.999 b	107.9 a

D. salt marshes = dry upper zones of salt marshes

Table 5: SDS-PAGE patterns of total soluble proteins among the seven studied *Suaeda* genotypes*

MW	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7	Band types
147	1	1	1	1	1	1	1	monomorphic
130	1	1	1	1	1	0	1	polymorphic
101	0	0	0	1	1	0	0	polymorphic
87	0	0	1	1	1	1	1	polymorphic
80	1	1	1	1	1	1	1	monomorphic
79	1	1	1	1	1	1	1	monomorphic
55	1	1	1	1	1	1	1	monomorphic
42	1	1	1	1	1	1	1	monomorphic
35	1	1	1	1	1	1	1	monomorphic
30	0	0	0	1	1	0	0	polymorphic
28	0	0	0	0	0	0	1	Unique
21	0	0	0	1	1	1	1	polymorphic

*(M) Maker; *Suaeda vera* from coastal plain = (1), *Suaeda vera* from sand dunes = (2), *Suaeda vera* from salt marshes = (3), *Suaeda pruinosa* from sand dunes= (4), *Suaeda pruinosa* from salt marshes = (5), *Suaeda vermiculata* from upper zones salt marshes = (6) and *Suaeda vermiculata* from salt marshes = (7).

Table 6: Number, types and polymorphism percentage of leaf soluble protein bands extracted from the seven studied Suaeda genotypes.

	Polymorphic bands			
Monomorphic Bands	Non-unique bands	Unique bands	Total bands	Polymorphism%
6	5	1	12	50%

Table 7: Polymorphism percentages by eight isozyme systems among the seven studied *Suaeda* genotypes*.

Types of isozyme	No of monomorphic bands	No of polymorphic bands	Total bands	Polymorphism%
Acph	1	3	4	75
Adh	0	4	4	100
α-est	1	2	3	66
β-est	0	2	2	100
Ao	0	2	2	100
Malic acid	1	0	1	0
Mdh	1	0	1	0
Px	1	3	4	75
Total	5	16	21	76

**Suaeda vera* from coastal plain = (1), *Suaeda vera* from sand dunes = (2), *Suaeda vera* from salt marshes = (3), *Suaeda pruinosa* from sand dunes= (4), *Suaeda pruinosa* from salt marshes = (5), *Suaeda vermiculata* from upper zones salt marshes = (6) and *Suaeda vermiculata* from salt marshes = (7).

Table 8: Primer sequences, total bands number of monomorphic bands, number of polymorphic bands and polymorphism percentages of RAPD primers among the seven studied *Suaeda* genotypes *.

				Polymorphic bands			
No	RAPD Primer	Primer Sequences	Monomorphic bands	Non-unique bands	Unique bands	Total bands	Polymorphism%
1	A4	5-AATCGGGCTG-3	0	13	5	18	100
2	A14	5-TCTGTGCTGG-3	0	11	6	17	100
3	B10	5-CTGCTGGGAC-3	6	4	1	11	45
4	B5	5-TGCGCCCTTC-3	7	5	1	13	46
5	Z10	5-CCGACAAACC-3	4	0	0	4	0
Total			--		33	13	63

**Suaeda vera* from coastal plain = (1), *Suaeda vera* from sand dunes = (2), *Suaeda vera* from salt marshes = (3), *Suaeda pruinosa* from sand dunes= (4), *Suaeda pruinosa* from salt marshes = (5), *Suaeda vermiculata* from upper zones salt marshes = (6) and *Suaeda vermiculata* from salt marshes = (7).

Table 9: Primer sequence, total bands, number of monomorphic fragments, number of polymorphic bands and polymorphism percentages of ISSR primers among the seven studied *Suaeda* genotypes *

				Polymorphic bands			
No	ISSR Primer	Primer Sequences	Mono-morphic bands	Non-unique bands	Unique bands	Total bands	% Polymorphism
1	17898A	$(CA)_6$ AC	0	20	5	25	100
2	17898B	$(CA)_6$ GT	0	12	16	28	100
3	17899B	$(CA)_6$ GG	1	14	1	16	94
4	HB9	$(GT)_6$ GG	0	12	8	20	100
5	HB10	$(GA)_6$ CC	0	20	6	26	100
Total			1	78	36	115	99

**Suaeda vera* from coastal plain = (1), *Suaeda vera* from sand dunes = (2), *Suaeda vera* from salt marshes = (3), *Suaeda pruinosa* from sand dunes= (4), *Suaeda pruinosa* from salt marshes = (5), *Suaeda vermiculata* from upper zones salt marshes = (6) and *Suaeda vermiculata* from salt marshes = (7).

in plants at sand dunes (6.50% and 76.39%) respectively, associated with the lowest sulfur content (0.31%) while the reverse was observed in plants at coastal plain the lowest values of both Cl^- and water content (1.85% and 64.82%) associated with the highest sulfur (0.52%).

Data in Table 3 distinguished that, ash, K, Ca, Mg, Cl and S affected significantly in *S. pruinosa* growing at sand dunes and salt marshes while Na and water content had non-significant. Ash, K, Cl and S were higher in *S. pruinosa* inhabiting salt marshes, while Ca and Mg were lower than those in *S. pruinosa* inhabiting sand dunes. From the data are shown in Table 3, it is clear that all analyses in *S. vermiculata* had significant effect except Na. Ash, K, Ca and water content increased in plants growing at dry upper zones of salt marshes while Mg, Cl and S increased in plants growing at salt marshes. Data in Table 4 illustrated that, all studied organic compounds except glycine betaine had significant effect on *S. vera* growing at different habitats. Total carbohydrates recorded the highest value (30.31%) in *S. vera* at coastal plain, while soluble carbohydrates recorded the highest value in plants inhabiting salt marshes (2.19%). Crude protein recorded the highest amounts in plants inhabiting sand dunes (15.05%) while the lowest value in plants inhabiting salt marshes (12.72%). Ascorbic acid recorded the maximum value in plants inhabiting salt

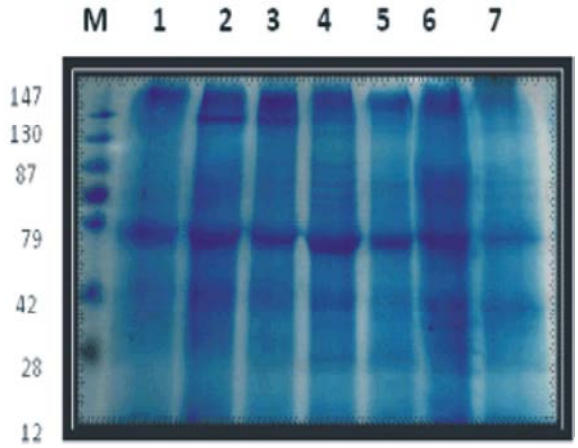

Fig 1: SDS-PAGE profiles of soluble among the seven studied Suaeda genotypes *.
*(M) Maker; *Suaeda vera* from coastal plain = (1), *Suaeda vera* from sand dunes = (2), *Suaeda vera* from salt marshes = (3), *Suaeda pruinosa* from sand dunes = (4), *Suaeda pruinosa* from salt marshes = (5), *Suaeda vermiculata* from upper zones salt marshes = (6) and *Suaeda vermiculata* from salt marshes = (7).

marshes (7.1 mg/100g FW), while proline recorded the maximum value in plants inhabiting sand dunes (331.9 µmol/g FW).

Concerning *S. pruinosa*, the studied organic compounds except total carbohydrates and crude protein affected significantly on plants growing at sand dunes and salt marshes as illustrated in Table 4. Glycine betaine was higher in plants at sand dunes, while soluble carbohydrates, ascorbic acid and proline were higher in plants at salt marshes. From the data are shown in Table 4, it is cleared that, the examined organic compounds except total carbohydrates affected significantly on *S. vermiculata* growing at dry upper zones of salt marshes and salt marshes. Crude protein, ascorbic acid and proline decreased by more salinity under salt marshes while soluble carbohydrates and glycine betaine increased.

SDS-PAGE Leaf Proteins: Leaf protein analysis was carried out on seven genotypes of *Suaeda.* (Tables 5 and 6) and illustrated in Fig. 1. The results indicated that a total number of twelve protein bands were observed among the seven studied *Suaeda* genotypes. Concerning band intensity, there was detectable change in band intensity for all seven *Suaeda* genotypes. It is quite clear from data that bands of molecular weight 35, 42, 55, 79 and 80 kDa in *S. vera* from sand dunes and *Suaeda vermiculata* from dry upper zones of salt marshes gave the highest band intensity as compared with the other genotypes. On the contrary, *S. pruinosa* from sand dunes was stable in band intensity. Moreover, six bands were monomorphic, one unique and five bands were polymorphic revealing 50% of polymorphism. A negative marker was detected by *S. vermiculata* from dry upper zones of salt marshes at 130 kDa. Also, a negative marker was detected by *S. vera* genotypes at 21 kDa. While, two positive markers were detected by *S. pruinosa* from sand dunes and salt marshes at 101 and 30 kDa. Moreover, a unique band was detected by *S. vermiculata* from salt marshes at 28 kDa.

Isozyme Analysis: Eight isozyme systems including *Acp (*acid phosphatase), *Adh* (alcohol dehydrogenase), α and β *Est (*esterases), *Ao* (aldehyde oxidase), *Ma* (malic acid)**,** *Mdh* (malate dehydrogenase) and Prx (peroxidase) were used to study the genetic variability among seven studied *Suaeda* genotypes (Fig 2 and Table 7). The results showed a total of 36 bands and revealed 76% of polymorphism.

RAPD Analysis: Five RAPD primers were used in the present study to investigate the genetic relationships among the seven studied *Suaeda* genotypes as shown in Table 8 and Fig. 3. Seventeen monomorphic and forty six polymorphic distinct fragments produced 73% of polymorphism among the seven studied *Suaeda* genotypes using these five primers. These results showed that **A4** and **A14** primers were highly polymorphic revealed 100% of polymorphism. Moreover, **B5** and **B10** primers were less polymorphic revealed 46% and 45% of polymorphism, respectively. On the other hand, **Z10** primer showed no polymorphism. **A4**, **A14**and **B5** Primers produced the highest total number of bands from eighteen to thirteen bands. While, **B10** and **Z10** primers produced the lowest total number of bands from eleven to four bands.

ISSR Analysis: Five ISSR primers were used to investigate the genetic relationships and diversity among the seven studied *Suaeda* genotypes as shown in Fig. 4 and Table 9. One monomorphic band and 114 polymorphic distinct fragments revealed 99% of polymorphism among the seven studied *Suaeda* genotypes. The results showed that 17898A, 17898B, HB9 and HB10 primers were highly polymorphic which revealed 100% of polymorphism while, 17899B primer revealed the lowest polymorphism 94%.

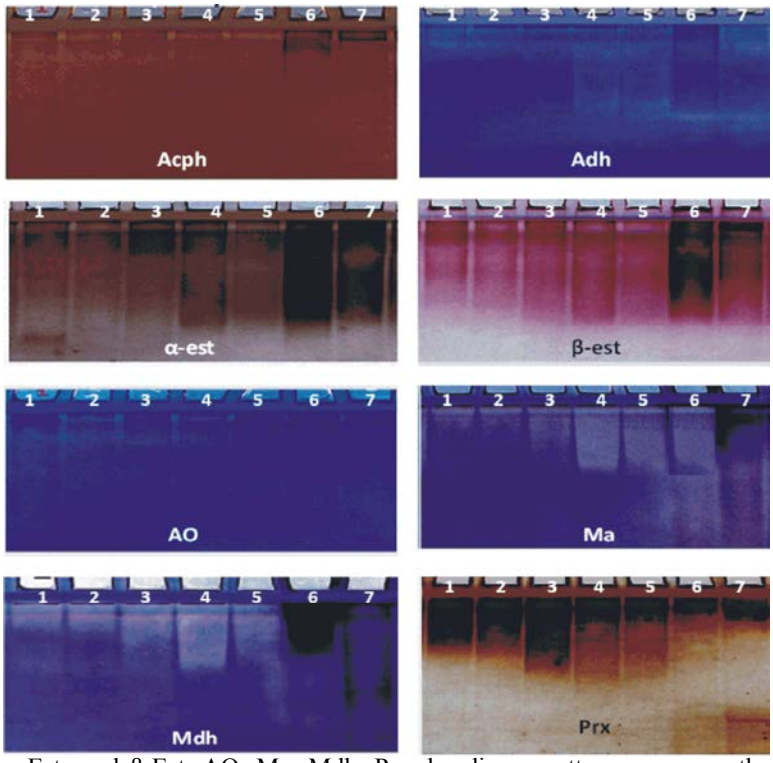

Fig. 2: Acph, Adh, a-Est and ß-Est, AO, Ma, Mdh, Prx, banding patterns among the seven studied Suaeda genotypes *.

*(M) Maker; *Suaeda vera* from coastal plain = (1), *Suaeda vera* from sand dunes = (2), *Suaeda vera* from salt marshes = (3), *Suaeda pruinosa* from sand dunes = (4), *Suaeda pruinosa* from salt marshes = (5), *Suaeda vermiculata* from upper zones salt marshes = (6) and *Suaeda vermiculata* from salt marshes = (7).

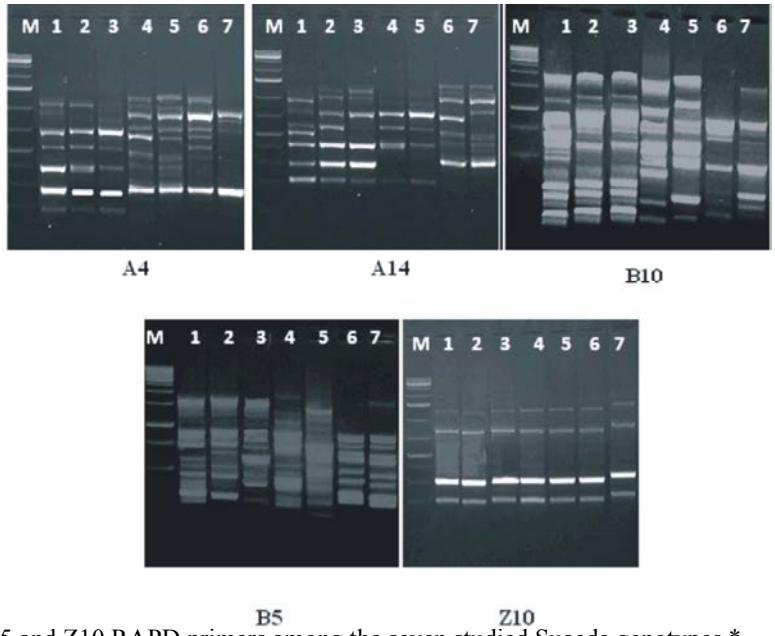

Fig. 3: A4, A14, B10, B5 and Z10 RAPD primers among the seven studied Suaeda genotypes *

*(M) Maker; *Suaeda vera* from coastal plain = (1), *Suaeda vera* from sand dunes = (2), *Suaeda vera* from salt marshes = (3), *Suaeda pruinosa* from sand dunes = (4), *Suaeda pruinosa* from salt marshes = (5), *Suaeda vermiculata* from upper zones salt marshes = (6) and *Suaeda vermiculata* from salt marshes = (7).

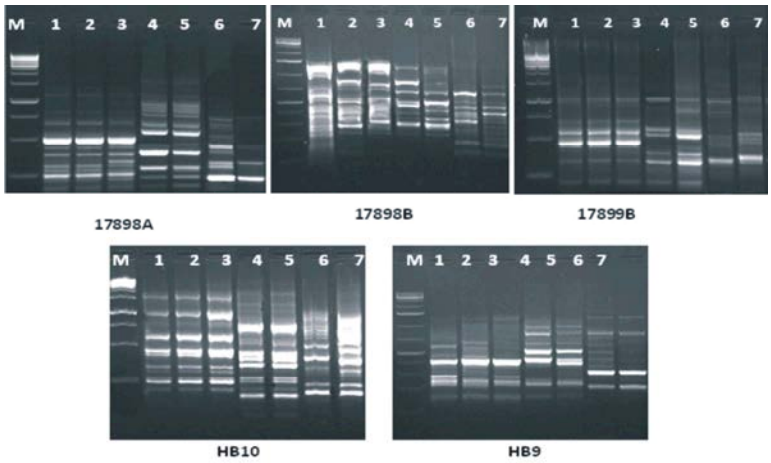

Fig. 4: 17898A, 17899B, 17899B, HB9 and HB10 ISSR primers among the seven studied Suaeda genotypes. *(M) Maker, *Suaeda vera* from coastal plain = (1), *Suaeda vera* from sand dunes = (2), *Suaeda vera* from salt marshes = (3), *Suaeda pruinosa* from sand dunes = (4), *Suaeda pruinosa* from salt marshes = (5), *Suaeda vermiculata* from upper zones salt marshes = (6) and *Suaeda vermiculata* from salt marshes = (7).

Table 10: ISSR primers for the seven *Suaeda* species* specific marker.

No. of genotype	Genotypes	ISSR Primer	Unique bands	Total
1	*S. vera* from sand plain	17899B	1	6
		17898B	5	
2	*S. vera* from salt marches	17898A	2	4
		17898B	2	3
	S. vera from sand dune	17898B	1	1
4	*S. pruinosa* from salt marches	17898A	1	6
		17898B	2	
		HB9	3	
5	*S. pruinosa* from sand dune	HB9	1	3
		17898B	2	
6	*S. vermiculata* from upper salt marches	17898A	1	8
		17898B	1	
		HB9	2	
		HB10	4	
7	*S. vermiculata* from salt marches	17898B	4	8
		HB9	2	
		HB10	2	
				36

**Suaeda vera* from coastal plain = (1), *Suaeda vera* from sand dunes = (2), *Suaeda vera* from salt marshes = (3), *Suaeda pruinosa* from sand dunes= (4), *Suaeda pruinosa* from salt marshes = (5), *Suaeda vermiculata* from upper zones salt marshes = (6) and *Suaeda vermiculata* from salt marshes = (7).

17898A, HB10 and 17898B Primers produced the highest total number of bands ranged from twenty five to twenty eight bands. Moreover, 17899B primer revealed the lowest total number of amplified fragments, sixteen bands.

Species –Specific –Markers: Some specific markers for some of the seven studied *Suaeda* genotypes across ISSR analyses which were listed in Table 10. The five ISSR primers were found to be species specific. *Suaeda vera* from coastal plain had six specific markers, one was scored by 17899B and the other five specific markers were scored by 17898B. Meanwhile, *Suaeda vera* from salt marshes had four specific markers, two were scored by 17898A and two were scored by 17898B that could be used as salinity tolerance marker for *Suaeda vera* under saline habitats. In addition, *S. vera* from sand dunes had only one specific marker scored by 17898B. Moreover, *S. pruinosa* from salt marshes had six specific markers, one was scored by17898A, two were scored by17898B and three were scored by HB9. Meanwhile, *S. pruinosa* from sand dunes had three specific markers, one specific marker was scored by HB9 and two specific markers were

Table 11: Sequence and annealing temperature of PCR primers used in amplification of (CMO) gene and (BADH) gene.

Product Size	Annealing temperatures	Primer Nucleotide Sequences	Specific Primers
500 bp	60	F 5-TAATGATGGCAGCAAGCGCAA-3	CMO
		R 5 -AATTACTTCAAAGTTTGTTGCAAC-3	
1000 bp	50	F 5-TCCTCTCGTCTCCAGTCCAC-3	BADH
		R 5- AATGCAGACTAACAACCCATGA-3	

Table 12: Selective nucleotides of AFLP primer combinations, number of total bands, polymorphic bands and polymorphism percentages.

	Selective nucleotides		Number of bands		
Primer combination	*EcoRI*	*MseI*	Total	Polymorphic	% of polymorphism
EcoRI / MseI	ACC	CAC	42	20	47%

Table 13: Similarity matrix of total analysis (protein, isozymes, RAPD, ISSR and AFLP) markers among the seven studied *Suaeda* genotypes*.

No.	1	2	3	4	5	6	7
1	100						
2	64	100					
3	64	75	100				
4	35	39	39	100			
5	40	41	41	77	100		
6	38	24	30	40	39	100	
7	28	29	33	32	33	48	100

**Suaeda vera* from coastal plain = (1), *Suaeda vera* from sand dunes = (2), *Suaeda vera* from salt marshes = (3), *Suaeda pruinosa* from sand dunes= (4), *Suaeda pruinosa* from salt marshes = (5), *Suaeda vermiculata* from upper zones salt marshes = (6) and *Suaeda vermiculata* from salt marshes = (7).

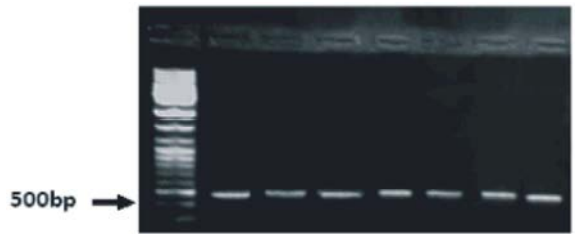

Fig 5: The PCR product using specific primer of CMO gene indicated that appearance of one band with fragment size 500bp among the seven studied Suaeda genotypes*.

Fig. 6: The PCR product using specific primer of BADH indicated that appearance of one band with fragment size 1000bp among the seven studied Suaeda genotypes* .

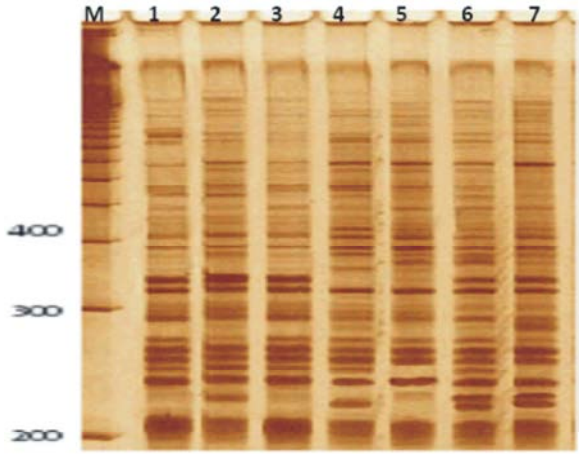

Fig. 7: AFLP fingerprints one combination among the seven studied Suaeda genotypes*.
*(M) Maker, *Suaeda vera* from coastal plain = (1), *Suaeda vera* from sand dunes = (2), *Suaeda vera* from salt marshes = (3), *Suaeda pruinosa* from sand dunes = (4), *Suaeda pruinosa* from salt marshes = (5), *Suaeda vermiculata* from upper zones salt marshes = (6) and *Suaeda vermiculata* from salt marshes = (7).

scored by 17898B. *S. vermiculata* from upper zones of salt marshes had eight specific markers, one specific marker was scored by 17898A, one specific marker was scored by 17898B, two specific markers were scored by HB9 and four were scored by HB10 which indicated that, *S. vermiculata* from upper zones of salt marshes produced the highest number of specific markers for species and for salinity tolerance. Finally, *S. vermiculata* from salt marshes had

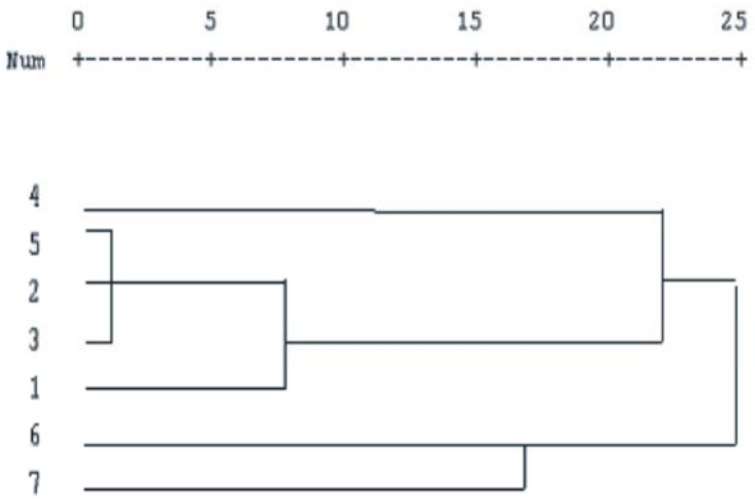

Fig. 8: Dendrogram based on total analysis (protein ,isozymes, RAPD, ISSR and AFLP) among the seven studied Suaeda genotypes*.
*(M) Maker, *Suaeda vera* from coastal plain = (1), *Suaeda vera* from sand dunes = (2), *Suaeda vera* from salt marshes = (3), *Suaeda pruinosa* from sand dunes = (4), *Suaeda pruinosa* from salt marshes = (5), *Suaeda vermiculata* from upper zones salt marshes = (6) and *Suaeda vermiculata* from salt marshes = (7).

eight specific markers, four of which were specific markers scored by 17898B, two specific markers were scored by 17898B, two specific markers were scored by HB9 and two were scored by HB10, indicated that *S. vermiculata* from salt marshes produced the highest number of specific markers for species and for salinity tolerance.

Gene Detection: The PCR product using specific primer of CMO and BADH genes indicated that the appearance of one band for each with fragment sizes 500 and 1000 bp, respectively as shown in Fig. 5, 6 and Table 11.

AFLP Analysis: A total number of 42 amplified fragments were obtained by using this primer pair within a fragment size range of 200 to 550 bp (Fig.7 and Table 12). Twenty polymorphic bands were obtained representing 48%. Common bands resulted were 22 amplified fragments.

Combined Analysis Based on Total Analysis: Based on total analysis (protein, isozymes, RAPD, ISSR and AFLP) similarity matrix was developed by SPSS computer package system in Table (13) and shown in Fig (8). The closest relationship was scored between *S. pruinosa* from sand dunes and *S. pruinosa* from salt marshes producing similarity of 76%. While, *S. vera* from sand dunes and *Suaeda vermiculata* from upper zones of salt marshes revealed the lowest similarity of 24%. The dendrogram based on (protein, isozymes, RAPD, ISSR and AFLP) separated the seven studied *Suaeda* genotypes into two main clusters. Moreover, *S. vermiculata* from different habitats were separated into the first cluster while *S. pruinosa* and *S. vera* from different habitats was cluster together in the second main cluster.

DISCUSSION

Coinciding soil properties of studied habitats; coastal plain supporting *Suaeda vera* by low salinity and moderate amounts of $CaCO_3$, sand dunes supporting both *S. vera* and *S. pruinosa* which is moderate salinity and high $CaCO_3$, salt marshes supporting *S. vera* and *S. pruinosa* associate with high in both of salinity and $CaCO_3$, dry upper zones of salt marshes supporting *S. vermiculata* which is moderate in salinity associate with low $CaCO_3$ and salt marshes supporting *S. vermiculata* which is the highest in salinity and low $CaCO_3$. The accumulation of ash, Na^+ and Cl^- in *S. vera* inhabiting sand dunes which is moderate salinity, then decreased under more salinity at salt marshes illustrate that under high salinity the excess of Na^+ and Cl^- may be excluded by roots, while water is taken up from the soil. The highest Na in *S. vera* associate with the lowest K illustrate the competition between monovalent cations. The decrease of Ca under salt marshes in the three studied species, K^+ in both *S. vera* and *S. vermiculata* and Mg^{++} in both *S. vera* and *S. pruinosa* agreed with Khan

et al. [2] who found that Ca^{++}, Mg^{++} and K^{+} concentration decreased with increasing salinity in *S. fruticosa*. Regarding the increase of Mg under salt marshes in *S. vermiculata*, Abd El-Maboud [42] reported that Mg^{++} displaces Ca^{++} under saline condition for *Salsola tetrandra* (Chenopodiaceae). Also, Kinraide [43]reported that, higher condition of competitive cations such as Na^{+}, K^{+} and Mg^{++} have been shown to displace cell membrane associated Ca^{++}. Concerning the increase in Cl^{-} in both *S. pruinosa* and *S. vermiculata* growing at salt marshes, Song *et al*. [44] found that concentration of Cl^{-} increased with increased application of NaCl in *S. salsa*. The increase in water content in *S. vera* at sand dunes to dilute the toxicity effect of excess of Na^{+} and Cl^{-}. Compartmentalization of Na^{+} into vacuoles allows plants to use Na^{+} as an osmoticum, maintaining the osmotic potential that increases the water content within the cell [45, 42]. *S. vermiculata* tends to decrease in water content by more salinity at salt marshes. In this trend, Khan *et al*. [2] found that water content in *Suaeda fruticosa* (L.) increased at low salinities up to 200 mole/m^3 NaCl and decreased with a further increase in salinity.

The accumulation of total carbohydrates in *S. vera* at low salinity (coastal plain) is favorable condition for building up carbohydrates. Munns and Tester [46] and Abd El-Maboud [42] reported that, salts may build up in the apoplast and dehydrate the cell; they may build up in the cytoplasm and inhibit enzymes involved in carbohydrate metabolism, or may be build up in the chloroplast and exert a direct toxic effect on photosynthetic processes. Soluble carbohydrate plays effective role as organic osmolytes in the studied species growing under salt marshes. This result is in agreement with those obtained by Tawfik *et al*. [47], who found that soluble carbohydrates increased in *Kochia indica* when had irrigated with 50% fresh water mixed with saline Qaroon lake compared to that irrigated with fresh water. The decrease in crude protein under salt marshes in both *S. vera* and *S. vermiculata are* in agreement with those obtained by Tawfik *et al*. [47], who found that *Kochia indica* had higher crude protein when irrigated with moderate salinity 25% saline Qaroon lake mixed with fresh water compared to those irrigated with fresh water and high salinity 50% from Qaroon lake. The reduction in proteins level under salinity due to low uptake of nitrate ion [48]. The stability of crude protein in *S. pruinosa* associate with the same number and molecular weight of protein bands in both inhabiting sand dunes and salt marshes with zero polymorphic.

S. vermiculata has rich ascorbic acid (AsA) tends to decrease under salt marshes. This result is in agreement with those reported by Tammam *et al*. [49], who found that ascorbic acid content in *Dunaliella tertiolecta* significantly decrease at 1.0 and 0.5 NaCl by about 50% then increase at 0.1 M NaCl by 6%, whereas at 4 M NaCl it decrease by 77% relative to the control value. ASA in *S. vera* and *S. pruinosa* tends to accumulate with increasing salinity under salt marshes to dilute deleterious effect caused by free radicals under salinity stress. AsA functions as a reductant for many free radicals, thereby minimizing the damage caused by oxidative stress. Plant with higher amount of AsA content showed better protection against oxidative stress. Ascorbate influences many enzyme activities, minimizing the oxidative damage through synergic function with other antioxidants [50, 51].

The role of proline is not obvious in *S. vera* which increase under moderate salinity at sand dunes and decrease under high and low salinity (salt marshes and coastal plain), while in *S. pruinosa* it plays a vital role in osmotic adjustment under salinity stress. Glycine betaine is the most effective as osmoregulator and antioxidant in *S. vermiculata* under salinity stress. There is a reversible relation between proline and glycine betaine among *Suaeda* species and their habitats. Tipirdamaz *et al*. [52] reported that species that behave as glycine betaine accumulators behave as poor proline accumulators and vice versa. Ben-Hassine *et al*. [10] reported that accumulation of compatible solutes such as glycine betaine and proline during salinity and drought are reported to improve PS-II efficiency and antioxidant enzyme activity.

Concerning protein band intensity, five protein bands were detected in *Suaeda vera* from sand dunes and *S. vermiculata* from upper zones of salt marshes which gave the highest band intensity as compared with of other genotypes and in relation to the increased crude protein amount. SDS- PAGE marker distinguished among the three studied *Suaeda* species by the bands with molecular weight 101, 30 and 21kDa. Two positive markers 101 and 30kDa can be considered as specific markers for *S. pruinosa* while, the band 21kDa can be considered as negative marker for *S. vera*. Moreover, a unique band 28kDa was detected by *S. vermiculata* from salt marshes could be considered as species specific band. The presence of 50% polymorphism among the seven studied *Suaeda* genotypes agreed with the results obtained by Jery *et al*. [53] who studied the genetic diversity and relationships of 11 species and cultivars belonging to different Angiosperms families using seed

protein SDS-PAGE marker. The protein pattern resolved into 36 bands (for soybean), 41 (for *Chenopodium quinoa*) (*Chenopodiaceae*) and 28 to 39 bands of Amaranth (*Amaranthaceae*) species. All species and cultivars can be distinguished from each other. Soybean and quinoa species had a characteristic protein pattern showing a high degree of polymorphism.

Based on isozyme markers, polymorphism percentages were estimated for the eight isozyme systems among the seven studied *Suaeda* genotypes. The highest percentage of polymorphism of 100% was detected in Adh, β-est and AO followed by75% in Acph and Prx, 66% of polymorphism in α-esterase while the lowest polymorphism was 0% in Ma and Mdh. *S. vermiculata* from salt marshes and *S. vera* from coastal plain exerted specific band for each by α-esterase as compared with the other genotypes associated with the increase in total carbohydrates metabolites to confirm that esterases hydrolyzes ester bond in lipid to produce plant energy. These results were in accordance to the results obtained by Haddioui and Baaziz [54] who used seven isozyme systems to investigate genetic diversity in Atriplex halimus L. with a high polymorphism percentages among populations from different location in Morocco and in accordance with the results obtained by Khalil *et al*. [55] who successfully detected the genetic variability in ten *Brassicaceae* species collected from North coast in of Egypt and Sinai, revealed by α-β-est, Acph, Prx, Adh and Mdh which produced 73% of polymorphism. Comparing ISSR with RAPD makers, forty six polymorphic distinct fragments (73% of polymorphism) were revealed by RAPD and 114 polymorphic distinct fragments (99% of polymorphism) were revealed by ISSR produced higher polymorphism than RAPD among the seven studied *Suaeda* genotypes. These results are in agreement with those obtained by Ajithkumar and Panneerselvam [56] who evaluated the genetic diversity between five landraces of *Setaria italica* collected from various places in India. They used twenty five RAPD and ten ISSR primers. ISSR primers produced 35% polymorphic bands between the landraces of *Setaria italica* when compared to 27.5% by RAPD analysis. ISSR marker systems were found to be more accurate for the genetic diversity studies in the seven studied *Suaeda* genotypes when compared to RAPD marker. On the other hand, Al-Salameen *et al*. [57] studied the genetic diversity of *Haloxylon salicornicum* (*Chenopodiaceae*) collected from nine locations by RAPD and ISSR techniques. 24 RAPD and 25 ISSR primers produced 946 and 1016 bands, respectively. RAPD produced higher polymorphism (49%) compared with ISSR that produced (47%).

The PCR product indicated the appearance of one band of BADH gene and one band represented CMO gene in all of the seven studied *Suaeda* genotypes. These results agreed with the results obtained by Qiuli *et al*. [12, 58] who isolated and characterized CMO and BADH genes from halophyte *Suaeda liaotungensis* and *Salicornia europea,* respectively. Twenty polymorphic bands from forty two bands were obtained representing 48% of polymorphism. The results agreed with the results obtained by Prinz *et al*. [59] who used AFLP molecular marker in 120 individual plants *Suaeda maritima* (L.) from 40 coastal and inland populations in Central Europe with nine primer combinations and scored a total of 243 AFLP bands. Genetic diversity values were not significantly different in populations from natural inland salt sites as compared to coastal habitats.

CONCLUSION

In the light of these results, we could conclude that biochemical and molecular markers have different discriminating capacity among the seven studied *Suaeda* genotypes in different habitats and they are very useful in exploring the genetic diversity and relationships among them which add ga new dimension to plant taxonomy. Detecting salinity tolerance genes for biosynthesis of glycine betaine which can be used in breeding programs.

REFERENCES

1. El-Shaer, H.M. and M.H. El-Morsy, 2008. Potentiality of salt marshes in Mediterranean coastal zone of Egypt. Biosaline Agriculture and High Salinity Tolerance, pp: 207-219.
2. Khan, A.M., I.A. Ungar and A.M. Showalter, 2000. The effect of salinity on the growth, water status and ion content of a leaf succulent perennial halophyte, *Suaeda fruticosa* (L.) Forssk. Journal of Arid Environments, 45: 73-84.
3. Flowers, T.J., 2004. Improving crop salt tolerance. Journal of Experimental Botany, 55: 307-319.
4. Navarro, A. and F. Rubio, 2006. High-affinity potassium and sodium transport systems in plants. Journal of Experimental Botany, 57: 1149-1160.
5. Choi, S., S. Lim, S. Kim, D. Choi, J. Kim and Y. Choo, 2012. Growth and solute pattern of *Suaeda maritima* and *Suaeda asparagoides* in an abandoned salt field. Journal of Ecology and Field Biology, 35: 351-358.

6. Hoque, M.A., M.N. Banu, Y. Nakamura, Y. Shimoishi and Y. Murata, 2008. Proline and glycinebetaine enhance antioxidant defence and methylglyoxal detoxification systems and reduce NaCl-induced damage in cultured tobacco cells. Journal of Plant Physiology, 165: 813-824.
7. Abdul Jaleel, C., K. Riadh, R. Gopi, P. Manivannan, J. Ines, H. Al-Juburi, Z. Cheng-Xing, S. Hong-Bo and R. Panneerselvam, 2009. Antioxidant defense responses: Physiological plasticity in higher plants under abiotic constraints. Acta Physiologiae Plantarum, 31: 427-436.
8. Apel, K. and H. Hirt, 2004. Reactive oxygen species: metabolism, oxidative stress and signal transduction. Annual Review of Plant Biology, 55: 373-99.
9. Ashraf, M. and M. Foolad, 2007. Roles of glycinebetaine and proline in improving plant abiotic stress resistance. Journal of Environmental and Experimental Botany, 59: 206-216.
10. Ben Hassine, A., M. Ghanem, S. Bouzid and S. Lutts, 2008. An inland and a coastal population of the Mediterranean xero-halophyte species *Atriplex halimus* L. differ in their ability to accumulate proline and glycinebetaine in response to salinity and water stress. Journal of Experimental Botany, 59: 315-1326.
11. Khan, A.M., I.A. Ungar, A.M. Showalter and H.D. Dewald, 1998. NaCl- induced accumulation of glycine betaine for subtropical halophytes from Pakistan. Physiologia Plantarum, 102: 487-492.
12. Qiuli, L., H. Yin, D. Li, H. Zhu, Y. Zhang and W. Zhu, 2007. Isolation and characterization of CMO gene promoter from halophyte *Suaeda liaotungensis*. Journal of Genetics and Genomics, 34: 355-361.
13. Sharifi, G., S.M. Kouhsari, H. Ebrahimzadeh and M. Khatamsaz, 2006. Isozyme analysis of seeding samples in some species of *Hyoscyamus* from Iran. Pakistan Journal of Biological Science, 9: 1685-1692.
14. Wang, Z., S. An, H. Liu, X. Leng, J. Zheng and Y. Liu, 2004. Genetic structure of the endangered plant *Neolitsea sericea* (Lauraceae) from the zhoushan archipelago using RAPD markers. Annals of Botany, 95: 305-313.
15. Aguilera, J.G., L.A. Pessoni, G.B. Rodrigues, A.Y. Elsayed, D.J. Da Silva and E.G. De Barros, 2011. Genetic variability by ISSR markers in tomato (*Solanum lycopersicon* Mill). Revista Brasileira de Ciências Agrαrias Recife, 6: 243-252.
16. Vos, P., R. Rogers, M. Leeker, M. Reijans, L. Van, T. Hornes, M., Fijters, A. Pot, J. Peleman, J. Kuiper and M. Zabeau, 1995. AFLP, a new technique for DNA fingerprinting. Nucleic Acids Research, 23: 4407-4414.
17. Yuan, L.X.Y., J.F. Warburton, M., L.X. Zhang, S. Khairallah, M. Liu, X. Peng and L. Li, 2000. Comparison of genetic diversity among maize inbred lines based on RFLP, SSRs, AFLPs and RAPDs. Yi Chuan Bao, 2: 723-733.
18. Rowell, D.L., 1994. Soil Science: Methods and Applications. Dept of Soil Science, Univ. of Reading. Copublished in the US with John Willey and Sons Inc.; New York, pp: 350.
19. Jackson, M.L., 1967. Soil Chemical Analysis. Prentice. Hall of India Private, New Delhi.
20. Rainwater, F.H. and L.L. Thatcher, 1960. Methods for Collection and Analysis of Water Samples. U.S. Geol. Survey. Water Supply.
21. Reitemeier, R.F., 1943. Semimicro analysis of saline soil solutions. Industrial and Engineering Chemistry Analytical Education, 15: 393-402.
22. A.O.A.C., 1990. Official Methods of Analysis of the Association of Official Analytical Chemists. 15th Ed. Washington D.C., USA.
23. Yoshida, S., D.A. Frono, J.H. Cook and K.A. Gomez, 1976. Laboratory Manual for Physiological Studies on Rice. 3rd Ed. The International Rice Research Institute, Los Baños, Phillipines.
24. Jackson, W.A. and G.W. Thomas, 1960. Effect of KCl and dolometic limestone on growth and ion uptake of sweet potato. Soil Science, 89: 347-352.
25. Chaplin, M.F. and J.F. Kennedy, 1994. Carbohydrate analysis: A practical Approach. 2nd Ed. Oxford Univ., Press Oxford, New York, Tokyo, pp: 344.
26. Dubois, M., K. Gilles, J. Hamilton, P. Rebers and F. Smith, 1956. Colorimetric method for determination in sugars and related substances. Analytical Chemistry, 28: 350-356.
27. Hussain, I., I. Khan and G. Marwat, 2011. Analysis of vitamin C in selected medicinal plants. Journal Chemistry Society of Pakistan, 33: 260-262.
28. Bates, L., R. Waldren and I. Teare, 1973. Rapid determination of free proline for water-stress studies. Plant Soil, 39: 205-207.
29. Greive, C.M. and S.R. Grattan, 1983. Rapid assay for determination of water-soluble quaternary amino compounds. Plant Soil, 70: 303-307.
30. Snedecor, G.W. and W.G. Cochran, 1969. Statistical Methods. 6th Ed. Iowa State Press.
31. Duncan, D.B., 1955. Multiple Range and Multiple F Test; Biomctircs,11: 1-42.
32. Laemmli, U.K., 1970. Cleavage of structural proteins during assembly of head bacteriophage T_4. Nature, 227: 680-685.

33. Stegemann, H., A.M.R. Afifiy and K.R.F. Hussein, 1985. Cultivar Identification of dates (*Phoenix dectylifera*) by protein patterns. 2nd International Symposium of Biochemical Approaches to Identification of Cultivars. Braunschweig, West Germany, pp: 44.
34. Wendel, J.F. and N.F. Weeden, 1989. Visualization and Interpretation of Plant Isozymes. In: Isozymes in Plant Biology. Soltis D. E. and P. S. Soltis (eds). Chapman and Hall Publishers, London, pp: 18.
35. Scandalios, J.C., 1964. Tissue-specific isozyme variations in maize. Journal of Heredity, 55: 281-285.
36. Jonathan, F.W. and N.F. Wendel, 1990. Visualization and Interpretation of Plant Isozyme. In: Isozymes in Plant Biology. D. E. Sdtis and P.S. Sottis (Eds). London Chapman and Hall, pp: 5-45.
37. Heldt, W.H., 1997. A Leaf Cell Consists of Several Metabolic Compartments Plant Biochemistry and Molecular Biology. Institute of Plant Biochemistry, Gottingen with the Collaboration of Fiona.
38. Doyle, J.J. and J.L. Doyle, 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemistry Bullten, 19: 11.
39. Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Research, 18: 6531-6535.
40. Sambrook, J., E.F. Fritsch and T. Maniatis, 1989. Molecular Cloning. A Laboratory Manual, Second Edition, 6: 3-9.
41. Sharma, P.C., B. Huttel, P. Winter, G. Kahl, R.C. Gardner and K. Weising, 1995. The potential of microsatellites for hybridization and polymerase chain reaction based DNA fingerprinting of chickpea (*Cicer arietinum* L.) and related species. Electrophoresis, 16: 1755-1761.
42. Abd El-Maboud, M.M., 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, Bot. Dept. Fac. Sci., Al- Azhar Univ.
43. Kinraide, T., 1999. Interactions among Ca^{++}, Na^{+} and K^{+} in salinity toxicity: Quantitative resolution of multiple toxic and ameliorative effects. Journal of Experimental Botany, 50: 1495-1505.
44. Song, I., M. Chen, G. Feng and Y. Jia, 2009. Effect of salinity on growth, ion accumulation and the roles of ions in osmotic adjustment of two populations of *Suaeda salsa*. Plant Soil, 314: 133-141.
45. Blumwald, E., G.S. Aharon and M.P. Abse, 2000.Sodium transport in plant cells. Biochemica et Biophysica Acta, 1465: 140-151.
46. Munns, R. and M. Tester, 2008. Mechanisms of salinity tolerance. Annual Review of Plant Biology, 59: 651-681.
47. Tawfik, M.M., A.T. Thalooth, Nabila M. Zaki, M.S. Hassanein, Amany A. Bahr and Amal G. Ahmed 2013. Sustainable production of *Kochia indica* grown in saline habitat. Journal of Environmental Treatment Techniques, 1: 56-61.
48. Agastian, P., S. Kingsley and M. Vivekanandan, 2000. Effect of salinity on photosynthesis and biochemical characteristics in mulberry genotypes. Photosynthetica, 38: 287-290.
49. Tammam, A.A., E.M. Fakhry and M. Mostafa El-Sheekh, 2011. Effect of salt stress on antioxidant system and the metabolism of the reactive oxygen species in *Dunaliella salina* and *Dunaliella tertiolecta*. African Journal of Biotechnology, 10: 3795-3808.
50. Foyer, C.H. and G. Noctor, 2005a. Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. Plant Cell, 17: 1866-1875.
51. Foyer, C.H. and G. Noctor, 2005b. Oxidant and antioxidant signaling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. Plant Cell and Environment, 28: 1056-1071.
52. Tipirdamaz, R., D. Gagneul, C. Duhazé, A. Aïnouche, C. Monnier, D. Özkuma and F. Larher, 2006. Clustering of halophytes from an inland salt marsh in Turkey according to their ability to accumulate sodium and nitrogenous osmolytes. Environmental and Experimental Botany, 57: 139-153.
53. Jery, D., E.D. Licon, R. Haruenkit, E. Pawelizik, O.M. Belloso and Y.S. Park, 2003. Identification and differences of total proteins and their soluble fractions in some pseudocereals based on electrophoretic Patterns. Journal Agriculture Food Chemistry, 51: 7798-7804.
54. Haddioui, A. and M. Baaziz, 2001. Genetic diversity of natural populations of *Atriplex halimus* L. in Morocco: an isoenzyme-based overview. Euphytica, 121: 99-106.
55. Khalil, M.A., Rasha, K.A. Soliman, N.K.F. Rashed and S.A. Ibrahim, 2010. Genetic polymorphism of some medicinal plants belonging to *Brassicaceae* using molecular markers. Egyptian Journal of Genetic and Cytology, 39: 41-52.

56. Ajithkumar, I.P. and R. Panneerselvam, 2013. Analysis of intra specific variation in *Setaria italica* (L.) P. Beauv landraces using RAPD and ISSR markers. International Journal of Research in Biochemistry and Biophysics, 3: 15-20.
57. Al-Salameen, F., H. AL-Hashash and S. Al-Amad, 2013. Assessment of genetic diversity of *Haloxyn salicornium genotypes,* a native plant of Kuwait. Kuwait Journal of Science, 40: 149-164.
58. Wu, W., R. Zhou, Y. Huang, D. Boufford and S. Shi 2010. Molecular evidence for natural intergeneric hybridization between Liquidambar and Altingia. Journal of Plant Research, 123: 231-239.
59. Prinz, K., K. Weising and I. Hensen, 2009. Genetic structure of coastal and inland populations of the annual halophyte *Suaeda maritima* (L.) in Central Europe, inferred from amplified fragment length polymorphism marker. Plant Biology, 11: 812-820.