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BIOCHEMICAL AND MOLECULAR GENETICS IDENTIFICATION OF *Salicornia sp.* AND *Sarcocornia sp*. IN THE NORTH COAST OF EGYPT

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ABSTRACT

Because water scarcity anticipated to increase within the destiny in particular with growing global population and the rise in prosperity problem of the shortage of water suitable for cultivation of meals plants inside the global is growing in arid and semi-arid regions. There is the call to discover some other plant resource that doesn't need freshwater *i.e.* able to grow using seawater. It is worth to note that *Salicornia sp*. and *Sarcocornia sp*. may be grown at the seawater.

Currently, considered one of the most crucial issues dealing with Egypt is a way to provide food within the frame of limiting to be had soils for cultivation, limitation of water resources, especially after Ethiopian Nahda Dam and growing in population. Accordingly, the use of halophytes forage plants (*Salicornia* and *Sarcocornia*) using seawater has emerged as one in all the most exciting and intelligent research points. Therefore, a case observe was carried out in 2018 and 2019 to evaluate the nutritional status of *Salicornia* and *Sarcocornia* plants which can be grown on salty water in the North Coast of Egypt. Five samples of *Salicornia* and *Sarcocornia* amassed from Damietta Port Said coastal road and identified depends on phenotypic homes to *Sarcocornia perennis* (DA), *Sarcocornia perennis* (PS), *Sarcocornia fruticosa, Salicornia europaea* and *Salicornia herbacea*. Proximate composition analyses were carried out. It turned into obtrusive that, among dry biomass, carbohydrate has become in the most important proximate compositions in the *Sarcocornia sp.* and *Salicornia sp*. Tissues observed through ash. Molecular evaluation by SCoT techniques turned into done for *Salicornia* and *Sarcocornia*. The SCoT molecular marker techniques reach producing reproducible and dependable amplicons. Even though that the SCoT technique became higher in assessment for molecular variety and discrimination ability for all studied *Salicornia* and *Sarcocornia.*

The results obtained have shown that the high nutritional value of the plant in terms of protein content, carbohydrates content and as a result, it is suitable for food.

Keywords: *Salicornia*, *Sarcocornia* salinity, nutritional, North Coast of Egypt, SCoT Molecular Markers, genetic diversity, cluster analysis and molecular distance.

INTRODUCTION

Sarcocornia sp. and *Salicornia sp.* are currently experiencing a disaster of dwindling freshwater elements and salinization of soil and groundwater **(Ventura and Sagi, 2013 and Singh et al 2014).** This water scarcity is predicted to grow in the future because of a developing global population and an upward thrust in prosperity **(De Vos et al 2010)**. The hassle of the shortage of water appropriate for the cultivation of economic plants in the international is growing in arid and semi-arid regions. In its record of 2006, the World Bank noted that the annual in line with capita water resources decreased from 3430 m3 in 1960 to 667 m3 in 2025 **(Qadir et al 2007).** It was, therefore, essential to try

to plant coastal and barren location land with saltwater, where the first-rate plant life was decided to be halophyte vegetation, together with *Salicornia* plant and *Sarcocornia*, which proved they may be monetary, environmental and food software to consist of seeds for 30% of the oil much like the meals oil for holding Linoleic and oleic acids, high protein content (30-35%), validity as animal feed, the possibility of extract biodiesel and effective materials to deal with many diseases **(Fan et al 2013 and Buhmann et al 2015).** The cultivation of *Sarcocornia sp.* and *Salicornia sp.* are the answer to many agricultural, food and medical problems, at the same time as the cultivation of *Salicornia* flora and *Sarcocornia* plays a crucial role within the exploitation of the coastal and wasteland soil, without coming into the system of crop cultivation **(Akinshina et al 2016).**

SCoT markers, just like others, are one of the reliable techniques due to hundreds of advantages collectively with efficient, informative, and even inexpensive**.** Primers applied in this technique are designed in line with the short-conserved place surrounding the ATG translation start (or initiation) codon, displaying the correlation between realistic genes and their corresponding traits **(Collard et al 2009; Bhattacharyya et al 2013 and Singh, 2014).** Hence, this method has been efficaciously carried out in medicinal flora to discover their genetic variability **(Tiwari, 2016 and Mao et al 2018).** Using this approach, *Salicornia* and *Sarcocornia* **(Steffen et al 2015)** could probably be implemented to discriminate among genera. The SCoT primers are based on conserved areas flanking the initiation codon sequences of genes. It stocks the precept of the usage of a single primer like RAPD and ISSR. The marker tool has been efficiently hired in genetic diversity assessment and fingerprinting of some of the rural and horticultural crop species **(Xiong et al 2011 and Mulpuri et al 2013).** As a simple and novel marker system, start codon targeted (SCoT) marker modified into evolved based mostly on the fast conserved vicinity flanking the begin codon (ATG) in plant genes **(Collard and Mackill, 2009).** SCoT marker requires no sequence data and is correlated with useful genes and corresponding traits **(Mulpuri et al 2013).** The targets of this look at were to assess the nutritional characterization of *Sarcocornia sp.* and *Salicornia sp.* test out the genetic relationships amongst *Salicornia* and *Sarcocornia* genotypes that grow surely in distinct web sites of soil and saltwater sources in alongside Port Said- Damietta coastal avenue and the north-west coast of Egypt through molecular and biochemical fingerprinting for characterizing and detecting polymorphism.

MATERIALS AND METHODS

Fresh samples of aerial elements of the studied plant species had been accrued from two salt marshes sites along Port Said- Damietta coastal road, Egypt with the aid of Dr Mohamed Abd El-Maboud, Ecology and Range Management Dept., Desert Research Center, Egypt. At the first site, the two species *Sarcocornia fruticosa* and *Sarcocornia perennis* were collected; the GPS studying is 31 12.259N - 32 16.923E. At the second one, *Salicornia europaea* and *Salicornia herbacea* had been accumulated; the GPS studying is 31 17.618N - 32 09.680E.

Plant material

Fresh samples of *Sarcocornia perennis (*DA*), Sarcocornia perennis (*PS*), Sarcocornia fruticosa, Salicornia europaea* and *Salicornia herbacea* **(Fig. 1)** were transported to the laboratory within 8h after collection. The samples had the offshoots in 10–15 cm long. About 5–10 cm of the youngest fully expanded branch tips were selected used in all experimentations. For nutritional characterization, *Sarcocornia sp*. and *Salicornia sp.* samples were first washed with deionized water. Sample homogenates were then obtained using a common kitchen homogenizer and finally stored at -70°C before further uses. To determine the quality degradation during storage, each 150 g S&S samples were randomly selected and packaged in the polyethylene perforated bags, and stored in the dark at -20°C. All experiments were conducted in triplicate.

Proximate composition analyses

Moisture content was determined by drying the sample in an oven at 105°C for 4 h until a constant weight was obtained **(AOAC, 1990).** Total lipids were determined according to the Soxhlet extraction methodology **(James, 1995).** Crude protein content was calculated from total nitrogen content determined by the Kjeldahl method **(AOAC, 1990)** using a conversion factor of 6.25. Crude fiber content was determined using the neutral detergent reagent method described by **(Guevara et al 2003).** Ash content was determined by burning the sample in a muffle furnace at 600°C for 5 h. according to **(Jones et al 1991).** Total carbohydrate content was estimated by the difference between 100 and the sum of the percentages of moisture, crude protein, total lipid, and ash contents **(Enujiugha, 2003).**

Salicornia herbacea

Salicornia europaea

Sarcocornia fruticosa

Sarcocornia perennis (DA)

*Sarcocornia perennis (*PS*)*

Fig. 1. Plant materials of *Sarcocornia sp.* and *Salicornia sp.* used in the study.

RESULTS AND DISCUSSION

Proximate compositions

The proximate compositions of *Sarcocornia perennis (*DA*), Sarcocornia perennis (*PS*), Sarcocornia fruticosa, Salicornia europaea* and *Salicornia herbacea* are summarized in **Table (1).** Moisture represented the largest single content among the proximate compositions of *Sarcocornia sp*. and *Salicornia sp.* tissue (fresh weight). It was evident that, among dry biomass, carbohydrate was in the biggest proximate compositions in the *Sarcocornia sp.* and *Salicornia sp.* tissues, followed by ash. While compared to corresponding data for several local common vegetables reported by **(Yang et al 2002),** the protein level in *Sarcocornia sp.* and *Salicornia sp*. were higher than that in celery leaf and spinach (2.6%) and not similar that in lettuce (1.3%) and Chinese cabbage (1.4%). Although the lipid content was relatively low, it was characterized by a high degree of unsaturation mainly for the sake of alpha-linolenic and linoleic acids (data not shown here) (**Tikhomirova et al 2008**). The table illustrates that the proximate composition of *Sarcocornia perennis* (DA) and *Sarcocornia perennis (PS) and Sarcocornia fruticosa,* not differences also *Salicornia europaea and Salicornia herbacea* don't have any differences. The proximate composition in *Sarcocornia Sp. and Salicornia sp.* collected from Port Said-Damietta coastal road, Egypt differs from *Salicornia bigelovii* collected from sea-beans in Chinese, It has 1.54 %, 0.37%, 0.83, 4.48 and 4.36% crude protein, total lipids, crude fiber, total carbohydrate and ash respectively **(Donghe et al 2010).**

Molecular procedures

DNA isolation

Genomic DNA was isolated from freshly *Salicornia* and *Sarcocornia* by DNeasy plant mini kit (bio

basic). The DNA quality was checked employing absorbance ratios A_{260}/A_{280} through a UV-spectrophotometer where DNA is pure with a ratio A_{260}/A_{280} from 1.8- 2.0. Moreover, using electrophoresis in 1.2 % agarose gel with ethidium bromide, a qualitative check for DNA samples was done.

Polymerase Chain Reaction (PCR) and Sequencing

Genomic DNA was used as a template for Polymerase Chain Reaction (PCR) amplification the use of 6 SCoT primers in molecular evaluation for the 5 collected samples. SCoT primers procured from Operon Technology, Alameda, U.S.A. On the opposite hand, SCoT primers had been designed from a consensus sequence derived from the previous studies through **Joshi et al (1997)** and **Sawant et al (1999).** All SCoT primers had been 18-mer **(Table 2)** for SCoT primers design, the begin codon ATG (+1, +2, and +3), 'G' at position +4, 'C' at position +5, and A, C, C and A at positions +7, +8, +9 and +10, respectively, had been fixed (5´-ATGGCTACCA-3´). Amplification reactions for SCoT technique were completed as described by way of **Fathi et al (2013)** and **Xiong et al (2011)** and were completed in Techni TC-512 Thermal Cycler as follows: One cycle at 94ºC for 4 min observed by using 40 cycles of 1 min at 94ºC, 1 min at annealing temperature 57ºC and a couple of min at 72ºC, followed through 72º C for 10 min, the reaction was finally stored at 4 ºC.

Gel Electrophoresis

Amplified products were loaded and separated on a 1.2% agarose gel with ethidium bromide and 100 bp to 1.5 kb ladder markers. The run was carried out for about 30 min at 100 V in mini-submarine gel Bio-Rad.

Gel reading and analysis

DNA banding pattern photos were photographed using the Bio-1D Gel Documentation system and were analyzed by GelAnalyzer3 software which scoring clear amplicons as the present (1) or absent (0) for each primer and entered in the form of a binary data matrix. From this matrix, DNA-profiles were performed for SCoT techniques according to **Adhikari et al (2015)**.

Molecular diversity assessment

This is the first report of studying the genetic variability in *Salicornia sp.* and *Sarcocornia sp.* using SCoT markers. Where 15 primers were tested on samples of apricot rootstock, six SCoT primers gave prominent and reproducible bands. These primers were selected for final amplification and data analysis. Banding patterns and DNA profiles of these techniques were shown in **Figs. 2** and **Table 3.** Evidently, from these figures that SCoT techniques revealed polymorphic patterns and confirmed to be valid in discriminating among *Salicornia sp*. and *Sarcocornia sp*.

Table 3 exhibited that SCoT primers generated less scorable and polymorphic amplicons per primer. As well as, amplicons molecular size (bp) of each SCoT techniques were ranged from 160: 1175. The rate of genetic diversity, unique marker % and polymorphism average % among *Sarcocornia perennis (*DA*), Sarcocornia perennis (*PS*), Sarcocornia fruticosa, Salicornia europaea* and *Salicornia herbacea* based on SCoT markers were nearly not equal **(Table 4).** Primer SCoT 2 gave the lowest percentage of polymorphic (16.5%) while SCoT 6 primer gave the highest percentage of polymorphic (66.66%). More importantly that the SCoT marker is generated from the functional region of the genome, the genetic analyses using this marker would be more useful for crop improvement programs such as genotype identification, considering genetic diversity, construction of linkage maps and QTL mapping **(Hajibarat et al 2015).**

Table 4 illustrated that all successfully SCoT primers in this study, which target highly expressed genes as described by **Sawant et al (1999)**, were different in the last three nucleotides at the 3 ends and were similar in the last five nucleotides at the 5 ends. However, all of these primers showed different data and marker profiles (**Fig. 2)**, this was in agreement with those results obtained by **Aswathy et al (2016).**

Table 1. Proximate compositions of *Sarcocornia perennis (*DA*), Sarcocornia perennis (*PS*), Sarcocornia fruticosa, Salicornia europaea* and *Salicornia herbacea* [a]

Proximate composition (g.100 g-1 FW)[b]	*Sarcocornia perennis(DA)*	*Sarcocornia perennis(PS)*	*Sarcocornia fruticosa*	*Salicornia europaea*	*Salicornia herbacea*
Moisture	87.35±1.32 [B]	87.33±1.31 [B]	84.97±1.2 [B]	79.05±1.25 [A]	78.95±1.26 [A]
Crude protein	2.75± 0.1[A]	2.80±0.1[A]	2.94±0.11 [A]	4.70±0.05 [B]	4.62±0.06 [B]
Total lipids	0.63±0.01 [A]	0.52±0.01 [A]	0.62±0.02 [A]	0.75±0.02 [B]	0.75±0.03 [B]
Crude fiber	1.26±0.12 [A]	1.10±0.15 [A]	1.30±0.15 [A]	1.40±0.14 [B]	1.49±0.16 [B]
Total carbohydrate	3.40±0.42 [A]	3.50±0.38 [A]	3.01±0.32 [A]	5.79±0.38 [B]	6.04±0.32 [B]
Ash	4.62±0.32 [A]	4.75±0.31 [A]	7.15±0.32 [B]	8.31±0.46 [C]	8.15±0.54 [C]

a Values were mean ±S.D. over three replicates. The same Cubital mean no different at 5%
b FW, fresh weight.

Table 2. List of the primer names and their nucleotide sequences used in the study for SCoT procedure

No	Name	Sequence	No	Name	Sequence
1	SCoT 2	ACC ATG GCT ACC ACC GGC	**4**	SCoT 6	CAA TGG CTA CCA CTA CAG
2	SCoT 3	ACG ACA TGG CGA CCC ACA	**5**	SCoT 8	ACA ATG GCT ACC ACT ACC
3	SCoT 4	ACC ATG GCT ACC ACC GCA	**6**	SCoT 10	ACA ATG CTA CCA CCA AGC

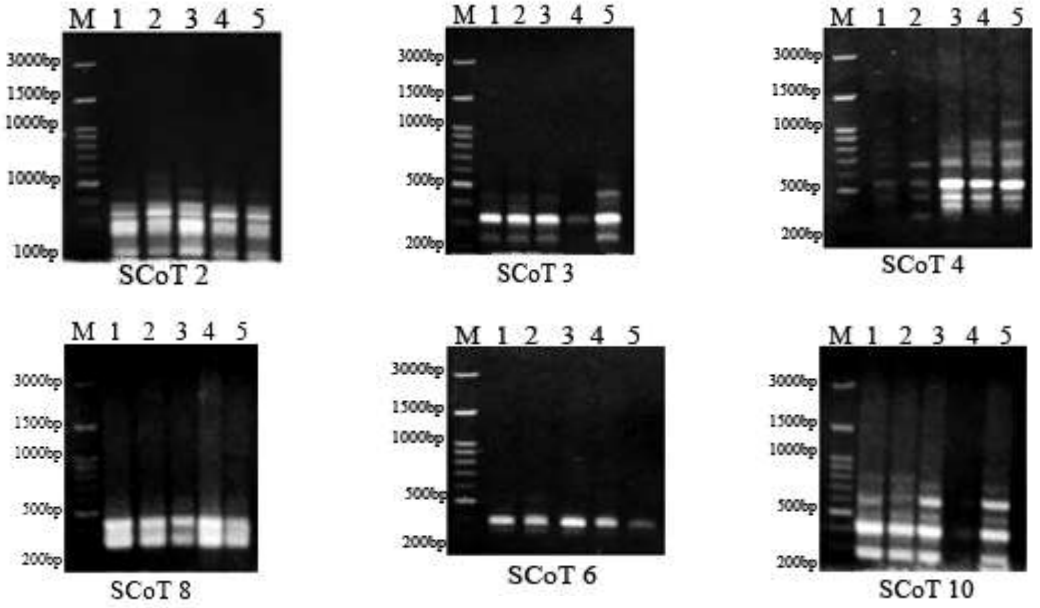

Fig. 2. Banding patterns of SCoT-PCR products for *Sarcocornia perennis (*DA*), Sarcocornia perennis (*PS*), Sarcocornia fruticosa, Salicornia europaea* and *Salicornia herbacea* produced with 6 primers. L, 1.5 kbp ladder and lanes 1 to 4 represent the five genotypes.

Table 3. DNA-profile representation of SCoT fingerprints of *Sarcocornia perennis (*DA*), Sarcocornia perennis (*PS*), Sarcocornia fruticosa, Salicornia europaea* and *Salicornia herbacea* based on 31 amplicons 124 of them were marker loci.

	Band No	M.W (bp)	1	2	3	4	5
SCoT 2	1	580	0	1	0	0	0
	2	445	1	1	1	1	1
	3	400	1	1	1	1	1
	4	365	1	1	1	1	1
	5	270	1	1	1	1	1
	6	160	1	1	1	1	1
	Total		**5**	**6**	**5**	**5**	**5**
SCoT 3	1	545	0	0	0	0	1
	2	475	1	1	1	1	1
	3	380	0	1	0	0	0
	4	340	1	1	1	1	1
	5	245	1	1	1	0	1
	Total		**3**	**4**	**3**	**2**	**4**
SCoT 4	1	1175	1	0	0	0	1
	2	940	1	0	1	1	1
	3	765	1	0	0	1	0
	4	675	1	1	1	1	1
	5	545	1	1	1	1	1
	6	460	1	1	1	1	1
	7	315	0	0	1	1	1
	8	265	1	1	0	0	0
	Total		**7**	**4**	**5**	**6**	**6**
SCoT 6	1	640	0	1	0	1	0
	2	400	1	1	1	1	1
	3	370	1	1	1	0	0
	Total		**2**	**3**	**2**	**2**	**1**
SCoT 8	1	485	1	1	1	1	1
	2	420	1	0	0	1	1
	3	360	1	1	1	1	1
	Total		**3**	**2**	**2**	**3**	**3**
SCoT 10	1	825	1	1	0	0	0
	2	760	0	0	1	0	1
	3	600	1	1	1	1	1
	4	500	0	1	1	0	1
	5	400	1	1	1	1	1
	6	270	1	1	1	1	1
	Total		**4**	**5**	**5**	**3**	**5**
	Total		24	24	22	21	24

Table 4. Molecular data estimated from banding patterns of SCoT technique

Primer Name	Sequence (5´→ 3`)	Total Band	Monomorphic Band	Polymorphic band	Unique Band	Polymorphic %
SCoT 2	CAACAATGGCTACCACCC	6	5	1	1	16.66%
SCoT 3	CAACAATGGCTACCACCG	5	2	3	3	60%
SCoT 4	CAACAATGGCTACCACCT	8	3	5	1	62.50%
SCoT 6	CAACAATGGCTACCACGC	3	1	2	-	66.66%
SCoT 8	CAACAATGGCTACCACGT	3	2	1	-	33.33%
SCoT 10	ACAATGCTACCACCAGC	6	3	3	-	50%
Total		31	16	15	5	48.38%

On the other hand, molecular similarity (MS) matrix between all *Sarcocornia sp.* and *Salicornia sp.* based on SCoTs, data was recorded in **Table 5**.

On the other hand, molecular similarity (MS) matrix between all *Sarcocornia sp.* and *Salicornia sp.* based on SCoTs, data was recorded in **Table 5**.

This matrix indicates that the range of molecular similarity (MS) based on SCoT markers ranged from 0.75 (between *Salicornia herbacea* and *Sarcocornia perennis* (PS) to 0.91 (between *Salicornia herbacea* and *Sarcocornia fruticosa*).

Fig. 3 showed the dendrogram of the SCoT techniques analysis derived from the UPGMA method using the Dice-dissimilarity index according to **Xanthopoulou et al (2015)**. This dendrogram divided into three groups according to the truncated line at a coefficient of similarity= 0.76, group one contains *Sarcocornia fruticosa* (3) and *Salicornia herbacea* (5) group two contains *Sarcocornia perennis* (MD) (1) and *Salicornia europaea* (4) and *Sarcocornia perennis* (PS) is separate in group three.

This confirms that the data of SCoT techniques were suitable for evaluating the genetic relationships among *Sarcocornia perennis (*DA*), Sarcocornia perennis (*PS*), Sarcocornia fruticosa, Salicornia europaea* and *Salicornia herbacea* because of the delicate the information about genetic diversity. This result was in agreement with **Baghizadeha and Dehghan (2018)**, who reported that cluster analysis based on SCoT data discriminated the Iranian pistachio cultivars in terms of their genetic characterizations.

Also, **Table 3** showed that some of the *Sarcocornia perennis* (DA), *Sarcocornia perennis* (PS), *Sarcocornia fruticosa*, *Salicornia europaea* and *Salicornia herbacea*. The *Sarcocornia perennis* (PS), has two positive marker loci with **SCoT 2 and SCoT 3 primers** with size 580 bp and 280 bp respectively and one negative marker loci generated through **SCoT 4 primer** at marker loci with 940 bp. The *Sarcocornia fruticosa* has one negative marker loci generated by **SCoT 3 primer** at marker loci with 245bp and *Salicornia herbacea* has one positive marker loci generated by **SCoT 3 primer** at marker loci with 545bp. The five *Sarcocornia sp.* and *Salicornia sp.* characterized by 5 unique markers, were 3 positive and 2 negatives. On the opposite hand, from **Table 4 & 5** and **Fig. 3** indicated that out of total amplicons number (31), 5 with ratio 16%, 16 amplicons are monomorphic and 15 amplicons polymorphic. Hence, previous results illustrate the advantage of the SCoT technique in terms of genetic diversity assessment in *Sarcocornia sp.* and *Salicornia sp.* These results had been in agreement with **Gorji et al (2011**) in Potato and **Etminan et al (2016)** in durum wheat, at the same time as **Baghizadeha and Dehghan (2018)** advocated it is higher to use this method in conjunction with each other for distinctive different fingerprinting.

In conclusion, the molecular analysis of the tested *Salicornia* and *Sarcocornia* has revealed clear differences at the molecular level among these plants.

Table 5. Molecular similarity (MS) between *Sarcocornia perennis (*DA*), Sarcocornia perennis (*PS*), Sarcocornia fruticosa, Salicornia europaea* and *Salicornia herbacea* based on Dice dissimilarity index for SCoT data

	1	**2**	**3**	**4**	**5**
***Sarcocornia perennis* (MD) (1)**	1.0				
***Sarcocornia perennis* (PS)(2)**	0.83	1.0			
***Sarcocornia fruticosa* (3)**	0.83	0.83	1.0		
***Salicornia europaea* (4)**	0.84	0.76	0.84	1.0	
***Salicornia herbacea* (5)**	0.83	0.75	0.91	0.84	1.0

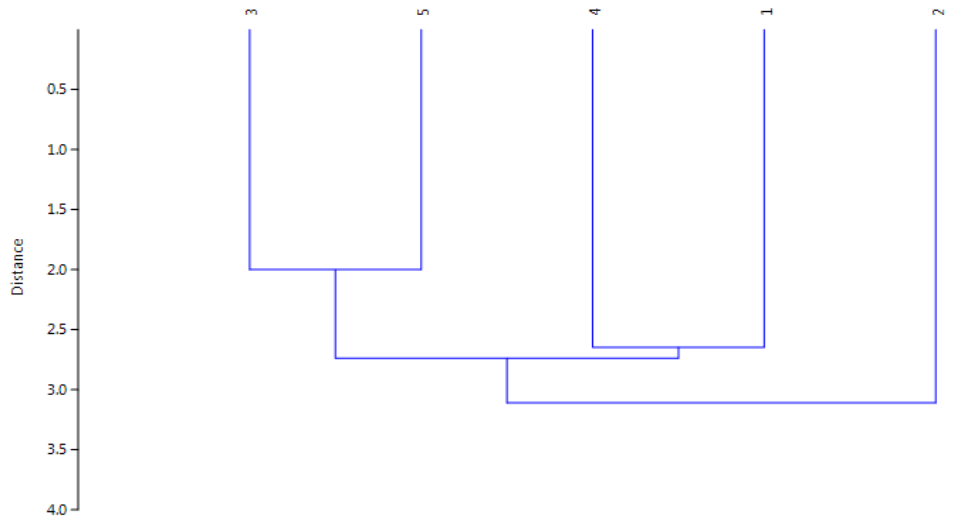

Fig. 3. Dendrogram derived by UPGMA method using Dice-dissimilarity coefficient for data of SCoT techniques for *Sarcocornia perennis (*DA*), Sarcocornia perennis (*PS*), Sarcocornia fruticosa, Salicornia europaea* and *Salicornia herbacea*

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تحديد الوراثة البيوكيميائية والجزيئية للساليكورنيا والساركوكورنيا في الساحل الشمالى لمصر

[42]

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الموجـــــز

نظرا لندرة المياه حاليا مع تزايد عدد السكان في العالم ونقص حاد في المياه في المناطق والشبه القاحلة زادت جهود الباحثين للبحث واكتشاف لموارد نباتية لا تحتاج الي مياه عذبة و لها القدرة علي استخدام المياه المالحة او مياه البحر ونظرا لأن نبات *Salicornia sp.* و*Sarcocornia sp.* يمكن ان تنمو جيدا علي مياه البحر وفي مصر في هذه الظروف وانخفاض المخصصات المائية للفرد و مشكلة سد النهضة الإثيوبي يجب الإعتماد علي النباتات الملحية للاعلاف (Halophytes forage plants) مثل (*Salicornia* and *Sarcocornia*) والإعتماد عليها في إنتاج الأعلاف الخضراء خاصة في السواحل الشمالية وتم تجميع عينات من طريق الطريق الساحلي بين دمياط وبورسعيد وهذه العينات تم تعريفها وهي *Sarcocornia perennis* (DA), *Sarcocornia perennis* (PS), *Sarcocornia fruticosa, Salicornia europaea* and *Salicornia herbacea*. وتم إجراء تحليل الكيميائئ لتقدير البروتين والكربوهيرات والدهون والرماد وتم الإعتماد علي بادئات Scot في التفريق الوراثي بين العينات التي تم جمعها وأظهرت النتائج المتحصل عليها بإرتفاع القيمة الغذائية للنباتات محل الدراسة وإرتفاع محتواها من البروتين والكربوهيدرات مما يجعلها إختبار جيد في إنتاج الأعلاف خاصة مع ندرة المياه وتوافر مياه البحر في السواحل الشمالية كما امكن التفريق الوراثي بين العينات المجمعه من الأماكن المختلفة لعمل شجرة قرابة بينهم .

الكلمات المفتاحية: الساليكورنيا، الساركوكورنيا الملوحة، العناصر الغذائية ،الساحل الشمالى في مصر، الواسمات الجزيئية، التنوع الوراثي، تحليل المجموعات والمسافات الجزيئية

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