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AGRO-PHYSIOLOGICAL AND GENETIC CHARACTERIZATION OF HALOPHYTE SPECIES AND THEIR IMPACT ON SALT-AFFECTED SOIL

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SUMMARY

The adverse effects of climate change and heightened soil salinity on agricultural production are definite. Halophytes serve to remove salts from soil effectively and economically. Consequently, the presented work has evaluated the impact of three halophytic species on salt-affected soil. The study used inter simple sequence repeats (ISSRs) and start codon targeted (SCoT) markers to examine the genetic variations. Field experiments progressed on salt-affected soils around Qarun Lake's coastal region for two consecutive seasons (2019 and 2020). The soil and plants underwent analysis using established methodologies. The findings indicated that after the fifth cutting for the three halophytic species, there was a drop in salinity indices, implying an improvement in soil quality assessments. On the other hand, six ISSR and 10 SCoT primers amplified 96 and 190 bands with 84.14% and 88.29% polymorphism, respectively. Additionally, they demonstrated numerous positive and negative markers linked to some phenotypic traits. Polymorphic information content (PIC) values were 0.51 (ISSRs) and 0.48 (SCoT), indicating that these markers were moderately informative. Heterozygosity index (He) values were 0.59 (ISSRs) and 0.57 (SCoT), implying a substantial degree of genetic diversity present within the studied species.

Keywords: Halophytic species, forage production, bioethanol, remediation, ISSR, SCoT

Key findings: *Leptochloa fusca* was more effective in salinity remediation, having the highest productivity and protein content (CP), hence, considered a good source for forage production. Meanwhile, *Sporobolus virginicus* (Smyrna) produced the utmost lignocellulosic biomass, making it a potential candidate for bioethanol production in the future. Overall, the ISSR and SCoT markers generated reliable banding patterns to evaluate the genetic variation among halophytic species.

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INTRODUCTION

Abiotic stress seriously jeopardizes the growth and development of plants and results in massive crop losses globally. The most prevalent type of abiotic stress is salt stress. It limits crop productivity worldwide, posing a severe danger to agriculture, particularly in areas with arid climates (Sayed *et al*., 2021). Egypt is one of the countries most affected by salinity. The harmful effects of salinity can be reduced by removing salts from affected areas and finding plant varieties with high salt tolerance (Hossain, 2019).

Halophyte plants can reproduce and grow in soils with high salt concentrations, making them the ideal model for comprehending the complex genetic and physiological processes involved in coping with salinity stress (Fan, 2020). These plants have evolved several defense mechanisms to withstand salinity and survive in situations with a lot of salt. When present at the exact concentrations, salt promotes halophyte vegetative growth while preventing non-halophyte growth, which reflects the ability of plants to produce different phenotypic traits in response to shifting environmental circumstances (Yuan *et al*., 2019; Rittirongsakul *et al*., 2020). In addition, Garcia *et al*. (2020) noted that halophytes can adjust high salt concentrations depending on physiological, environmental, and genetic factors.

Physical, chemical, and biological remediation techniques have helped reduce soil salinity, depending on how efficiently they can uptake Na^+ and Cl^- ions (Flowers *et al*., 2015). One of the biological techniques is "halophytoremediation," which relies on halophytes' capacity to accumulate and/or exclude salts by storing salt ions they get through their roots in their leaves. It is also an inexpensive and easy technique. Therefore, halophytes can be beneficial companion plants with salt-sensitive crops because they have quick metabolic responses that promote osmotic correction to prevent salinity stress. Different halophyte species are applicable in the remediation procedure because they have variable tolerance mechanisms (Meng *et al*., 2018; Li *et al*., 2019). Halophytes have a substantial potential to produce biofuels because of their lignocellulose biomass and seed oil, a plentiful energy source (Joshi *et al*., 2020a).

Knowledge of genetic variability is necessary to comprehend how species adapt to biotic and abiotic conditions, changing their genetic makeup. Using DNA-based molecular markers to estimate genetic variability is highly effective because the information directly comes from the genome without interference from environmental factors (Environment, 2017). One of the molecular markers used in previous research on Halophytes' phylogeny and genetic diversity was ISSR markers (Aghaei *et al*., 2022). SCoT markers have also been helpful in the study of genetic diversity and fingerprinting of halophytes (Osmonali *et al*., 2023). ISSRs-PCR is a technique that uses primers (16–18 bp) to amplify inter-microsatellite sequences at multiple loci throughout the genome (Marwal and Gaur, 2020). The SCoT marker specifically targets the region flanking the start codon ATG, a highly conserved region in plant genes. Therefore, it can distinguish genetic changes in a particular gene connected to a specific trait (Vanijajiva, 2020; Rai, 2023).

The present study sought to learn the effect of growing three halophytic species on salt-affected soil and utilize the ISSRs and SCoT markers to investigate the genetic differences and species characterization.

MATERIALS AND METHODS

Two-field experiments materialized on a private salt-affected farm near Qarun Lake in Fayum, Egypt (29° 28′ 19.92′′ N, 30° 36′ 51.12′′ E) to study the effect of growing three halophyte species on salt-affected soil under a drainage water irrigation. These are *Sporobolus virginicus* (Smyrna) and *Sporobolus virginicus* (Dixie), as exotic species introduced by the late Prof. Dr. N.I. Ashour from Delaware, USA (with the sample’s kind donation by Prof. Dr. J.L. Gallagher, College of Marine Studies, University of Delaware, Lewes,

Table 1. Chemical analysis of irrigation water in the experiment. (Average values of two seasons).

Analysis parameters	Analysis results
pH	7.98
EC (dSm^{-1})	12.32
Soluble cations (Meq /L^{-1})	
K^+	0.52
Na^+	155.22
Mg^{2+}	14.65
Soluble anions (Meq /L^{-1})	
Ca^{2+}	18.76
SO_4^{2-}	26.04
Cl^-	154.62
HCO_3^-	2.81

EC = Electrical conductivity

DE 19958, USA). Additionally, *Leptochloa fusca* (L.) as a local species, collected from salt-affected areas around Qarun Lake, where the Lake's salinity has surpassed saltwater due to sanitation, climate change, and agricultural drainage (Elgamal *et al*., 2017). Rhizomes' transplanting commenced every March 27 for 2019 and 2020 in a randomized complete block design with three replications. The mechanical and chemical analysis of the soil occurred at depths of 0 to 30 and 30 to 60 cm, according to Carter and Gregorich (2007). Applying five equal doses of calcium superphosphate (15.5% P_2O_5), 1.5 g potassium sulfate (48.0% K_2O), and 6.75 g urea (46.5% N) at the rates of 32 kg P_2O_5/fed, 24 kg K_2O/fed, and 105 kg N/fed, respectively, added before transplanting, and after each cutting at 50 days intervals (Tawfik *et al*., 2015). Drainage water was utilized for irrigation (EC 12.32 dSm^{-1}), with the analysis of water irrigation presented in Table 1. Irrigation ran every five days using a sprinkler irrigation system. Total irrigation water for each cutting was 492, 515, 528, 507, and 478 m^3/fed, calculated from the meteorological data of the Central Laboratory for Agri-cultural Climate (CLAC) depending on the Penman-Monteith Equation (Allen *et al*., 1989).

Documenting the fresh and dry weight, chlorophyll a + b, proline, the osmotic potential, K^+/Na^+, and Ca^{2+}/Na^+ ratios followed the standard methods. Crude fiber (CF), ether extract (EE), ash, lignin, hemicellulose, and cellulose attained estimation according to AOAC (2010). The sodium adsorption ratio (SAR) and exchangeable sodium percentage (ESP) calculations employed the formula according to Martins-Noguerol *et al*. (2021).

Statistical analysis

Data scrutiny was by analysis of variance (ANOVA). Bartlett's test revealed homogeneity of error, with the combined analysis of the two seasons conducted using the computer-based statistical package MSTATC. The Least Significant Difference (LSD) helped compare the means (LSD at 5%).

Genomic DNA extraction

The DNA extraction process from halophyte leaves commenced at the National Research Centre using the i-genomic DNA plant DNA Extraction Mini kit (iNtRON Biotechnology, Inc., Korea, Cat. No. 17371) according to the manufacturer's instructions.

ISSRs and SCoT analyses

A total of 16 primers (six ISSRs and 10 SCoT) became subjects for PCR amplification and synthesis by Willowfort, UK (Table 2). A 6.3 µL of COSMO PCR RED Master Mix (Willowfort, Birmingham, Cat. No. WF10203001), 1 µL of DNA, 1 µL of primer, and sterile ddH_2O for a final volume of 12.5µL constituted for PCR amplifications. The PCR reaction conducted a Labcycler thermal cycler (Sensoquest, Germany). A PCR program for amplifyingDNA

Table 2. Nucleotide sequences of SCoT and ISSRs primers used in this research.

No.	Primer name	Sequence (5′-3′)	No.	Primer name	Sequence (5′-3′)
1	SCoT-1	CAACATGGCTACCACCA	1	HB10	GAGAGAGAGAGACC
2	SCoT-3	CAACAATGGCTACCACCG	2	UBC-844	CTCTCTCTCTCTCTCTRC
3	SCoT-5	CAACAATGGCTACCACGA	3	17899-A	CACACACACACAAG
4	SCoT-13	ACGACATGGCGACCATCG	4	UBC-809	AGAGAGAGAGAGAGAGG
5	SCoT-15	ACGACATGGCGACCGCGA	5	UBC-880	GGAGAGGAGAGGAGA
6	SCoT-18	ACCATGGCTACCACCGCC	6	UBC-892	TAGATCTGATATCTGAATTCCC
7	SCoT-19	ACCATGGCTACCACCGGC			
8	SCoT-21	ACGACATGGCGACCCACA			
9	SCoT-28	CCATGGCTACCACCGCCA			
10	SCoT-34	ACCATGGCTACCACCGCA			

included the following: initial denaturation at 94 °C for 3 min, then 35 cycles of denaturation at 94 °C for 45 s, 1 min of annealing at a temperature specific for each primer, and 1.5 min of extension at 72 °C. Finally, a 7 min step at 72 °C, using the BERUS 100-bp DNA ladder (Willowfort, Birmingham, Cat. No. WF10407001). The final amplified products incurred 1 h of electrophoresis at 100 V on agarose gel (1.5%) with 1 µL of EtBr (10 mg/L) in 1X TBE buffer. Then, the DNA profiles visualized on a UV transilluminator.

ISSRs and SCoT data analyses

The analysis of gel images used the GelAnalyzer 19.1 software (https://www.gelanalyzer.com) to establish the amplified fragments' molecular sizes. Amplified fragments bore classification as present (1) or absent (0). using the Gene-Calc (https://gene-calc.pl/pic) for calculating polymorphic information content (PIC) and heterozygosity (He) values.

RESULTS AND DISCUSSION

Evaluation of the three halophytic species for remediation of salt-affected soil

Analysis of soil samples had depths of 0 to 30 and 30 to 60 cm before and after transplantation. The soil analysis results differed after the fifth cutting of halophyte plants from before their transplantation, as shown in Table 3. There was a notable improvement in soil quality indicators, and soil salinity decreased. The halophytes reduced the Electrical conductivity (EC), SAR, ESP, Na^+, HCO_3^-, SO_4^{2-}, and Cl^- values. Data also showed a rise in silt percentage and organic C, K^+, and Mg^{2+} values. The three halophytic species successfully reduced salinity, but *Leptochloa fusca* was the highest. The cultivation of halophytes indicated not affecting the pH values of the soil. Halophytes were also an effective biological method of reclaiming salt-affected soils because of their ability to expel salts through certain glands and are also palatable to farm animals. These results are consistent with those of Tawfik *et al*. (2015), who found that *Leptochloa fusca* exhibited a typical halophyte behavior, making it a valuable plant for removing excess salt from the root zone and enhancing the physical properties of the soil. Farzi *et al*. (2017) also found that these plants reduced the measured salinity parameters to acceptable values. Likewise, Joshi *et al*. (2020b) showed that halophytes can undergo phytoextraction, shedding light on how halophytes can help reclaim degraded saline soils. With osmotic adjustment, most halophytes can remarkably accumulate salt ion amounts in their vacuoles (Li *et al*., 2019). Hence, these plants can be phytoremediation (Arrekhi *et al*., 2021; Ahmadi *et al*., 2022).

Fresh and dry weight for five cuttings of the three halophytic species

The data in Table 4 and Figure 1 revealed that the three halophytes were extremely salt-tolerant and produced different amounts of biomass that could serve as feeds. However,

Table 3. Soil analysis of the experiment site before and after transplantation of the three halophytic species.

Soil characters	Before transplantation		*Sporobolus virginicus* (Dixie)		*Sporobolus virginicus* (Smyrna)		*Leptochloa fusca*	
	0-30 cm	30-60 cm	0-30 cm	30-60 cm	0-30 cm	30-60 cm	0-30 cm	30-60 cm
Chemical analysis								
EC (dSm^{-1})	015.44	009.78	011.27	006.36	012.68	009.70	010.14	005.69
HCO_3^- %	013.37	012.70	012.90	011.67	012.00	010.70	012.58	011.60
SO_4^{2-} %	080.87	062.06	073.72	056.87	072.69	055.01	071.69	055.39
Cl^- %	201.12	179.06	182.83	164.07	181.94	162.46	179.94	161.82
Ca (ppm)	079.05	076.34	074.69	071.72	073.84	071.23	073.80	070.99
Mg (ppm)	027.94	025.72	028.33	027.68	027.82	026.90	028.72	027.57
K (ppm)	001.91	001.79	002.00	001.88	001.92	001.80	002.03	001.90
Na (ppm)	302.38	223.80	271.73	187.73	270.36	185.52	266.41	181.02
pH	007.55	007.19	007.50	007.17	007.54	007.14	007.43	007.04
Organic C	002.17	002.03	002.67	002.52	002.45	002.31	002.57	002.42
SAR	041.34	031.33	037.86	026.63	037.92	026.49	037.21	025.79
ESP	074.00	068.00	072.00	065.00	072.00	065.00	072.00	064.00
Mechanical analysis								
Sand %	022.56	023.73	021.62	022.51	21.49	022.52	021.29	022.39
Silt %	016.42	015.50	017.12	016.24	16.99	015.97	017.31	016.45
Clay %	061.94	061.81	061.46	061.45	61.72	061.71	061.60	061.36

EC = Electrical conductivity, SAR = Sodium adsorption ratio, ESP = Exchangeable sodium percentage.

Table 4. Fresh and dry weight (Kg/m^2) for five cuttings of the three halophytic species.

Halophytic species	First cutting		Second cutting		Third cutting		Fourth cutting		Fifth cutting	
	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.
Sporobolus virginicus (Dixie)	0.405	0.110	0.454	0.126	0.513	0.141	0.575	0.157	0.399	0.111
Sporobolus virginicus (Smyrna)	0.375	0.099	0.423	0.119	0.479	0.126	0.533	0.150	0.382	0.104
Leptochloa fusca	0.424	0.119	0.451	0.148	0.534	0.150	0.568	0.165	0.459	0.128
LSD 5%	0.032	0.011	0.035	0.010	0.040	0.013	0.044	0.014	0.038	0.009

LSD = Least significant difference.

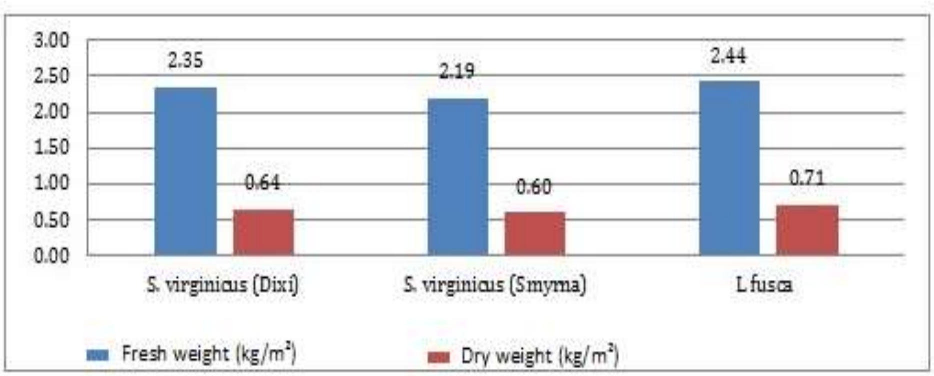

Figure 1. Total productivity for the five cuttings of the three halophytes.
Fresh weight (LSD = 0.14), Dry weight (LSD = 0.09).

Leptochloa fusca had the best growth, increasing total productivity. This variation might be due to genetic diversity, salt tolerance, and bio-drainage ability. On the other hand, the fourth cutting was superior to the other cuttings. Tawfik *et al*. (2015) reported similar findings and said that the halophytes are suitable for planting in saline habitats; thus, these halophytes have immense economic value as nonconventional forage and fodder plants. Also, Bahr *et al*. (2017) stated that halophytic plants showed high salinity- and drought-stress tolerance. These plants can grow well in poor soils and arid and semi-arid regions with their physiological characteristics, exhibiting growth and development with adequate soil surface coverage. Tawfik *et al*. (2018) described that saline irrigation could encourage the growth of several halophytic species. It may refer to an improvement in shoot osmotic status brought on by a rise in ion uptake metabolism.

Some physiological aspects of the three halophytic species

The pertinent findings revealed significant differences in the three halophytes' proline and chlorophyll a + b contents, while these differences were insignificant for other measurements (Table 5). The highest value for chlorophyll a + b (3.06 mg/g dry wt.) and the lowest value of proline (437.91 μg/g dry wt.) came from *Leptochloa fusca*. The chlorophyll a + b content in all halophytes was significant, indicating that the halophytes tolerated the negative influence of salt. The highest proportion for proline (484.10 μg/g dry wt.) was evident in *Sporobolus virginicus* (Smyrna). This result follows the increases in proline content in plants under salinity stress, increasing salt tolerance (Kavi Kishor *et al*., 2015; Khanna-Chopra *et al*., 2019). It indicates that the three species are salinity tolerant. According to Koyro *et al*. (2013), proline showed significant amounts in many halophytic plants under salt stress. Slama *et al*. (2015) and Delavar *et al*. (2020) mentioned that halophytes can regulate soluble substances like glycine betaine and proline to increase cell volume and maintain osmotic balance under salinity. These organic compounds likely play a role in leaf osmotic adjustment and the protection of membrane stability at severe salinity (Feng *et al*., 2021). Salt-marsh halophytes cope with salt by excluding entry into roots, sequestering salts intracellular (leading to succulence), and excreting salt via glands, usually on leaf surfaces (Nhu *et al*., 2020). Furthermore, the findings demonstrated no appreciable variations in the halophytic species' Ca^{2+}/Na^{+} and K^{+}/Na^{+} ratios. But, compared with the studied species, *Leptochloa fusca* had a higher K^{+}/Na^{+} ratio. Sun *et al*. (2019) showed that higher K^{+}/Na^{+} ratios increase salinity tolerance.

Nutritional values of different halophytic species grown in the salt-affected soil

The examined halophytic species significantly differed in terms of their crude protein (CP), crude fiber (CF), Neutral detergent fiber (NDF), and ether extract (EE) contents (Table 6). The maximum levels of CF, Ash, ADF, NDF, and EE amounted to 23.54%, 25.21%, 22.43%, 15.76%, and 3.21%, respectively, in *Sporobolus virginicus* (Dixie). Meanwhile, the highest value of CP (9.94%) was in *Leptochloa fusca*. The moderate protein level of these halophytes (8.95%–9.94%) makes them suitable as forage crops and have excellent nutritional value for feeding ruminant animals. Results are consistent with the findings of Arrekhi *et al*. (2021), who indicated that ruminant cattle require a minimum level of 7%–8% CP for optimal feed consumption. Thus, these plants have a substantial nutritional value suited as traditional fodder plants (Alzarah, 2021). Our results were less than those reported by Tawfik *et al*. (2015), who revealed that the CP content of some halophytes ranges from 9.68% to 13.68%, as well as CF, Ash, ADF, NDF, and EE, which were 25.57%, 30.11%, 27.36%, 16.35%, and 4.32%, respectively.

Table 5. Some physiological aspects for the fifth cutting of the halophytic species.

Characters	halophytic species			
	Sporobolus virginicus (Dixie)	*Sporobolus virginicus* (Smyrna)	*Leptochloa fusca*	LSD 5 %
Chlorophyll a + b (mg/g dry wt.)	002.77	002.87	003.06	0.23
Soluble carbohydrates %	043.67	044.65	042.87	NS
Proline (μg/g dry wt.)	470.92	484.10	437.91	34.21
Potassium content (mg/g dry wt.)	011.06	010.82	011.09	NS
Sodium content (mg/g dry wt.)	011.27	011.07	010.78	NS
Calcium content (mg/g dry wt.)	003.49	003.26	003.47	NS
K^+/Na^+ ratio	000.98	000.98	001.03	NS
Ca^{2+}/Na^+ ratio	000.32	000.30	000.31	NS
Succulence (fresh wt. /dry wt.)	003.64	003.63	003.44	NS
Osmotic potential	009.36	009.57	009.13	NS

LSD = Least significant difference, NS = Not significant.

Table 6. Nutritional values for the fifth cutting of the three halophytic species.

Plant species	CP (%)	CF (%)	Ash (%)	ADF (%)	NDF (%)	EE (%)	NFE (%)
Sporobolus virginicus (Dixie)	8.95	23.54	25.21	22.43	15.76	3.21	39.09
Sporobolus virginicus (Smyrna)	9.40	22.92	24.92	21.50	14.96	2.91	39.85
Leptochloa fusca	9.94	23.24	25.14	21.10	14.90	2.98	38.71
LSD 5%	0.58	01.42	NS	NS	00.95	0.18	NS

CP = Crude protein, CF = Crude fat, ADF = Acid detergent fiber, NDF = Neutral detergent fiber, EE = Ether extract, NFE = Nitrogen-free extract, LSD = Least significant difference, NS = Not significant.

Lignocellulosic biomass of the three halophytic species grown in salt-affected soil

With the study's target of a species that has ideal cellulose, hemicellulose, and minimal lignin concentrations, they can produce a higher sugar and, as a result, increased ethanol production during fermentation. *Sporobolus virginicus* (Smyrna) is a promising species for the generation of bioethanol since it has a significant amount of cellulose/hemicellulose and low lignin content (Figure 2). According to this study, halophytes can effectively compete with other traditional biofuel sources. These results agree with Joshi *et al*. (2020a), who indicated that halophytes are economical sources of lingocellulosic biomass.

Molecular analysis

DNA markers have been eminent in revealing the genetic variations in species (Hailu and Asfere, 2020). ISSR markers served in numerous studies on hereditary differences of halophytes (Kim *et al*., 2017; Aghaei *et al*., 2022; Paica *et al*., 2022). SCoT markers helped evaluate genetic differences and characterize halophytes (Rittirongsakul *et al*., 2020; Abd El-Moneim *et al*., 2021; Osmonali *et al*., 2023). Based on this, the related study used ISSRs and SCoT markers to estimate the genetic variability among the three halophytic species studied.

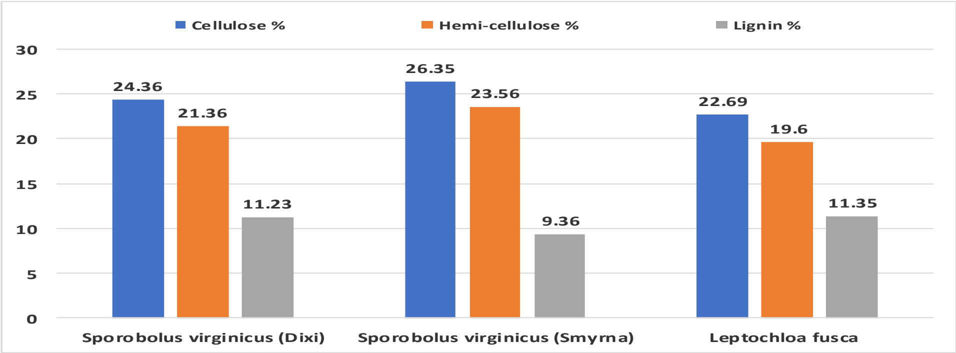

Figure 2. Content of cellulose, hemicellulose, and lignin for the fifth cutting of the three halophytic species.

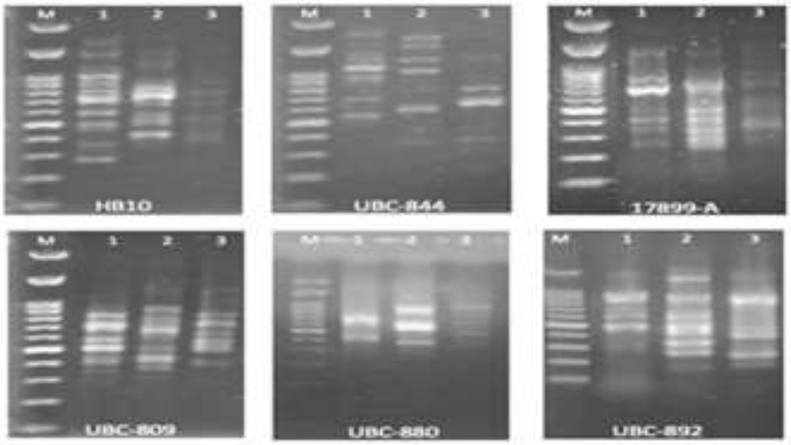

Figure 3. ISSRs amplification profile using six primers with three halophytic species. M: Ladder 100 bp, 1: *Sporobolus virginicus* (Dixie), 2: *Sporobolus virginicus* (Smyrna), and 3: *Leptochloa fusca.*

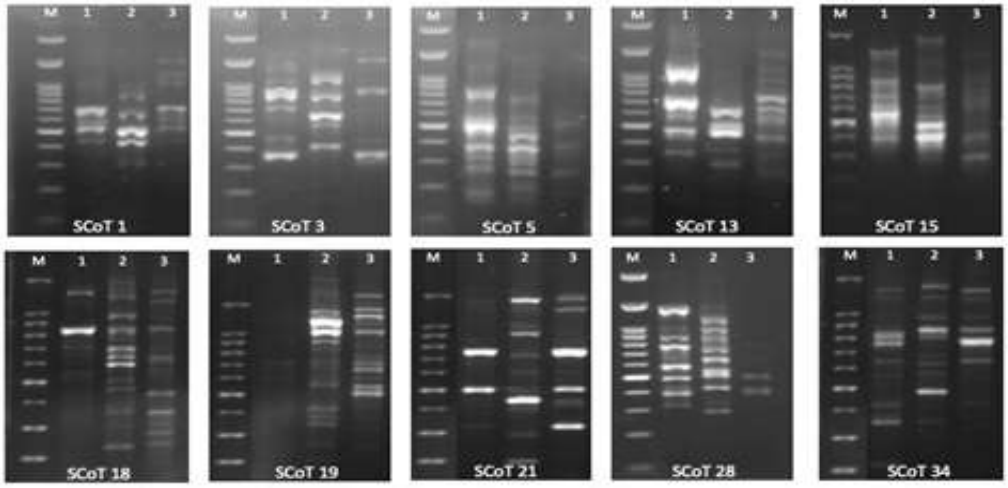

Figure 4. SCoT amplification profiles using 10 primers with the three halophytic species. M: Ladder 100 bp, 1: *Sporobolus virginicus* (Dixie), 2: *Sporobolus virginicus* (Smyrna), and 3: *Leptochloa fusca*.

Polymorphism as detected by ISSRs and SCoT – PCR techniques

Six ISSR primers incurred assessment for the genetic variation among the studied halophyte species, generating 96 total bands with molecular sizes ranging from 216 to 2450 bp (Figure 3). The number of scored bands varied from nine to 25, with a mean of 16 bands per primer (Table 7). The overall polymorphic bands were 81, representing 84.4% of the whole bands. The polymorphism percentage ranged from 62.50% (UBC-809) to 100% (UBC-880), with a mean of 84.14%. The recorded polymorphism percentage in this research was higher than 83.12% in *N. schoberi L.* (Paica *et al*., 2022) and 65.69% in Wild *Salicornia* (Aghaei *et al*., 2022) but lower than 90.91% in Quinoa (Abd El-Moneim *et al*., 2021).

For SCoT, the 10 primers produced 190 bands (Figure 4), wherein 167 were polymorphic (87.9%) and varied in size (164–3020 bp). The number of scored bands differed from 12 to 28 (Table 8). The polymorphism percentage ranged from 71.43% (SCoT-19) to 100% (SCoT-13), with an average of 88.29%. This percentage was higher than 85.26% in Quinoa (Abd El-Moneim *et al*., 2021) and 48.38% in halophytes forage (Hussein *et al*., 2020).

The PIC value varied from 0.037 (HB10) to 0.57 (UBC-892), with an average of 0.51 by ISSRs. For SCoT, the PIC ranged from 0.35 (SCoT-19) to 0.59 (SCoT-1), with an average of 0.48, indicating that these markers had a high level of polymorphism. PIC in this search had a lower value than 0.77 (ISSRs) and 0.62 (SCoT), which Abd El-Moneim *et al*. (2021) obtained in Quinoa. Furthermore, the He values range from 0.498 (HB10) to 0.645 (UBC-892), with an average of 0.59 for ISSRs and 0.455 (SCoT-19) to 0.664 (SCoT-1), with an average of 0.57, for SCoT markers. It suggests a relatively high level of genetic diversity within the three halophytes, which is attributable to their different origins. This study's estimated He values are higher than 0.378 (Aghaei *et al*., 2022) and 0.2 (Paica *et al*., 2022) for ISSRs.

Species identification by unique ISSRs and SCoT markers

ISSRs and SCoT markers ably differentiated between the studied species by generating unique positive and negative bands (Table 9). The overall number of distinct bands produced by ISSRs was 42 (positive) and 38 (negative), while SCoT created 90 positive and 77 negative unique strips. Based on the results from soil analysis, the three halophytic species successfully reduced salinity, and *Leptochloa fusca* was superior; additionally, with nutritional values, *Leptochloa fusca* was the highest in CP. Also, it displayed nine positive and 10 negative markers through ISSRs primers, plus 23 negative and 20 positive indicators within SCoT primers, assuming a linkage with salinity remediation and protein content. Otherwise, *Sporobolus virginicus* (Smyrna) showed the highest proportion of proline as a salinity-tolerance indicator; it also has a significant amount of cellulose/hemicellulose and low lignin content. *Sporobolus virginicus* (Smyrna) had 13 negative and 16 positive markers within ISSRs primers and 49 positive and 35 negative cursors related to the production of lignocellulosic biomass. These positive and negative unique bands are beneficial to identify species and can correlate to specific species traits (Al-Naggar *et al*., 2013; Abd El-Moneim *et al*., 2021). However, it is necessary to analyze and sequence these distinct bands for future work. These markers may also help in genotyping and identifying polymorphisms that directly connect to gene function.

For ISSRs primers (HB10 and UBC-844) and SCoT primers (SCoT-18 and SCoT-34), 12 distinct positive bands emerged as the highest. However, the maximum negative unique band was 10 with primer HB10 for ISSRs and 15 with primer SCoT-34 for SCoT. Although the species studied were very diverse, the existing monomorphism of 16% in ISSRs and 12% in SCoT indicates the presence of regular bands that may link to salt-tolerant traits. There is a need to probe the relationship between these monomorphic bands and genes involved in salt tolerance in the future.

Table 7. Results of ISSR markers used to study the genetic differences of the halophytic species.

No.	Primer name	Size range (bp)	Total No. of bands	Monomorphic bands	Polymorphic bands	Unique bands		Polymorphism %	PIC	He
						+M	-M			
1	HB10	248-2380	25	2	23	12	10	092.00	0.374	0.498
2	UBC-844	339-2450	18	1	17	12	05	094.44	0.494	0.583
3	17899-A	242-1630	13	4	09	02	07	069.23	0.550	0.626
4	UBC-809	249-1469	16	6	10	06	04	062.50	0.536	0.604
5	UBC-880	300-2084	09	0	09	05	04	100.00	0.530	0.596
6	UBC-892	216-1640	15	2	13	05	08	086.67	0.572	0.645
	Total	-	96	15	81	42	38	-	-	-
	Average	-	16	2.5	13.5	07	6.3	084.14	0.510	0.590

PIC = Polymorphic information content, He = Expected heterozygosity, +M = Positive marker, -M = Negative marker.

Table 8. Results of SCoT markers used to study the genetic differences of halophyte species.

No.	Primer name	Size range (bp)	Total No. of bands	Monomorphic bands	Polymorphic bands	Unique bands		Polymorphism %	PIC	He
						+M	-M			
1	SCoT-1	267-1753	12	1	11	07	04	091.67	0.590	0.664
2	SCoT-3	306-2342	18	3	15	10	05	083.33	0.494	0.583
3	SCoT-5	164-1973	18	3	15	11	04	083.33	0.495	0.584
4	SCoT-13	230-2406	16	0	16	08	08	100.00	0.563	0.640
5	SCoT-15	315-1460	15	1	14	08	06	093.33	0.589	0.663
6	SCoT-18	226-1520	26	4	22	12	10	084.62	0.371	0.492
7	SCoT-19	226-2178	21	6	15	07	08	071.43	0.351	0.455
8	SCoT-21	229-1650	20	3	17	07	10	085.00	0.365	0.480
9	SCoT-28	276-3020	16	1	15	08	07	093.75	0.563	0.639
10	SCoT-34	230-1725	28	1	27	12	15	096.43	0.374	0.497
	Total	-	190	23	167	90	77	-	-	-
	Average	-	19	2.3	16.7	09	7.7	088.29	0.480	0.570

PIC = Polymorphic information content, He = Expected heterozygosity, +M = Positive marker, -M = Negative marker.

Table 9. Halophytic species identification by unique ISSR and SCoT markers.

Species	ISSR		SCoT	
	Positive marker/ species	Negative marker/ species	Positive marker/ species	Negative marker/ species
Sporobolus virginicus (Dixie)	17	15	21	19
Sporobolus virginicus (Smyrna)	16	13	49	35
Leptochloa fusca	9	10	20	23
Total	42	38	90	77
Average	7	6.3	9	7.7

CONCLUSIONS

The findings of this research suggested halophytes can adapt to rising salinity and a dwindling supply of freshwater. Also, it has the potential for reclaiming salt-affected soils and producing forage and bioethanol. Furthermore, ISSRs and SCoT can help estimate the genetic variations between the halophytes. These markers can also be beneficial to finding common DNA fragments among salt-tolerant plants and relate the amplified bands with those genetic elements that may have some role in salt tolerance.

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