

Genetic Characteristics Comparison Among *Suaeda* spp. (Amaranthaceae) Species in the Western Azbar Plateau - Cameroun

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ABSTRACT

This study was embarked on to identify the genetic characteristics among species *Suaeda* (Amaranthaceae) in Anbar Plateau region. The genetic diversity of the *Suaeda* species was studied after DNA extraction and using the Inter Simple Sequence Repeats Reaction method to recorded in the genetic aspects. DNA was extracted from young leaves of the studied species. The concentration and purity of the DNA were determined, and the detection of genetic relationships between the studied plant species as well as finding this genetic fingerprint by ISSR Reaction. The results showed that the plant samples collected during study belong to species of the genus *Suaeda*, and these species were: *S. aegyptiaca*, *S. altissima*, *S. carnosissima*, *S. fruticosa*, *S. monoica*, *S. vera* and *S. vermiculata* symbolized by (S1, S2, S3, S4, S5, S6, S7) respectively for the purpose of brevity. The results showed that there was the highest close between *S. aegyptiaca* and *S. vera* which amounted to 0.6319, and the results showed that there was the least closeness between *S. aegyptiaca* and *S. carnosissima* which amounted to 0.4231. Genetic traits are among the stable traits that can be adopted in separating the studied species. The genetic study, especially at the DNA level, is one of the most important modern taxonomic studies that rely on PCR technology for the accuracy of its results and the speed of obtaining results. There are at least seven species of the genus *Suaeda* in the western province of Azbar, Cameroun.

Keywords: Amaranthaceae, Azbar plateau, genetic characteristics, *Suaeda* spp.

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INTRODUCTION

The *Suaeda* genus of plants is also known as seaweeds as well as seablites (1). Most species are confined to saline or alkaline soil habitats, such as coastal salt flats and tidal wetlands. They contain a trait seen in different plant genera that thrive in saline habitats (halophiles). There are about 110 species of the genus *Suaeda* (2).

The genus *Suaeda* includes plants that use the carbon-fixing pathway (C3 or C4), and the C4 pathway evolved independently in the same genus, and there are now about 40 species of the genus *Suaeda* that use the C4 pathway. *Suaeda aralocaspica*, classified in its Borszczowia section, uses a specific type of C4 photosynthesis without the typical Kranz anatomy leaf anatomy (3)(4)(5).

Suaeda can synthesize natural substances of strong antioxidant activity. It is considered a renewable source of energy, food and edible oil for a large number of people who live in a harsh environment with high salinity and drought conditions. This is due to its relatively large amounts of fixed oils, minerals and vitamins, which make it a potential renewable source for foods. These plants are also of great benefit because they are used as alternative medicines. In addition, *Suaeda* is used to treat various diseases due to its high content of polyphenols and flavonoids (6)(7).

Despite the multiplicity of the importance of the sage family, it has not been studied well in the world from a taxonomic point of view, due to the limited available taxonomic characteristics and the succulent nature of several types of them, and the delay in flowering and fruiting time as well as the unattractiveness of many of its types (8).

The Inter Simple Sequence Repeats reaction is used to find the contrast between different organisms (9)(10). It did not receive the required attention in Iraq and the world. However, there are recent contributions to cover the lack of information on the classification of family members, especially in Africa and Asia (11).

Based on what has been mentioned and the scarcity of local studies on this genus and the absence of information about it and its relationship to different photosynthesis pathways on the one hand, and its relationship to the taxonomic and evolutionary aspect on the other hand, the current study aims to record as much information as possible about several species of the genus *Suaeda* spp. In Azbar province, western Cameroun.

MATERIALS AND METHODS

Sample collection

Plants were collected from the western plateau of Azbar Province, where several tours were conducted in the region and the tours were between periods of time ranging between 15/8/2020 and 15/9/2021 and through it the study samples were collected, which represented seven species belonging to the genus *Suaeda*.

Sample preservation

The samples were preserved after collection for the purpose of preparing them for the study. Parts of the sample were pressed with a wooden piston to be dried and to study the morphological and taxonomic characteristics. Parts of the samples were also placed in plastic bags, they were kept at a temperature of 4°C until conducting the genetic study..

Method of crushing plant leaves of the studied species)

Weigh (1 g) of the leaves of the studied samples and cut them into several small pieces using sterile scissors and put them in the pre-cooled ceramic mortar after which liquid nitrogen is added. The temperature is -20o until the start of the DNA extraction process.

Isolation of Genomic DNA

DNA was isolated from the young leaves of the above study species using the Genomic DNA Kit Plant supplied by SCIENTIFIC (USA) IBI. Electrophoresis was carried out using a 1% agarose gel, where the samples were carried over with a voltage difference of 100 millivolts for an hour, and the DNA was investigated by exposing it to a UV transilluminator with a wavelength of 256 nanometers.

Determination of the extracted DNA concentration and purity

The DNA concentration was estimated by measuring the absorbance of the UV spectrum using a spectrophotometer and at a wavelength of (260) nm. A DNA sample was added to 1980 µl of the solute solution and then placed in a Spectrophotometer at a wavelength of (260) nanometers, and after reading the screen of the device, the following equation was applied to calculate the concentration of DNA:

DNA concentration µg/µL = absorbance reading per 1 ml of sample at wavelength (260) x inverse of dilution (100) x (50/1000)

As for the purity of each sample of the study, it was estimated by dividing the absorbance reading at the

wavelength (260) nm by the reading of the absorbance at the wavelength (280) nm (12).

ISSR reactions

ISSR reactions were performed on 7 samples from the studied family, based on the (13).

The Substances and solutions required for ISSR reactions:

1-PCR PreMix.

2- Random Primers: Table 1 supplied by IDT (Integrated DNA Technologies) (Korea).

3- DNA template.

RESULTS AND DISCUSSION

Genomic DNA extraction

The agarose gel electrophoresis at a concentration of (1%) showed the emergence of a single bund representing the chromosomal DNA of the different species of *Suaeda* (Fig. 1).

Table (2) shows the concentrations of DNA extracted from the studied species and extracted using (Kit) and it ranged between (160.55 ± 148.25) ng. μ l⁻¹, purity ranged between (1.55 ± 1.77), DNA samples used in the PCR reaction were diluted using (TE) in order to obtain a concentration of (25-50) ng. μ l⁻¹, symbols were given to represent the studied samples (S1) *S. aegyptiaca*, (S2) *S. altissima*, (S3) *S. carnosissima*, (S4) *S. fruticosa*, (S5) *S. monoica*, (S6) *S. vera*, (S7) *S. vermiculata*.

Table 3 and Fig. 2 shows the results of the genetic study. In this study, 10 primers were used, as all the primers showed doubling. The results of doubling were appropriate to reveal the genetic relationships between the studied plant species as well as finding their genetic fingerprint. The results data were recorded on the basis of the presence or absence of DNA doubling sites, association sites, total bundles, and the percentage of total bundles, as well as the variation in the sizes and numbers of loci, the highest and lowest value. For link sites in every type and in every primer, the presence of unique sites and absent sites.

Table 3 and Fig. 2 shows the results of ISSR reactions. The highest primer in terms of the number of binding sites was (SSK7) with (55) bundles, and the lowest primer in terms of the number of binding sites was the primer (SSK2) with (27) bundles, the highest molecular size in binding sites were (1200bp) in the primer (SSK4) and the lowest molecular size was (100bp) in the primer (SSK6).

Table 4 and Fig. 2 shows the use of the ISSR index to analyze genetic variation among eight types of cruciferous plants, determine the genetic dimension and find genetic fingerprints for some species. Primarily, as well as the lowest and highest molecular size, the number of unique and absent sites for each species.

Table 1: ISSR primers sequences, the temperatures used and the time duration for electrophoresis

NO	Primers	primer sequence 5' → 3'	Tm °C	Time Electrophoresis
1	SSK1	GAGAGAGAGAGACC	48°C	45 minutes
2	SSK2	GTGTGTGTGTGTCC	48°C	45 minutes
3	SSK3	CTCCTCCTCGC	48°C	45 minutes
4	SSK4	TCTCTCTCTCTCTCC	48°C	45 minutes
5	SSK5	AGCAGCAGCAGCAGCAGCG	48°C	45 minutes
6	SSK6	GAGAGAGAGAGAGAGAGAC	48°C	45 minutes
7	SSK7	GAGCAACAACAACAACAA	48°C	45 minutes
8	SSK8	AGAGAGAGAGAGAGAGCG	48°C	45 minutes
9	SSK9	CTCTCTCTCTCTCTTT	48°C	45 minutes
10	SSK10	CACACACACACACAG	48°C	45 inutes

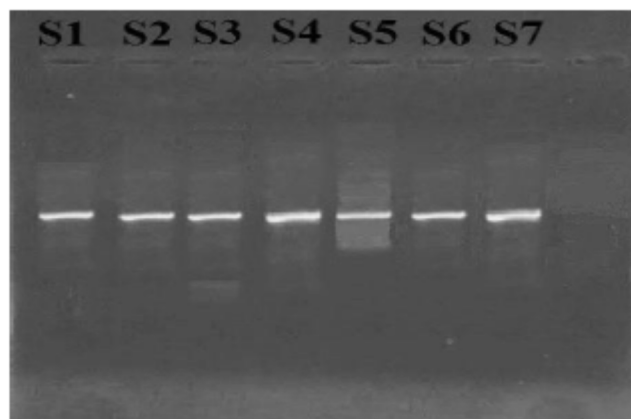


Fig. 1: Electrophoresis of DNA samples extracted from Suaeda plant species on agarose gel at a concentration of 1% and a voltage difference of 100 mA for 45 minutes

Table 2: DNA concentration and purity of the studied plant species

Type sample	Abs 260	Abs 280	260/280	con(ng/ml sample)	
S1	2.965	1.711	1.73290473	148.25	dsDNA
S2	3.154	1.898	1.66174921	157.70	dsDNA
S3	3.211	1.811	1.77305356	160.55	dsDNA
S4	3.089	1.934	1.59720786	154.45	dsDNA
S5	3.043	1.855	1.64043127	152.15	dsDNA
S6	3.017	1.887	1.59883413	150.85	dsDNA
S7	2.987	1.933	1.54526643	149.35	dsDNA

Table 3: Prefixes and some replication results of ISSR technology with the studied species

location and size of unique packets (bp)	location and size of absent packets (bp)	number and proportion of dissimilar packets	number and proportion of identical packets	lowest and highest molecular size (bp)	number and proportion of packets	Primers
950bp (S4) 900bp (S3) 850bp (S4) 750bp (S4) 725bp (S7) 675bp (S7) 450bp (S1) 425bp (S2) 325bp (S5) 275bp (S3)	0	36 100%	0	275bp-950bp	36 8.144%	SSK1
900bp (S5) 850bp (S6) 350bp (S1) 325bp (S5)	0	27 100%	0	325bp-900bp	27 6.108%	SSK2
850bp (S4) 750bp (S3) 600bp (S7) 275bp (S1) 250bp (S2)	400bp (S3)	43 100%	0	250bp-950bp	43 9.728%	SSK3
1200bp (S5) 1000bp (S3) 900bp (S1) 150bp (S3)	0	40 100%	0	150bp-1200bp	40 9.049%	SSK4
750bp (S3) 700bp (S1) 325bp (S3)	0	44 100%	0	250bp-950bp	44 9.956%	SSK5
900bp (S5) 150bp (S7) 100bp (S6)	0	49 100%	0	100bp-900bp	49 11.087%	SSK6
1000bp (S2) 850bp (S4) 700bp (S4) 225bp (S6)	0	48 87.273%	7 12.727%	225bp-1000bp	55 12.445%	SSK7
600bp (S3) 200bp (S7) 175bp (S3) 150bp (S7)	0	54 100%	0	150bp-950bp	54 12.217%	SSK8
900bp (S4) 575bp (S2) 550bp (S5) 350bp (S2) 300bp (S3) 275bp (S6) 225bp (S7)	0	48 100%	0	225bp-900bp	48 10.859%	SSK9
900bp (S5) 800bp (S5) 625bp (S7) 225bp (S5) 175bp (S7)	0	46 100%	0	175bp-900bp	46 10.407%	SSK10
		435 98.416%	7 1.584%		442 100%	Total

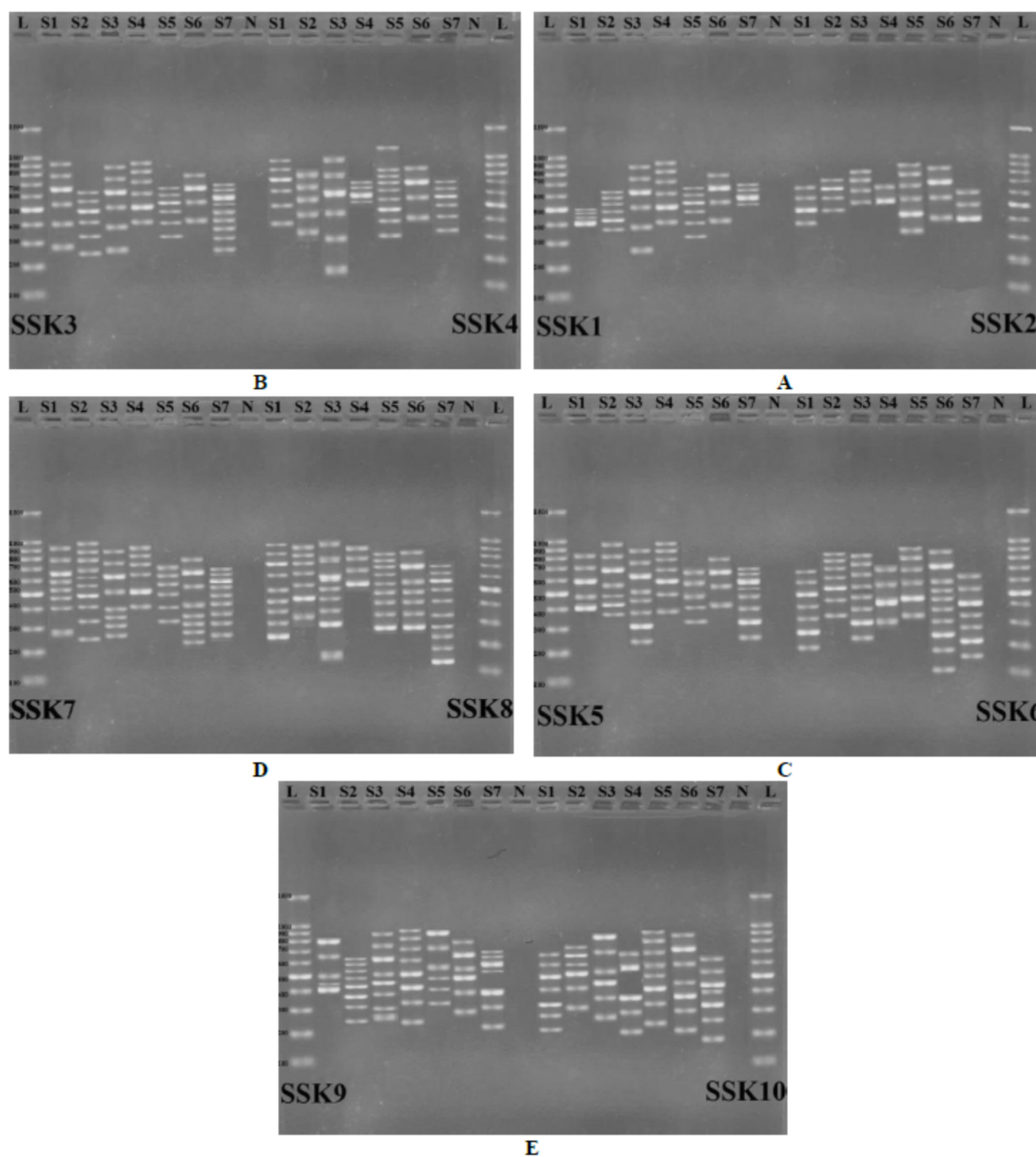


Fig. 2: Electrophoresis of PCR reaction products of Primers SSK1, SSK2, SSK3, SSK4, SSK5, SSK6, SSK7, SSK8, SSK9 and SSK10 by ISSR indicators of *Suaeda* plant species on agarose gel at a concentration of 1% and at a voltage difference of 100 mA for 45 minutes

Table 4: Some results of ISSR reactions, depending on the studied species

location and size of unique packets (bp)	location and size of absent packets (bp)	number and proportion of dissimilar packets	number and proportion of identical packets	lowest and highest molecular size (bp)	number and proportion of packets	Species
450bp (SSK1) 350bp (SSK2) 275bp (SSK3) 900bp (SSK4) 700bp (SSK5)	0	60 98.361%	1 1.639%	200bp- 950bp	61 13.801%	<i>S. aegyptiaca</i> (S1)
425bp (SSK1) 250bp (SSK3) 1000bp (SSK7) 575bp (SSK9) 350bp (SSK9)	0	65 98.485%	1 1.515%	250bp- 1000bp	66 14.932%	<i>S. altissima</i> (S2)
900bp (SSK1) 275bp (SSK1) 750bp (SSK3) 400bp (SSK3) 1000bp (SSK4) 150bp (SSK4) 750bp (SSK5) 325bp (SSK5) 175bp (SSK8) 300bp (SSK9)	400bp (SSK3)	67 98.529%	1 1.471%	150bp- 1000bp	68 15.385%	<i>S. carnosissima</i> (S3)
950bp (SSK1) 850bp (SSK1) 750bp (SSK1) 850bp (SSK3) 850bp (SSK7) 700bp (SSK7) 900bp (SSK9)	0	51 98.077%	1 1.923%	200bp- 950bp	52 11.765%	<i>S. fruticose</i> (S4)
325bp (SSK1) 900bp (SSK2) 325bp (SSK2) 1200bp (SSK4) 900bp (SSK6) 550bp (SSK9) 900bp (SSK10) 800bp (SSK10) 225bp (SSK10)	0	66 98.507%	1 1.493%	225bp- 1200bp	67 15.158%	<i>S. monoica</i> (S5)
850bp (SSK2) 100bp (SSK6) 225bp (SSK7) 275bp (SSK9)	0	57 98.276%	1 1.724%	100bp- 850bp	58 13.122%	<i>S. vera</i> (S6)
725bp (SSK1) 675bp (SSK1) 600bp (SSK3) 150bp (SSK6) 200bp (SSK8) 150bp (SSK8) 225bp (SSK9) 625bp (SSK10) 175bp (SSK10)	0	69 98.571%	1 1.429%	150bp- 725bp	70 15.837%	<i>S. vermiculata</i> (S7)
		435 98.416%	7 1.584%		442 100%	Total

Table 5: values of genetic affinity between the studied species according to the results of ISSR

ISSR	S1	S2	S3	S4	S5	S6	S7
S1	1.0000						
S2	0.5110	1.0000					
S3	0.4231	0.5385	1.0000				
S4	0.5989	0.5934	0.5385	1.0000			
S5	0.6154	0.5879	0.4560	0.5879	1.0000		
S6	0.6319	0.5385	0.5055	0.5604	0.5989	1.0000	
S7	0.5440	0.5385	0.5055	0.4945	0.5330	0.5495	1.0000

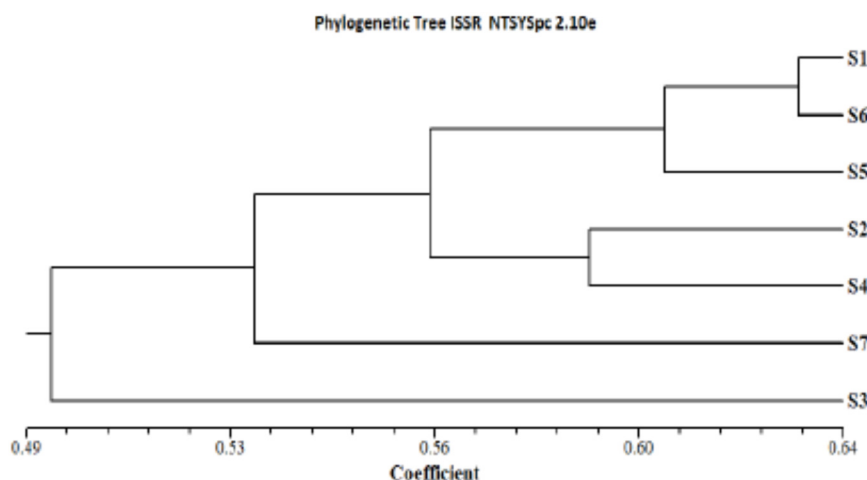


Fig. 3: Genetic relationship between the studied species according to ISSR indicators. Symbols represent the studied samples

Table 4 and Fig. 2 shows that the species (S7) had the highest number of binding sites (70) sites and a percentage (15.837%) and the lowest number of binding sites (52) sites and a percentage (11.765%) appeared in the type (S4), which had the highest molecular size It appeared in the type (S5) (1200bp) and the lowest molecular size (100bp) appeared in the type (S6), the highest number of binding sites for unique packages (10) sites appeared in the type (S3), and the lowest number of binding sites for unique packages (4) sites In the type (S6), and the type (S3) was distinguished by one link site for an absent band,

while the results of the other studied types did not show any absent bands.

The genetic affinity between the studied species was estimated using the genetic program (NTSYSpc 2.10e) based on the presence of bundles common to each of the studied species. The results were analyzed by the Nei equation (14).

Table 5 and Fig. 3 shows the genetic affinity between the studied species, which ranged between (0.4231 - 0.6319). When the genetic material (DNA) between the two species matches, it indicates that the value of

the genetic distance between them should be zero, and the percentage of genetic similarity between the two species was (100%) (15).

Previous studies of molecular and morphological evolution on the genus *Suaeda* by (3) contributed to clarifying the taxonomic system of the sub-genus and the order of divisions in the genus *Suaeda*, while recent molecular genetic studies have found unclear relationships between *Suaeda* spp. At the species level (16)(17).

CONCLUSION

Genetic traits are among the stable traits that can be adopted in separating the studied species. The genetic study, especially at the DNA level, is one of the most important modern taxonomic studies that rely on PCR technology for the accuracy of its results and the speed of obtaining results. There are at least seven species of the genus *Suaeda* in the western province of AZbar, Cameroun.

Author's Contribution:

IWARLA, R., AYINUB, H., and EDIBA, A.: Carried out the experimental work, wrote the manuscript

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REFERENCES

1. USDA.. *Suaeda*. Natural Resources Conservation Service, PLANTS Database. Retrieved 4 December (2015).
2. FNA, Flora of North America, Entry for *Suaeda*. (2019); 4: 260, 360, 389, 390.
3. Schütze P, Freitag H, Weising K. "An integrated molecular and morphological study of the subfamily Suaedoideae Ulbr. (Chenopodiaceae)". *Plant Systematics and Evolution*. (2003); 239(3-4): 257–286.
4. Sage RF. A portrait of the C4 photosynthetic family on the 50th anniversary of its discovery: species number, evolutionary lineages, and Hall of Fame. *Journal of Experimental Botany*. (2016); 67(14): 4039-4056.
5. Kapralov MV, Akhani H, Voznesenskaya EV, Edwards G, Franceschi V, Roalson EH. Phylogenetic Relationships in the Salicornioideae / Suaedoideae / Salsoloideae s.l. (Chenopodiaceae) clade and a clarification of the phylogenetic position of *Bienertia* and *Alexandra* using multiple DNA sequence datasets. *Systematic Botany*. (2006); 31(3): 571–585.
6. Mohammed H A. The valuable impacts of halophytic genus *Suaeda*; nutritional, chemical, and biological values. *Medicinal Chemistry*, (2020); 16(8): 1044-1057.
7. Li Q, Song J. Analysis of widely targeted metabolites of the euhalophyte *Suaeda salsa* under saline conditions provides new insights into salt tolerance and nutritional value in halophytic species. *BMC Plant Biology*, (2019); 19(1): 1-11.
8. Akhani H. Halophytic vegetation of Iran: towards a syn- taxonomical classification. *Ann Bot (Rome)*. (2004); 4: 66-82.
9. Hammadi SY, Hussein AS, Mageed DM, Dheeb BI, Ismail EN. RAPD and ISSR analyses of *Saccharomyces cerevisiae* isolates from different sources. *Journal of Biotechnology Research Center*. (2018); 12(2): 40-50.
10. Mezher MA, Mohammad SH, Wahab KM, Salman ZA, Rashid SA, Tuama BA. Study genetic diversity between trichophyton rubrum isolates using ISSR and RAPD markers. *Journal of Biotechnology Research Center*. (2018); 12(2): 5-13.
11. Takhtajan AL. Outline of the Classification of flowering plants (Magnoliophyta). *Bot. Rev.*, (1980); 46: 225-359.
12. Maniatis T, Fritsch FF, Sambrook J. In vitro application of DNA by the polymerase chain Reaction in molecular cloning : A Laboratory Manual. 2nd ed. , Cold Spring Harbor Laboratory Press, New York, USA, (2001); p : 691.
13. Zietkiewicz E, Rafalski A, Labuda D. Genome fingerprinting by simple sequence repeat

- (SSR)-anchored polymerase chain reaction amplification. *Genomics*, (1994); 20(2): 176-183.
14. Nei M, Li WH. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences*, (1979); 76(10): 5269-5273.
15. Esselman EJ, Crawford DJ, Brauner S, Stuessy T F, Anderson GJ, Mariosilva O. RAPD marker diversity within and divergence among species of *Dendroseris* (Asteraceae: Lactuceae). *American Journal of Botany*, (2000); (4): 591-596.
16. Brandt R , Lomonosova M , Weising K , Wagner N., Freitag H. Phylogeny and biogeography of *Suaeda* subg. *Brezia* (Chenopodiaceae/Amaranthaceae) in the Americas. *Pl. Syst. Evol.* (2015); 301: 2351–2375.
17. Kim S., Chung SO. Phylogenetic study of the Genus *Suaeda* (Chenopodiaceae) based on chloroplast and nuclear DNA sequences from Korea. *Korean J. Environ. Ecol.* (2018); 32: 566–574.