Comparison of genetic characteristics among species of *Suaeda* spp. (Amaranthaceae) in the western Anbar plateau - Iraq

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Abstract

Background: Comparison of genetic characteristics among species of Suaeda spp.

Objective: identifying the genetic characteristics among species *Suaeda* (Amaranthaceae) in Anbar Governorate. (From August 2020 to September 2021 fresh plant samples were collected in the flowering stage for the studied species).

Materials and methods: 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.

Results: 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. carnosissma, 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. carnosissma* which amounted to 0.4231.

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 Anbar Governorate - Iraq.

Key words: genetic characteristics, *Suaeda*, Amaranthaceae, western Anbar plateau.

1. 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 Anbar Governorate, western Iraq.

Materials and Methods

2.1. Sample collection

Plants were collected from the western plateau of Anbar Governorate (Ramadi, Fallujah, Khalidiya, Habbaniyah, Al-Muhammadi, Hit, Al-Baghdadi, Haditha), 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*.

2.2. 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 ^{o}C until conducting the genetic study.

2.3. 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 -20^{o} until the start of the DNA extraction process.

2.4. 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.

2.4.1. 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).

2.4.2. 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.

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

NO	Primers	primer sequence $5^{-} \rightarrow 3^{-}$	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	TCTCTCTCTCTCTCTCC	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	CTCTCTCTCTCTCTCTT	48˚C	45 minutes
10	SSK10	CACACACACACACACAG	48˚C	45 inutes

3.Results:

3.1. 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).

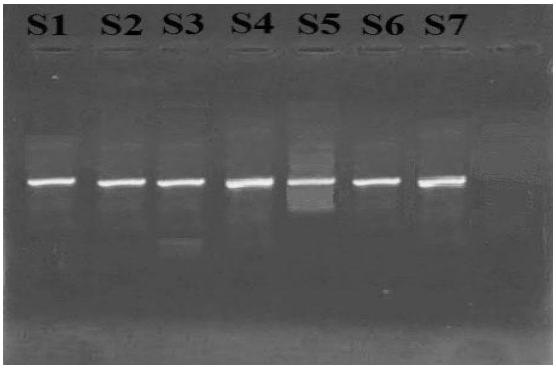

Figure (1) : Electrophoresis of DNA samples extracted from *Suaeda* plant species on agarose gel at a concentration of 1% and a voltage difference of 100 mA for 45 minutes

Table (2) shows the concentrations of DNA extracted from the studied species and extracted using (Kit) and it ranged between (160.55-148.25) ng. μl^{-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. μl^{-1}, symbols were given to represent the studied samples (S1) *S. aegyptiaca*, (S2) *S. altissima*, (S3) *S. carnosissma*, (S4) *S. fruticose*, (S5) *S. monoica*, (S6) *S. vera* , (S7) *S. vermiculata*.

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

3.2. Results of ISSR reactions

Table (3) and Figure (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.

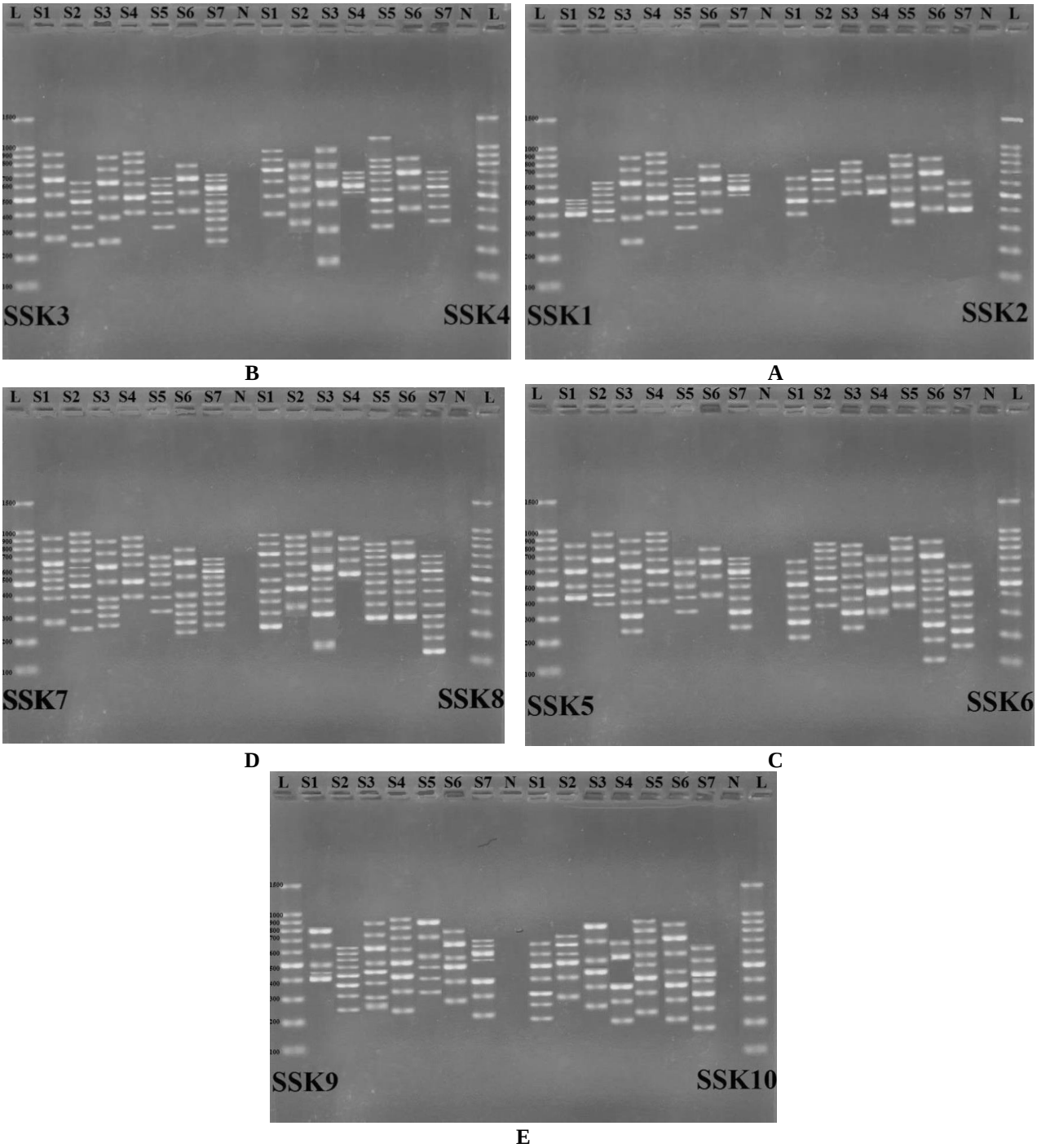

Figure (2): Electrophoresis of PCR reaction products of Primers SSK1, SSK2, SSK3, SSK4, SSK5, SSK6, SSK7, SSK8, SSK9 and SSK10 by ISSR indicators of *Suaeda* plant species on agarose gel at a concentration of 1% and at a voltage difference of 100 mA for 45 minutes

•Inferring the ISSR reactions for the prefixes used

Table (3) and Figure (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 (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

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%	*S. aegyptiaca* (S1)
425bp (SSK1) 250bp (SSK3) 1000bp (SSK7) 575bp (SSK9) 350bp (SSK9)	0	65 98.485%	1 1.515%	250bp- 1000bp	66 14.932%	*S. altissima* (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%	*S. carnosissma* (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%	*S. fruticose* (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%	*S. monoica* (S5)
850bp (SSK2) 100bp (SSK6) 225bp (SSK7) 275bp (SSK9)	0	57 98.276%	1 1.724%	100bp- 850bp	58 13.122%	*S. vera* (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%	*S. vermiculata* (S7)
		435 98.416%	7 1.584%		442 100%	Total

3.2.1. Results of ISSR reactions for the studied species

Table (4) and Figure (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.

• Inferring the ISSR reactions for the studied species

Table (4) and Figure (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.

3.3. Estimation of genetic affinity based on the results of the ISSR .

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): 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

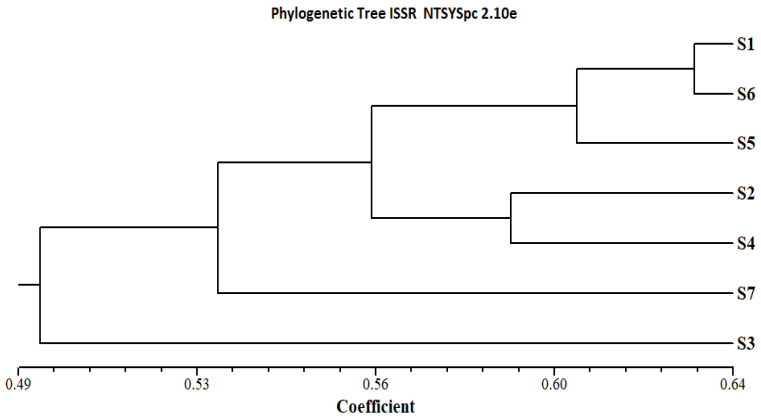

Figure (3): Genetic relationship between the studied species according to ISSR indicators. Symbols represent the studied samples

Table (5) and Figure (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).

4. Discussion:

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).

5. 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 Anbar Governorate – Iraq.

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مقارنة الخصائص الوراثية بين أنواع جنس *Suaeda* spp. (Amaranthaceae) في هضبة الأنبار الغربية - العراق

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الخلاصة:

خلفية عن الموضوع : مقارنة الخصائص الوراثية بين أنواع *Suaeda* spp.

الهدف من البحث: التعرف على الخصائص الوراثية بين انواع جنس *Suaeda* (Amaranthaceae) في محافظة الانبار. (من أغسطس 2020 إلى سبتمبر 2021 تم جمع عينات نباتية طرية في مرحلة التزهير للأنواع المدروسة).

المواد وطرق العمل: تم دراسة التنوع الجيني لأنواع *Suaeda* بعد استخلاص الحمض النووي واستخدام طريقة التفاعل البسيط المتكرر لتكرار التفاعل لتسجيلها في الجوانب الجينية. تم استخراج الحمض النووي من الأوراق الصغيرة للأنواع المدروسة. تم تحديد تركيز ونقاوة الحمض النووي ، وكشف العلاقات الوراثية بين الأنواع النباتية المدروسة وكذلك اكتشاف البصمة الوراثية بواسطة تفاعل ISSR.

النتائج: أظهرت النتائج أن عينات النبات التي تم جمعها أثناء الدراسة تنتمي إلى أنواع من جنس *Suaeda* ، وهذه الأنواع هي: *S. aegyptiaca*، *S. altissima*، *S. carnosissma*، *S. fruticosa*، *S. monoica* و *S. vera* اعطيت الرموز (S1، S2، S3، S4، S5، S6، S7) على التوالي لغرض الإيجاز. أظهرت النتائج أن هناك أعلى تقارب بين *S. aegyptiaca* و *S. vera* بلغ 0.6319 ، وأظهرت النتائج أن أقل تقارب بين *S. aegyptiaca* و *S. carnosissma* بلغ 0.4231.

الاستنتاجات: الصفات الوراثية من الصفات المستقرة التي يمكن تبنيها في فصل الأنواع المدروسة. تعد الدراسة الجينية وخاصة على مستوى الحمض النووي من أهم الدراسات التصنيفية الحديثة التي تعتمد على تقنية PCR لدقة نتائجها وسرعة الحصول على النتائج ، وهناك ما لا يقل عن سبعة أنواع من جنس *Suaeda* في غرب محافظة الانبار - العراق.

الكلمات المفتاحية: الخصائص الوراثية، *Suaeda*, Amaranthaceae، هضبة الانبار الغربية.