Determination of genetic diversity using SDS-PAGE and RAPD markers and active compound gene in *Zygophyllaceae* Genotypes

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ABSTRACT

Biochemical variability was conducted by protein electrophoretic patterns among ten genotypes of Zygophyllaceae. RAPD molecular markers were used to estimate the polymorphism among these genotypes. SDS-PAGE revealed 36 total bands (67% polymorphism). Five RAPD primers revealed 80 bands with the highest polymorphism (95%). Based on similarity matrix of overall analysis (SDS-PAGE and RAPD), the dendrogram was constructed and separated the ten Zygophyllaceae genotypes into two main clusters. The first included the Zygophyllum coccineum genotypes from two locations, Z. simplex and Nitraria retusa from three locations, the second included Fagonia indica from two locations, Peganum harmal and Tribulus terrestris. The molecular marker was better than biochemical marker to detect genetic diversity among Zygophyllaceae genotypes. Results showed that the length of the TDC (tryptophan decarboxylase) gene for alkaloid production at the fragment of 1770 bp. BLAST sequence alignment and phylogenetic tree analyses revealed that the TDC fragment is genetically close to Rauvolfia verticillata and Catharanthus roseus. This study can be used in the future for conservation, breeding and improvement programs

Key words: *Zygophyllaceae, Isozymes, ISSR, TDC gene, sequence alignment and phylogenetic tree.*

INTRODUCTION

Zygophyllaceous family comprises about 28 species in Egypt (Batanouny and Ezzat; 1971), representing a group of succulent plants that are drought and/or salt tolerant, present in oases, eastern Egyptian desert, coastal region and Sinai. Plants are annual or perennial herbs such as: *Zygophyllum* sp., *Nitraria retusa, Peganum harmal* L., *Fagonia indica* and *Tribulus terrestris* L. with wide medicinal uses such as ; treatment of fever and skin problems, anti-inflammatory, analgesic, antipyretic, antioxidant, anti-microbial, antiviral, anticancer, urinary tract infection. Important active compounds, e.g., alkaloids, saponins, flavonoids and phenolic compounds have been reported in such plant (Li *et al.,* 2006) Electrophoretic separation of proteins is a powerful and efficient tool in addressing taxonomic and evolutionary relationships at both species and subspecies levels (Ladizinsky and Hymowitz, 1979) and are useful in re-assessing the species relationships in a number of genera; *Zygophyllum* L. (EL-

Ghamery *et al*., 2003) and *Brassicaceae* (Nelly, 2015).

Molecular markers could reflect the difference between species. RAPDs are dominant molecular markers developed by Williams *et al*., (1990). RAPD provides rapid results, is less time-consuming and less expensive (Arif and Khan, 2009) and gives information about genomic variability below the species level. The RAPD technique had been successfully used in a variety of taxonomic and genetic diversity studies and it was found suitable for use with some *Zygophyllaceae* plants. A few studies have used RAPDs to assess levels and patterns of variation with different plants of *Zygophyllaceae* (Arghavani *et al*., 2010, Kumar, and Verma, 2012 and Patil *et al*., 2014). The obtained dendrogram revealed, that *Z. simplex* represents the closest relationship with *Z. migahidii* Al-arjany, (2011). Phylogenetic distances show that *Fagonia glutinosa* and *F. indica* are far from *Tribulus terrestris* and *T. macropterus* and have completely different pattern of RAPD profiles compared to other genotypes in Saudi Arabia. Tryptophan decarboxylase (TDC), cleaves the carboxyl group of tryptophan and catalyzes the conversion of tryptophan into tryptamine and in plants operates at the interface between primary and secondary metabolism of alkaloid. Tryptophan decarboxylase has been found and detected in several plants such as *Camptotheca acuminata* (López-Meyer and Nessler, 1997), *Rauvolfia verticillata* (Gerasimenko *et al*., 2002), *Ophiorrhiza pumila* (Yamazaki *et al*., 2003), rice (Sei *et al*., 2007) and overexpression of a Catharanthus tryptophan decarboxylase (tdc) gene leads to enhanced terpenoid indole alkaloid production in transgenic hairy root lines of *Rauwolfia serpentine* (Shakti *et al*., 2013). The aims of this study were to investigate the genetic relationships among *Zygophyllaceae* genotypes by molecular and biochemical fingerprints for characterizing and detecting polymorphism. Alkaloid gene and sequence homology on NCBI database were detected among *Zygophyllaceae* genotypes.

MATERIALS AND METHODS

Zygophyllaceae species were collected from ten locations in Egypt during February 2015-2017, as shown in (Table 1).

Table (1): Geographical locations of the studied Zygophyllaceae species.

No	Species	Locations
1	*Zygophyllum coccineum* L.	Hammam Cleopatra
2	*Zygophyllum coccineum* L.	Burg El-Arab
3	*Zygophyllum simplex*	Siwa Oasis
4	*Nitraria retusa*	Siwa Oasis
5	*Nitraria retusa*	Burg El-Arab
6	*Nitraria retusa*	North Sinai
7	*Peganum harmal* L.	Siwa Oasis
8	*Fagonia indica*	Burg El-Arab
9	*Fagonia indica*	Quatamia
10	*Tribulus terrestris* L.	Quatamia

Extraction of total protein and SDS-PAGE analysis

The protein extraction technique employed was similar to the extraction technique described by Saraswati *et al*., (1993). Sample buffer was added to 0.2 g bulked leaf sample of each genotype which was then ground with liquid nitrogen and mixed with extraction buffer thoroughly in an Eppendorf tube by vortexing. The extraction buffer: contained 0.5 M Tris-HCl, pH 6.8, 2.5% SDS, 5% urea, and 5% 2-mercaptoethanol. Before centrifugation at 10,000 *g* for 5 min at 4°C, the sample buffer was boiled for 5 min. SDS-PAGE was performed using a standard method on a vertical slab gel. Bromophenol blue was added to the supernatant as a tracking dye to watch the movement of proteins on the gel. Protein profiling of samples was performed using SDS- PAGE as described by Laemmli (1970). Total proteins were analyzed by SDS-PAGE on 12% polyacrylamide gel. After electrophoresis, the protein bands were visualized by staining with Coomassie brilliant blue G-250. Marker proteins Fermentas (Massachusetts, USA) were used as references. The bands produced in the electropherogram were scored, and their molecular weights were compared to the standard Pharmacia protein marker (Massachusetts, USA).

Protein imaging and data analysis

Gel photography and documentation were carried out using the Bio-Rad gel documentation system. The number of bands revealed on each gel lane was counted and compared using the Gel Pro-Analyzer software. Quantitative variations of bands number were estimated using the Bio- RAD Model Gel Doc 2000 (California, United States). With regard to variation in protein banding patterns, electrophoregrams of each genotype were scored for the presence or absence of bands and used to construct a Similarity matrix and dendrogram was developed by the statistical pakage for SPSS (10) software program.

DNA extraction

Young leaves were harvested and placed in a sealable plastic bag with appropriate label. The collected leaves were used for DNA extraction, while excess leaf materials were stored at -80 ˚C for future DNA extraction. Total genomic DNA was extracted from leaves using a modified CTAB method based on the protocol of Doyle and Doyle (1990). Quality and concentration of total DNA was verified by Spectrophotometer 300UV-Visible (United States) at 260 and 280 nm. Further quality of DNA was tested by submerged horizontal agarose gel (1.2%) electrophoresis containing ethidium bromide (0.5 µg ml^{-1}) in 1X TBE buffer at 100 volts for one hour and visualized under UV light, gel documentation system. The experiment was repeated three times for RAPD analysis.

RAPD analysis for DNA amplification

Twenty random decamer primers (Operon 2Technologies, USA) of OPA, OPB, OPE, OPG, and OPZ series were used individually as primers for RAPD analysis. The PCR amplification was carried out at Genetics Department Laboratory, Ain Shams University, and Cairo, Egypt. Five arbitrary 10-base reproducible primers out of twenty were selected for PCR amplification. Amplification reactions were performed with 25µL 10X assay buffer, 2.0 µL 1.25 mM each dNTP, 15 ng of the primer, 1X Taq polymerase buffer, 0.5 units Taq DNA polymerase TaKaRa, (Dalian, Co., Ltd.

China), 2.5 mM MgCl , and 30 ng genomic DNA. DNA amplification was performed in a Perkin Elmer Cetus 480 DNA Thermal Cycler (Midland, Canada). programmed for 45 cycles as follows: denaturation, (one cycle) 94˚C for 4 minutes, annealing (40 cycles) 94 ˚C for 1 minute, 36 ˚C for 1 minute and 30 seconds, 72 ˚C for 2 minutes and 30 second, extension, (one cycle) 72 ˚C for 7 minutes. The amplification products were separated by electrophoresis on 1.2% (w/v) agarose gels with 0.5X TBE buffer, stained with 0.2 mg/ml ethidium bromide. All reactions were repeated twice. A DNA ladder was used as molecular standards and the bands were visualized and analyzed by UV-transilluminator and photographed by gel documentation system (Bio-Rad® Gel Doc-2001, Germany).

Data analysis

Reproducible bands were scored manually as '1' or '0' for presence or absence of the bands. The data were used for similarity-based analysis using the social science programme SPSS (10) software program.

Detection of TDC gene

Polymerase chain reaction (PCR) and Sequencing

PCR was conducted using forward and reverse primers, which were designed based on the sequence of TDC obtained from the GenBank (BLAST). The sequences of the primers were 5'- GCA TCT AGA ATG GGC AGC ATT-3' (forward) and 5'- CGG GGA TCC AGC TTC TTT -3' (reverse).A mixture of 20 µl solution consisting of 10x Ex Taq polymerase buffer (Promega Madison, USA), 2 mM MgCl2, 200 uM dNTPs, 25 pmol primers, 1U Taq polymerase TaKaRa, (Dalian, Co., Ltd. China), and distilled water was used for each PCR reaction. The PCR program was set on 93 ˚C for 2 minutes preheating continued with 35 cycles consisting of 1 minute denaturation at 93 ˚C, 1 minutes annealing at 58 ˚C, and 90 seconds extension at 72 ˚C. The PCR product was visualized on 1,5% agarose gel and subjected to 100 volts for 1hr and then photographed using UV gel documentation system, (UVP corporation, UK).

Purification of PCR Product and Sequencing

PCR products were purified using High Pure PCR Product purification Kit (Roche-Switzerland) and sequenced (MWG, Germany).

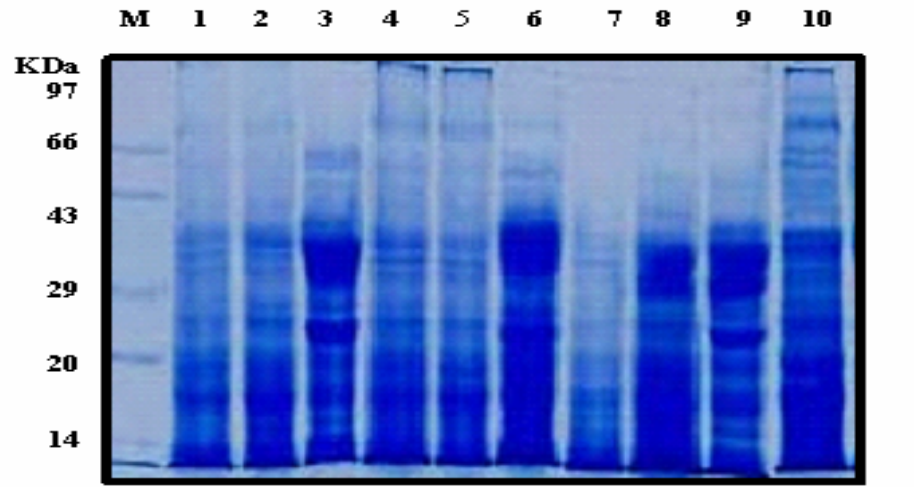
Bioinformatic Analysis

Primer Premier 5 software (http://www.Premierbiosoft.com) was used to design all the primers. rSequences were aligned using the software CLUSTAL W. The phylogenetic tree was constructed by Neighbour-Joining method using MEGA4.0 (Molecular Evolutionary Genetics Analysis, version4.0) software program.

RESULTS AND DISCUSSION

Leaf protein analysis was carried out on the ten *Zygophyllaceae* genotypes (Table 2) and illustrated in (Fig. 1). The results indicated a total number of 36 protein bands were present among the ten *Zygophyllaceae* genotypes (ranging from 14 - 99 kDa). Concerning band intensity, there was detectable change in band intensity for all ten *Zygophyllaceae* genotypes. Moreover, twelve bands were monomorphic, twenty four bands were polymorphic revealing 67% of polymorphism indicating the genetic variation among the ten *Zygophyllaceae* genotypes. Seventeen unique bands were detected, seven for *Tribulus terrestris* L. from

Quatamia (molecular weights 40, 43, 46, 48, 70, 86 and 97 kDa), four for *Fagonia indica* from Quatamia with molecular weights (23, 29, 30 and 38 kDa), three for *Nitraria retusa* from Siwa Oasis (molecular weights 32, 34 and 45 kDa) and finally, two for *Zygophyllum simplex* from Siwa Oasis of the molecular weights (24 and 50 kDa) which could be considered as species specific bands. These results agreed with Rnjan (2013) who studied seven varieties of leguminous spices belonging to three different major tribes (*Phaseoleae*, *Viciae* and *Cicereae*) for their evolutionary relationships using SDS-PAGE. All seven species utilized in that study were clearly identifiable from the protein banding pattern. SDS-PAGE of total seed protein profiles was an efficient procedure for differentiating these legume species. Additionally, Lukong *et al*. (2014) studied six species of *Legumenoseae* in eastern Nigeria to assess their genetic variability and relationship. The polymorphism of these species was 10.71% by SDS-PAGE in the range of 20-28 kDa which confirmed the usefulness of SDS-PAGE procedure in plant taxonomic, evolutionary and genetic relationships.



Fig(1): SDS-PAGE profile of soluble proteins among the ten Zygophyllaceae genotypes . *(M) = Maker, (Zygophyllum coccineum L. from Hammam Cleopatra) = 1, (Z. coccineum L. from Burg El-Arab) =2, (Z. simplex from Siwa Oasis) = 3, (Nitraria retusa from Siwa Oasis) = 4, (N. retusa from Burg El-Arab) = 5, (N. retusa from North Sinai) = 6, (Peganum harmal L. from Siwa Oasis) =7, (Fagonia indica from Burg El-Arab) = 8, (F. indica from Quatamia) = 9 and (Tribulus terrestris L. from Quatamia) = 10.

Table (2): SDS-PAGE patterns of leaf soluble proteins extracted from the ten Zygophyllaceae genotypes *.*(Zygophyllum coccineum L. from Hammam Cleopatra) = 1, (Z. coccineum L. from Burg El-Arab) =2, (Z. simplex from Siwa Oasis) = 3, (Nitraria retusa from Siwa Oasis) = 4, (N. retusa from Burg El-Arab) = 5, (N. retusa from North Sinai) = 6, (Peganum harmal L. from Siwa Oasis) =7, (Fagonia indica from Burg El-Arab) = 8, (F. indica from Quatamia) = 9 and (Tribulus terrestris L. from Quatamia) = 10.

Polymorphism	Lane10	Lane9	Lane8	Lane7	Lane6	Lane5	Lane4	Lane3	Lane2	Lane1	MW
Polymorphic	0	0	0	0	0	1	1	0	0	0	99
Unique	1	0	0	0	0	0	0	0	0	0	97
Unique	1	0	0	0	0	0	0	0	0	0	86
Unique	1	0	0	0	0	0	0	0	0	0	70
Polymorphic	0	0	0	0	1	1	1	0	0	0	68
Polymorphic	0	1	1	0	0	0	0	0	0	0	66
Unique	0	0	0	0	0	0	0	1	0	0	50
Unique	1	0	0	0	0	0	0	0	0	0	48
Polymorphic	0	0	0	0	0	0	1	1	0	0	47
Unique	1	0	0	0	0	0	0	0	0	0	46
Unique	0	0	0	0	1	0	0	0	0	0	45
Polymorphic	1	1	1	0	0	0	0	0	0	0	44
Unique	1	0	0	0	0	0	0	0	0	0	43
Polymorphic	1	1	1	0	0	0	0	0	0	0	42
Unique	1	0	0	0	0	0	0	0	0	0	40
Polymorphic	1	0	1	0	1	0	0	0	0	0	39
Unique	0	1	0	0	0	0	0	0	0	0	38
Monomorphic	1	1	1	1	1	1	1	1	1	1	37
Monomorphic	1	1	1	1	1	1	1	1	1	1	36
Monomorphic	1	1	1	1	1	1	1	1	1	1	35
Unique	0	0	0	0	1	0	0	0	0	0	34
Unique	0	0	0	0	0	0	0	1	0	0	33
Unique	0	0	0	0	1	0	0	0	0	0	32
Unique	0	1	0	0	0	0	0	0	0	0	30
Unique	0	1	0	0	0	0	0	0	0	0	29
Monomorphic	1	1	1	1	1	1	1	1	1	1	28
Monomorphic	1	1	1	1	1	1	1	1	1	1	26
Unique	0	0	0	0	0	0	0	1	0	0	24
Unique	0	1	0	0	0	0	0	0	0	0	23
Monomorphic	1	1	1	1	1	1	1	1	1	1	22
Monomorphic	1	1	1	1	1	1	1	1	1	1	20
Monomorphic	1	1	1	1	1	1	1	1	1	1	19
Monomorphic	1	1	1	1	1	1	1	1	1	1	18
Monomorphic	1	1	1	1	1	1	1	1	1	1	16
Monomorphic	1	1	1	1	1	1	1	1	1	1	15
Monomorphic	1	1	1	1	1	1	1	1	1	1	14

RAPD analysis

A total of 20 RAPD primers were used to screen ten *Zygophyllaceae* genotypes. Out of these, only five RAPD primers showed reproducible results as shown in Fig (2) and Tables (3 and 4). A total of 80 bands were amplified among the ten *Zygophyllaceae* genotypes, where sixteen were monomorphic, 19 species- specific bands and 76 were polymorphic distinct bands (95% polymorphism). The results showed that primers OPA1 and OPB8 were highly polymorphic (92% and 100% polymorphism) and produced wide molecular band length

that ranged from (225 to 1200bp), (200 to 500bp), respectively. Moreover, primer OPZ15 showed the least polymorphism 68.4% and produced molecular band length that ranged from 168 to 686bp. OPA1 revealed the highest species-specific bands, seven bands and OPB8 revealed the least species specific bands (three bands). While, *Zygophyllum coccineum* L. from Hamman Cleopatra and *Tribulus terrestris* L. from Quatamia, each revealed five specific bands. Moreover, *Z. simplex* and *Nitraria retusa* from Siwa Oasis revealed two specific bands. Finally, *Fagonia indica* from Burg El-Arab *Peganum harmal* L. from Siwa Oasis revealed one specific band. Sources of polymorphism in RAPD assay may be due to deletion, addition or substitution of base within the priming site sequence. High diversity is the reflection of adaptation to environment, which is beneficial to its propagation, conservation of resources, the domestication of wild species, and the screen of specified locus. Similar findings were obtained with few molecular studies of *Zygophyllaceae* (Hammad and Qari 2010) who assessed genetic diversity using RAPD among 12 populations of *Z. coccineum*, *Z. album* and *Z. aegyptium* collected from various locations in Egypt and Saudi Arabia. Analysis with RAPD markers revealed genetic variation between and within populations of *Zygophyllum*. A total of 54 bands were amplified among the three species of *Zygophyllum*, 83.3% with thirteen bands commonly detected in all the samples, which could be the genus-specific bands for *Zygophyllum*. *Zygophyllum coccineum* showed higher levels of genetic variation and more unique alleles than the other species. Moreover, Elkholy *et al*. (2011) investigated genetic variability in *Nepeta septemcrenata* populations from six locations in Saint Katherine Protectorate (SKP), using RAPD and detected total of 122 DNA bands, 54 polymorphic bands, 44 monomorphic and 24 unique with 40.69% polymorphism. These results may be explained as the effect of environmental condition which varies from location to another. Finally, Adeeba *et al*. (2014) investigated the genetic diversity among the *Asclepiadaceae* species using RAPD markers. Sixty RAPD primers of OPA, OPC, OPE and OPD series produced 78 polymorphic bands with 69% polymorphism.

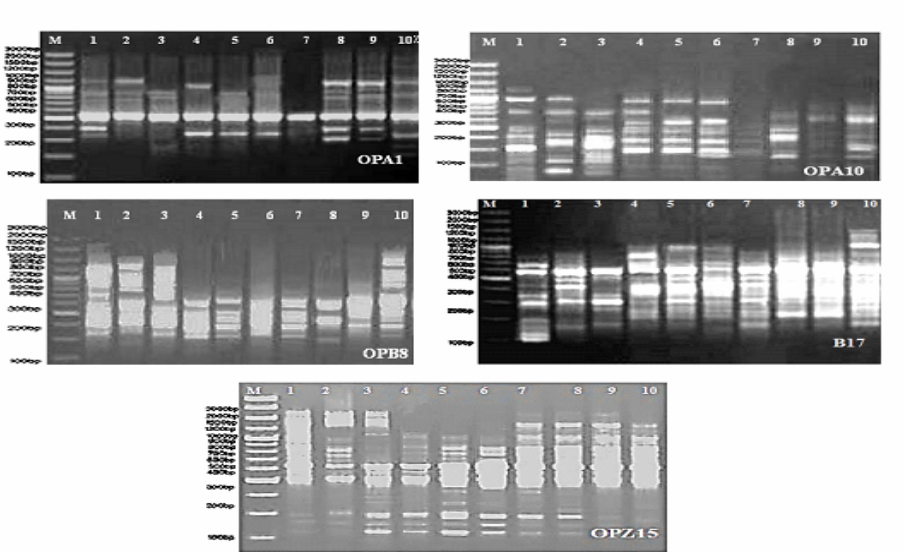


Fig.(2):OPA1, OPA10, OPB8, OPB17 and OPZ15, RAPD primers among the ten Zygophyllaceae genotypes under different habitats.* *(M) = Maker, (Zygophyllum coccineum L. from Hammam Cleopatra) = 1, (Z. coccineum L. from Burg El-Arab) =2, (Z. simplex from Siwa Oasis) = 3, (Nitraria retusa from Siwa Oasis) = 4, (N. retusa from Burg El-Arab) = 5, (N. retusa from North Sinai) = 6, (Peganum harmal L. from Siwa Oasis) =7, (Fagonia indica from Burg El-Arab) = 8, (F. indica from Quatamia) = 9 and (Tribulus terrestris L. from Quatamia) = 10.

Table (3): OPA1, OPA10, OPB8, OPB17 and OPZ15, RAPD primers among the ten Zygophyllaceae genotypes under different habitats.** (Zygophyllum coccineum L. from Hammam Cleopatra) = 1, (Z. coccineum L. from Burg El-Arab) =2, (Z. simplex from Siwa Oasis) = 3, (Nitraria retusa from Siwa Oasis) = 4, (N. retusa from Burg El-Arab) = 5, (N. retusa from North Sinai) = 6, (Peganum harmal L. from Siwa Oasis) =7, (Fagonia indica from Burg El-Arab) = 8, (F. indica from Quatamia) = 9 and (Tribulus terrestris L. from Quatamia) = 10.

Polymorphism percentages	Total amplified bands	Unique bands	Polymorphic bands	Monomorphic bands	Length range(pb)	Primer sequences	Primer code
92%	14	7	13	1	225-1200	5’-CAGGCCCTTC-3’	OPA1
87%	15	4	13	2	100-600	5’-GTGATCGCAG-3’	OPA10
100%	15	3	9	7	200-500	5’-GTCCACACGG-3’	OPB8
82%	17	3	14	0	198-1200	5’-AGGGAACGAG-3’	OPB17
68.4%	19	1	13	6	168-686	5’-CAGGGCTTTC-3’	OPZ15
95%	80	18	76	16			Total

Table (4): RAPD primers for Zygophyllaceae species - specific marker.

Unique bands	Plant species	Primer Code NO.
3	*Zygophyllum coccineum* L. - Hammam Cleopatra	
2	*Tribulus terrestris* L. - Quatamia	OPA1
1	*Z. simplex* - Siwa Oasis	
1	*Nitraria retusa* - Siwa Oasis	
2	*Zygophyllum coccineum L.* - Burg El-Arab	OPA10
1	*Fagonia indica* from Burg El-Arab	
1	*Tribulus terrestris* L. - Quatamia	
1	*Zygophyllum simplex* - Siwa Oasis	OPB8
2	*Tribulus terrestris* L. - Quatamia	
2	*Zygophyllum coccineum* L. - Hammam Cleopatra	
1	*Nitraria retusa* - - Siwa Oasis	OPB17
1	*Peganum harmal L.*- Siwa Oasis	
1	*Nitraria retusa* - North Sinai	OPZ15
19		TOTAL

Combined analysis

Based on total analysis (protein and RAPD), similarity matrix was developed by SPSS computer package system in Table (5) and shown in Fig (3). The closest relationship was scored between *Fagonia indica* from two locations with similarity of 95%, while *Fagonia indica* from Burg El-Arab and *Zygophyllum coccineum* from Hammam Cleopatra gave the least similarity of 12%. The dendrogram based on overall analysis separated the ten *Zygophyllaceae* genotypes into two main clusters. Moreover, *Zygophyllum coccineum* from two locations , *Z. simplex* and *Nitraria retusa* from three locations were separated together in the first main cluster. While, *Fagonia indica* from two locations, *Peganum harmal* and *Tribulus terrestris* were separated in the second cluster.

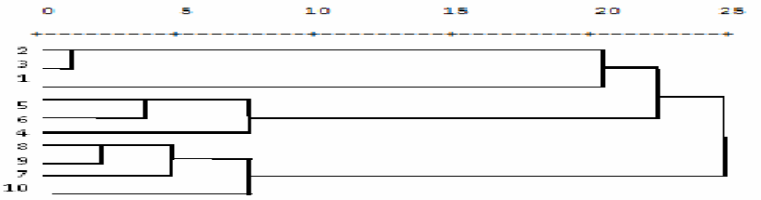


Fig. (3) : Dendrogram based total analysis (protein and RAPD) markers among the ten studied Zygophyllaceae genotypes under different habitats* *(Zygophyllum coccineum L. from Hammam Cleopatra) = 1, (Z. coccineum L. from Burg El-Arab) =2, (Z. simplex from Siwa Oasis) = 3, (Nitraria retusa from Siwa Oasis) = 4, (N. retusa from Burg El-Arab) = 5, (N. retusa from North Sinai) = 6, (Peganum harmal L. from Siwa Oasis) =7, (Fagonia indica from Burg El-Arab) = 8, (F. indica from Quatamia) = 9 and (Tribulus terrestris L. from Quatamia) = 10.

Table (5): Similarity matrix of total analysis (protein and, RAPD) markers among the ten studied Zygophyllaceae genotypes under different habitats*. *(Zygophyllum coccineum L. from Hammam Cleopatra) = 1, (Z. coccineum L. from Burg El-Arab) =2, (Z. simplex from Siwa Oasis) = 3, (Nitraria retusa from Siwa Oasis) = 4, (N. retusa from Burg El-Arab) = 5, (N. retusa from North Sinai) = 6, (Peganum harmal L. from Siwa Oasis) =7, (Fagonia indica from Burg El-Arab) = 8, (F. indica from Quatamia) = 9 and (Tribulus terrestris L. from Quatamia) = 10.

10	9	8	7	6	5	4	3	2	1	
									100	1
								100	30	2
							100	38	33	3
						100	48	48	33	4
					100	92	55	55	40	5
				100	96	88	52	52	37	6
			100	16	18	19	29	29	13	7
		100	69	16	18	17	12	17	12	8
	100	95	91	77	18	13	17	22	16	9
100	77	66	68	64	27	30	14	19	13	10

TDC Gene detection

The PCR product using specific primer of TDC gene indicated that the appearance of one band for each with fragment size 1770 bp as shown in (Fig. 4). These results agreed with the results obtain by Gerasimenko *et al*., (2002) who isolated and characterized TDC genes from *Rauvolfia verticillata* and Shakti *et al*., (2013) who isolated and characterized TDC genes from *Catharanthus roseus.*

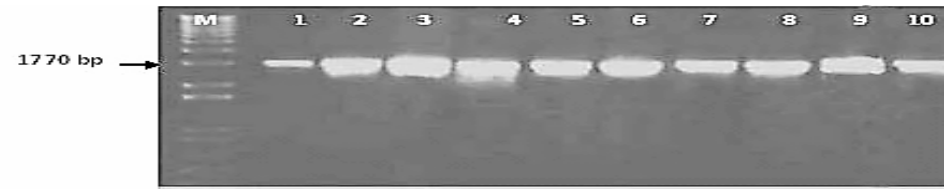


Fig. (4): PCR product of TDC gene sequence from ten Zygophyllaceae genotypes

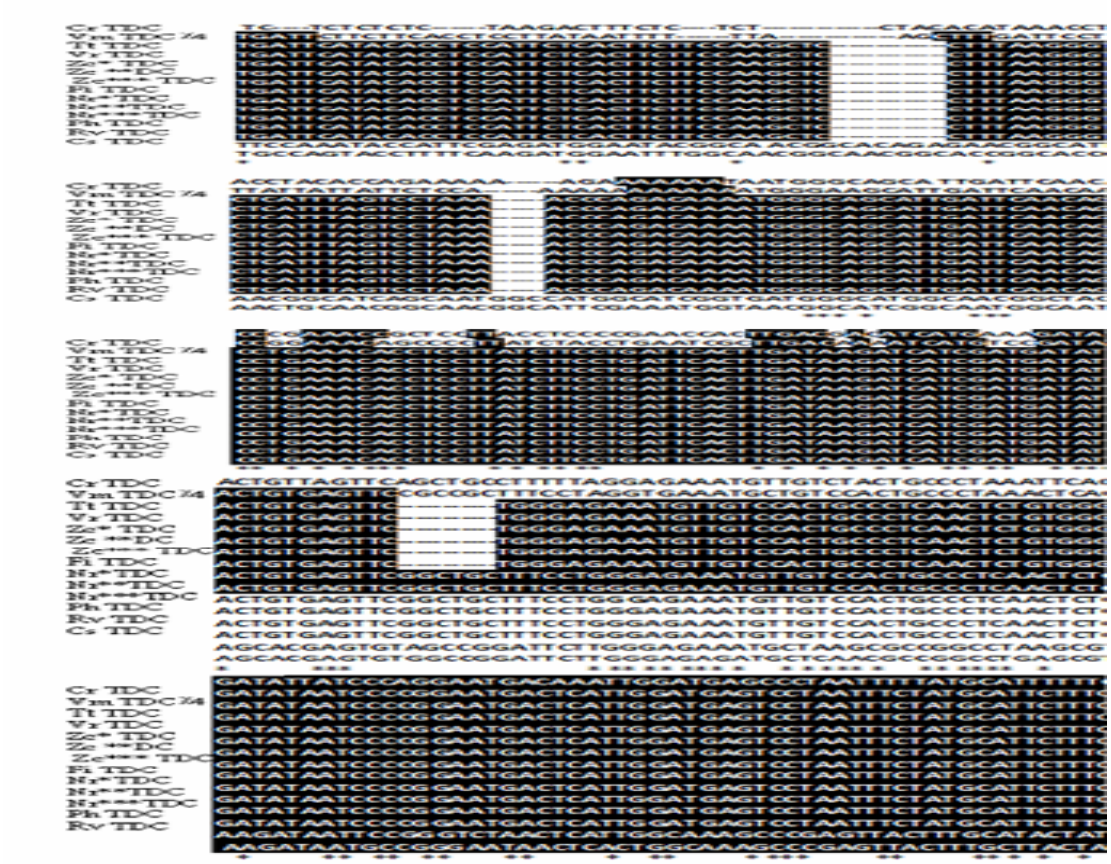


Fig. (5): Sequence alignment of TDC gene in zygophyllaceae genotypes: (TtTDC= Tribulus terrestris* L. from Quatamia), (Zc*TDC= *Zygophyllum coccineum* L. from Hammam Cleopatra), (ZcTDC= *Z. coccineum* L. from Burg El-Arab),(Zc*TDC= *Z. simplex* from Siwa Oasis), (FiTDC= *Fagonia indica* from Burg El-Arab), (Nr*TDC= *Nitraria retusa* from Siwa Oasis), (Nr**TDC= *N. retusa* from Burg El-Arab), (Nr***TDC= *Nitraria retusa* from Siwa Oasis), (PhTDC= *Peganum harmal* L. from Siwa Oasis) all were in relation to those of some other plants including TDC DNA sequences of (Cr TDC= *Catharanthus roseus* ,GenBank accession no. M25151.1), (Vm TDC = *Vinca minor* GenBank accession no. JN644946.1), (RvTDC = *Rauvolfia verticillata* GenBank *accession no. HM067439.1) and (CsTDC= Camelina sativa GenBank accession no. XM_019233363.1) and sequences show conserved domains with black colour.***

Description	Max score	Total score	Query cover	E value	Ident	Acession
Vinca minor tryptophan decarboxylase mRNA, complete cds	1600	1600	92%	4e-99	90%	JN644946.1
C.roseus tryptophan decarboxylase mRNA, complete cds	1099	1099	93%	4e-98	97%	M25151.1
PREDICTED: Camelina sativa probable tyrosine decarboxylase 2 (LOC104730467), transcript variant X3, mRNA	1400	1400	90%	4e-98	96%	XM019233363.1
PREDICTED: Raphanus sativus probable tyrosine decarboxylase 2 (LOC108840444), mRNA	1797	1797	88%	4e-98	89%	XM018613271.1
Rauvolfia verticillata tryptophan decarboxylase (TDC) mRNA, complete cds	3273	3273	99%	4e-99	98%	HM067439.1

Fig. (6): NCBI- BLAST analysis of TDC gene homology among Zygophyllaceae genotypes.

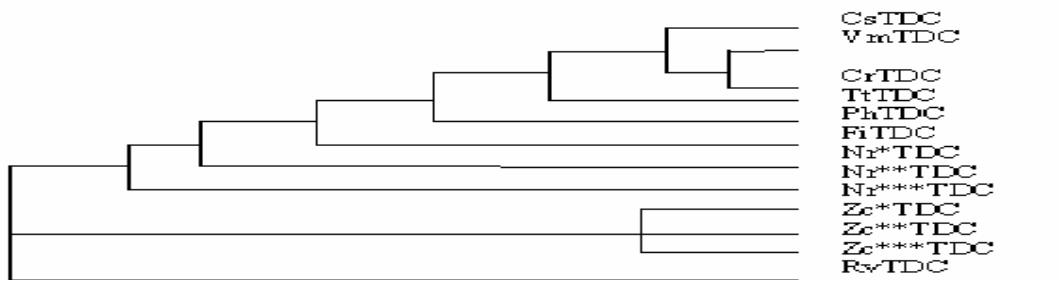


*Fig. (7): Phylogenetic tree of the TDC gene sequences in zygophyllaceae genotypes: (TtTDC= Tribulus terrestris L. from Quatamia), (Zc*TDC= Zygophyllum coccineum L. from Hammam Cleopatra), (Zc***TDC= Z. coccineum L. from Burg El-Arab),(Zc*TDC= Z. simplex from Siwa Oasis), (FiTDC= Fagonia indica from Burg El-Arab), (Nr*TDC= Nitraria retusa from Siwa Oasis), (Nr**TDC= N. retusa from Burg El-Arab), (Nr***TDC= Nitraria retusa from Siwa Oasis), (PhTDC= Peganum harmal L. from Siwa Oasis) all were in relation to those of some other plants including TDC DNA sequences of (Cr TDC= Catharanthus roseus ,GenBank accession no. M25151.1), (Vm TDC = Vinca minor GenBank accession no. JN644946.1), (RvTDC = Rauvolfia verticillata GenBank accession no. HM067439.1) and (CsTDC= Camelina sativa GenBank accession no. XM_019233363.1).*

Analysis of the TDC gene nucleotide sequence alignment

Sequencing and BLAST analysis showed that the length of TDC fragment shares high homology with the other known TDC gene as shown in Fig. (5 and 6). Homology search results in GenBank (NCBI) showed that TDC nucleotide had high identity to other plants such as *Raphanus sativus* (89% identities, accession number XM018613271.1), *Vinca minor* (90% identities, accession number JN644946.1), *Catharanthus roseus* (97% identities, accession number M25151.1), *Camelina sativa* (96% identities, accession number XM_019233363.1) and *Rauvolfia verticillata* (98% identities, accession number HM067439.1).

Phylogenetic analysis of DNA sequence of TDC gene

Phylogenetic analysis was done by aligning DNA sequences using software ClustalW and Mega4.0 software to construct a tree (Fig. 7).This study discovered that the TDC gene of *Zygophyllum coccineum* from two locations and *Z. simplex* were grouped together and were closer to *Rauvolfia verticillata* and all species belongs to *Zygophyllacae* family. The TDC genes of *Vinca minor, Catharanthus roseus* and *Camelina sativa* were also grouped together in one group have a close genetic relationship.

CONCLUSION

This study could pave the way for detailed research to understand all the aspects of this divergence to solve a lot of taxonomical problems of *Zygophyllaceae* species which can provide certain reference values, such as distinguishing genetic resources and judging genetic relationships. RAPD marker was an effective method for the identification of *Zygophyllaceae* germplasm than protein. Sequence comparison and bioinformatics analysis of TDC genes were conducted to explore the reason for genetic diversity and genetic relationships among ten *Zygophyllaceae* genotypes. Results showed that the length of the TDC fragment is 1770 bp. Phylogenetic tree analyses revealed that the TDC fragment is genetically close to *Rauvolfia verticillata* and *Catharanthus roseus*. The complete gene needed for further gene transformation for crop improvement. Information generated from this study can be used in the future for conservation, breeding and improvement programs.

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الملخص العربي

تحديد التباين الوراثي باستخدام دلائل RAPD، SDS-PAGE و الجين المسئول عن المادة الفعالة في بعض التراكيب الوراثية للعائلة الزيجوفيلية

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تمت دراسة التباين الوراثى البيوكيماوى باستخدام التفريد الكهربى للبروتينات بين عشرة تراكيب وراثية للعائلة الزيجوفيلية.كما تم استخدام الكاشفات الجزيئية RAPD لمعرفة الاختلافات الوراثية. أعطت SDS-PAGE ستة وثلاثون حزمة كلية بنسبة إختلافات سبعة وستون فى المئة ، بينما أعطت RAPD ثمانين حزم كلية بنسبة إختلافات خمسة و وتسعين فى المئة وهى الأعلى. إستنادا الى التحليلات الكلية تم تصميم شجرة القرابة الوراثية للعشرة تراكيب وراثية التى فصلتهم الى مجموعتين رئيسيتين: الاولى *Z.simplex,* (*Zygophyllum coccineum*) *Nitraria retusa* و الثانية (*Tribulus terrestris* *Peganum, harmal,* و *Fagonia indica*) ، تفوقت الدلائل الجزيئية عن البيوكيماوية فى اظهار الاختلافات الوراثية بين العشر تراكيب وراثية من الزيجوفيلية وأظهرت النتائج وجود الجين المسئول عن انتاج القلويداتTDC بطول حزمة ١٧٧٠زوج نيوكلتيدية، بمضاهاة التتابعات باستخدام ال BLAST وتحليل شجرة القرابة (تحاليل المعلوماتية الحيوية) تشابه التتابع فى العائلة الزيجوفيلية مع *نباتات Catharanthus roseus* و*Rauvolfia verticillata* . نتائج الدراسة يمكن استخدامها فى برامج الحفظ الوراثى والتربية والتحسين الوراثى.