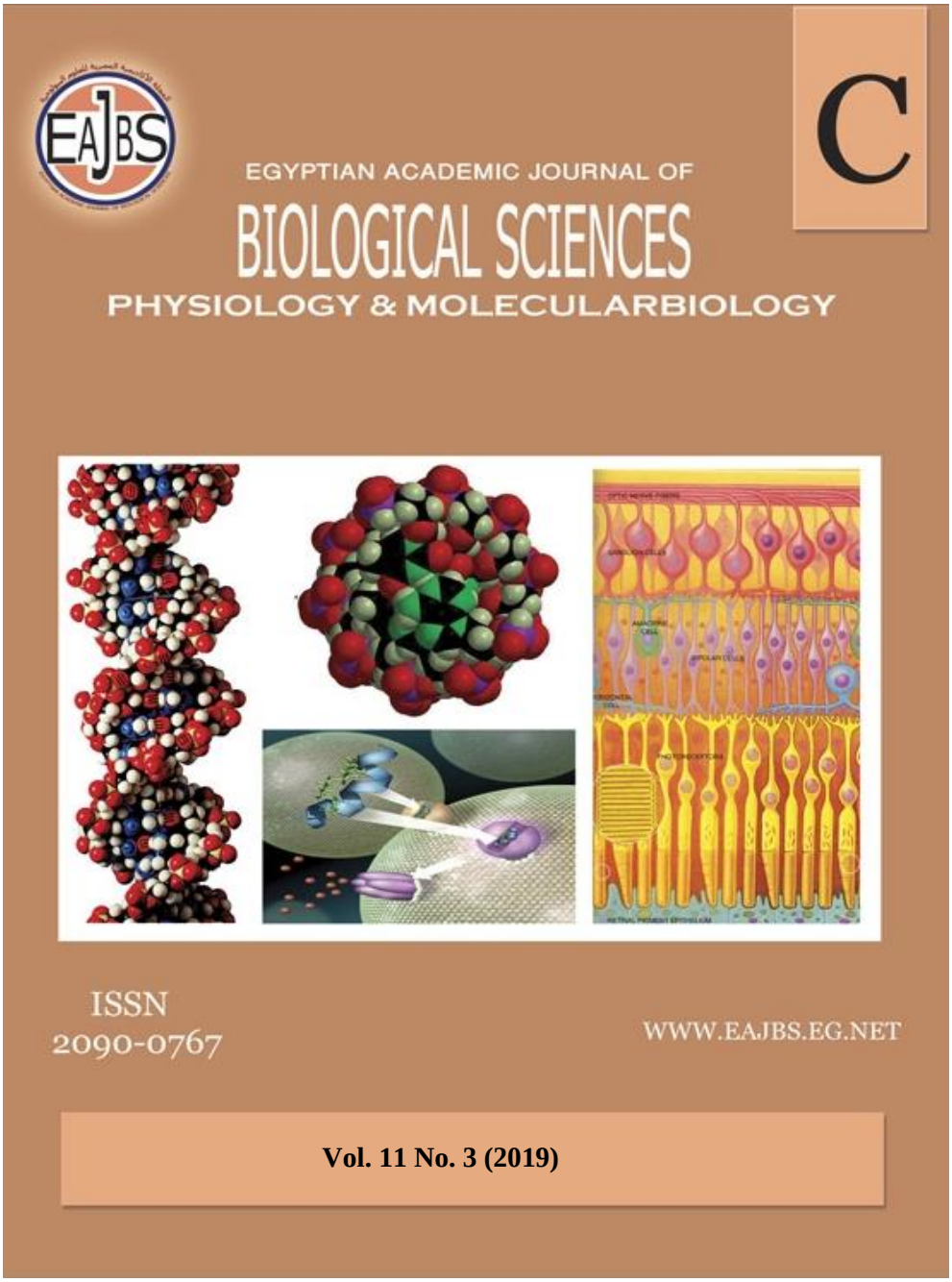

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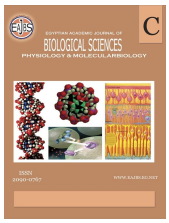
Egypt. Acad. J. Biolog. Sci., 11(3): 69- 76 (2019)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767
http://eajbsc.journals.ekb.eg



Isolation and Identification of Some Genes for Drought Tolerance in *Suaeda* Sp Plant

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ARTICLE INFO
Article History
Received:18/8/2019
Accepted:15/10/2019

***Keywords*:**
Suaeda vera, Suaeda pruinosa, P5CS, BADH and DREB gene, phylogenetic tree

ABSTRACT

In the present study, two species of the drought-tolerant genus Suaeda (*Suaeda vera* and *Suaeda pruinosa*) were investigated for detection of some genes responsible for drought resistance. Three genes (P5CS, BADH, and DREB) were chosen and detected using specific primers producing bands of different sizes 1500bp, 700bp and 430bp, respectively. The obtained fragments of the three genes were Sequenced, and phylogenetic tree constructed. The results revealed the efficiency of BADH to clarify the closest relatedness of Suaeda species with other species on National Center for Biotechnology Information (NCBI) database. In addition, one can conclude that BADH gene may be included in drought resistance mechanism of Suaeda species. This study can be used in the future for breeding and crop improvement programs..

INTRODUCTION

Environmental stress, such as low temperature, high temperature, high salinity, and drought restrict the distribution and productivity of plants. When subject to salt stress or drought, some vascular plants typically respond with increased accumulation of proline and glycine betaine, an important osmoprotectant that is produced in response to salt and other osmotic stresses Zhou *et al*., (2008). (Abd El-Maboud and Khalil 2013) detected an increase of glycine betaine and proline in *Suaeda fruticosa* and *S. vera* under salinity and drought stress. In higher plants, proline is synthesized from glutamate or arginine /ornithineIn higher plants, P5CS is encoded by a nuclear gene from *Vigna aconitifolia* (Hu *et al*., 1992), *Arabidopsis thaliana* (Strizhov *et al*., 1997), *Glycine max* and *Lactuca sativa* (Porcel *et al*., 2004) and other species. The last step in betaine synthesis in plants is catalyzed by betaine aldehyde dehydrogenase (BADH), a nuclear-encoded chloroplastic enzyme. To date, BADHs have been isolated from several species, viz spinach (*Spinacia oleracea* L.) (Shirasawa *et al*., 2006), barley (*Hordeum vulgare* L.) (Nakamura *et al*., 2001) and mangrove (*Avicennia marina*) (Wu *et al*., 2008). The Dehydration- responsive element-binding proteins (DREBs) are members of the APETALA2/ethylene-responsive element- binding factor (AP2/ERF) family of transcription factors in the promoters of stress-inducible genes (Yamaguchi and Shinozaki 2006). Genes included in the DREB subfamily are divided into six small subgroups (A-1 to A-6) based on similarities in the binding domain. DREB from *Salicornia brachiata* was induced by NaCl, drought, and heat stress (Gupta *et al*., 2014). In the present study, three identified drought-responsive genes were detected in two Suaeda species in order to clarify their role in plantresistance.

MATERIALS AND METHODS

Suaeda species were collected from two locations in the North Coast, Egypt during March 2017-2018. *Suaeda vera was* collected from Hammam *Cleopatra while Suaeda pruinosa* was collected from EL-Maktala as shown in Fig. (1).

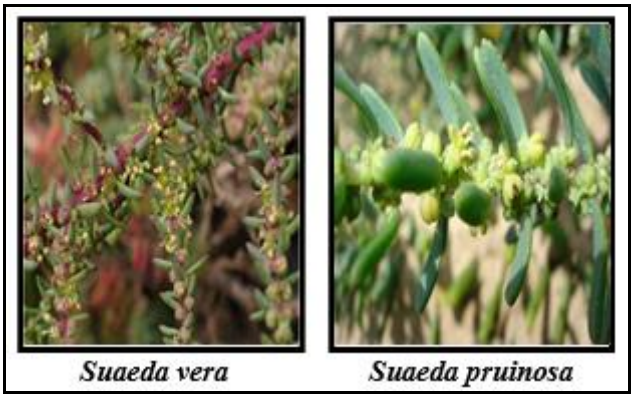

Fig. (1): *Suaeda vera* and *Suaeda pruinosa* collected from two locations in North Coast, Egypt.

DNA Extraction:

The young leaves were collected and stored at -80 ˚C until used for DNA extraction. Total genomic DNA was extracted using DNeasy Plant Mini Kit (QIAGEN). Quality and concentration of total DNA was verified by Spectrophotometer 300UV-Visible (United State) 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.

Table (1): Sequences of the three specific designed primers used for the detection of drought stress genes in Suaeda sp.

Primer	Sequence
P5CS (Δ1-pyrroline-5-carboxylate synthetase)	5'-TACTGAGACTGTGAAGTCGC-3' (forward) 5'-ATGGCATTGCAGGCTGCCG-3' (reverse)
BADH (Betaine aldehyde dehydrogenase)	5'-TCCTCTCGTCTCCAGTCCAC-3' (forward) 5'-AATGCAGACTAACAACCCATGA-3' (reverse)
DREB (Dehydration- responsive element-binding) proteins)	5'-ATGGAAGAAGCGTTAGGTGGAGA-3'(forward) 5'-TGGAGGACGTCGAGTATTGTGG-3' (reverse)

Polymerase Chain Reaction (PCR):

The designed primers **(Table 1)** were used in PCR reaction under the following conditions: 94º C for 10 min, then 45 cycles at 94º C for 30 s, 62º C for 30 s, and at 72º C for 1 min and the final extension at 72º C for 5 min. 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 the High Pure PCR Product Purification Kit (Roche-Switzerland) and sequenced (MWG, Germany).

Bioinformatic Analysis*:*

Primer 3 software (http://www. Premierbiosoft.com) was used to

design all the studied primers. Sequences were aligned and the phylogenetic tree was designed using the software ClustalW**.**

RESULTS AND DISCUSSION

P5CS Gene Detection:

The PCR product using a specific primer of P5CS gene resulted in a band of size1500 bp for each species as shown in (Fig. 2). These results agreed with the results of Abou Gabal *et al*., (2013) who isolated and characterized P5CS genes from *Alhagi Maurorum.*

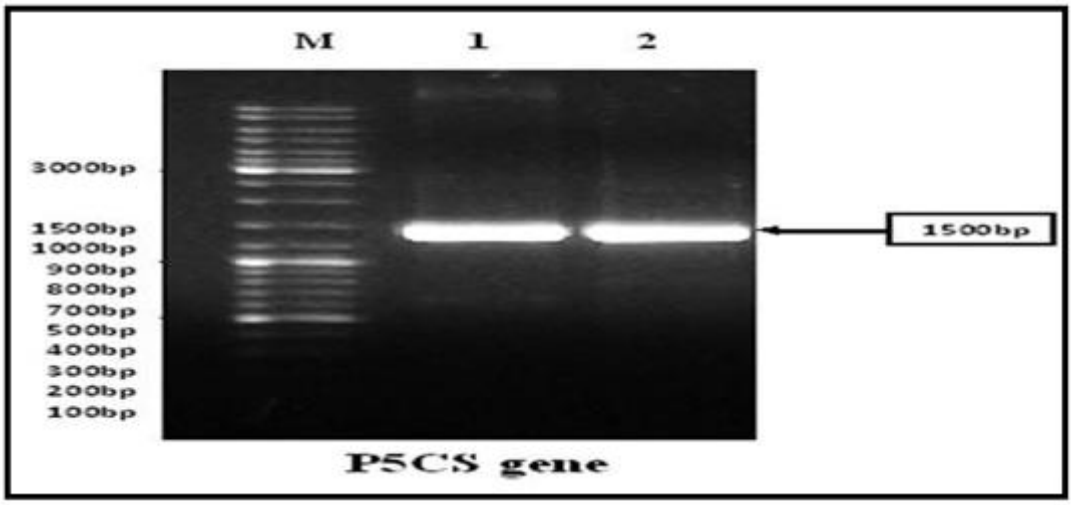

Fig. (2): PCR product of **P5CS** gene in the studied Suaeda sp.

Analysis of the P5CS Gene Nucleotide Sequence Alignment:

Sequencing and Basic local alignment search tool (BLAST) analysis showed that the length of P5CS fragment shared high homology with the other known P5CS gene as shown in **Table (2)**. Homology search results in (NCBI) showed that P5CS nucleotide had high identity to other plants such as *Spinaca dencea* (98% identities, accession number XM 021989924.1), *Actinida deliciosa* (97% identities, accession number U92286.1), *Mesembryanthemum crystallinum* (96% identities, accession number AF067967.1), *Lycium chinense* (96% identities, accession number KF771023.1), *Amborella trichopoda* (95% identities, accession number XM 011625851.2) *Atriplex nummularia* (95% identities, accession number EF160132.1) *Morus alba* (94% identities, accession number XM 024173484.1) *Cucumis melo* (93% identities, accession number XM 00413842.2).

Table (2): NCBI- BLAST analysis of P5CS gene sequence homology of the studied Suaeda sp.

Description	Max score	Total score	Query cover	E value	Ident	Accession
Spinaca dencea (p5CS)gene	266	266	99%	4e-67	98%	XM 021989924.1
Actinida deliciosa (p5CS)gene	304	304	92%	2e-78	97%	U92286.1
Mesembryanthemum crystallinum (p5CS)gene	269	269	98%	3e-68	96%	AF 067967.1
Lycium chinense (p5CS)gene	452	452	99%	5e-123	96%	KF 771023.1
Amborella trichopoda (p5CS)gene	278	278	98%	4e-70	95%	XM 011625851.2
Atriplex nummularia (p5CS)gene	313	313	98%	3e-81	95%	EF160132.1
Morus alba (p5CS)gene	275	275	99%	4e-70	94%	XM 024173484.1
Cucumis melo (p5CS)gene	279	279	96%	2e-75	93%	XM 00413842.2

Phylogenetic Analysis of P5CS Gene Sequence in the Studied *Suaeda sp:*

Phylogenetic analysis was done by aligning DNA sequences using ClustalW software to construct a phylogenetic tree (Fig. 3). This analysis has grouped P5Cs gene of plant species under study, *Suaeda vera* and *Suaeda pruinosa* in one cluster with P5CS gene of *Spinaca dencea, Actinida deliciosa,* and *Atriplex nummularia.* While, the P5CS gene of the other plant species was far enough to be grouped in another cluster.

BADH Gene Detection:

The PCR product using a specific primer of BADH gene produced a band of size700 bp for the studied suaeda sp. as shown in **(**Fig. 4). These results agreed with the results obtained by (Shanthi *et al.*, 2013) who isolated and characterized BADH genes from Atriplex spp

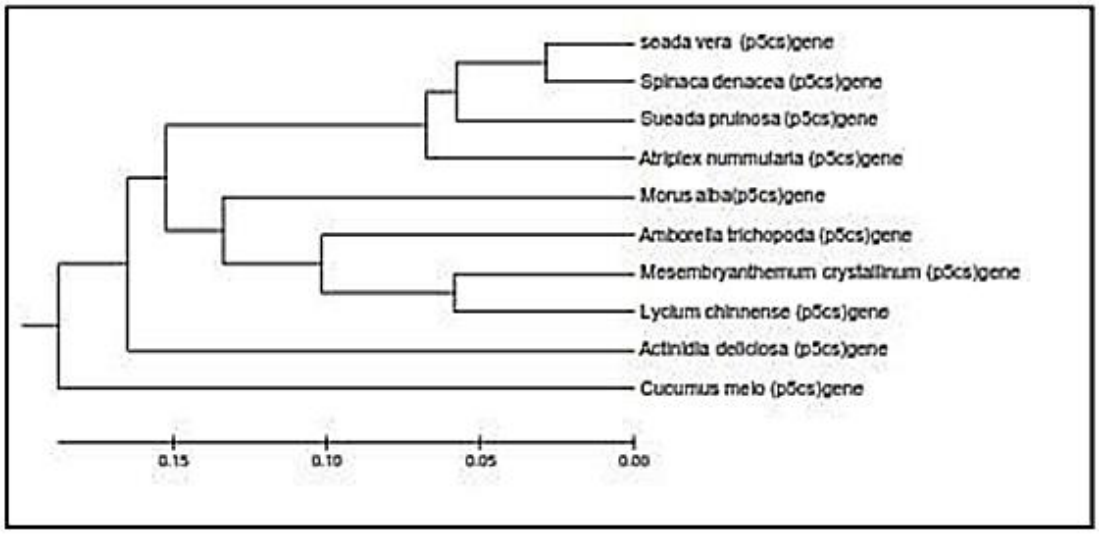

Fig. (3): Phylogenetic tree of the **P5CS** gene sequences of the studied Suaeda sp. with other **P5CS** genes submitted in NCBI database.

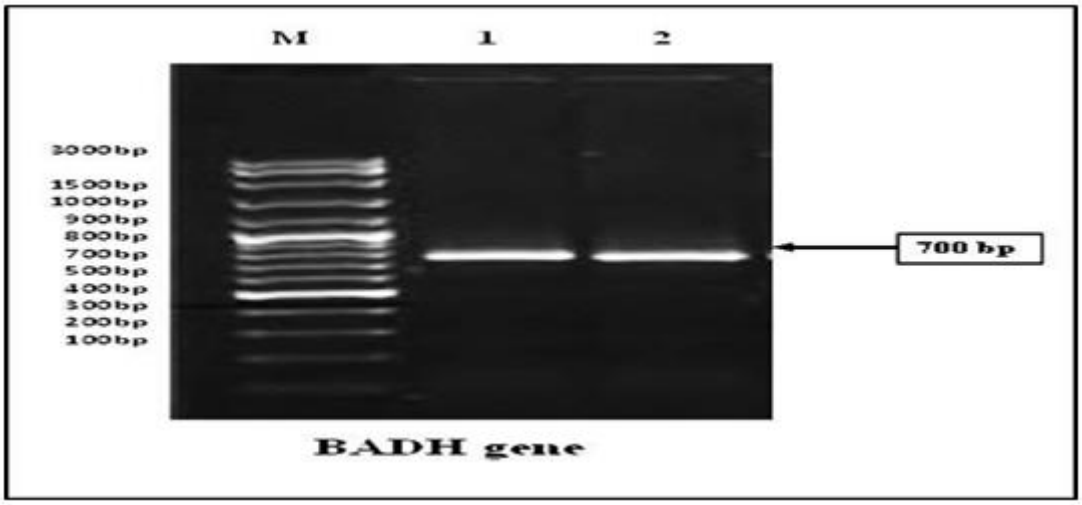

Fig. (4): PCR product of **BADH** gene in the studied Suaeda sp.

Analysis of the BADH Gene Nucleotide Sequence Alignment:

Sequencing and BLAST analysis showed that the length of BADH fragment shared high homology with the other known BADH genes as shown in Table (3). Homology search results in NCBI showed that BADH gene had high identity to other plants such as *Suaeda galuca* (98% identities, accession number KF594413.1), *Halocnemum strobilaceum* (97% identities, accession number

JN9698912.1), *Salicornia biglovii* (96% identities, accession number KU875306.1), *Vitis vinifera* (95% identities, accession number FQ393912.1), *Gossyppium raimondi* (95% identities, accession number XM 012613938.1) *Panax ginsng* (94% identities, accession number AY31099131) *Jatropha curcas* (90% identities, accession number JX860301.1) and *Chenopodium quinoa* (89% identities, accession number KP774603.1).

Table (3): NCBI- BLAST analysis of **BADH** gene sequence homology of the studied Suaeda sp.

Description	Max score	Total score	Query cover	E value	Ident	Accession
Sueada galuca (BADH) gene	2660	2660	99%	0.0	98%	KF 594413.1
Halocnemum strobilaceum (BADH) gene	2107	2107	99%	0.0	97%	JN9698912.1
Salicomia biglovii (BADH) gene	2024	2024	99%	0.0	96%	KU875306.1
Vitis vinifera (BADH) gene	1023	1023	97%	0.0	95%	FQ393912.1
Gossyppium raimondi (BADH) gene	1003	1003	98%	0.0	95%	XM 012613938.1
Panax ginsng (BADH) gene	1012	1012	98%	0.0	94%	AY31099131
Jatropha curcas (BADH) gene	1007	1007	93%	0.0	90%	JX860301.1
Chenopodium quinoa (BADH) gene	1687	1687	90%	0.0	89%	KP774603.1

Phylogenetic Analysis of BADH Gene:

Phylogenetic analysis was created by aligning DNA sequences using ClustalWsoftware to construct a phylogenetic tree (Fig. 5). This study elucidated that the BADH gene of *Suaeda vera* and *Suaeda pruinosa,* was succeded to group the two plant species together in sub-cluster. In addition another species of the same genus *Suaeda galuca* has closest relationship to *Suaeda species* under study. Meanwhile, *Halocnemum strobilaceum* and *Salicornia biglovii* were also grouped in the same cluster with suaeda species. In contrast, the remainder species clustered together in another group.

DREB Gene Detection:

The PCR product using a specific primer of DREB gene showed one band for each Suaeda species with size 430bp as shown in (Fig. 6). These results were in agreement with Gupta *et al.*, 2014 who isolated and characterized DREB genes from *Salicornia brachiate*.

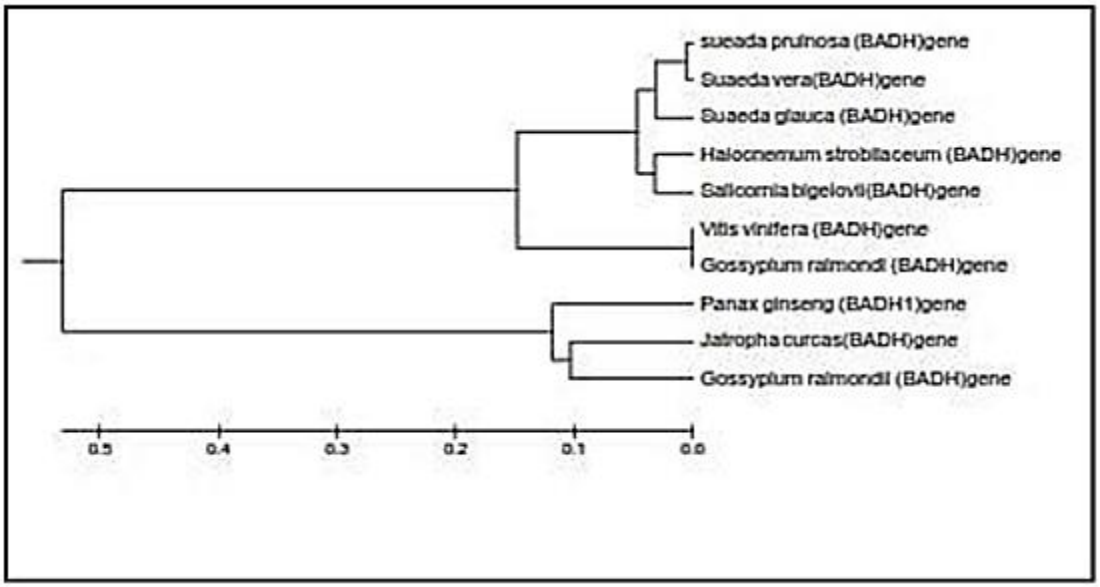

Fig. (5): Phylogenetic tree of the BADH gene sequences of the studied Suaeda sp. with other BADH genes submitted in NCBI database.

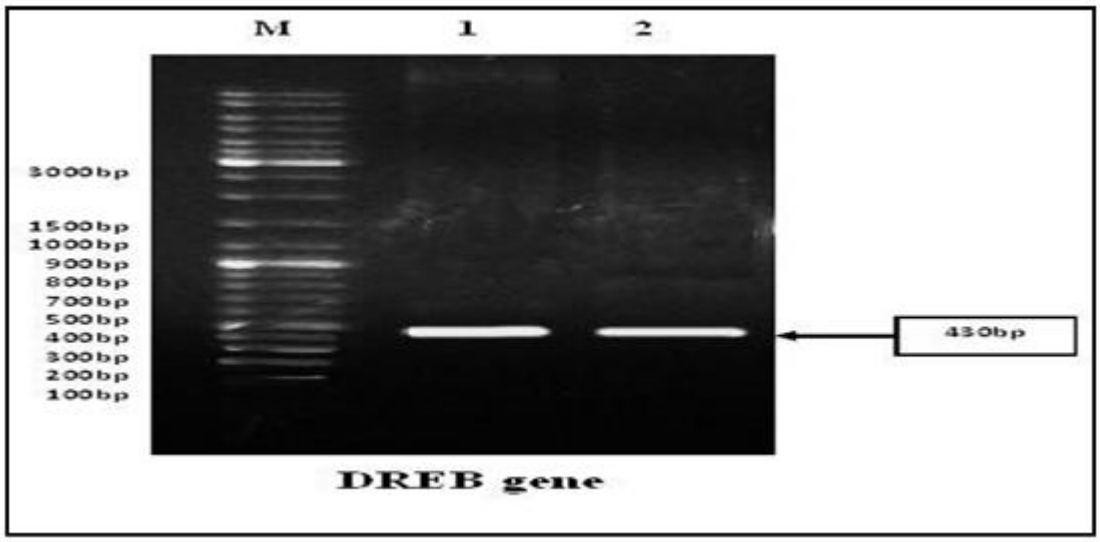

Fig. (6): PCR product of DREB gene in the studied Suaeda sp

Analysis of the DREB Gene Nucleotide Sequence Alignment:

Sequencing and BLAST analysis showed that the length of DREB fragment revealed high homology with the other known DREB gene as shown in Table (4). Homology search results in NCBI showed that DREB gene had high identity to other plants such as *Glycine max* (96% identities, accession number KT031079.1), *Vigna radiata* (94% identities, accession number XM 01464.1), *Lupinus albus* (94% identities, accession number CP023115.1), *Phaselous vulgaris* (92% identities, accession number FQ393912.1), *Cajans cajan* (90% identities, accession number XM020383974.1) *Carica papaya* (90% identities, accession number KU O65116.1) *Rosa drinensis* (89% identities, accession number XM024308992.1).

Table (4): NCBI- BLAST analysis of DREB gene sequence homology gene sequence homology of the studied Suaeda sp.

Description	Max score	Total score	Query cover	E value	Ident	Accession
Glycine max (DRB) gene	829	829	99%	0.0	96%	KT 031079.1
Vigna radiata (DRB) gene	529	529	99%	0.0	94%	XM 01464.1
Lupinus albus (DRB) gene	255	255	95%	0.0	94%	CP 023115.1
Phaselous vulgaris (DRB) gene	1023	1023	97%	0.0	92%	FQ393912.1
Cajans cajan (DRB) gene	545	545	98%	0.0	90%	XM 020383974.1
Carica papaya (DRB) gene	279	279	89%	0.0	90%	KU O65116.1
Rosa drinensis (DRB) gene	232	232	86%	0.0	89%	XM024308992.1

Phylogenetic Analysis of DNA Sequence of DREB Gene:

Phylogenetic analysis was undertaken by aligning DNA sequences using ClustalW software to construct a phylogenetic tree (Fig. 7). than *Suaeda pruinosa* .On the other hand, *Phaselous vulgaris, Carica papaya,* and *Cajans cajan* were distant from Suaeda sp.

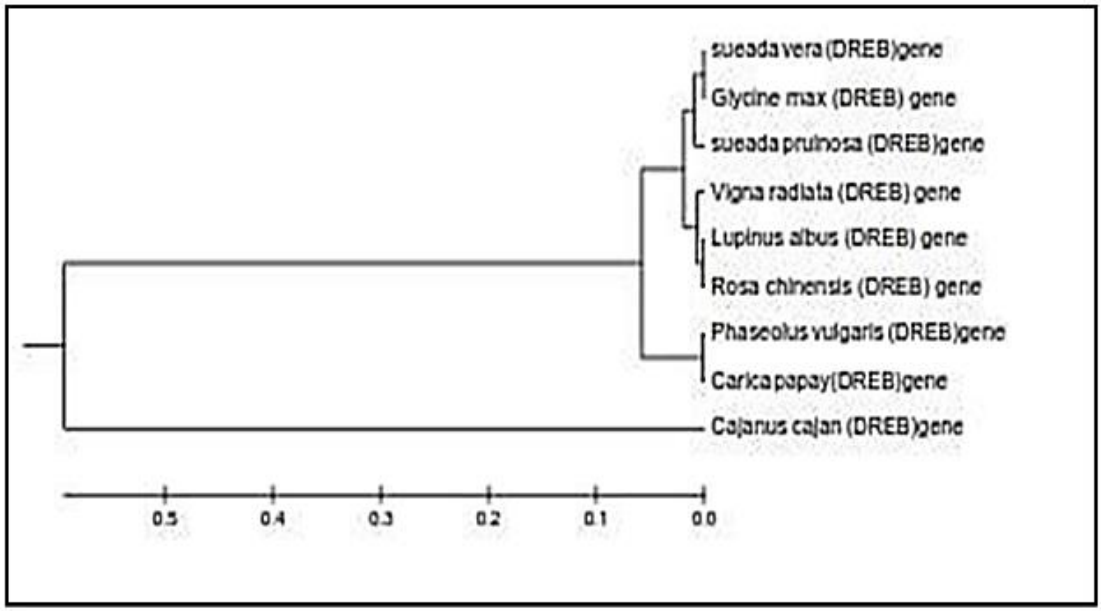

Fig. (7): Phylogenetic tree of the DREB gene sequences of the studied Suaeda sp. with other DREB genes submitted in NCBI database

CONCLUSION

This investigation revealed that BADH gene was the most efficient in clarifying the closest genetic relationships between Suaeda plant species under study compared to P5CS and DREB genes. In addition, it may have a key role in drought stress resistance mechanisms in Suaeda plant species and can be used for crop improvement strategies.

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