INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 10, No. 2, p. 81-88, 2017

RESEARCH PAPER OPEN ACCESS

Molecular genetic relationships among *Haloxylon salicornicum* plant and its closely related taxa in Saudi Arabia

Sanad M. Al Sobeai*

Sajir College of Arts and Science, Shaqra University, Saudi Arabia, Saudi Arabia

Key words: Haloxylon, Medicinal plant, ITS1, Saudi Arabia.

http://dx.doi.org/10.12692/ijb/10.2.81-88 Article published on February 24, 2017

Abstract

The present study encompasses molecular identification, genetic distance and genetic relationships among *Haloxylon salicornicum* plants collected from the Saudi Arabia and its closely related taxa was investigated by sequencing the amplified ITS region including the 3` end 40 bp from 18S gene, the complete ITS1, 5.8S rDNA and ITS2 as well as 104 bp from the 5` end of 26S gene. The results showed that the *H. salicornicum* of Saudi Arabia desert has low level of genetic distance and genetic diversity when compared with its closely related taxa. These high level of genetic similarities of *H. salicornicum* and its closely related taxa might be due to the controlled gene flow and small size of samples. Further studies at the molecular level are also required to elucidate and provide intimations for more understand the interplay of genetic distance and genetic relationships among *H. salicornicum* with closely associated plants.

* **Corresponding Author:** Sanad M. Al Sobeai ✉ Salsobaei@su.edu.sa

Introduction

Haloxylon salicornicum belongs to the Chenopodiaceae family, and it is widely distributed in Northern Africa Central Asia, Middle East, adapted in temperate and tropical areas (Boulos, 1999; Ashraf *et al.,* 2012). *H. salicornicum* has been used in traditional or folklore medicine for cure of many diseases such as cold (Ghazali *et al*., 2010; Abdallah and El-Ghazali, 2013), hepatobiliary diseases and diabetes (Butnik *et al.* 1991; Stuart Chapin, 2001; Ghazali *et al*. 2010; Ahmad and Eram, 2011; Soliman *et al*., 2012), and gynecological complications in women (Saleem, 2012). It is known to contain an assortment of bioactive such as piperidine alkaloid (Ajabnoor *et al.,* 1984), haloxynine, and haloxine (El-Shazly *et al.,* 2005; Muhammad *et al.,* 2012; Singh *et al.,* 2015). *H. salicornicum* is a fodder plant, mostly grazed by the camels, has high salt contents (Ashraf *et al.,* 2012; Akhani *et al., 2007*). It is an almost leafless, much-branched plant, growing to approximately 100 centimeters in height, woody at base. Stem and branches are pale yellow with lacking of the large leaves, jointed, joints produces into two short triangular points which take the place of leaves and are woolly within, flowers and fruits not observed (Fig. 1). The fruit with wings is about 0.8 centimeters in diameter. The seed is about 10 centimeters in diameter (Hedge, 1997; Akhani *et al.,* 2007). The flowers are in short spikes up to 6 centimeters long (en.wikipedia.org).

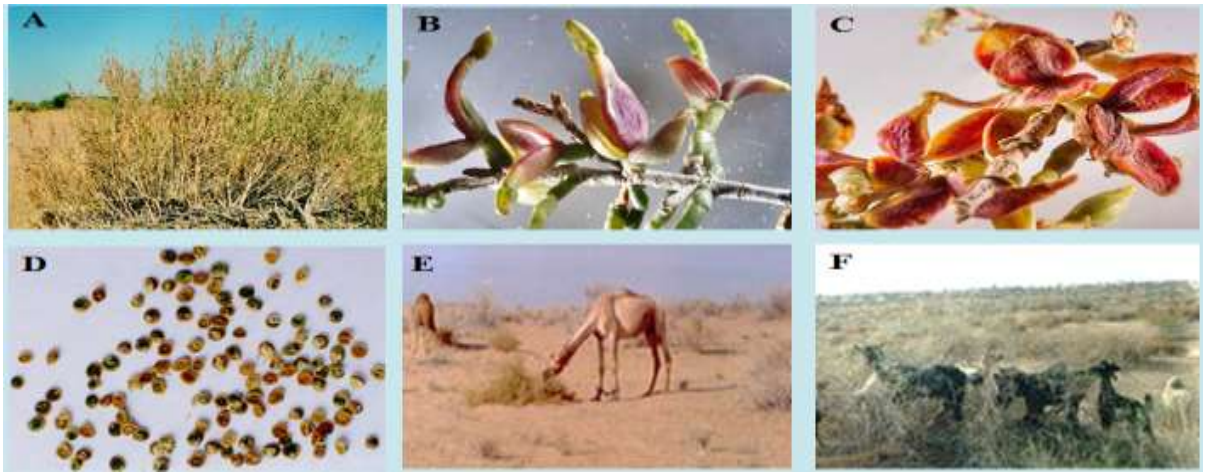

Fig. 1. A: Flowering stage of the *H. salicornicum* (Singh *et al.,* 2015); **B:** *H. salicornicum* branch with flower galls (bird-beak-shaped galls); **C:** Colorful fruit-like galls (flower, or bird-beak-shaped galls); **D:** *H. salicornicum* seeds*;* **E:** Camel grazing on *H. salicornicum* (Singh *et al.,* 2015); **F:** Goats and sheeps browsing on *H. salicornicum* (Singh *et al*., 2015). (www.asergeev.com*).*

H. salicornicum propagates primarily through seeds. Seeds remain viable for about 1 year (Singh *et al.,* 2015) with 50 % decrease in viability at room temperature after one year; small seeds lose their viability faster than larger seeds. The optimum epigeal germination is <20°C and germination is severely inhibited >30°C (Zaman *et al.,* 2006). Highest proportion of seedlings emerged from seeds placed on the surface and the greatest depth from which seedlings emerged was 20 mm (Brown and Al-Mazrooei, 2001). *Haloxylon* consist of about 25 species including *H.salicornicum* (AL-Sobeai *et al.,* 2015). In Saudi Arabia, only those species are found, namely *H. persicum* and *H. salicornicum* (AL-Sobeai *et al.,* 2015, Singh *et al.,* 2015). *H. salicornicum* is used as antidiabetes (Baldwin *et al*., 1995). as antiseptic and anti-inflammatory (Pyankov *et al.,* 2001). The plant is known locally as Rimth possessing medical importance and is widely distributed throughout the Kingdom. The phytochemical analysis of the aerial parts of *H. salicornicum* revealed the presence of alkaloids, cardiac glycosides, anthraquinones, flavonoids, saponins, coumarins, sterols, tannins, volatile oils and volatile bases (Baldwin *et al.,* 1995). *H. salicornicum* is a persistent tree with a great potential for restoration of degraded arid lands,

re-vegetation and promotes the biodiversity (Omar *et al.,* 2000). During October to November month the *H. salicornicum* is flowering, It can withstand environmental stresses and can be grown on marginal lands. Having good abiotic stresses (drought, high temperature and salinity) tolerance, it should be considered as a potential species adapted to anticipated climate change (AL-Sobeai *et al.,* 2015).

Relatively slight attention has been given to this plant in Saudi Arabia, which hinders its molecular genetic diversity studies and utilization of it is beneficial potential restoration of degraded arid lands. Besides summarizing information on taxonomy, nomenclature, distribution of *H. salicornicum* to motivate concentration in this species, *H. salicornicum* was selected for molecular genetic diversity studies for its mass distribution throughout Saudi Arabia. This study aims to draw extra consideration to this species as a potential multipurpose plants.

Materials and methods

Plant materials

In this study, samples of *H. salicornium* plant have been collected from the region around AL-Khormah city 200 km east to Taif city at the western Saudi Arabia. The samples were labeled, sealed in sterilized polythene bags, brought to the laboratory and were stored at -20°C till their use for DNA extraction.

Morphological identification

The morphology details of the plant height, number of branches, stem color, flower color, seed shape, seed moisture and seed weight were carefully recorded to determine the diversity for morphological characters.

DNA isolation

Total genomic DNA was extracted directly from plant samples. Each sample was ground separately in a pestle and the DNA were extracted with the standard protocol of the DNeasy Plant Mini Kit (QIAGEN). All samples of DNA were stored at -20°C until used.

Primer design

The following primers were designed after comparing numerous *H. salicornium* sequences in the Gen Bank database for amplification of a target regions,

The sequences were aligned with Clustal W followed by manual adjustments with a text editor; forward primer (ITS4) 5`- TCCTCCGCTTATTGATATGC-3`and the reverse primer (ITS5) 5`- GGAAGTAAAA GTCGTAACAAGG -3`, Primers were obtained in a lyophilized form and the reconstitution of the primers was attempted in nuclease-free water to prepare concentrated stocks of 100µM (according to their concentrations). Working solutions of 10µM were prepared by individual dilution of the primer stocks in nuclease free water.

PCR amplification, and sequencing analysis

A total volume of 25µL PCR mixture (1µL genomic DNA, 12.5µL PCR Master Mix, 0.5µL of each primer and 10.5 nuclease free water) was used. PCR amplification was carried out in a Techne thermocycler. The PCR conditions were 94°C for 5 min as initial denaturation step followed by 35 cycles of 94°C for 60 s denaturation, 56°C for 60 s annealing and 72°C for 60s extension, the final extension was at 72°C for 4 min. PCR products were run on 1% agarose gel containing ethidium bromide and visualized. The PCR products were sequenced using Taq Dye Deoxy Terminator Cycle Sequencing kit (Applied Biosystems, Gouda, The Netherlands) using the same two primers used in the PCR amplification. The obtained sequences were aligned and adjusted.

Data analyses

Six hundred ninety one nucleotides spanning ITS1, 5.8S and 26S genes from the nuclear DNA for the collected samples were sequenced in this study. Comparisons with sequences in the Gen Bank database were achieved in BLASTN searches at the National Center for Biotechnology Information site (www.ncbi.nlm.nih.gov). The sequenced data were multiple sequences aligned with their counterparts found in the Genbank database for *H. persicum* (FR775277.1), *Salsola kali* (AY514843.1), *Allenrolfea vaginata* (AY514828.1), *Bassia hirsuta* (AY514831.1), *Chenopodium album* (HE855664.1) and *Calicorema capitata* (AY514807.1).

The aligned data were used for phylogenetic analyses and the sequence for *Haloxylon salicornicum* was used for tree rooting and the tree were constructed. The gap-containing sites and unambiguous nucleotides were deleted so that 898bp were left for phylogenetic analyses. The phylogenetic analyses and multiple sequence alignment were conducted by using CLUSTALW software (www.genome.jp/tools/clustalw/).

Results and discussion

The amplified and sequenced ITS region, in this study, included the 3` end 40bp from 18S gene, the complete ITS1, 5.8S rDNA and ITS2 as well as 104bp from the 5` end of 26S gene. The sequenced fragment was aligned with its counterparts from the closely related species from *Haloxylon, Allenrolfea vaginata, Bassia hirsute, Chenopodium album and Calicorema capitate* genera found in the Genbank database.

H. salicornicum is an arid regions shrub species possessing multiple beneficial applications in food, fuel and medicine besides it is highly endangered. Very recent study (Snigh *et al.,* 2015) has surveyed its distribution, ecology, uses and diversity to stimulate interest to promote its domestication in arid lands. However, the molecular studies on this respect are very rare or absent.

Most of the published studies, regarding the genetic variability within *H. salicornicum*, have focused on data obtained from RAPD-PCR and/or ISSR (Al-Qurainy, 2007; AL-Salameen *et al.,* 2013; Meghwal *et al.,* 2014). Within the Saudi populations of this species (Al-Qurainy, 2007) has found that the genetic variability was higher within rather than between populations.

The results of the present study revealed that the *H. salicornicum* of Saudi Arabia desert has low level of genetic diversity when compared with its closely related taxa. The majority of RAPD variation in *Halyloxon salicornicum* was found within rather than between populations and Dahna has more variation than Thumama as estimated by genetic distance. This low variation between *H. salicornicum* and its six closely related taxa (Al-Qurainy, 2007), could be due to the controlled gene flow and small size of samples. When compared to present results, more or less similar ranges of genetic dissimilarities have also been reported in previous studies (Ali *et al.,* 2007; Abbas *et al.,* 2009). A number of studies have revealed lower levels of genetic diversity in many crops species (George *et al.,* 2006; Wang *et al.,* 2007; Tang *et al.,* 2007; Leegesse *et al.,* 2007). Fig. 2 and 3 shows a distance tree of the region corresponding to the mat K gene of the *H. salicornicum* and its six closely related taxa from the Gen Bank.

The present study; therefore, could be considered the first molecular investigation using sequence data for assessing the genetic relationships among *Haloxylon* tax on and its closely related taxa in Saudi Arabia. The molecular comparison was made between these samples collected from Saudi environment and that collected from the Gen bank (non-Saudi population). The compared data showed low genetic diversity when compared with its closely related taxa.

```
AY514843.1    CAATCCTCTTATTTACGATCAANATCTTTTGGAGTCCTTCTTGAACGAATCTATTTCTAC
AY514831.1    CAATCCTCTTATTTACGATCAACGTCTTTTGGAGCTCTTATTGAAAGAATCTATTTCTAC
FR775277.1    CAATCCTCTTATTTACGATCAACATCTTTTGGAGCTCTTCTTGAACGAATCTATTTCTAC
AY514828.1    CAATCCTCTTATTTACAATCAACATCTTTTGGAGCCCTTCTTGAACGAATCTATTTCTAC
AY514807.1    CAATGCTCTTATTTAAGATCAATATCTTTTGGAGCTCTTCTTGAACGAATCCATTTCTAC
HE855664.1    CAATCCTCTTATTTACGATCAACCTCTTTTGGAACCCTTATTGAACGAATTTTTTTCTAC
FR2016.1      ATATACT-TTATTCGATACAAACTTTTTTT-----TCTTGAAGAGCCACTATAATAATGA
              ** ** *****  * ** * ****   *** **  * *  * *

AY514843.1    GGAAAAGTCAAATATCTAGGAAAAAATTTTCCTAAGGATTTTAGGGTTATCCTATGGTTT
AY514831.1    GGAAAAGTAAAATATCTAGTCAAAAATTGGACGAAGGATTTTGGGGTTATCCTATGGTTT
FR775277.1    GGAAAAGTCAAATATCTAGGAAAAAAATTTCCTAAGTATTTTGGGGTTATCCTACGGTTT
AY514828.1    GGAAAGGTAAAATATCTAGTAAAAGCTTTTACCAAGGATTCTGGGGTTATCCTATGGTTG
AY514807.1    GTAAAGTTAAAATATCTAGTTAAAA------TTAAGGCTTTTGGGGTTATCCTATGGTTT
HE855664.1    GGAAAACTAAAATATCTAGTAAAAGTTTTTACTAAGGATTTTGGGGTTATCCTATGGCTT
FR2016.1      GAAAACTTTCTACATATACGTCCAAA-TCGATCAATAATCTCAGAATC-TGATAAATCGG
              * *** *  * ** **   *     **  *  * * * **

AY514843.1    TTCAAANAACCTTTTCTGCATTATGTTAGGTATCAAGGAACAATTCTTCTGGCTTCAAAA
AY514831.1    TTCAAAGAACCTTTTCCACATTATGTTAGGTATCAAGGAAATTGTTTTTTGGCTTCAAAA
FR775277.1    TTCAAAGAACCTTTCCCGCATTATGTTAGGTATCAAGGAAAAATTTTTCTGGCTTCAAAA
AY514828.1    TTCAAAGAACCTTTTCCGCATTATGTTAGGTATCAAAAAAAATCAATTCTGGCTTCAAAA
AY514807.1    TTCAAAGAACCTTTCCCGCATTATGTTAGGTATCAAGGAAAATCCCTTCTGGCTTCAAAA
```

```
HE855664.1    TTCAGAGAACCTTTCCCGCATTATGTTAGGTATCAAGGAAAATCAATTCTAGCCTCAAAA
FR2016.1      TCCAGACCGACTTACTAATAGGATGTCCTAATACGTTACAAAATTTTTCTTTAGCCAACG
              * ** *  ***    * ****     *   *    ** *   ***

AY514843.1    GGGACATCTCTTCTGATACATAAATGGAAATATTATCTTATCTATTTCTGGCAATGCGAT
AY514831.1    GGTGCATCTCTTCTGATACATAAATGGAAATTTTATTTTATATATTTCTGGCAATGTCAT
FR775277.1    GGTGCATCTATTCTGATACATAAATGGAAATATTATCTTATCTATTTCTGGCAATGTAAT
AY514828.1    GGTACATCTCTTCTGATGCATAAATGGAAATATTACCTTATCCATTTCTGGCAATGTCAT
AY514807.1    GGGACATCTCTTCTGATGCATAAATGGAAATATTACTTTATCTATTTCTGGCAATGTTAT
HE855664.1    GGGACATCTCTTTTGATGCATAAATGGAAAAATTACCTTATCCATTTCTGGCAATGTAAT
FR2016.1      -----ATCCAACCAGAGGAAGAATTGGAACTATGGTATCAAAT-TTCTTAATAATATTCT
                 ***    **  * ** ***** *  *  * *  ** *  ***  *

AY514843.1    TTTTCTGTGTGGNCTCAACCAAGAA--GAATCTATATCAATCGATTATCAAACCATTCGC
AY514831.1    TTTTCTGTGTGGTCTCAGCCAAGAA--GAATCTATATCAATCGATTATCAAACCATTCTC
FR775277.1    TTTTCTGTGTGGTCTCAAACAAGAA--GAATCTATATCAATCGATTATCAAACCAGCCCC
AY514828.1    TTTTCTGTGTGGCCTCAACCAAGAA--AAATCTATATCAATCGATTATCAAACCATTCCC
AY514807.1    TTTTCTGTGTGGTCTCAACCAAGAA--GAATCTATATCAATCAATTATCAAACTATTCCC
HE855664.1    TTTTCTGTGTGGTCTCAACCAAAAA--GACTCTATATCAATCGATTATCAAACCATTCCT
FR2016.1      CTATTAGAAATGAATTTTCTAACATTTGACTCCGTATTACTGAAGAATTTATTCGCACAC
              * * *  * *   ***  *** ******* * * ** *    *

AY514843.1    TCGACTTTATGGGTTTTCTTTCAAGTGTACGAATGAATTCTTCAGTAATACGGAGTCAAA
AY514831.1    TCGACTTTATGGGTTTTCTTTCAAGTGTGCGACTGAATTCTTCAGTAATACGGAGTCAAA
FR775277.1    TCGACTTTATGGGTTTTCTTTCAAGTGTGCGAATAAATTCTTCAGTAATACGGAGTCAAA
AY514828.1    TTGACTTTATGGGTTTTCTTTCAAGTGTGCGACTCAATTCTTCAGTAATACGGAGTCAAA
AY514807.1    TCGACTTTATGGGTTTTCTTTCAAGTGTGCGATTCAATTCGTCAGTAATACGGAGTCAAA
HE855664.1    TAGACTTTATGGGTTTTCTTTCAAATGTGCGACTCAATTCTTCAGTAATACGGAGTCAAA
FR2016.1      TTGAAAGAAAACCCATAAAGTCGAGGGGCTGGTTTGATAATCGATTGATATAGATTCTTC
              * **  *   *  *** * * * **   * **** ** **

AY514843.1    T-GTCACAAAATTCATTTCTAATAGAGAATATTATTAAGAA-GTTTGATNCCATAGTTCC
AY514831.1    T-GTTAGAAAATTCATTTCTAATAGAGAATATGATTAAGAA-GTTTGATACCATAGTTCC
FR775277.1    T-GTTAGAAAATTCATTTCTAATAGAGAATATTATTAAGAA-ATTTGATACCATAGTTCC
AY514828.1    T-GTTAGAAAATTCATTTCTAATAGCAAATATTATTAAGAA-GTTTGATACTATAGTTCC
AY514807.1    T-GTTAGAAAATTCATTTCTATTAGAGAATATTCGTAAGAA-GTTCGATACCATAGTTCC
HE855664.1    T-GTTCGAAAATGCATTTCTAATAGAGAATATTATTAAGAA-ATTCGATGCCACAGTTCC
FR2016.1      TTGTTTGAGACCACA---CAGAAAAATTACATTGCCAGAAATAGATAATATAATATTTCC
              * **  * *  **  *  *  * **   * **    **  * ******

AY514843.1    AATTATTCCTCTGGTTGGAT-CGTTGGCTAAAGCGAAATTTTGTAACGTATTAGGACCTC
AY514831.1    AATTATTCCTCTGGTTGGGT-CATTGGCTAAAGAAAAATTTTGTAACGTATTAGGGCATC
FR775277.1    AATTCTTCCTCTGGTTGGAT-CGTTGGCTAAAGAAAAATTTTGTAACGTATTAGGACATC
AY514828.1    AATTATTCCTCTGGTTGGAT-CGTTGGCTAAAGCGAAATTTTGTAGCGTATTAGGACATC
AY514807.1    AATTATTCCTCTAGTTGGCT-CGTTGGCTAAAGAGAAATTTTGTACCGTATTAGGACATC
HE855664.1    AATTATTCCTCTGGTTGGAT-CGTTGGCTAAAGCGAAATTTTGTAATGTATTAGGGCATC
FR2016.1      ATTTATGTATCAGAATAGATGCACCTTTTGAAGCCAGA----AAAATTTTTCCTTGATAC
              * *** *  **  * * * *    * *** * *    *  * *     *

AY514843.1    CTATTAGTAAGTCGGCCTGGACCGATTTATCAGATTCGGAGATTATTGATCGATTTGGAC
AY514831.1    CTATTAGTAAGTCTGTCTGGACTGATTTATCAGATTCTGATATTATTGATCGATTTACAC
FR775277.1    CTATTAGTAAGTCGGTCTGGACCGATTTATCAGATTCTGAGATTATTGATCGATTTGGAC
AY514828.1    CTATTAGTAAGTCGGTCTGGACCGATTTATCAGATTCTGAGATTATTGATCGATTTGGAC
AY514807.1    CTGGTAGTAAGTCGGTTTGGACCGATTTATCTGATTCTGACATTATTGACCGATTTGGAC
HE855664.1    CCGTTAGTAAGTCGGTCTGGACCGATTTATCCGATTCTGATATTATTGATCGATTTGGAC
FR2016.1      CTAACA-TAATGCGG----GAAAGGTTCTTTGAA-----AAACCGTAGGATAACCCCAAA
              *  * *** * *  ** * ** *  *  *  ** **  *  *  *

AY514843.1    ATATATGCAGAAAGGTTTCTCATTANNATAGTGGTTCTTCAAGAAAAAAAAGTTTGTATC
AY514831.1    GTATATGCAGAAAGTTTTCTCATTATTATAGCGGTTCTTCAAGAAAGAAAAGTTTGTATC
FR775277.1    GTATATGTAGAAAGTTTTCTCATTATTATAGTGGCTCTTCAAGAAAAAAAAGTTTGTATC
AY514828.1    GTATATGCAGAAAGCTTTCTCATTATTATAGTGGCTCTTCAAGAAAAAAAAGTTTGTATC
AY514807.1    GTATCTGCAGAAATCTTTCTCATTATTATAGTGGCTCCTCAAGAAAAAAAAGTTTGTATC
HE855664.1    GTATATGTAGAACTATTTTTCATTATTATAGTGGCTCTTCAAGAAAAAAAAGTTTGTATC
FR2016.1      ATACTTA-GGAAATTTTTTTCCTAGATATT-TGACTTTTCCGTAGAAAAAAATTCGTTCA
              ** *  ***  *** ** *  **  * * **  * * ***** ** **

AY514843.1    GAATAAAGTATATACTTCGACTTTCTTGTGCTAGAACTTTGTCTCGT-----AAACACAA
AY514831.1    GAATAAAGTATATACTTCGACTTTCTTGTGCGAGAACTTTGTCTCGT-----AAACACAA
FR775277.1    GAATAAAGTATATACTTCGACTTTCTTGTGCTAGAACTTTGTCTCGT-----AAACATAA
AY514828.1    GAATAAAGTATATACTTCGACTTTCTTGTGCTAGAACTTTGTCTCGT-----AAACACAA
AY514807.1    GAATAAAGTATATACTTCGACTTTCTTGTGCTAGAACTTTGTCTCGT-----AAACACAA
HE855664.1    GAATAAAGTATATACTTCGATTTTCTTGTGCTAGAACTTTGTCTCGT-----AAACACAA
FR2016.1      AGAAGGGCTCCAAAAGATGTTGATCGTAAATACGAGGATTGGTTAAGGAGAAAAACAAAA
              *   * * *  *  ***    **  *** *     ***** **

AY514843.1    AAGTACT-GTACGTGCTTTTTTGAAAA--GATTAGGTTCAGAATTTTTGGAAGAATTTTT
AY514831.1    AAGTACT-GTACGTGCTTTTTTGAAAA--AATTAGGTTCCGAATTTTTGGAAGAATTTTT
FR775277.1    AAGTACT-GTACGGGCTTTTTTGAAAA--GATTAGGTTCAGAATTTTTGGAAGAATTTTT
AY514828.1    AAGTACT-GTACGTGCTTTTTTGAAAA--GATTAGGTTCAGAATTTTTGGAAGAGTTTTT
AY514807.1    AAGTACT-GTACGTGCTTTTTTGAAAA--GATTAGGTTCAGAATTTTTGGAAGAATTCTT
HE855664.1    AAGTACT-GTACGTGCTTTTTTGAAAA--GATTAGGTTCAGAATTTTTAGAAGAATTCTT
FR2016.1      AGGGATTCGTATTCCCATACATGGAAATTATATAGGAACAAGAATAATCTTTGATTCCTT
              * * * ****  **  ** ****   **** *  * * *  *** **

AY514843.1    TACGGAGGAAGAAAAAGTTCTTTCTTTGACCTTACCCAGGGATTCTTCGACCTCATGGGG
AY514831.1    TACGGAGGAAGAAAAAGTTATTTCTTTGGTCTTACCCAGGGATTCTTCTACCTCATGGGA
FR775277.1    TACGGAGGAAGAAAAAGTTCTTTCTTTGATCTTGCCCAGGGATTCTTCTACCTCATGGGG
AY514828.1    TACGGGGGAAGAAAANGTTCTTTCCTTGATCTTACCTAGGGACTCTTCTACCTCACGGGG
AY514807.1    TACAGAGGAAGAAAAAGCTCTTTCTTTGATCTTACCAAGGGATTCTTCTACTTCAGGGAG
HE855664.1    TACAGAGGACGAAAAAGTTCTTTCTTTGATCTTACCAAGAGATTCTTCTATCTCACGGGG
FR2016.1      TTTGAAAAAAAGGAAATGAATTCTTTTGGAGTAAT---AAGACTATTCCAATT-ACGATA
              *    *  **   **  *** *     ** **** * * **  *

AY514843.1    ATTCTATATATATAGAAGGCGTATTTGGTATTTGGATGTTATTTGTATCCATAATTTGAC
AY514831.1    ATTCTATATA------GGACGTATTTGGTATTTTGATATTCTTTTTATCCATAATTTGAC
FR775277.1    ATTATATAGA------GGGCGTATTTGGTATTTGGATATTATTTGTATCCATAATTTGAC
AY514828.1    ATTATATAGC------GGGCGTATTTGGTATTTGGATATTATTTGTATTCATAATTTCAT
```

```
AY514807.1    ATTATATAGA------GGGTGTGTTTGGTATTTGGATATTATTTGTATTCATAATTTAGC
HE855664.1     ATTCTATAGA------GGGCGCATTTGGTATTTGGATATTATTTGTATCCATAATTTGTC
FR2016.1      CTCATAAAGA---------AATAATCGTAAT--AAATGCAAAGAAGAGGCATCTTTCAAC
               * *** *          ** **  **      * *** **
AY514843.1    CAACGATGAATGA-----TTTGTTATGAGACTGTTGAAATGAAATGGAAATTTTATCTAA
AY514831.1    CAACGATGAATGA-----TTTGTTATGAGACTGTTGAAATGAAATCGAAATTTTACCTAA
FR775277.1    CAACGATGAATGA-----TTTGTTATAAGACTGTTGAAATTAAATGGAAATTTTACCTAA
AY514828.1    CAACGATGAATGAAATGATTGGTTATGAGACTGTTGAAATGAAATGGAAATTCTACCTAA
AY514807.1    TAATCATCAATGA---TTTTAGTTATAAGACTATCGAAATGAA----------------
HE855664.1    CAACGATGAATGA-----TTGATTATGAGACTGTCTAAATGAAATTGAAATTCTATCTAA
FR2016.1      CAACAGCGGAGAG-----TTTGAATCAAGATT-TCTAGATGGACGGGGTAGGGTATTAAT
               **    *     **    *** * * *** *
AY514843.1    NTG---AATGNATGGATAAGATAAAAGANAATTCATT----TCTATACTGAA
AY514831.1    ATG---AA---ACGGATAAGATAAAACAAAATTCATTCGTTTCTATACTGAA
FR775277.1    ATG---AATGAAGGGATAAGATAAAA-AAAATTCATTCATTTCTAGACTGAA
AY514828.1    ATG---AATGGATGGATAAGATAAAACAAAATTTATTCATTTATATACTGAA
AY514807.1    -TG---AA----GGGATAAGATAAAA---AATTCATTTATTTTTATACTGAA
HE855664.1    ACG---AATG-GGGGATAAGAAAAAAATTCATTTTTCTAC-TTTCTACTGAA
FR2016.1      ATATCTAACACATAATTTAGATGTAA--GAATTTGTCC---TCTAAAAAAGG
 **       * ***  **   *** *    * *  *
```

Fig. 2. Aligned sequences of mat K gene for *Haloxylon* taxon [FR2016.1] and its closely related taxa. Each taxon is referred to by its accession number on the left column. Dashes denote gabs and stars refer to the sequence identity. [*H. persicum* (FR775277.1), *Salsola kali* (AY514843.1), *Allenrolfea vaginata* (AY514828.1), *Bassia hirsuta* (AY514831.1), *Chenopodium album* (HE855664.1) and *Calicorema capitata* (AY514807.1)].

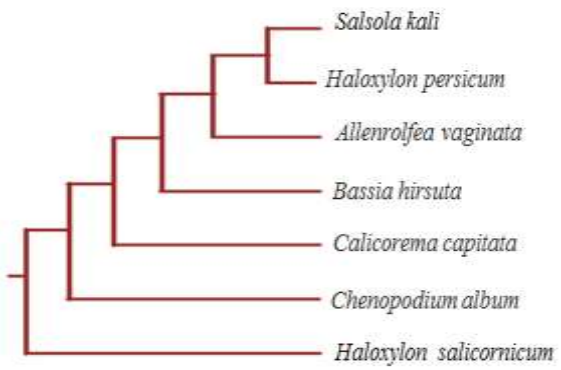

Fig. 3. The Tree UPGMA dendrogram based Jaccard's similarity showing genetic relationships among *Haloxylon* taxon and its closely related taxa.

Conclusion

Haloxylon salicornicum is an important source of feed, bioactive phytochemical implication, a persistent tree with a great potential for restoration of degraded arid lands, re-vegetation and promotes the biodiversity *H. salicornicum* is genetically relatively identical and to some extent genetic difference between its closely related taxa, the environmental and geographic factors might be playing a role in this influencing similarity. More intensive molecular studies are recommended to profound further understanding of genetic similarity among populations of *Halyloxon salicornicum* and its closely related taxa.

Acknowledgment

The author is extremely grateful to the Dr. Maaweya E. H. Awadalla. Department Botany and Microbiology, College of Science, King Saud University, and Dr. Sayed A. M. Amer. Department of Biology, Faculty of Science, Taif University. Saudi Arabia, for their assistance and advice in the collection of the plant material used in this study.

References

Abbas S, Farhatullah J, Marwat KB, Khan IA. Munir I. 2009. Molecular analysis of genetic diversity in Brassica species. Pak. J. Bot **41,** 167-176.

Abdallah EM, El-Ghazali GE. 2013. Screening for antimicrobial activity of some plants from Saudi folk medicine. Glob J Res Med Plants Indigen Med **2,** 189-197.

Ahmad M, Eram S. 2011. Hepatoprotective studies on *Haloxylon salicornicum*: a plant from Cholistan Desert. Pak J Pharm Sci **24,** 377-382.

Ajabnoor MA, Al-Yahya MA, Tariq M, Jayyab AA. 1984. Antidiabetic activity of *Hammada salicornica*," Fitoterapia, vol. **55,** no. 2, pp. 107-109.

Akhani, Hossein, Edward, Gerald, Roalson, Eric H. 2007. Diversification of the Old World Salsoleae S.L. (Chenopodiaceae): Molecular Phylogenetic Analysis of Nuclear and Chloroplast Data Sets and a Revised Classification". International Journal of Plant Sciences 168(6), 931-956.

Ali W, Munir I, Ahmad, MA, Muhammad W, Ahmed, Durrishahwar N, Ali S. Swati ZA, 2007. Molecular characterization of some local and exotic *Brassica juncea* germplasm. Afr. J. Biotechnol **6,** 1634-1638.

AL-Sobeai, Sanad M, Alamer, Khalid H, Amer, Sayed AM. 2015. A preliminary molecular variability within *Haloxylon salicornium* accessions growing in Saudi Arabia . Plant Omics, Vol. **8, No. 6,** 604-608.

Ashraf MA, Karamat M, Shahnaz K, Abdul W, Ismail Y. 2012 . Study of chemical and mineral constituents of *Haloxylon salicornicum* collected from Cholistan Desert, Bahawalpur, Pakistan," Wlfenia Journal, vol. **19, no. 10,** pp. 306-327.

Baldwin BG, Sanderson MJ, Porter JM, Wojciechowski MF, Campbell CS, Donoghue MJ. 1995. The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. Ann. Missouri. Bot. Gard **82,** 247-277.

Boulos L. 1999. Flora of Egypt, vol. 1, Al-Hadra, Cairo, Egypt.

Brown G, Al-Mazrooei S. 2001. Germination ecology of *Haloxylon salicornicum* from Kuwait. Bot Jahrb Syst Pflanzengesch Pflanzengeogr **123,** 235-247.

Dagla HR, Shekhawat NS. 2005. In vitro multiplication of *Haloxylon recurvum* (Moq.)- a plant for saline soil reclamation, J Plant Biol **7(3),** 155-160.

El-Shazly AM, Dora G, Wink M. 2005. Alkaloids of *Haloxylon salicornicum* (Moq.) Bunge ex Boiss. (Chenopodiaceae)," Pharmazie, vol **60, no. 12,** pp. 949-952.

Ghazali GE, Khalifa S, Gameel AS, Abdallah EM. 2010. Traditional medicinal plants indigenous to Al-Rass province, Saudi Arabia. J Med Plant Res **4,** 2680-2683.

Gibbons S, Denny BJ, Ali-Amine S, *et al.* 2000. NMR spectroscopy, X-ray crystallographic, and molecular modeling studies on a new pyranone from *Haloxylon salicornicum*," Journal of Natural Products, vol **63, no. 6,** pp. 839-840.

Hedge IC. 1997. "Haloxylon". In Rechinger, Karl Heinz et al. Flora Iranica Bd. 172, Chenopodiaceae. Graz: Akad. Druck. pp. 315-326. www.asergeev.com/pictures/archives/compress/2015/1578/17.htm

Leegesse BW, Myburg AA, Pixley KV, Botha M. 2007. Genetic diversity of African maize inbred lines revealed by SSR markers. Hereditas **144,** 10-17.

Omar S, Al-Mutawa Y, Zaman S. 2000. Vegetation of Kuwait. Kuwait Institute for Scientic research, Al-Assriya Printing press, publishing and distribution company, Kuwait pp. 159.

Pyankov VI, Artyusheva EG, Edwards GE, Black CC, JR, Soltis PS. 2001 Phylogenetic analysis of tribe Salsoleae (Chenopodiaceae) based on ribosomal ITS sequences: Implications for the evolution of photosynthesis types. Am. J. Bot **88(7),** 1189-1198.

Saleem N. 2012. An ethno-pharmacological study of Egyptian Bedouin women's knowledge of medicinal plants. 2015. Ph.D. Thesis, University of Strathclyde, Glasgow UK.

Singh JP, Rathore VS, Roy MM. 2015. Notes about *Haloxylon salicornicum* (Moq.) Bunge ex Boiss., a promising shrub for arid regions. Genet Resour Crop Evol **62,** 451-463.

Soliman GA, Donia Ael R, Awaad AS, Algasoumi SI, Yusufoglu H. 2012. Effects of *Emex spinosa*, *Leptadeniapyro technica*, *Haloxylon salicornicum* and *Ochradenus baccatus* extracts on the reproductive organs of male rats. Pharm Biol **50,**105-112.

Tackholm V. 1974. Students' Flora of Egypt, Cairo University Press, Cairo, Egypt, 2nd edition.

Tang RG, Gao L, He Z, Han S, Shan, Zhong R, Zhou C, Jiang J, Li Y. Zhuang W. 2007. Genetic diversity in cultivated groundnut based on SSR markers. J. Genet. Genom **34,** 449-459.

Wang HY, Wei YM, Yan ZH, Zheng YL. 2007. EST-SSR DNA polymorphism in durum wheat (*Triticum durum* L.) collections. J. Appl. Genet **48,** 35-42.

Yokoo Y, Kohda H, Kusumoto A, *et al.* 1999. Isolation from beer and structural determination of a potent stimulant of gastrin release," Alcohol and Alcoholism, vol **34, no. 2,** pp. 161-168.

Zaman S, Padmesh S, Bhat NR, Tawfiq H. 2006. Germination of some Kuwait's native plants under saline conditions. Am Eurasian J Agric Environ Sci **1(2),** 146-148.