Molecular characterization of *Haloxylon salicornicum* Moq. in Saudi Arabia

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**Article info**

**Abstract**

The purpose of writing this paper is to study the genetic variability between and within different *Haloxylon salicornicum* populations in different regions of Saudi Arabia Kingdom, using the determination of genetic fingerprint method by Inter Simple Sequence Repeat (ISSR). Because this plant highly vulnerable to depletion by humans in all places of existence, it is an economically valuable plant where raft is an important pastoral resource in central and northern Arabia. It also has multiple medical uses. It is a plant that can withstand abiotic stresses such as drought and high temperature, making it suitable for cultivation in marginal lands in arid areas. All of the above was a catalyst for plant characterization using a number of *Haloxylon salicornicum* samples, collected from different regions of Saudi Arabia to find out the genetic variation of species, genetic diversity in knowing the plant community is an essential step towards the design of programs for plant breeding as well as preserved from extinction. This was done using the Inter Simple Sequence Repeat (ISSR). The results showed there were significant differences and molecular differences between plant samples. The average polymorphism between the genetic inputs of the studied *Haloxylon salicornicum* samples was 53.7%, and this percentage of genetic variability is significant for progress in growth and plant regeneration in the face of unfair practices against it, in addition to adverse environmental conditions in most years. As evidenced by the percentage of matrix similarity. The ISSR results indicate that the genotype between five different regions genotypes ranged from 0.365 to 0.527, indicating that *Haloxylon salicornicum* is a local plant capable of surviving and adapting to the environmental conditions in Saudi Arabia through the positive change in the genetic makeup of this species.

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**0. Introduction**

*Haloxylon salicornicum* Moq. (Amaranthaceae). One of economic plants. It is an important pastoral source in the central and northern Arabian Peninsula (Chaudhary and Al-Jowaid, 1999; Taia and El-Ghanem, 2001) Used as firewood (Shahid et al., 2000). It is one of the five main woods species in Saudi Arabia which include *Haloxylon salicornicum*, *acacia tortilis*, *Haloxylon salicornicum*, *Artica Calligonum comosum* and *Anabasis articulate* (Abdul Aziz Mohammed and Qarawi, 1996). Undoubtedly, the effects of logging are not limited to damage to the species, but also to the disruption of the structure of the vegetative society (Thalen, 1979). In the worst case, soil erosion, sand drain and destruction of natural resources occur (Abdul Aziz Mohammed and Qarawi, 1996). *Haloxylon salicornicum* is of a great environmental importance in arid and semi-arid ecosystems in Saudi Arabia, where it helps to stabilize and not degrade soil, stabilize sand dunes and reduce wind intensity (Zaman et al., 2006). It is also used as an antiseptic, wound dresser and rheumatic anti-inflammatory (Nazia et al., 2010). Currently, *Haloxylon salicornicum* bushes are deteriorating, decreasing their numbers and facing major environmental problems such as desertification and soil degradation, which puts them at risk of extinction if they do not pay attention to protecting and preserving them in their natural places of spread. Due the economic and environmental importance of the plant, it deserves further studies where it is still needed for further studies to know the self-environment and how to thrive and know the factors that affect Germination of seeds and growth of seedlings in order to preserve and benefit from it. Study of variance and genetic diversity in the plant community is an essential step towards designing plant propagation programs as well as preserving them from extinction.
The genetic diversity of a society forms the basis of its ability to cope with environmental conditions. In society leads to increased zygote symmetry in community samples characterized by lack of individual fitness (Ellstrand and Elam, 1993). The greater genetic variation of the organism the neighborhood has increased its ability to develop and natural selection and to cope with the surrounding conditions which may be unsuitable for its growth and reproduction (Tarabain et al., 2013).

1. Materials and method

1.1. Collect samples

Seeds of *Haloxylon salicornicum* were collected from different regions of Saudi Arabia (Althumamah “Th”, Diriyah “D”, Tabuk “T”, Jouf “J” and Hail “H”) Table 1 to determine the genetic variation of *Haloxylon salicornicum*. Seeds were collected through the National Center for Agriculture and Livestock Research, Ministry of Environment, Water and Agriculture, Riyadh, Saudi Arabia, https://www.naarrc.sa/ except Riyadh region seeds which were collected by the researcher. Seeds were given symbols indicating the area which they belong to facilitate calculation of their genetic analysis.

2. DNA extraction

Experiments were conducted in the laboratories of King Saud University, Genomic DNA extraction and purification were performed according DNAeasy Plant Mini Kit, Germany; Cat # 69104) from Qiagen following the instructions of the manufacturer. DNA quality was checked by electrophoresis in an Agarose gel 1% and quantified using NanoDrop 2000c Spectrophotometer, Thermo scientific company.

3. ISSR analysis

A group of 24 ISSR random primers were used (supplied by Thermo Fisher) sequences and names of the primers are illustrated in Table 2. PCR amplifications were performed by Peltier Thermal...
Cycler PTC-200 (MJ Research) step at 95 °C for 1 min, followed by 35 cycles of nature change at 94 °C for 30 secs, hardening temperature up to 50 °C for 30 sec and elongation at 72 °C for 2 min, finally, an additional stretch was used for 10 min at 72 °C.

4. Agarose gel electrophoresis

After DNA amplification process, are separated on a gel agarose 1%, is set up e adding 1 g of pure agarose to 100 ml of 1X TBE Buffer and heated in the microwave one minute to about until completely dissolved agarose, and then cooled to 70 °C and added 4 µl of Ethidium Bromide 10 mg/ml. The agarose solution is poured into the gel cast and the comb is placed in its proper place. It is left for 30 min to an hour at room temperature. It can be placed in the refrigerator to harden the gel at a shorter time.

5. Statistical analysis

The results were statistically analyzed by recording present or absent band (1, 0) of each samples. The class genetic relationship and similarities were calculated and Dendrogram was found among plant samples using Unweighted Pair Group Mean Arithmetic average (UPGMA) to generate a matrix via the using Statistica program Multivariate Statistical Package (MVSP 3.22). This matrix was used to calculate similarity/genetic distance jaccard’s coefficient.

6. Results

Products from amplification of ISSR primers are listed in Table 3 in terms of the total bands, polymorphic and monomorphic bands and the percentage of Polymorphism. 22 from 24 primers which gave the best amplification results with the entire sample DNA. final ISSR-PCR analysis show up 125 clearly identifiable bands Figs. 1–4, 59 of them have identical genetic bundles, while 66 appear to be different. And percentage of polymorphism was was 53.7%. Fig. 5 represents dendrogram obtained by using Jaccard UPGMA dendrogram coefficient, on the two main groups. First group consists of one subgroup (H) with a similarity of 36% with the second group which in turn includes two subgroups with similarity 49% between samples of Al-Jawf (J) and Tabuk (T). The second group consists of two subgroups also with similarity 53% between sampels of Diriyah (D) and Thumama (Th).
7. Discussion

In the beginning the current study, as found by the only researchers of its kind in the Arab region, is the study of the genetic variability of *H. Salicornicum* ash, except for a study (Al-Qurainy, 2007). He studied genetic distances between individuals within the same group and between two groups of *H. Salicornicum* in the areas of garbage and denuding in Saudi Arabia using RAPD technology. The results of the DNA obtained during this study using ISSR technique were able to distinguish between the patterns of heredity well. Where the average polymorphism of genetic variation between species was 53.7%. This ratio is an approach to what reached (Al-Samal et al., 2013) which studied the genetic variation between *H. salicornicum* samples collected from nine desert sites of Kuwait where the rate of genetic diversity was 47%. Thus, this variation is likely to be caused by different genotypes that may reach the species level (Al-Walaibi, 2013). It is may be due to environments variation in the environmental and climatic conditions in terms of temperature, rain, wind and terrain This is consistent with the findings of (Kumar and De Britto, 2011; Besher et al., 2014). By studying the genetic relationship between the genotypes studied by applying the similarity matrix, the higher values of this matrix indicate the existence of genetic convergence. It was noted that the degree of genetic kinship between the convergent genotypes in the geographical location, as in the samples collected from the province of Diriyah (D) and Thumama Park (E), as well as the proportion of genetic similarity between the samples collected from Tabuk and Al-Jawf due to the convergence of their geographical locations, however. Like most studies in this area such as study, which indicates the existence of a genetic variation between them and large The results of this study can be correlated with the geographical distribution of plants and some environmental characteristics of collection sites. This result is consistent with studies of (Al-Samman et al., 2013; Sarwat and Srivastava, 2008; Besher et al., 2014; Baktash and Al-Hameed, 2015), They pointed out that the single-stranded inputs in the Dendrogram tree are genetically similar and that cluster analysis is one of the best mechanisms for determining the genetic relationships of endemic plants for different regions. If we look at the isolation of the (H) cluster from the rest of the clusters and record it with the highest genetic variation with the rest of the genetic inputs, the variation is likely to be the environmental adaptation adopted by the plant to survive in a somewhat different environment (mountain environment). Studied Which led to the possibility of genetic mutations contributed to the differentiation of this genetic input from the rest of

Fig. 3. PCR-ISSR profile for primers (13–18) with all samples collected from different region of saudi arabia kingdom, Th: Thumama, D: Diriyah , T: Tabuk, J: AL-Jawf, H: Hail.
the inputs used in the study and this result is consistent with what I mentioned (Shuaib et al., 2010). As a final result, *Haloxylon salicornicum* can be said to have high genetic diversity, that increased genetic diversity of plant populations leads to increased plant adaptability and resilience to different environmental conditions and even propagation, this is consistent with (Besher et al., 2014; Hegazi et al., 2011; Russell, 2002). This study also showed that use of ISSR technique was able to distinguish between genotypes well where it comes from the contrast ratio between the genotypes.

### 8. Conclusion

This study also showed that use of ISSR technique was able to distinguish between genotypes well where it comes from the contrast ratio between the genotypes is 53.7%, this percentage is almost accepted in growth processing, and renewal of the plant under unsuitable conditions in most of the time. As evidenced by the percentage of matrix similarity, the results of ISSR genetic distance between the genotypes are ranged from 0.365 to 0.527. This indicates the ability of *Haloxylon salicornicum* to face the around condi-

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**Fig. 4.** PCR-ISSR profile for primers (19–24) with all samples collected from different region of saudi arabia kingdom, Th: Thumama, D: Diriyah, T: Tabuk, J: Al -Jawf, H: Hail.

**Fig. 5.** Dendrogram of UPGMA illustrate the relationships among the studied samples of *Haloxylon salicornicum* Ward's hierarchical cluster analysis based on distance jaccard's coefficient matrix.

**Table 1**

| Sample code | Place of collection | Localities |
|-------------|---------------------|------------|
| Th          | Wadi Althumamah     | N24°39"E46°44' |
| D           | Diriyah             | N29°49"E39°52' |
| T           | Tabuk               | N28°23"E36°32' |
| J           | Jouf                | N30°31"E38°12' |
| H           | Hail                | N27°33"E41°40' |

This study also showed that use of ISSR technique was able to distinguish between genotypes well where it comes from the contrast ratio between the genotypes.
The authors extended their appreciation to Deanship of Scientific Research at King Saud University for funding the work through the research group project No. RG-1435-086.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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