Kuwait J. Sci. **Vol. 40 (1) June 2013** pp. 149-164, 2013

Assessment of genetic diversity of *Haloxylon salicornicum* genotypes, a native plant of Kuwait

F. AL-SALAMEEN*, H. AL-HASHASH*, AND S AL-AMAD*

**Biotechnology Department, Kuwait Institute for Scientific Research, P.O. Box 24885, 13109 Safat, Kuwait; Phone: 2498-9157; email: fslamian@kisr.edu.kw*

ABSTRACT

Haloxylon salicornicum is one of the most important native desert flora scattered in Kuwait. Despite its ecological and economical importance, *H. salicornicum* is endangered by severe loss. Unless great attention is paid towards its protection and documentation, it faces the danger of extinction. Due to lack of information on the genetic diversity of *H. salicornicum,* this study attempts to analyse samples collected from nine representative locations from the Kuwait desert. Diversity analysis was performed using RAPD and ISSR technique. Twenty five RAPD and 24 ISSR primers produced 946 and 1016 bands respectively. RAPD produced a relatively higher proportion of polymorphic bands (49.4%) compared with ISSR (47%). However, Mantel test shows a very good degree of fit (r = 0.96) between RAPDs and ISSRs data. Thus, the data of both marker systems were combined to produce single dendrogram. This showed that samples collected from regions 4, 5, 7, 8A, and 8B, are the most related (PDV = 0.13- 0.15), whereas those collected from regions 2 and 6 are the most genetically similar (PDV = 0.28- 0.25) respectively.

Keywords: Genetic diversity, *H. salicornicum*, RAPD, ISSR.

INTRODUCTION

The native vegetation of Kuwait is unique with diverse species of desert plants that are adapted to the harsh climate and ecological system. The species that exist in this ecosystem have enormous scientific value because they offer a valuable genetic pool for drought, heat and salt-tolerance and plant material for research (Omar & Bhat, 2008). In addition, these plants have potential for use in phyto-remediation, ornamented landscaping and erosion prevention. Unfortunately the native plant biodiversity of Arabian Peninsula is being rapidly depleted in recent years. Over 90% of the total land area now suffers from some form of desertification, and 44% is severely or very severely degraded (Ghazanfar & Fisher, 1998). Recently, Kuwait has suffered a severe loss of its native desert flora and species are facing the danger of extinction. In addition, serious damage was inflicted on the natural environment during the

Iraqi invasion of Kuwait during 1990 (Abou el-Nil *et al*., 2000; Omar & Bhat 2008). Furthermore, heavy pollution with crude oil is a serious threat to wildlife (Karrar *et al.,* 1991), and causes massive degradation of ground cover and loss of flora and fauna. Therefore, there is an urgent need to conserve the flora of Kuwait for future generation. The understanding of genetic variation within and among population is essential for the establishment of effective and efficient conservation practice for native desert plants. Recently, Kuwait has embarked on a huge restoration plan to enhance the greenery of Kuwait, including a major plan for restoration of several species such as *Salicornicum* community (Omar & Bhat, 2008). For this reason, assessment of genetic variation within and among population of Kuwaiti desert plant species is necessary for formulating conservation management strategies.

Haloxylon salicornicum in Chenopodiaceae is one of the main structural elements in Eastern Arabian vegetation associations (Böer & Warnken, 1996; Roshier *et al.,* 1996). It is a perennial shrub or small tree measuring 1.5-12 m. tall. The fragile young shoots are forked, branching and segmented. The dense or open crown is green in the summer and gray or brownish in the autumn. The flowers are bisexual and borne singly by short twigs in the axils of the scale like bracts. Saxauls regenerate readily by suckers and reproduce by seed. *Haloxylon salicornicum* is widely distributed in Egypt, Palestine, Jordan, Iraq, Iran, Pakistan and northeast of Kuwait Bay and southern coastal areas of Kuwait (Ali & Qaiser, 2001; Zaman, *et al.,* 2006). The plant is a food source for domestic stock and wildlife, stabilizes the soil surface, and is a factor in the distribution and abundance of other flora and fauna. In particular, it provides suitable micro climates, camouflage and refuge for various animals (Böer & Sargeant, 1998). Traditionally it has been applied externally on insect stings (Ali & Qaiser, 2001), and the ash of the plant is used for internal ulcers (Nazia, *et al.,* 2010). *H. salicornicum* is considered as one of the most promising species for use in revegetation and sand dune fixation (Zaman, *et al.,* 2006). Omar *et al.,* (2001) pointed out that *H. salicornicum* communities are under pressure from overgrazing leading to reduction in percentage of distribution of *H. Salicornicum* community from 2.2% in the old vegetation map to 22.7% in the new vegetation map in Kuwait. Halwagy & Halwagy (1974) explained that the non-existence of *H. Salicornicum* community in the south of Kuwait was due to changes in the borderline with Saudi Arabia. Moreover, the *Haloxyletum* map unit showed that the community has retreated in the west and northwest of Kuwait. Therefore, there is a realistic and urgent need to conserve *H. Salicornicum* community and other plants in Kuwait for future generations. Thus, evaluations of genetic diversity within and among population are needed

to determine and understand the genetic variation. Such information are crucial for establishment of an effective and efficient conservation practice for endangered plants (FAO, 1998; Engles *et al.,* 2002). In addition, understanding the extent and distribution genetic diversity provides predictive estimates of genetic variation within a species, and also facilitates breeding material selection (Qi *et al.,* 2008).

Molecular techniques based on polymerase chain reaction (PCR), such as Random Amplified Polymorphic of DNA (RAPD) (Williams *et al.,* 1990) and Inter-Simple Sequence Repeats (ISSR) (Zietkiewicz *et al.,* 1994) can provide an accurate assessment of genetic variation. RAPD has been successfully used in investigating genetic diversity within and between populations for many plant species including *H. salicornicum* (Al-Qurainy, 2007). It requires short primers of arbitrary sequences and produces random polymorphic segments with band sizes from 100 to 3000 bp depending upon the genomic DNA and the primer (Ibrahim, *et al.,* 2010). ISSR is a technique that can differentiate between related individuals. It involves primers consisting of a di- or tri-nucleotide sequence repeats with anchoring sequence of 1-3 nucleotides (Salimath *et al.,* 1995). ISSR DNA markers have been applied widely in genetic characterization of various plant species (Kantely *et al.,* 1995; Tsumura *et al.,* 1996) such as tea (Mondal, 2002), and *cultivated* and wild species of *Oryza* (Joshi, *et al.,* 2000). Both techniques require low quantities of template DNA, and do not require prior knowledge of target sequences. They are simple, fast and relatively low cost (Salimath *et al.,* 1995).

The aim of this study was to analyse representative samples collected from populations of *H. salicornicum* in the Kuwait desert using RAPD and ISSR techniques in an effort to assess the genetic diversity of this important native plant in Kuwait.

METHODS

Plant materials

Samples of *H. salicornicum* collected from nine locations in desert of Kuwait were used for RAPD and ISSR analysis. The locations are presented in Appendix 1 and Figure 1.

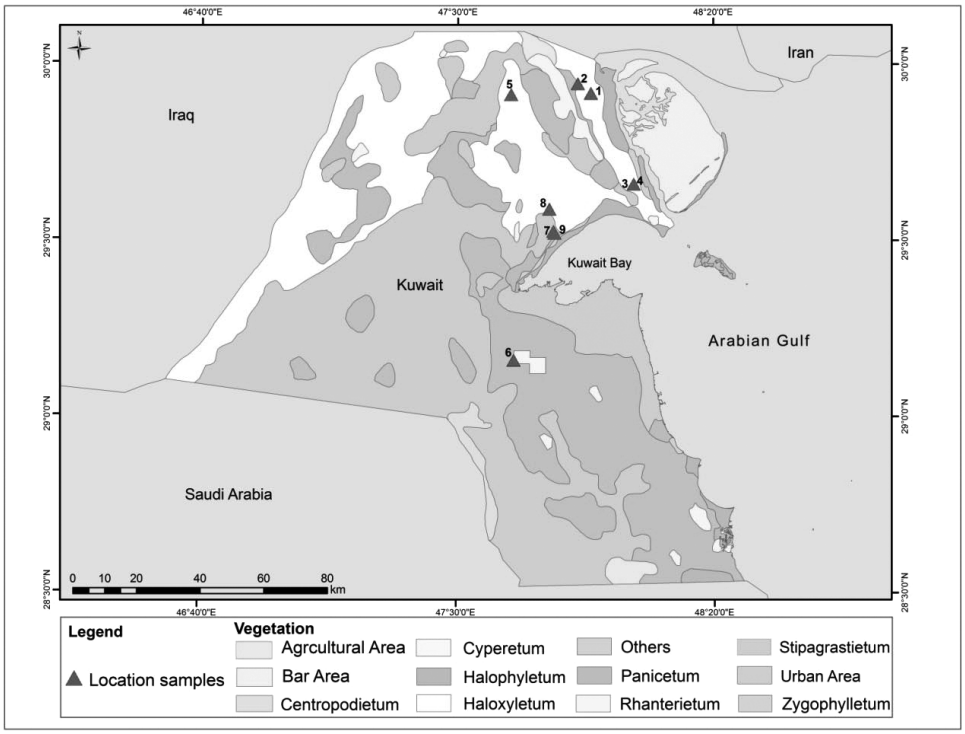

Fig. 1. Locations of *Halexylon salicornicum* population used in this study. Numbers correspond to the populations. (Appendix 1)

Analysis of DNA using RAPD and ISSR

DNA extraction. Total genomic DNA was extracted from fresh leaves of three pooled individuals collected from each of nine locations according to the method described by Dellaporta *et al*. (1983). The DNA samples were quantified using a Nanodrop-1000 spectrophotometer (US), and their size and integrity were assessed by 1% agarose gel electrophoresis according to Sambrook *et al*. (1989).

RAPD analysis. Amplification of RAPDs fragments was performed on isolated DNA samples according to Williams *et al*. (1990) with some modifications. PCR reactions were carried out in 25 μl volumes containing 7.5 mM Tris-HCl [pH 9 (at 25^{o}C)], 50 mM KCl, 2 mM $(NH_4)_2SO_4$, 3 mM $MgCl_2$, 0.6 mM of each dNTP (Vivantis), 80 ng primer, 1.5 unit of Taq polymerase

(Biotools), and 20 ng of genomic DNA. A total of 25 decamer oligonucleotide primers (Appendix 2) (Operon Technologies Inc. USA and Amersham). Amplification was performed in a Genius Hybrid Thermal Cycler (Techne, UK) with the following program: 94 ^{o}C for 1 min, followed by 45 cycles, each of which consists of 10 sec at 94^{o}C, 10 sec at 35 ^{o}C and 70 sec at 72^{o}C. A final extension cycle was performed at 72^{o}C for 2 min. At least two replicates were prepared for each PCR reaction.

ISSR analysis. Using 24 selected primers (Appendix 3), ISSR analysis (Bornet & Branchard 2001) was carried out on the isolated DNA samples. PCR reaction was performed in a total volume of 25 μl containing 100 mM Tris-HCl (pH 8.8 at 25^{o}C), 50 mM $(NH_4)_2SO_4$, 3.2 mM $MgSO_4$,0.2 mM of each dNTP (Roche), 1U of Taq DNA polymerase (Fermentas), 25 ng of genomic DNA, and 125 Pmol from each primer (Invitrogen). Amplification reactions were subjected to a thermocycling profile composed of an initial hot start and denaturation step at 94^{o}C for 5 min, followed by 40 cycles of 94^{o}C for 10 sec, 50^{o}C for 10 sec, and 72^{o}C for 10 sec and a final extension step at 72^{o}C for 7 min. The PCR products of RAPD and ISSR were analysed by electrophoresis in 1.8% agarose (Q-BIOgene) gels in 0.5 $\times$ TBE buffer stained with ethidium bromide. A 100bp DNA ladder (Vivantis) was used as a reference.

Statistical analysis. Amplification profiles generated from RAPDs and ISSRs were photographed under UV light, screened and compiled into a binary data matrix. All the reactions were repeated at least twice and only distinct, reproducible and bright bands were scored as present (1) or absent (0). Based on the collective data generated from RAPD and ISSR markers, the unweighted pair group arithmetic average (UPGMA) method and percent disagreement values (PDV) of the STATISTICA program (STATSOFT, Inc. 2003) were used to construct the binary data matrix and the dendrogram.

RESULTS AND DISCUSSION

RAPD analysis

All tested RAPDs generated reproducible and easily scorable amplification profiles. Amplification of *H. salicornicum* using RAPDs produced a total number of 946 unambiguous DNA fragments with an average of 37.8 fragments/primer. The total number of polymorphic bands produced by the 25 primers was 48, thus representing a level of polymorphism of 49.4% (Appendix

2). Primers produced multiple band profiles with a number of amplified DNA fragments ranging from 9 (OP-I14) to 60 (OP-D03). All except one primer (OP-I14) produced polymorphic bands ranging from 2% (OP-F10) to 100% (Amersham5, OP-C04, OP-D03, OP-F02 and OP-F16). Based on the profiles generated from all primers, fragment size ranged from 300-3000 bp.

ISSR analysis

The 24 primers used for the ISSRs amplification produced good reproducible and scorable patterns (Appendix 3). The amplification profiles were screened for the presence of polymorphisms among the *H. salicornicum* samples. A total of 1016 bands were generated by those primers, with primers A8 and B10 yielding the highest number of bands and primer A23 and A45 the lowest (75 and 18, respectively). Of all the 1016 amplified bands, 503 were polymorphic (47%). The number of polymorphic bands ranged from 0 (only monomorphic bands, A23, A45 and C30) to 47 (A41). Primers B3 an B7 produced 100% polymorphic bands. In profiles generated from all primers, band size ranged from 200-2000 bp.

The results of the present study revealed that the average polymorphism detected by the RAPD assay (65.2%) was slightly higher than that of the ISSR (63%).

The RAPD and ISSR matrices were subjected to the Mantel Test to verify the level of conformity between the data obtained. Results showed that there was a very good degree of fit with a 0.96 r matrix correlation value between RAPDs and ISSRs data which led us to combine the data in one matrix. (Appendix 4).

PDV mean average for the combined data of all plant samples studied was 0.22. The most closely related genotypes were those from the same region 8 A and B (PDV = 0.13), followed by samples from regions 7 and 8 (PDV = 0.15) with the same value being obtained between the samples from regions 4 and 5. On the other hand, the most distantly related genotypes were those collected from region 2 and 8/B, and between those from regions 3 and 6 (PDV = 0.28 and 0.25, respectively). The dendrogram constructed (Fig. 2) for the analyzed samples was in a very good agreement with the PDV matrix. The samples collected from region 2 were the most distal in the tree followed by samples from region 6. The average value for these samples was the highest among all others (PDV = 0.28 and 0.25 respectively).

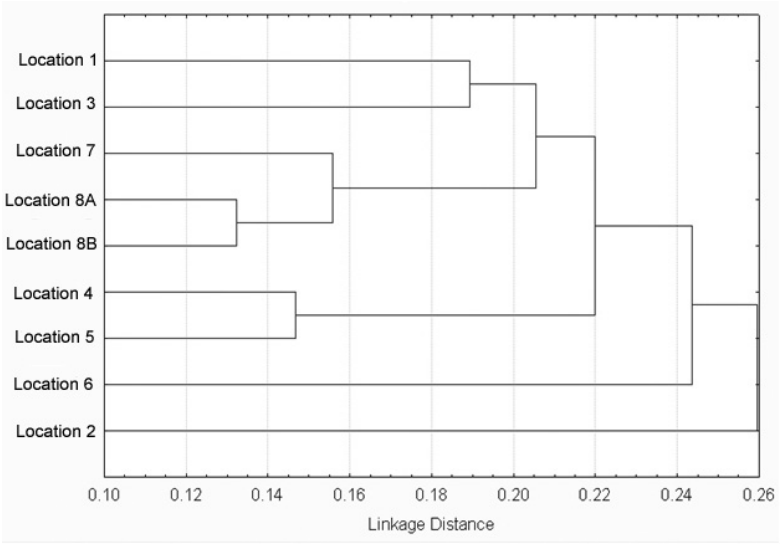

Fig. 2. Dendrogram showing the degree of relationship among *Halexylon Salicornicum* samples based on Combined RAPDs and ISSR data.

In this work we studied the genetic variations in *Halexylon salicornicum* collected from different locations in Kuwaiti desert using RAPD and ISSR marker techniques. Despite the availability of phenotypic and ecological studies on *Halexylon salicornicum* in Kuwaiti desert (Daoud, 1985), there is a lack of information concerning the genetic diversity of this plant species. Thus, an attempt has been made to characterize the genetic variation of *H. salicornicum* collected from different locations in Kuwait. Our results showed that RAPDs produced a slightly higher percentage of polymorphism than that of ISSRs. However, both techniques gave a very similar distance matrices for the samples studied allowing us to combine the data in one dendrogram to obtain more balanced values for genetic variation among genotypes and a representation of their relationships in a single dendogram. This allowed us to explore the DNA polymorphism in the collection of genotypes analyzed, and generated many polymorphic markers ensuring a good coverage of the genome.

Haider *et al,* (2012) found that the overall polymorphism among date palm genotypes identified by RAPD markers was higher than that observed for the ISSR markers, and suggested that RAPD markers are very effective for assessing the molecular polymorphism of date palm. It should be noted that RAPD is less specific than ISSR because it uses shorter primers that need lower annealing temperature which makes annealing of primers more random. A study on *H. ammodendron* carried out by Sheng *et al,* (2004), showed that both RAPD and ISSR analyses revealed a high level of genetic diversity in natural

populations of *H. ammodendron* with the proportion of variation attributable to within-population differences being higher than that among populations. In the undertaken study, no attention was paid to the within- population differences, rather, from each population a representative sample from 2-3 plants was bulked for DNA extraction. All populations were easily differentiated, with a range of PDV between 0.13 (regions 8A and 8B) and 0.29 (regions 2 and 6). With an average of PDV $=0.22$, among all regions, this study indicates that there is a low to moderate level of genetic variation among *H. salicornicum* populations. With reference to the map in Figure 1, the locations 1 and 3 are geographically far away from 5 and 6 regions and this could be the reason for genetic variation as represented in figure 2. Genetic differences between locations 2 and 1 could be due to within population differences because they were collected from geographically close locations .

CONCLUSION

Both RAPD and ISSR techniques were very effective in detecting the genetic diversity among Kuwaiti populations of *H. salicornicum*. The combined dendrogram of the two techniques revealed that there is a low to moderate level of genetic diversity. Further studies are needed to analyze and compare the within-population differences to that among populations of *H. salicornicum*.

ACKNOWLEDGMENT

The authors are grateful to the top management of the Kuwait Institute for Scientific Research (KISR) for their support. Mr Hamad Alaqeel and Mr. Ahmed Ben Hajji (Research assistant, Biotechnology Department, KISR) are also acknowledged for their contribution in the lab work.

REFERENCES

Abou El-Nil, M., Sudhersan, C., Hussain, J. & Al-Melhem, S. 2000. Mass propagation of native desert plants Phase II: development of tissue culture technique. Kuwait Institute for Scientific Research, Report No. KISR5937, Kuwait.

Ali, S. I. & Qaiser, M. 2001. Flora of Pakistan, Chenopodiaceae Jointly published by Department of Botany, University of Karachi, Karachi, Pakistan & Missouri Botanical Press: Missouri Botanical Garden, St. Louis, Mussouri, USA **204**: 185.

Al-Qurainy, F. H. 2007. Genetic distance within and between two populations as revealed by RAPD markers. Saudi Journal of Biological Sciences **14**: 221-226.

Böer, B. & Sargeant, D. 1998. Desert perennials as plant and soil indicators in Eastern Arabia. Plant and Soil **199**: 261-266.

Böer, B. & Warnken, J. 1996. Flora of the Jubail Marine Wildlife Sanctuary, Saudi Arabia. In 'A Marine Wildlife Sanctuary for the Arabian Gulf. Environmental Research and Conservation Following the 1991 Gulf War Oil Spill'. (Eds. F Krupp, A. H. Abuzinada and I. A. Nader.). pp 290--301. (National Commission for Wildlife Conservation and Development: Riyadh, KSA). Senckenbergische Naturforschende Gesellschaft: Frankfurt).

Bornet B, & Branchard, M. 2001. Nonanchored inter simple sequence repeat (ISSR) markers: reproducible and specific tools for genome fingerprinting. Plant Molecular Biology Reporter **19**: 209-215

Daoud, H. S. 1985. Flora of Kuwait. London: KPI Limited.

Dellaporta, S. L., Wood, I. & Hicks, I. B. 1983. Maize DNA mini-preparation: Version II Plant Molecular Biology Reporter **1**:19-21.

Engles, J. M. M., Rao, V. R., Brown, A. H. D. & Jackson, M. T. 2002. Managing plant genetic diversity, p. 487. CABI Publishing, UK.

FAO. 1998. The states of the world's plant genetic resources for food and agriculture, p. 510. FAO, Rome, Italy.

Ghazanfar, S. A. & Fisher, M. 1998. Vegetation of Arabian Peninsula. Geobotany **25**: 1-362.

Haider, N., Nabulsi, I. & MirAli, N. 2012. Phylogenetic relationships among date palm (Phoenix dactylifera L.) cultivars in Syria using RAPD and ISSR markers. Journal of Plant Biology Research **1**(2): 12-24.

Halwagy, R. & Halwagy, M. 1974. Ecological Studies on the Desert of Kuwait. II. The Vegetation. Journal of the University of Kuwait **1**: 87-95.

Ibrahim A. A., Mohammad A., Bakir, Haseeb A. K. Al Farhan, A.H., Al Homaidan, A.A., Ali H. B., Al Sadoon, M. & Mohammad S. 2010. A Brief Review of Molecular Techniques to Assess Plant Diversity. International Journal of Molecular Science. **11**, 2079-2096.

Joshi, S. P., Gupta,V. S., Aggarwal, K. R., Ranjekar, P. K. & Brar, D. S. 2000. Genetic diversity and phylogenetic relationship as revealed by inter simple sequence repeat (ISSR) polymorphism in the genus Oryza. Theoretical and Applied Genetics **100**: 1311-1320.

Kantely, R. V., Zeng, X., Bennetzen, J. L. & B. Zehr, 1995. Assessment of genetic diversity in dent and popcorn (*Zea mays L.*) inbred lines using inter-simple sequence repeat (ISSR) amplification. Molecular Breeding. **1**: 365-373.

Karrar. G., Batanounv, K. H., & Mian, M. A. 1991. A rapid assessment of the impact of The Iraq-Kuwait conflict on terrestrial ecosystems. New York, US: UNEP, MEPA publications.

Mondal, T. K. 2002. Assessment of genetic diversity of tea (*Camellia sinensis (L.)* O Kuntze) by inter-simple sequence repeat polymerase chain reaction. Euphytica **128**: 307-315.

Nazia, B., Sheraz, A., Tanoli, K., Farheen, S., Nighat, A., Salman, S., Ying, Z., Shahana U. K. & Abdul, M. 2010. In vitro antituberculosis activities of the constituents isolated from *Haloxylon salicornicum*. Bioorganic & Medicinal Chemistry Letters **20**: 4173-4176.

Omar, S. A. & Bhat, N. R. 2008. Alteration of the *Rhanterium epapposum* plant community in Kuwait and restoration measures. International Journal of Environmental Studies **65**(1)**:** 139-55.

Omar, S. A., Misak, R., King, P., Shahid, Sh. A., Abo-Rizq, H., Grealish, G. & Roy, W. 2001. Mapping the vegetation of Kuwait through reconnaissance soil survey. Journal of Arid Environments **48:** 341-55.

Qi X. H., Yang J. H. & Zhang M. F. 2008. AFLP-based genetic diversity assessment among Chinese vegetable mustards (*Brassica juncea (L.) Czern.*). Genetic Resources and Crop Evolution **55**:705-711

Roshier, D. A., Böer, B. B. & Osborne, P. E. 1996. The vegetation of Abu Dhabi and a preliminary classification of its plant associations. In 'Desert Ecology of Abu Dhabi'. (Ed. P. E. Osborne.) pp. 50-65. (National Avian Research Centre: Abu Dhabi).

Salimath, S. S., Oliveira, A. C., Godwin, I. D. & Bennetzen, J. I. 1995. Assessment of genome origins and genetic diversity in the genus Eleusine with DNA markers. Genome **38**: 757-763.

Sambrook, J., Fritsch, E. F. & Maniatis, T. 1989. Gel electrophoresis of DNA. In Molecular Cloning: A Laboratory Manual, 2nd Edition. Edited by J. Sambrook. New York: Cold Spring Harbor 601-619.

Sheng Y., Sheng W-H., Ke-Quan, P. E. I., & Ke-Ping, M. A. 2004. Population genetic structure of a dominant desert tree, *Haloxylon ammodendron* (Chenopodiaceae), in the Southeast Gurbantunggut Desert detected by RAPD and ISSR Markers. Acta Botanica Sinica **46**(6): 675-681

STATSOFT, Inc. 2003. STATISTICA (data analysis software system), version 6. www.statsoft.com.

Tsumura, Y., Ohba, K. & Strauss, S. H. 1996. Diversity and inheritance of Inter-simple sequence repeat polymorphism in Douglus fir (*Pseudotsuga menziessi*) and sugi (*Cryptomeria japonica*). Theoretical and Applied Genetics **92**: 40--45.

Williams J. G. K, Kubelik A. R, Livak K. J., Rafalski, J. A. & Tingey, S.V. 1990. DNA Polymorphism amplified by arbitrary primers as useful as genetic markers. Nucleic Acids Research. **18**: 6531-6535.

Zaman, S., Padmesh, S., Bhat, N. R., & Tawfiq, H. 2006. Germination of some

Kuwait's native plants under saline condition. American-Eurasian Journal of Agricultural And & Environmental science **1**: 146- 148.

Zietkiewicz, E., Rafalski, A. & Labuda, D. 1994.Genome fingerprinting by simple-sequence repeat (SSR) anchored polymerase chain reaction amplification. Genomics **20**: 176-183.

Appendix 1: Locations of *Haloxylon salicornicum* population used in this study for genetic diversity analysis.

Location	Latitude (N)	Longitude (E)
Location 1	29 49 46.6	047 41 4.9&
Location 2	29 56 51.4	047 53 29.4
Location 3	29 55 12.8	047 56 1.2
Location 4	29 54 58.6	047 40 25.9
Location 5	29 31 42.7	047 48 44.6
Location 6	29 35 30.2	047 47 56.6
Location 7	29 31 23.8	047 48 57.1
Location 8/A	29 39 45.2	048 04 24.2
Location 8/B	29 39 45.2	048 04 24.2

Appendix 2: RAPD primer sequences and polymorphic bands of *Haloxylon salicornicum*.

No.	Primer Name	Sequence	No. of Fragments Amplified	No. of Polymorphic Fragments	% Polymorphic Fragments
1	Amersham1	GGTGCGGGAA	25	7	28
2	Amersham2	GTTTCGCTCC	57	39	68
3	Amersham3	GTAGACCCGT	53	17&	32
4	Amersham4	AAGAGCCCGT	36	18	50
5	Amersham5	AACGCGCAAC	42	42	100
6	OP-C04	CCGCATCTAC	20	20	100
7	OPC-13	AAGCCTCGTC	29	11	38
8	OP-C14	TGCGTGCTTG	25	16	64
9	OP-C20	ACTTCGCCAC	53	26	49
10	OP-D02	GGACCCAACC	55	10	18
11	OP-D03	GTCGCCGTCA	60	60	100
12	OP-D08	GTGTGCCCCA	28	1	4
13	OP-D11	AGCGCCATTG	48	30	63
14	OPF-01	ACGGATCCTG	58	4	7
15	OP-F02	GAGGATCCCT	16	16	100
16	OP-F03	CCTGATCACC	27	18	67
17	OP-F09	CCAAGCTTCC	19	1	5
18	OP-F10	GGAAGCTTGG	46	1	2
19	OP-F11	TTGGTACCCC	33	24	73
20	OP-F13	GGCTGCAGAA	42	6	14
21	OP-F15	CCAGTACTCC	52	34	65
22	OP-F16	GGAGTACTGG	42	42	100
23	OP-I14	TGACGGCGGT	9	0	0
24	OP-Z01	TCTGTGCCAC	19	1	5
25	OP-Z19	GTGCGAGCAA	52	43	83
SUM			946	487	1235
Average			37.8	19.5	49.4

Appendix 3: ISSR primer sequences and polymorphic bands of *Haloxylon salicornicum*.

No.	Primer Name	Sequence	No. of Fragments Amplified	No. of Polymorphic Fragments	% Polymorphic Fragments
1	A1	CACACACACACARR	51	24	47
2	A4	CACACACACACARY	41	14	34
3	A8	CACACACACACARM	59	32	54
4	A10	CACACACACACARK	37	28	76
5	A14	CACACACACACARS	54	45	83
6	A16	CACACACACACAR	39	21	58
7	A20	CACACACACACAY	29	2	7
8	A23	CACACACACACAM	18	0	0
9	A26	CACACACACACAK	34	16	47
10	A30	AGCAGCAGCAGCR	51	42	82
11	A35	AGCAGCAGCAGCY	52	25	48
12	A38	AGCAGCAGCAGCM	49	13	27
13	A41	AGCAGCAGCAGCK	56	47	84
14	A42	AGCAGCAGCAGCS	51	24	47
15	A45	CACACACACACAS	18	0	0
16	B3	CTCTCTCTCTCTCTCTTG	25	25	100
17	B5	CACACACACACAGG	49	31	63
18	B7	GTGGTGGTGGC	36	36	100
19	B10	CAGCAGCAGCAGCAG	74	20	27
20	B13	CAACAACAACAACAA	30	12	40
21	C30	CAACAACAACAACAA	45	0	0
22	C31	AGAGAGAGAGAGAGAGT	47	20	43
23	C32	GAGAGAGAGAGAGAGAA	26	8	31
24	164-4	GACAGACAGACAGACA	45	18	40
SUM			1016	503	1138
Average			42	21	47.4

Appendix 4: Percent of Disagreement values (PDV) for *Haloxylon salicornicum* produced by combined RAPD and ISSR polymeric primers using UPGMA routine in Statistics

Location (Sample no.)	**Location 1**	**Location 2**	**Location 3**	**Location 4**	**Location 5**	**Location 6**	**Location 7**	**Location 8A**	**Location 8B**
Location 2	0.25								
Location 3	0.19	0.24							
Location 4	0.25	0.24	0.21						
Location 5	0.24	0.24	0.21	0.15					
Location 6	0.25	0.29	0.25	0.23	0.24				
Location 7	0.21	0.25	0.21	0.19	0.19	0.24			
Location 8A	0.21	0.28	0.21	0.24	0.21	0.25	0.15		
Location 8B	0.19	0.28	0.19	0.23	0.22	0.25	0.16	0.13	
Sum	**1.8**	**2.08**	**1.72**	**1.73**	**1.71**	**1.99**	**1.60**	**1.69**	**1.66**
Average	*0.23*	*0.26*	*0.21*	*0.22*	*0.21*	*0.25*	*0.2*	*0.21*	*0.21*

Submitted : *3/10/2012*
Revised : *3/1/2013*
Accepted : *3/1/2013*

تقييم التنوع الجيني لنبات الرمث، نبات فطري في دولة الكويت

د. فضيلة السلامين - هنادي الحشاش - سامي العمد

دائرة التكنولوجيا الحيوية - معهد الكويت للأبحاث العلمية

خلاصة

يعتبر نبات الرمث أحد أهم النباتات الصحراوية في دولة الكويت، وبالرغم من الأهمية الاقتصادية والبيئية لهذا النبات إلا إنه يعاني حالياً من التدهور والتقلص الفادح في الأعداد ومواجهة خطر الانقراض إذا لم يعطى اهتماماً لحمايته وتوثيقه علمياً. ونظراً لعدم توفر المعلومات حول التنوع الجيني لهذا النبات، فإن هذه الدراسة تقوم على تقييم التنوع الجيني باستخدام التقنيات الجزيئية مثل RAPD وISSR لعينات نبات الرمث في تسع مواقع من صحراء الكويت. وفي هذه الدراسة تم استخدام 25 مسبر RAPD انتخب 946 حزمة و24 مسبر ISSR انتخب 1016 قطعة. وبينت الدراسة أن تقنية الـ RAPD انتخبت أشكال متعددة 49,4٪ أكبر من تقنية الـ ISSR 47%. وبالرغم من ذلك فقد أظهر اختبار المانتل تطابقاً عالياً بين بيانات الـ RAPD والـ ISSR، لذلك تم الجمع بين بيانات التقنيتين لإنتاج شجرة وراثية واحدة بينت أن عينات نبات الرمث التي تم جمعها من المناطق 4, 8, 7, 8A, 8B متشابهة جينياً، بينما العينات التي تم جمعها من المناطق 6، 2، فإنها متباعدة جينياً.