Pak. J. Bot., 49(1): 201-206, 2017.

IMPACT OF ECOLOGICAL DIVERSITY ON GENETIC AND PHYTOCHEMICAL VARIATION IN*JUNIPERUS EXCELSA* FROM HIGH ELEVATION ZONES OF QUETTA VALLEY, PAKISTAN

SHAZIA SAEED[1], MUHAMMAD YOUNAS KHAN BAROZAI[1*], ALIA AHMED[1], RASOOL BAKSH TAREEN[1], SANIA BEGUM[2]AND GHULAM MUHAMMAD ALI[2]

[1]Department of Botany, University of Balochistan Quetta, Pakistan
[2] National Institute of Genomics and Advanced Biotechnology (NIGAB), NARC, Islamabad, Pakistan
[*]Corresponding author's email: barozaikhan@gmail.com

Abstract

Juniperusexcelsa (Cupressaceae) is an evergreen tree and the second most diverse group of the conifers distributed abundantly in high elevation zones of Balochistan. Genetic and phytochemical variations in three naturally occurring populations of *J.excelsa* were analysed. Genetic variability was assessed by different molecular markers (RAPD, ISSR and URP) with an objective to use genetic diversity as a key to conserve the taxon which is also known as living fossil as dominated in Mesozoic era. Genetic diversity was assessed by polymorphic bands to generate a dendrogram based on UPGMA. Using tested markers, 116 bands were amplified out of which 67 bands were polymorphic with an average value of 8.37 (57%) bands per primer. Based on data, a cluster dendrogram was prepared that exhibited the mean genetic similarity matrix as 0.57 and two major clusters diverge at 0.49. The genetic similarity coefficient among all accessions ranged from 0.35 to 0.90. In phytochemical analysis, total phenolic and flavonoid contents were estimated and compared among all accessions. Ecological characteristics of the study sites were measured to check their impact on genetic and chemical variation. Soil properties were analyzed for Principal Component Analysis. Chemical variation of *J. excelsa* of three sites revealed by dissimilarity matrix exhibiting genetic distance based on TPC and Flavonoids. Cluster analysis represent two major groups. Mean concentration of TPC and flavonoids were 56±9.15 and 150±27.9 mg/g respectively. PCA of soil considered three factors had Eigen values >1 and explain cumulatively 4.60 %, 26.02% and 10.36 % of the variance. First factor was positively correlated with second and fifth, but negatively correlated with other factors. In conclusion, molecular marker profiling together with phytochemical variation of total phenolic and flavonoid content in all accessions of *Juniperusexcelsa* and impact of ecological diversity on Genetic and chemical variation can be used as base line study for implementation of conservation strategies.

Key words: RAPD, URP, ISSR,Genetic variation, Chemical variation, *Juniperusexcelsa*, Quetta.

Introduction

Juniperus excelsa M. Bieb. (Cupressaceae) grows in various parts of the world; naturally it exists in Anatolia, central and south Balkons, central and south Asia and east Africa (Farjoon, 1992). Juniper tree is considered to be the second most diverse group of the conifers. The genus *Juniperus* consists of approximately 67 species, all native to northern hemisphere. *Juniperus excelsa* has been reported as a threatened tree in Pakistan by International Union for Conservation of Nature (IUCN) Red data List (Anon., 2006).

J. excelsa attains a height up to 20 m, trunk width up to 2m diameter at breast height. They can easily survive in harsh climatic condition (Fisher *et al.,* 1995). All *Juniperus* spp. grow slowly with meager seedling development. Some extensive 100,000 hectares area is covered adjacent to Quetta region in Zarghoon range and Ziarat by *Juniperus excelsa* (Khatak, 1963). These forests of *J. excelsa* are considered as one of the world's largest, oldest, drought resistant, enormously slow growing and long-lived trees which sometimes live more than 1000 years. Therefore, Sheikh (1985) termed them "Living Forest Fossil". However some natural isolated populations are also found near Quetta such as Takatu mountain range and Hazarganji Chiltan National Park.

Genetic diversity refers to genetic makeup of organism and plays a vital role in the survival of species and their adaptability in diverse ecosystems. For conservation strategies and breeding programs, genetic diversity and plant distribution is very important (Volis *et al.,* 2001; Meloni *et al.,* 2006; Bruschi *et al.,* 2003).

Molecular marker can be used to assess genetic variability within and among populations. Several marker systems are now available for genetic diversity analysis. RAPD markers are quite suitable for DNA fingerprinting as they are rapid and easy to assess however, amplification of bands can be effected by different factors such as variable sequence of primers, quantity of template and annealing site in genome (Kernodle *et al.,* 1993).The Inter Simple Sequence Repeat (ISSR) amplification assay was developed by Zietkiewicz *et al.* (1994) and did not need prior sequence and was less vulnerable (Adams *et al.,* 2003). Universal Rice Primer (URP) markers were first time used by Kang *et al.* (2002). These are repeated chains that were extracted of genome bank of the rice of local Korea and it canuse for genomes of plants, animal and microbes. URP markers with suitable PCR condition, produce high polymorphs. Additionally it has been proved that these markers are useful tools for genetic analysis in between and within species (Rashmi *et al.*, 2008; Abbas *et al.,* 2015).

In this view, the present study was conducted to check the genetic variability in three naturally occurring populations of *J. excelsa* and three elevation zones of Balochistan through molecular and phytochemical profiling. In parallel, the soil characteristics of the study sites were also analyzed and compared.

Materials and Methods

Study area: As the province of Baluchistan is rich in biodiversity, occupies a position between the two major Zoogeographical regions of the world namely, the Oriental and Palearctic. The geography of the region has an impact on the distribution of plants and animals (Anon., 2000). Sra-Ghurgai (Takatu-mountain range) found in Koh-i-Takatu mountain range its elevation ranges from 1660 to 3000 meterabove sea level (a.s.l). It is situated at 30º 17′ - 39º N latitude and 67º 01′- 02º E longitude (Saeed *et al.,* 2014). Zarghoon is another important high elevation zone adjacent to Quetta valley ranges from 2200 to 3,578 m (11738. ft. The highest peak). The area constitutes steep slopes, hills and narrow valleys. The Zarghoon range extended up to Ziarat and covered by thick Juniper forest approximately 200,000 acres. Hazarganji Chiltan National Park Comprised of diverse sandy and forest habitats. Common vegetation includes trees, shrubs and herbs with dominating *Pistachia* spp., *Cerassus* spp. *Fraxinusxanthoxyloides* and some fragmented *J. excelsa*. The Chiltanmarkhor (National animal of Pakistan) is also confined within the park boundaries. Temperature range is -10ºC to 35ºC, while Altitude ranges from 1600-3300m. Park is located at 30º 07'N latitude and 66º 58'E longitude (Saeed *et al.,* 2015).

Plant material: Young and healthy leaf buds were sampled (during 2012-2014) from three natural populations, including forest of Zarghoon range (ZR), Sra-Ghurgai (SG) and Hazarganji (HG). A total of nine genotypes i.e. three genotypes from three different sites of three populations were sampled for analysis (Table 1). Taxon was identified and dry samples were preserved. The voucher specimens with accession numbers were submitted in herbarium at Department of Botany, University of Balochistan Quetta. Leaves samples for DNA extraction and PCR amplification were immediately stored at ultra-low freezer.

DNA extraction and purification: For genomic DNA isolation, method described by Doyle & Doyle (1987) was used. To check the integrity of isolated DNA, 1% agarose gel stained with ethidium bromide was used to check the quantity of isolated DNA samples, was further checked on Biospec-Nano at 260/280 nm.

PCR reaction: A total of twenty two primers including RAPD, URP and 18-mer ISSR were used, 8 of them were polymorphic (Table2). The reaction for amplification was carried out in volume equal to 20*µl* reaction. PCR mixture containing, 3mM $MgCl_2$, 1X PCR buffer, 0.2mM dNTPs mix, 20 pmol primers, 0.01% gelatin, 1U/rxnDream *Taq*DNA polymeraseand 25 ng DNA template.

PCR amplification was performed using thermal cycler (Applied Biosystems 96 well USA). For RAPD primers, PCR was performed with initial denaturation at 94ºC 3min and then 36 cycles with annealing temperature 36ºC for 1 min, extension step at 72ºC for 2 minutes. An additional extension step for 5 minutes at 72ºC in the final cycle was also added. For URP primers, PCR was performed with initial denaturation at 94ºC 3min and then 40 cycles with annealing temperature 50-56ºC for 1 min, extension step at 72ºC for 2 minutes. For ISSR primers, PCR was performed with initial denaturation at 94ºC for 3min and then 40 cycles with annealing temperature 56ºC for 1 min and extension step at 72ºC for 2 minutes followed by an additional extension step for 5 minutes at 72ºC in the final cycle. The amplified products were checked by electrophoresis in 1.8% agarose gels containing ethidium bromide (0.5µg / ml) in 1X TAE buffer. The product was visualized by Gel documentation under UV light and the size of markers were estimated by comparing to the standard ladder (100bp BIORON 0.2mg/ml) in the gel.

Table 1. Ecological characteristics of the study sites.

S. No.	Sampling sites	Site code	Latitude (N)	Longitude (E)	Elevation (m)
1.	Sra-Gurgai (Takatu Mountain Range (SG)	A.1	30.17	67.01	1660-3000
		A.2	30.29	67.06	
		A.3	30.33	67.08	
2.	Zarghoon range (ZR)	B.1	30.21	67.12	2200-3200
		B.2	31.05	67.18	
		B.3	31.22	68.01	
3.	Hazarganji (HG)	C.1	30.21	66.54	1700-2600
		C.2	30.07	65.55	
		C.3	30.15	66.07	

Table 2. Details of polymorphic primers used in the study.

Primer	Sequence	TA (ºC)	TB	P	PB %
ISSR UBC 808	5' –(AG)8C- 3'	53	4	4	100
ISSR UBC 856	5' –(AC)8TG- 3'	52	20	20	100
URP (13-R)	3' –TACATCGCAAGTGACACACC- 5'	54	6	6	100
OPA-2	5' –TGCCGAGCTG- 3'	36	28	13	46
OPA-3	5' –AGTCAGCCAC- 3'	34	13	4	30
OPA-4	5' –AATCGGGCTG- 3'	34	15	8	53
OPC-2	5' –TGATCCCTGG- 3'	36	18	9	50
OPC-1	5' –GTTTCGCTCC- 3'	32	12	3	25
Total			116	67	58

Note: T_A(ºC) (annealing temperature), T (Total bands), P (Number of polymorphic bands), %*PB*, percentage of polymorphism, PIC Polymorphic Information Content)

Phytochemical variation: Spectrophotometric quantification to detect phytochemical variation of plants total phenolic and flavonoid contents was also carried out.

I. Flavonoids: For flavonoids estimation, method described by Ordonez *et al.* (2006) was used. Take 0.5 ml sample 0.5 ml 2 % $AlCl_3$ ethanol solution was added after one hour at room temperature the absorbance was measured at 420 nm. A yellow color indicated the presence of flavonoids. Total flavonoids contents was calculated as quercetin (mg/g) using equation ($Y=0.0255xR^2=0.9812$).

II. Total phenolic content (TPC): Total phenolic contents were measured by the method of Slinkard& Singleton (1977). An aliquot of the extract was mixed with 5ml FC reagent (previously diluted with water 1:10 v/v) and 4 ml of sodium carbonated (7%). The tubes were vortexed for 15 seconds and were allowed to stand for 30 minutes at 40ºC for color development. Absorbance was then measured at 765 nm. Total phenolic contents were expressed as mg/g tannic acid equivalent using the equation ($Y=0.1216xR^2=0.9365$).

Statistical analysis of data: For molecular markers, the amplified bands were scored as 1 (present) and 0 (absent), and data were clustered (dendrograms) based on similarity matrices using the paired group method with the help of software NTSYS 2.10 (Rohlf, 1998). For soil data and phytochemical parameters, Principal Component and Agglomerative hierarchical clustering were performed using XLSTAT 2012.

Results and Discussion

This is the first comprehensive report on molecular characterization and impact of ecosystem diversity on genetic diversity and phytochemical variation in populations of *J. excelsa* from high elevation zones of Quetta and adjacent areas ranging from 1600-3500m a.s.l.

The topographic factor affects the environmental variables and the soil properties. Geographical distance play key role in the variability of component. The principal component analysis considered the three factors had Eigen values >1 and explain cumulatively 4.60%, 26.02% and 10.36% of the variance. First factor is positively correlated with second and fifth but negatively correlated with others. Thus, the effect of the variation factor of the variable III led to a reduction of its values while the values of other variables increased. Badr *et al.* (2012) reported the genetic diversity in *Artemesiajudaica* was the result of different locations with different environmental heterogeneity.

Molecular markers play an important role in evaluating ecological consequences, such as climate change on plant response. It is evident that rapid warming can cause a decrease in the variability of alleles that response to climate. Hence the assessment and conservation of genetic variability by molecular markers can be an important source for analyzing adaptability to environmental changes (Jump & Penuelas, 2014). In present study, three different marker systems i.e. RAPD, URP and ISSR were used for the first time on this significant taxon from Balochistan. The amplified band size ranged from 395 to 1000 bp for RAPD, 400 to 1500 bp of ISSR and 285 to 1000 bp in URP (Fig. 2). We used combined RAPD, ISSR and URP markers to generate dendrogram by cluster analysis. A combined marker system approach to detect polymorphism could be useful in removing error and targeting various sites of genome as was previously used by Phong *et al.* (2011) in *Dalbergiaoliveri.* As *J. excelsa* was not characterized earlier by different markers combination so this study would give the amended pattern of genetic variation. Meloni *et al.* (2006) studied ISSRs and a few geographical regions and suggested that genetic variation was still harbored within populations and concluded the species was not endangered yet, and it probably will be sufficient, for conservation purposes, to maintain a few populations placed across the whole distribution range to ensure that the total genetic diversity was represented.

Out of tested primers, eight makers i.e. five RAPD, two ISSR and one URP exhibited polymorphism and showed reproducible bands among Nine *J. excelsa* accessions. Table 2 explains the characteristics of banding patterns obtained with primers. These eight primers amplified 116 loci and 67 were polymorphic (57% polymorphism). The total number of RAPD, ISSR, URP bands scored per primers also varied. Overall data revealed an average of 8.3 bands obtained per primer. The amplified band size ranged from 395 to 1000bp for RAPD, 400 to 1500 bp of ISSR and 285 to 1000bp in URP. ISSR and URP produced 100% polymorphism whereas RAPD primer OPA-4 and OPC-2 generated 53% and 50% polymorphism respectively. OPC-1 produced minimum 25% polymorphism. Similarly, Kasaian *et al.* (2011) has performed the molecular characterization of *Juniperus* species from Iran using 18 random primers and reported an average of 10.3 bands/primer. In other plant species with a large distribution, ISSRs yielded values of polymorphism between 72% and 95% in *Penstemon* (Wolfe *et al.*, 1998) and 81% in *Primulaobconica* (Nan *et al.*, 2003), but these studies were based on a greater number of individuals and ISSR markers.

Genetic similarity matrix and cluster analysis: Genetic similarity matrix was produced using combined data for RAPD, ISSR, and URP markers by similarity coefficient through the UPGMA, (NTSYS pc) (Fig. 3). The generated dendrogram of three populations of *J. excels* showed two major clusters that diverged at 0.49 similarity coefficients. First cluster sub divided into two main sub-clusters of which cluster I clearly represented the (SG) less diverse population with genetic distance 0.59 to 0.90. Whereas sub-cluster II comprised of B1, B2, B3 (ZR) with C1 (HG) cluster exhibited more diverse group with phylogenetic similarity coefficient of 0.67. Mean genetic similarity was calculated as 0.57 out of 36 possible comparisons. The highest genetic similarity occurred between A1 and A2 (SG) 0.900 depicted low genetic variability within population SG (Takatu Mountain range) (Table 3). In contrast, lowest genetic similarity coefficient of 0.35 was found between B2 (ZR) and C3 (HG) populations, indicating high genetic variability (Table 3).Previously it has been reported by Douaihy *et al.* (2011) for mountain ecosystems of the Basin that level of genetic diversity werehigh and observed among species and population. Whereas these results are in agreement with Genetic Diversity is high in all population except A1 and A2 found similar in one cluster. Other accessions from different sites are diverse making sub clusters (Fig. 3). However, Hasan *et al.* (2009) estimated genetic diversity in *Artemisia capillaris* and reported low genetic variability within population.

Table 3. Genetics similarity matrix of *J. excelsa* based on Euclidean distance.

	A.1	A.2	A.3	B.1	B.2	B.3	C.1	C.2	C.3
A.1	*								
A.2	0.9000	*							
A.3	0.7000	0.6000	*						
B.1	0.6000	0.5000	0.5000	*					
B.2	0.7500	0.6500	0.5500	0.7500	*				
B.3	0.6500	0.5500	0.4500	0.5500	0.7000	*			
C.1	0.7000	0.6000	0.6000	0.7000	0.7500	0.7500	*		
C.2	0.6000	0.5000	0.4000	0.5000	0.4500	0.5500	0.4000	*	
C.3	0.5000	0.5000	0.5000	0.5000	0.3500	0.4500	0.6000	0.6000	*

Table 4. Analysis of variance for TPC and flavonoids among different population of *J. excelsa.*

	TPC	Flavonoids
F-Value	37.05	0.56
P-Value	0.000	0.600
Significance level	***	NS

Significance level*,**,*** at p<0.05, p<0.01,p<0.001 respectively

Table 5. the TPC and flavonoid contents in *J. excelsa*for different poputions.

Area code	Phenolics (mg/g tannin equivalents)	Flavonoids (mg/g quercetin equivalents)
A	67.40 ± 1.05	147.38 ± 31.6
B	47.81 ± 2.06	139.34 ± 37.9
C	52.86 ± 4.42	164.31 ± 13.5
Overall	50.03 ± 9.15	150.34 ± 27.9

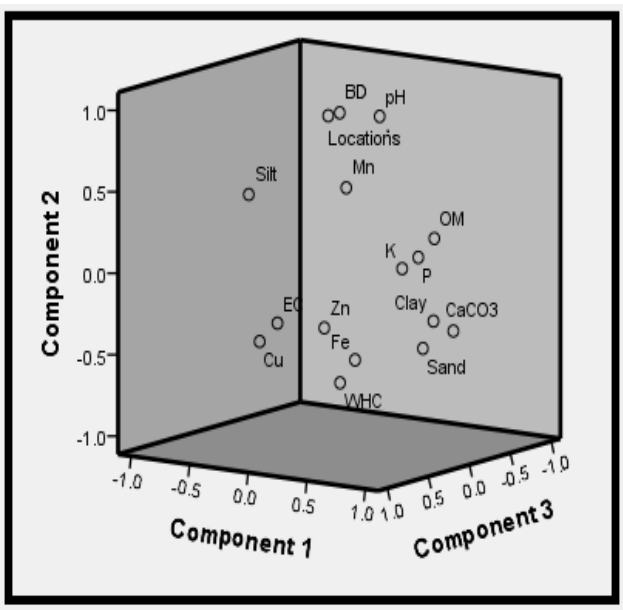

Fig. 1.3-D component plot of soil variables.

Phytochemical analysis: All accessions of *J. excelsa* were characterized to be having significantly varied levels of total phenolic contents (TPC) expressed as tannin equivalents and flavonoids contents as quercetin equivalent within and among population (Table 4). The TPC ranged from 66 to 68 mg/g with in population A (Sra-Gurgai), 45 to 49 mg/g with in population B (Zarghoon) and 49 to 57 mg/g within population C (Hazarganji) (Fig. 4). Variation was maximum among populations (ranged from 45 to 68 mg/g) than within population with highly significant difference (p<0.001). Mean concentration of TPC across the populations was 56±9.15 mg/g whereas flavonoid showed diverse pattern within and among population. It ranged from 113 to 175 mg/g within population A (Sra-Gurgai) in population B it ranged from 96-170 and population C from 149-175 mg/g amongst, flavonoid showed non-significant difference (Table 4). Mean concentration of flavonoids across the populations was 150±27.9 mg/g (Table 5). UV-B radiation and environmental factors can influence the flavonoids and phenolic contents (Dumas *et al.*, 2003). According to our results, high chemical variation in TPC was found in disturbed habitat of SG with genetically less diverse population of Juniper. Whereas in ZR and HG which are comparatively conserved regions, having more genetic diversity and less variation in TPC.

Based on TPC and Flavonoids contents data, the relationship among nine accession of *J. excelsa* from three sites was revealed by cluster analysis trough dissimilarity matrix (Fig. 5). Cluster analysis represented two major groups. The first group comprised of A1, B1, C1 and C3 whereas group two was represented by two sub-clusters including A3, B3 and C2. Where as B2 and A2 populations represented by a diverged cluster.The topographic factor affects the environmental variables and the soil properties. Geographical distance play key role in the variability of component. The principal component analysis considered the three factors which had Eigen values >1 and explained cumulatively 44.60%, 26.02% and 10.36 % of the variance. First factor is positively correlated with second and fifth but negatively co related with others. (Fig. 1) Thus, the effect of the variation factor of the variable III led to a reduction of its values while the values of other variables increased. Molecular markers are also used to assess plant response to climate change, which is a major issue at a global level. Changes, such as rapid warming, have been seen to cause a decrease in the variability of those loci controlling physical responses to climate. Climatic factors correlated with micro-geographical genetic differences, and the various molecular markers used for each study. They concluded that although phenotypic plasticity buffers against environmental changes over a plant's life cycle, it will weaken over time as climatic event become more extreme and over longer time spans. The assessment and maintenance of genetic diversity, through the use of molecular markers is crucial as it provides a repository of adaptability to environmental and other changes (Jump & Penuelas, 2014).

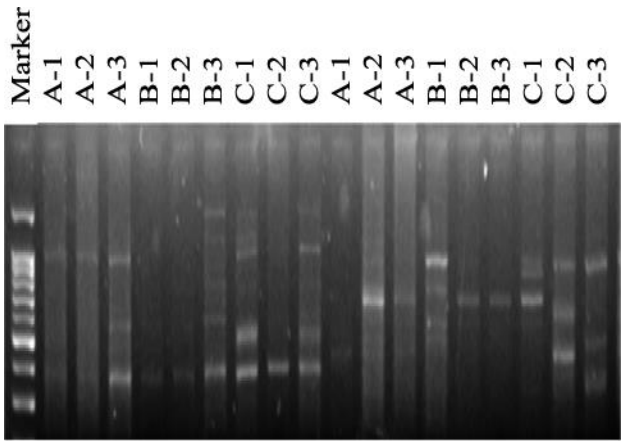

Fig. 2. PCR amplification pattern in*J. excelsa*with ISSR primer UBC 808 L1–L9 and URP primer 13R L10-L18 and L1 contains Marker of 100bp.

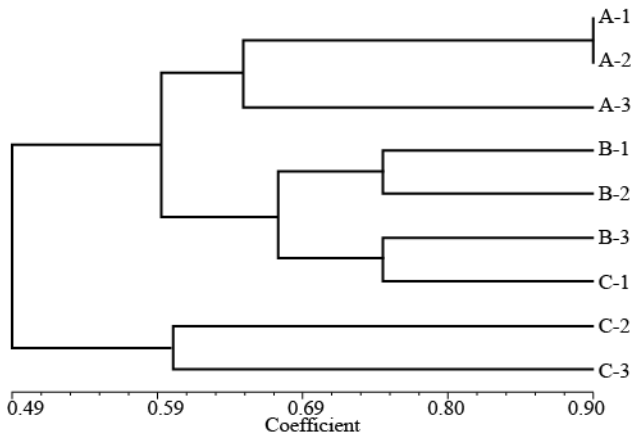

Fig. 3. NTSYS-dendrogram of cluster analysis based on data generated from RAPD, URP and ISSR among *J. excels* population (Listed in Table 1).

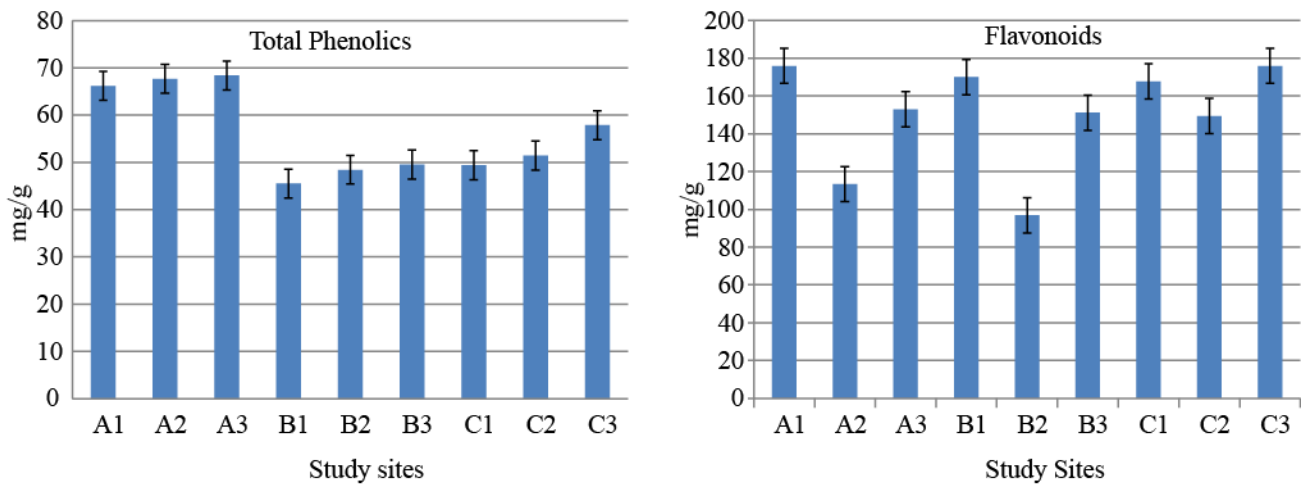

Fig. 4.Patterns of phytochemical variations among different populations and on different study sites.

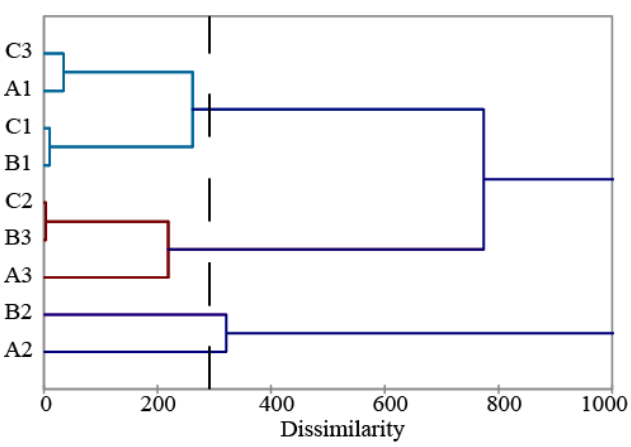

Fig. 5. Dendrogram estimating genetic divergence among *J. excelsa* populations based on TPC and Flavonoid contents on different study sites.

Conclusion

Based on our findings, it was concluded that there was an impact of ecological consequences on genetic diversity and phytochemical composition of *J. excelsa*. The *J. excelsa* populations have diverse genetic pattern on different sites included in this study. Due to fragmented habitat and anthropogenic effects, diversity within population found to be less than among populations in Takatu mountain range. Whereas semi-conserved and conserved regions of Zarghoon range and Hazarganji Chiltan National park still retained genetic diversity within populations, however, sustainable management plans are to be implemented. Furthermore it is also concluded that assessment and conservation of genetic diversity through molecular markers is essential that could be used as a key to conserve genetic variability and assess environmental changes on allelic frequency. Moreover, implementation of conservation strategies for such important taxa is highly recommended.

References

Abbas, G., A. Hameed, M. Rizwan, M. Ahsan, M.J. Asghar and N. Iqbal. 2015. Genetic Confirmation of Mungbean (*Vignaradiata*) and Mashbean (*Vignamungo*) Interspecific Recombinants using Molecular Markers. *Front. Plant Sci.*, 6: 1107.

Adams, P.A., A.E. Schwarzbach and P.R. Naresh. 2003. The concordance of terpenoid, ISSR and RAPD markers, and ITS sequence data sets among genotypes: an example from Juniperus. *Bioch. Sys. and Eco.*, 31: 375-387.

Anonymous. 2000. 1998. *District Census Report of Karak*. Publication Census Organization, Statistics Division, Government of Pakistan, Islamabad.

Anonymous. 2006. Conifer Specialist Group. 1998. *Juniperusexcelsa*. IUCN Red List of Threatened Species. IUCN 2006. www.iucnredlist.org. Retrieved on 12 May 2006.

Badr, A., Z. Abo-El-Khier, A. Ghada, A. Hegazi, Abd-El-Kawi and A. El-Sawy. 2012. Genetic variation in seven natural populations of *Artemisia judaica* L. in South Sinai using RAPD Markers. *World Applied Sciences Journal*, 18(10): 1475-1480.

Bruschi, P., G.G. Vendramin, F. Bussotti, and P. Grossoni. 2003. Morphological and molecular diversity among Italian populations of Quercuspetraea (Fagaceae). *Annals of Botany*, 91: 707-716.

Douaihy, B., G.G. Vendramin, A. Boratynski, N. Machon and B.M. Dagher-Kharrat. 2011. High genetic diversity with moderatedifferentiation in Juniperusexcelsa from Lebanon and the East Mediterranean Region. *AOB Plants*, 1-14.

Doyle, J.J. and J.L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf material. *Phytochem Bull*., 19: 11–15.

Dumas, Y, M. Dadomo, G.D Lucca and P. Grolier. 2003 Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. *Journal of the Science of Food and Agriculture*, 83(5):369-382.

Farjon, A. 1992. The taxonomy of multiseed Juniper (*Juniperus* sect. *Sabina*) in south west Asia and East Africa (Taxonomic notes on cuperassaceae I). *Edinburg J. Bot.*, 49: 251-283.

Fisher, M., A.S. Gardner and A.M. Sarangzai. 1995. The status and ecology of *Juniperus excels* subsp. *Polycarpos* from the northern mountains of Oman. *Vegetation*, 119: 35-51.

Hasan, S.M.Z., M.S.B. Shafie and R.M. Shah. 2009. Analysis of random amplified polymorphic DNA (RAPD) of Artemisia Capillaris (Worm Wood Capillary) in East Coast of Peninsular Malaysia. *World Appl. Sci J.*, 6(7): 976-986.

Jump, A.S. and Penuelas. 2014. Running to stand still: adaptation and the response of plants to rapid climate change. *Ecol. Lett*.,8: 1010-1020.

Kang, H.W., D.S. Park, Y.J. Park, CH. You, B.M. Lee, M.Y. Eun and S.J. Go. 2002. Printing of diverse genomes using PCR with universal rice primers generated from repetitive sequence of Korean weedy rice. *Mol. Cells*., 13(2): 72-81.

Kasaian, J., J. Behravan, M. Hassany, S.A. Emami, F. Shahriari and M.H. Khayyat. 2011. Molecular characterization and RAPD analysis of *Juniperus* species from Iran. *Genetics and Molecular Research*, 10(2): 1069-1074.

Kernodle, S.P., R.E. Cannon and J.G. Scandalios. 1993. Concentration of primer and template qualitatively affects product in random amplified polymorphic DNA-PCR. *Biotechniques*, 14: 362-364.

Khattak, G.M. 1963. Working plan for Juniper forests of Quetta civil division. Govt. Printing Press, West Pakistan.

Meloni, M., D. Perini, R. Filigheddu and G. Binelli. 2006. Genetic variation in five Mediterranean populations of *Juniper usphoenicea*as revealed by Inter-Simple Sequence Repeat (ISSR) markers. *Anna of Bot.*, 97: 299-304.

Nan, P., S. Shi, S. Peng, C. Tian and Y. Zhong. 2003. Genetic diversity in *Primulaobconica* (Primulaceae) from Central and South-west China as revealed by ISSR markers. *Annals of Botany*, 91: 329-333.

Ordonez, A.A.L., J.D. Gomez, M.A. Vattuone and M.I. Isla. 2006. Antioxidant activities of *Sechiumedule* (Jacq). *Food Chem*., 97: 452-458.

Phong, D.T., V.T.T. Hien, T.T.V. Thanh and D.V. Tang. 2011. Comparison of RAPD and ISSR markers for assessment of genetic diversity among endangered rare *Dalbergiaoliveri* (Fabaceae) genotypes in Vietnam. *Genet. Mol. Res.*, 10(4): 2382-2393.

Rashmi, A, V. Sharma and L. Lalit. 2008. Molecular characterization of *Chaetomium* species using URP-PCR. *Genetics and Molecular Bio.*, 4: 946-943.

Rohlf, J. 1998. NTSYSpc numerical taxonomy and multivariate analysis system version 2.0 User Guide F. Department of Ecology and Evolution State University of New York Stony Brook, NY 11794-5245.

Saeed, S., M.Y.K. Barozai, A. Ahmed and S.H. Shah. 2014. Impact of altitude on soil physical and chemical properties in SraGhurgai (Takatu mountain range) Quetta, Balochistan. *IJSER.*, 5(3): 730-735.

Saeed, S., Y.K. Barozai, A. Ahmed, R.B. Tareen, G.M. Ali, A. Shehzad and S. Begum. 2015. Genetic diversity of *Ephedra procera*from high altitudes of Quetta valley, Balochistan using RAPD and ISSR. *Pak. J. Weed Sci. Res.*, 21(2): 163-172.

Sheikh, M.I. 1985.Afforestration in Juniper forests of Ziarat, Balochistan. *Pak. J. For*., pp: 46.

Slinkard, K. and V.L. Singleton. 1977. Total phenol analyses: automation and comparison with manual methods. *Am. J. Enol. Viticult*., 28: 49-55.

Volis, S., S. Mendlinger, Y. Turuspekov, U. Esnazaov, S. Abugalieva and N. Orlovsky. 2001. Allozyme variation in turkmenian populations of wild barley, *Hordumspontaneum* Koch. *Ann Bot.*, 87:435-446.

Wolfe, A.D., Q.Y. Xiang and S.R. Kephart. 1998. Assessing hybridization in natural populations of Penstemon (Scrophulariaceae) using hypervariableinter simple sequence repeat (ISSR) bands. *Molecular Ecology*, 7: 1107-1125.

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

(Received for publication 15 January 2016)