NUSANTARA BIOSCIENCE
Vol. 13, No. 1, pp. 121-128
May 2021

ISSN: 2087-3948
E-ISSN: 2087-3956
DOI: 10.13057/nusbiosci/n130116

Genetic diversity and morphological variation of *Pinus gerardiana* along the environmental gradient from Zhob, Balochistan, Pakistan

JANAT GUL, SHAZIA SAEED, ALIA AHMED♥, SAADULLAH KHAN LEGHARI, ABDUL BASIT, ABDUL REHMAN, MUHAMMAD ZAHID KHAN
Department of Botany, University of Balochistan. Quetta 87300, Pakistan. Tel./fax.: +92-81-921-1264. ♥email: aliaahmed_botany@yahoo.com

Manuscript received: 29 March 2021. Revision accepted: 28 May 2021.

Abstract. *Gul J, Saeed S, Ahmed A, Leghari SK, Basit A, Rehman A, Khan MZ. 2021. Genetic diversity and morphological variation of* Pinus gerardiana *along the environmental gradient from Zhob, Balochistan, Pakistan. Nusantara Bioscience 13: 121-128. Pinus gerardiana*, an evergreen gymnosperm, is an economically- and ecologically valuable tree found in the Takhte Suleman Mountain Range, Zhob northeastern edge of the Balochistan, Pakistan. The present study is based on the assessment of the impact of environmental gradients with special reference to altitudinal gradient and soil variables on morphological, phytochemicals and genetic variation of *P. gerardiana*. A total of 27 genotypes of *P. gerardiana* were collected from the three elevation zones ranging from 2000 - 3500 m above sea level. The genetic diversity was assessed by combined markers, the random amplified polymorphic DNA (RAPD) and the Inter Simple Sequence Repeat (ISSR). Polymorphic bands analyzed the data to generate a dendrogram, based on the unweighted pair group method with arithmetic mean (UPGMA). Morphological characters assessed the morphological characters. Phytochemicals were investigated; the total phenolic content and total flavonoid contents were estimated and compared amongst all accessions. Our results revealed variations along altitudinal gradients and related to soil characteristics. The populations at the Middle altitude zone have greater diversity than populations at lower and higher altitudes. The resulting altitudinal variation can be attributed to various geographical and environmental conditions. These results can help in conservation and cultivation of this economically important tree.

Keywords: Altitudinal gradient, soil variables, phytochemicals, *Pinus gerardiana,* Zhob Forest, Pakistan

INTRODUCTION

The *Pinus gerardiana* Wall. ex D.Don, locally known as "Chilgoza" or "neoza pine", is considered one of the most promising trees with ecological and economical values around the world. This tree plays a very important role in the economic progress and livelihood of communities living close to the Forest (Shalizi and Khurram 2016). The tree is utilized for food, medicine and timber by the native communities of the area and wildlife. The tree contributes to the local income and revenue, sustains the soil surface, sustains a suitable microclimate, provides shelter, and is a refuge for animals. In Pakistan, 20% of the forest consists of Pinus trees, which can survive in harsh, cold environmental conditions, including excessive drought, high wind, and severe cold. The mountains of Sulaiman hold the world's largest expanse of Chilgoza (over 260 km^2) (Ahmed et al. 2011). The Chilgoza forests are under constant threats and pressure from the timber Mafia. This species is listed as highly threatened due to excessive cutting. During their explorations, many different analysts have identified the adverse anthropogenic activities in the area with regards to this tree, such as collecting for fuelwood, overgrazing and burning, which are responsible for the significant destabilizing influence in the forests (Ahmed et al. 2009; Beg and Mirza 1984; Hussain 2013).

The environmental factors, soil variables and amount of precipitation have influenced the growth of the Chilgoza pine tree. Natural regeneration of Chilgoza pine is very poor or entirely lacking in this zone (Kumar et al. 2013; Kumar et al. 2016). Genetic diversity provides the template for adaptation and evolution of populations and species.

Genetic variation is the key factor for the conservation of biodiversity (Thomas et al. 1999). In recent years, PCR-based molecular markers have allowed the use of DNA sequences in genetic analyses to provide a better understanding of the genetic diversity and differentiation of natural populations (Malik et al. 2008). Genetic make-up plays an important role in diverse ecosystems (Meloni et al. 2006). Recent research identifies better the role of molecular markers to assess the genetic diversity amongst and within the population. Nowadays, different molecular primers are in use for analysis of genetic diversity. RAPD markers are quite suitable for DNA fingerprinting as they are rapid and easy to assess (Kernodle et al. 1993). The Inter Simple Sequence Repeat (ISSR) is also used efficiently as no prior sequence is required (Adams et al. 2003). Loss of genetic variability is a major problem in biological diversity conservation because it inhibits a species from responding to natural selection and limits its evolutionary potential. If the harmful source of genetic diversity is known, different resources can be used efficiently in conservation.

In view of the aforementioned, the main objective of this study was, therefore, to assess the genetic diversity and population structure of economically important tree *P. gerardiana*. This would aid in defining conservation studies for the declining *P. gerardiana* population in Balochistan, Pakistan. Intraspecific variation at morphological and molecular level through morphological and molecular markers, assessment of ecological diversity of the area and its impact on the genetic diversity help for a better understanding of the genetic structure of the species.

MATERIALS AND METHODS

Study site and ecological diversity of site

Zhob Forest of Balochistan, Pakistan is part of the Sulaiman Mountain Range that located in the eastern edge of the Iranian Plateau, where the Indus River separates it from the subcontinent (Figure 1). Its elevation is approximately 3,380 m above sea level (Table 1). The study area exhibits a dry temperate climate characterized by long winters from October to April and short summers from June to August. Though rain is scarce, precipitation is received mostly in the form of snow during winter. The data pertaining to meteorological conditions of the area during the study period are presented (Figures 2.A-B).

Table 1. Geographical attributes of the study sites

Sampling site	Site no.	Code	Latitude (N)	Longitude (E)	Elevation (m asl.)
Lower elevation zone	1	L1	31.55°	69.91°	2000
	2	L2	31.56°	69.92°	2050
	3	L3	31.55°	69.92°	2090
Mid semi-arid zone	4	M1	31.58°	69.93°	2800
	5	M2	31.57°	69.92°	2950
	6	M3	31.57°	69.93°	2960
Upper arid zone	7	U1	31.53°	69.91°	3440
	8	U2	31.57°	69.82°	3365
	9	U3	31.54°	69.91°	3380

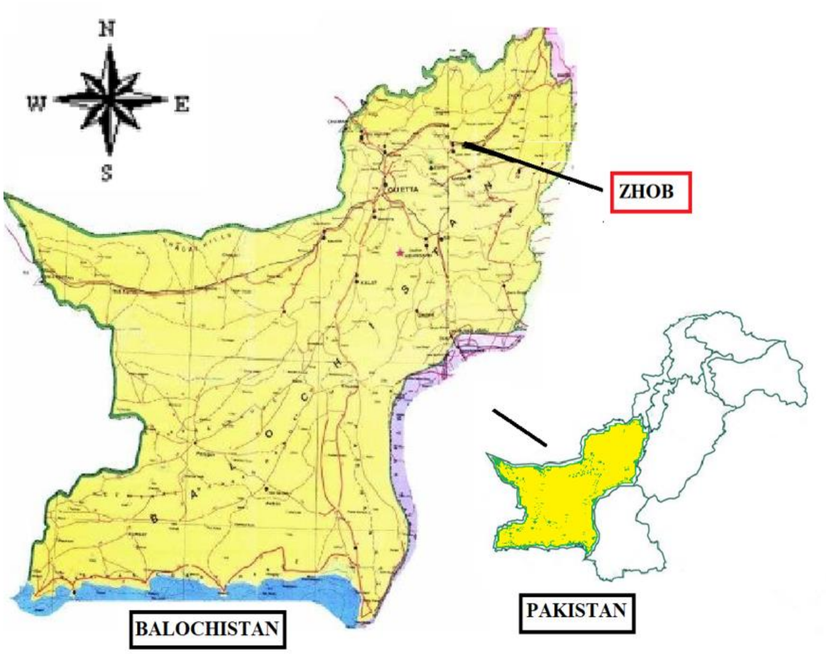


Figure 1. Map of study site in Zhob Forest, Balochistan, Pakistan

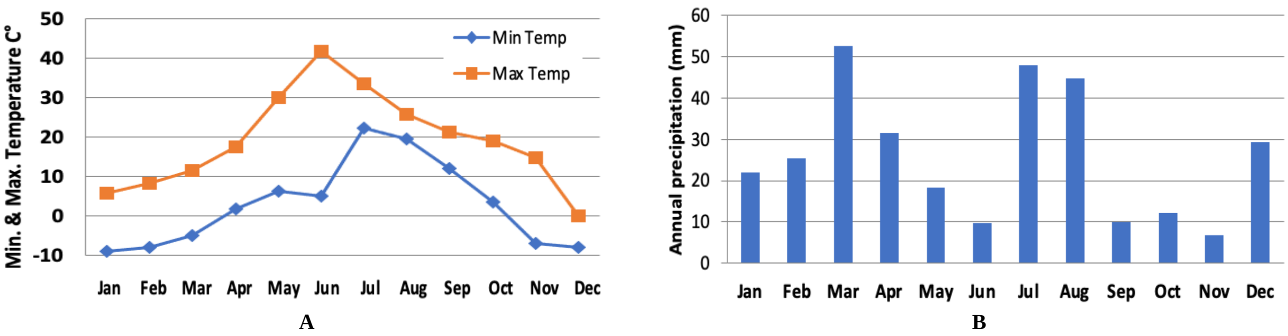


Figure 2.A. Temperature and B. Annual precipitation of the study site in 2018-2019

Sample collection

A survey was conducted during 2018-2019 to locate the populations of *P. gerardiana*. The whole distribution area of the species was divided into three zones, *viz*. lower elevation zone, mid semi-arid zone and upper arid zone. Data from three to five sites were recorded from each elevation zone. Soil samples were collected from each site for soil characterization. Fresh leaf samples for DNA extraction were taken from three randomly chosen trees in each site.

Ecological characteristics

Different ecological characteristics of the study sites were measured including microclimatic conditions and edaphic factors such as soil.

Elevation and aspects

Elevations of study sites were measured during sampling with the help of Global Positioning System (GPS). Aspects of the area were noted using Compass.

Temperature and annual precipitation

Temperature and annual precipitation data were collected for the years 2018-2019 from the metrological survey center, arid zone Quetta, Balochistan Pakistan.

Soil analysis

Composite soil samples were collected from the surface layer 0-30 cm depth. Samplings were made with systematically-randomized method (Zare et al. 2011). Samples were dried at room temperature, sieved with 2 mm sieve and then stored in zip bag for further analysis. Physical and other Characteristics were examined at "Peer Mehar Ali Shah Arid Agriculture University", Rawalpindi, Pakistan.

Survey of price list

To check the economic importance and economic value of plant, market survey was carried out. Price list was compared with open market (Table 2).

Morphological diversity analysis

To analyze the morphological diversity, tree samples were collected from nine sites at three different elevation zones. Samples were pressed and mounted on herbarium sheets. Data were analyzed by using morphological characters (S1).

Table 2. Market survey of *Pinus gerardiana* during 2018-2019

Year	Price in Zhob market	Price in open market
2018	1500-3000 PKR /kg (10-20 USD/kg)	6000-6500 PKR/kg (37-41 USD/kg)
2019	2500-3600 PKR/kg (16-24 USD/kg)	6200-7600 PKR/kg (39-48 USD/kg)

Phytochemical analysis

Total flavonoid content (TFC)

The TFC was estimated by Ordonez et al. (2006) technique using equation (Y=0.0255x, R^2=0.9812) absorbance at 420 nm.

Total phenolic content (TPC)

The TPC was calculated by the method of Slinkard and Singleton (1977) at 765 nm absorbance and expressed as mg/g tannic acid equivalent using the equation (Y=0.1216x, R^2=0.9365).

Genetic diversity analysis

Fresh pine needles were collected for DNA extraction and assessment of genetic diversity was undertaken by using molecular markers. The method of Saeed et al. (2017) was used for extraction and purification.

PCR reaction

A total of thirteen primers were used, including twenty RAPD and ten ISSR, amongst them thirteen were polymorphic (Table 3). The reaction mixture was used by also following the method of Saeed et al. (2017).

Statistical analysis

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, Agglomerative hierarchical clustering was performed using Minitab software.

Table 3. Details of polymorphic primers used in the study of *Pinus gerardiana*

Primer name	Sequence 3'—5'	TA (°C)	TB	P	PB %
OPA-2	TGCCGAGCTG	36	11	7	0.64
OPA-3	AGTCAGCCAC	34	7	3	0.43
OPA-4	AATCGGGCTG	34	12	5	0.42
OPA-7	GAAACGGGTG	34	6	3	0.50
OPA-8	GTGACGTAGG	36	4	2	0.50
OPA-13	CAGCACCCAC	36	11	8	0.73
OPA-17	GACCGCTTGT	36	10	7	0.70
OPA-20	GTTGCGATCC	36	7	6	0.86
UBC-810	(GA)8T	52	6	2	0.33
UBC-832	(AT)7 TYC	46	12	8	0.67
UBC-844	(CT)8RC	47	10	7	0.70
UBC-850	(GT)8YC	47	7	4	0.57
UBC-857	(ACA)5CYG	52	6	4	0.67
Total			109	66	0.61

Note: TA (°C): annealing temperature, T: Total bands, P: Number of polymorphic bands, PB: percentage of polymorphism

RESULTS AND DISCUSSION

This is the first comprehensive report on morphological, chemical and genetic diversity of an economically important tree, *Pinus gerardiana*, from the Zhob Forest, Balochistan, Pakistan. *P. gerardiana* is an ecologically- and economically valuable species, distributed in different parts of the world. In Pakistan, it is distributed in high mountainous zones of the Pakistani-Afghan border, Sulaiman Mountain Range, and Kashmir.

Economic importance

Pinus gerardiana is used as edible nuts and as medicinal plants in folk medicines by the local community of the study area. Economically, it is a very important nut. The local communities of the study area are categorized in the low-earning income bracket. No proper source of income has been identified, except for the collection of pine nuts, cutting of pine trees, and grazing in order to sustain their livelihood. The study area is in a remote, difficult geographical zone of the mountain, with a low literacy rate and under the threat of floods and cross-border disturbance. Moreover, no proper health facilities are available. A price survey was made in the year 2018-19. In 2018, it ranged from 1500-3000 PKR/kg (10-20 USD/kg) and increases to 2500-3600 PKR/kg ((16-24 USD/kg) in 2019. The price of nuts in the open market of Quetta and the rest of Pakistan is almost double that of the local market.

Environmental characteristics

The environmental parameters of the study sites varied along the altitudinal gradient (Table 4). The pH ranges from 6.64 to 7.45 from high elevation to low elevation. The total organic matter (2.63 ± 0.11%) was highest at high elevation. Soil at mid-elevation has high sand content (52.12 ± 4.18%), high elevation has high percentage of silt and clay (36.15 ± 2.65 and 18.12 ± 2.66, respectively). Sodium, potassium, and nitrogen were also reported highest at the high elevation zone. The concentrations of heavy metals, nickel and zinc (0.10 ± 0.04 and 0.04 ± 0.00 mg kg^{-1}, respectively) were high at the low elevation zone.

Morphological diversity

Morphology of the studied samples revealed that no variation was found in the studied population. In qualitative characters, no differences were found. The cone shape was nearly spherical, and cone color at maturation was dark brown, Seed shape is like a banana and the seed color was light to dark brown. Leaves were modified into needles that are dark green in color. Seed color varied along the altitudinal gradient. Seeds of high elevation were dark brown to black, while at low elevation, their color was light brown to reddish. Leaves were in fascicles of three needles. Quantitative characters showed a variation that may be due to the age of the tree or may be due to the impact of other environmental factors (biotic and abiotic factors). The height of tree was up to 40 ft (13 m) approximately. DBH of tree was up to 4 m. Leaf length 13 cm. Fruit size was 5 cm in length and 10 mm in width. The cone size ranged from 12 cm in length to 7 cm in width.

On the basis of quantitative characters (S1), cluster analysis was performed for all collected samples from three elevations. The cluster analysis revealed that there was a variation among the samples (Figure 2). This variation may due to anthropogenic activities or may be due to the impact of other environmental factors (biotic and abiotic factors). The age of the tree is the major factor in the variation of collected samples. Environmental factors and the soil type may also influence the size or growth of tree. As the moisture increases, the growth ring size also increases.

Genetic diversity

In the present results, 18 out of 30 primers produced clear bands in all collected samples. Two different marker systems, i.e., RAPD and ISSR were used for the first time on *P. gerardiana* from three elevation zones of Sulman Mountain Range. The amplified band size ranged from 200 to 1500 bp for RAPD and 150 to 1600 bp for ISSR (Figures 3 and 4). We used combined RAPD and ISSR markers to generate a dendrogram by cluster analysis. A combined marker system approach to detect polymorphism could be useful in removing errors and targeting various sites of genome as was previously used by (Saeed et al. 2020; Saeed et al. 2017).

Table 4. Mean values of environmental parameters along altitudinal gradient

Variables	Low elevation (Mean ± S.E)	Mid elevation (Mean ± S.E)	High elevation (Mean ± S.E)
Topographic variables			
Elevation (m)	2000 ± 50	2650 ± 45	3200 ± 45
Slope	32.50 ± 2.32	44.50 ± 5.75	48.45 ± 4.35
Edaphic variables			
pH	7.45 ± 0.12	7.23 ± 0.14	6.64 ± 0.17
Total organic matter (%)	1.62 ± 0.54	2.37 ± 0.14	2.63 ± 0.11
Sand (%)	50.3 ± 2.47	52.12 ± 4.18	45.32 ± 3.36
Silt (%)	33.21 ± 2.61	32.01 ± 2.57	36.15 ± 2.65
Clay (%)	16.07 ± 1.35	15.36 ± 1.45	18.12 ± 2.66
Sodium (mg kg-1)	11.45 ± 1.37	15.36 ± 2.34	17.33 ± 1.45
Potassium (mg kg-1)	6.74 ± 1.77	8.73 ± 1.75	11.18 ± 1.35
Nitrogen (%)	3.17 ± 0.44	3.28 ± 0.08	3.81 ± 0.74
Nickel (mg kg-1)	0.10 ± 0.04	0.08 ± 0.00	0.09 ± 0.03
Zinc (mg kg-1)	0.04 ± 0.00	0.02 ± 0.04	0.03 ± 0.01

Note: S.E.: standard error

Out of the twenty-five markers from the tested primers, thirteen primers had amplified polymorphic bands. Eight were from RAPD and five from ISSR and exhibited polymorphism, showing reproducible bands amongst nine *P. gerardiana* accessions. Table 4 identifies the characteristics of banding patterns obtained from the primers. Thirteen markers amplified 109 total bands and 66 were polymorphic (61% polymorphism). The total number of RAPD and ISSR bands scored per primer also varied. The overall data revealed an average of 8.38 bands obtained per primer.

Based on the UPGMA tree (Figure 5), the cluster is delimited into three main clusters showing the variation within the species along the altitudinal gradient. The Middle elevation zone retains the highest genetic diversity. It may be due to better environmental conditions and fewer anthropogenic activities.

The UPGMA clustering has been based on RAPD and ISSR populations. The cluster consists of two groups cluster A and cluster B. Cluster A consists of two subgroups of populations from the High elevation and Middle elevation zones, while cluster B comprised of populations from the low elevation zone.

Chemical diversity

All accessions of *P. gerardiana* were characterized as having significantly varied levels of total phenolic content (TPC), expressed as tannin equivalent and flavonoids contents as quercetin equivalent within and amongst populations (Figure 6). The TPC ranged from 40 to 58 mg^{-1} within the population. Flavonoids also showed a diverse pattern amongst all sites, ranging from 104 to 123 mg g^{-1}. Based on combined data of TPC and flavonoid contents, the dendrogram generated two main clusters: A and B. Cluster A comprised the population from high. Cluster B comprised of accessions from the middle elevation zone and low elevation zones (Figure 7).

Discussion

Pinus gerardiana grows in the tropical to subtropical regions of Pakistan. The seeds are used as edible nuts and medicine by the local people in the vicinity of the study sites. The *P. gerardiana* tree is facing the threat from anthropogenic activities like other conifers and conservation action needs to be undertaken on an urgent basis. The tree grows in high elevation cold climatic zones (Kumar et al. 2016). The present study aimed to investigate the genetic variability and the impact of environmental gradient with special reference to altitude and soil for this economically important tree. Molecular, phytochemical, and morphological variations were assessed on different samples collected along three elevation zones. From earlier reports, no evidence had been found on the genetic variation of *P. gerardiana* by using markers from a comparison of three different elevations. The regeneration pattern was studied for conservation as well as the population structures by (Akbar et al. 2014; Aziz et al. 2017).

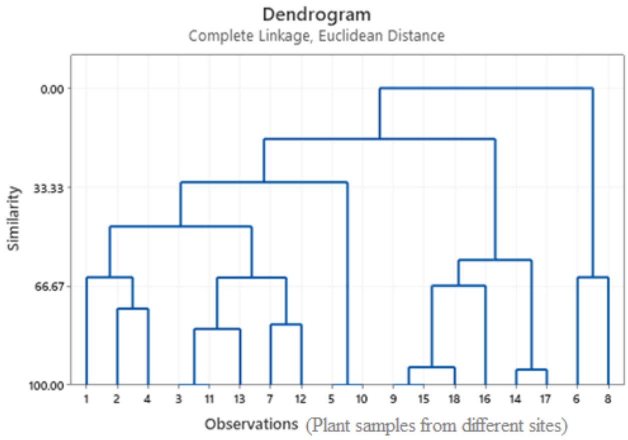
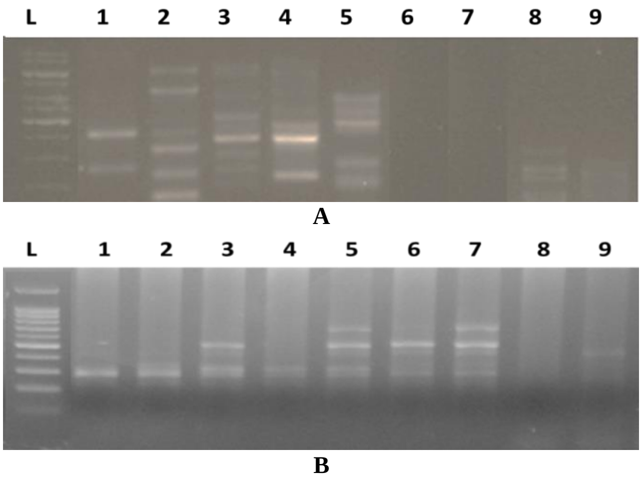


Figure 3. Tree diagram, based on quantitative morphological characters. Site number as coded in Table 1 (larger site numbers correspond to higher altitudes).



Figures 4. PCR amplification of *Pinus gerardiana*. A. RAPD OPA-1, B. ISSR- UBC-857 population from sites 1-9. With 1500 bp Leader.

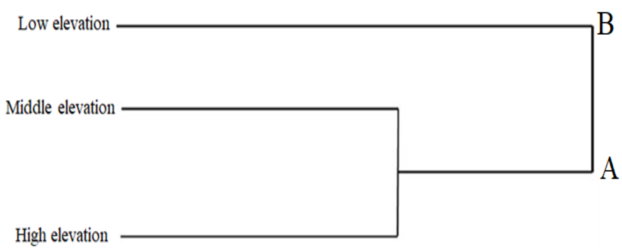


Figure 5. NTSYS- dendrogram of cluster analysis, based on data generated from RAPD and ISSR amongst three elevations

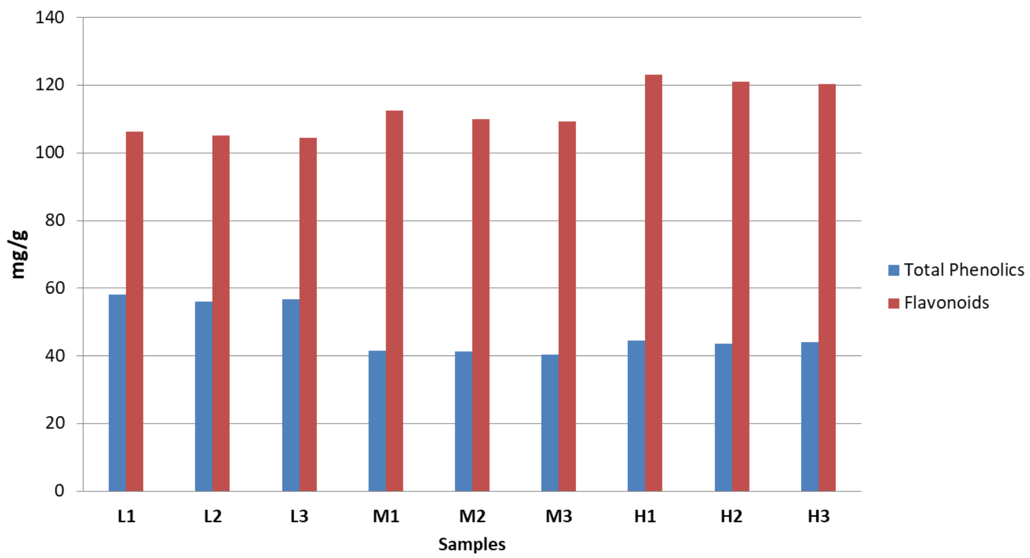


Figure 6. Patterns of phytochemical variations amongst different populations along elevation gradients. Site codes as: L: Low elevation, M: Middle, H: High.

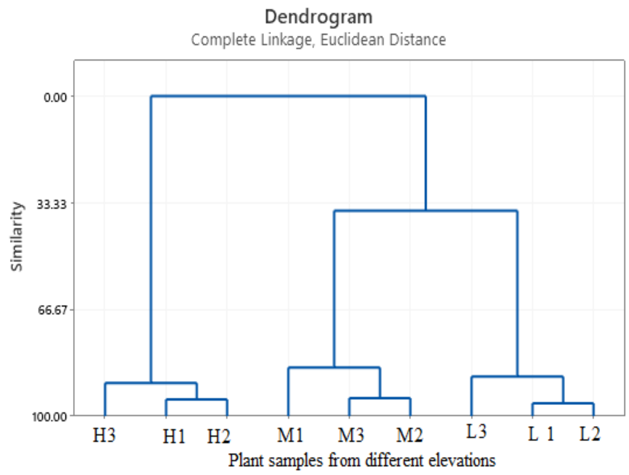


Figure 7. Dendrogram constructed using phytochemical diversity of samples from different populations along elevation gradients. Site codes as: L: Low elevation, M: Middle, H: High

Due to the importance of this valuable seed, it is greatly in demand, not only in the local market, but also from national and international markets (Peltier and Dauffy 2009). Earlier reports on an open market survey showed its prices to be approximately US$ 20-30 per kg. Open market prices are very high compared with the local market and best quality seed from the area is exported to other parts of the country and around the world (Akbar et al. 2013; Khan et al. 2015). In the global market, Pakistan, India and Afghanistan contribute the largest quantities of nut (Akbar et al. 2014; Kumar et al. 2014). Sample from different ecological sites shows the variation that may be the result of different climatic conditions and can be used to improve cultivation of the species. Conifers generally express a high degree of genetic diversity and are considered to be most variable group amongst the gymnosperms (Hamrick and Godt 1990). They express a high degree of variation amongst populations and a lesser extent of diversity (Bakshi and Konnert 2011). The genetic variation of *Pinus* may also be due to altitudinal gradients orchestrated with the change in climatic conditions associated with the variation in altitude (Loya-Rebollar et al. 2013).

Genetic variations were observed amongst the samples studied from different ecological regions that varied in altitude in our study. These variations of the population, along with altitudinal and environmental gradients, would be of great help in conserving the species for future climatic change (Sáenz-Romero et al. 2011). Species genetic resource conservation is required as geographically separated populations are expected to have different genetic compositions. Hamrick et al. (1992) stated that high genetic variation amongst the population may be due to woody plants with large geographical ranges with wind-assisted seed dispersal. Geographical distances and ecological consequences plays important role in the variability of component.

The present research shows intra-species genetic variation of *P. gerardiana* using RAPD and ISSR marker techniques. Eighteen genotypes from three elevation zones were assessed. Thirteen markers amplified 109 total bands and 66 were polymorphic (61%). The results suggest that RAPD markers show high genetic polymorphism in their capacity for producing polymorphic amplicons. Similar results were obtained by (Sinha et al. 2013) on the genetic polymorphism of *Pinus roxburghii*. Their study also justified the importance of genetic variations in both ex-situ *and* in situ conservations. In the present study, we found that chemical variation along altitudinal gradients may be a response to UV radiation. This may suggest the species adaptation to strong UV radiation and low-temperature environments at higher elevations. Flavonoid content is the main source for plant protection from UV radiation, and protects plant tissues. Moreover, many studies have provided new evidence that UV light induces the synthesis of flavonoids (Berli et al. 2010; Saeed et al. 2018).

Population structure of *P. gerardiana* is affected by different ecological factors like elevation, climate and soil variability. Similar findings were reported earlier (Kumar et al. 2013; Kumar et al. 2016; Sáenz-Romero et al. 2011; Sharma 2005) for *P. gerardiana.* In the present study, high genetic diversity within populations may be attributed to the effect of the environmental factors (Hahn et al. 2012; Sani et al. 2018) and anthropogenic activities in agreement with (Saeed et al. 2017). Climatic factors are associated with micro-geographical genetic differences, which may cause the phenotypic plasticity buffers against environmental changes over a plant's life cycle. Further, it weakens over time as climatic event changes.

Measurement of genetic diversity through molecular markers is difficult as it shows the adaptations of environment and other conditions. (Jump and Penuelas 2014). Earlier (Aziz et al. 2017) suggested the conservation of *P. gerardiana* for the future of this valuable Pine forest. This condition would have further broader implications, both for the ecosystem and livelihood of the local people.

In conclusion, based on our findings, it is concluded that there is an important genetic and phytochemical variation along the altitudinal gradient among the *P. geradiana* populations. Such patterning of genetic and phytochemical differentiation could result from the environmental (temperature, precipitation and soil characteristics) and the human disturbance variation along the altitudinal gradient. Our findings could help to design a conservation program that should include implementation of sustainable management plans, considering the large ecological and economic local importance of this pine species.

ACKNOWLEDGEMENTS

This research project was partially supported by University of Balochistan, UBRF Research Grant Program UBRF-17/018.

REFERENCES

Adams RP, Schwarzbach AE, Pandey RN. 2003. The concordance of terpenoid, ISSR and RAPD markers, and ITS sequence data sets among genotypes: An example from *Juniperus*. Biochem Syst Ecol 31: 375-387. DOI: 10.1016/S0305-1978(02)00157-6

Ahmed M, Khan N, Wahab M, Hamza S, Siddiqui MF, Nazim K, Khan MU. 2009. Vegetation structure of *Olea ferruginea* Royle forests of lower Dir district of Pakistan. Pak J Bot 41: 2683-2695.

Ahmed M, Palmer J, Khan N, Wahab M, Fenwick P, Esper J, Cook E. 2011. The dendroclimatic potential of conifers from northern Pakistan. Dendrochronologia 29: 77-88. DOI: 10.1016/j.dendro.2010.08.007

Akbar M, Ahmed M, Shaukat SS, Hussain A, Zafar MU, Sarangzai AM, Hussain F. 2013. Size class structure of some forests from Himalayan range of Gilgit-Baltistan. Sci Tech Dev 32: 56-73.

Akbar M, Khan H, Hussain A, Hyder S, Begum F, Khan M, Ali A, Hussian SA, Khan G, Abbas Q. 2014. Present status and future trend of chilghoza forest in Goharabad, District Diamer, Gilgit-Baltistan, Pakistan. J Biodiver Environ Sci 5: 253-261.

Aziz MA, Adnan M, Hussain SK, Hashem A, Alqarawi AA, Abd-Allah EF. 2017. Comparative regeneration status of *Pinus gerardiana* in two forest-use types of Sulaiman mountain range near Pak-Afghan border region: Historical, current and future perspectives. Pak J Bot 49: 227-236.

Bakshi M, Konnert M. 2011. Genetic diversity and differentiation through isozymes in natural populations of *Pinus wallichiana* A.B Jacks (Blue Pine) in India. Annals For Res 54: 23-37. DOI: 10.15287/afr.2011.95

Beg A, Mirza HK. 1984. Some more plant communities and the future of dry oak forest zone in Swat valley. Pak J For 34: 25-35.

Berli FJ, Moreno D, Piccoli P, Hespanhol-Viana L, Silva MF, bressan-Smith R, Cavagnaro JB, Bottini R. 2010. Abscisic acid is involved in the response of grape (*Vitis vinifera* L.) cv. Malbec leaf tissues to ultraviolet-B radiation by enhancing ultraviolet-absorbing compounds, antioxidant enzymes and membrane sterols. Plant Cell Environ 3 (1): 1-10. DOI: 10.1111/j.1365-3040.2009.02044.x

Hahn T, Kettle CJ, Ghazoul J, Frei ER, Matter P, Pluess AR. 2012. Patterns of genetic variation across altitude in three plant species of semi-dry grasslands. PloS One 7: e41608. DOI: 10.1371/journal.pone.0041608

Hamrick J, Godt M. 1990. Allozyme diversity in the grasses. Population biology of grasses, Cambridge University Press, Cambridge.

Hamrick JL, Godt MJW, Sherman-Broyles SL. 1992. Factors influencing levels of genetic diversity in woody plant species. Population genetics of forest trees. New Forest 6: 95-124. DOI: 10.1007/BF00120641

Hussain A. 2013. Phytosociology and dendrochronological study of Central Karakoram National Park, Northern Areas (Gilgit-Baltistan), Pakistan. Federal Urdu University Of Arts, Science & Technology, Islamabad.

Kernodle S, Cannon R, Scandalios J. 1993. Concentration of primer and template qualitatively affects product in RAPD-PCR. Biotechniques 14 (3): 362-364.

Khan H, Akbar M, Zaman M, Hyder S, Khan M, Nafees M, Raza G, Begum F, Hussain S, Khan S. 2015. Diameter size class distributions of *Pinus gerardiana* Wall. Ex D. Don from Gohar Abad Valley district Diamer, Gilgit-Baltistan. Pak J Biodivers Environ Sci 6: 50-56.

Kumar R, Shamet G, Avasthe R, Singh C. 2013. Ecology of chilgoza pine (*Pinus gerardiana* Wall) in dry temperate forests of northwest Himalaya. Ecol Environ Conserv 19: 1063-1066.

Kumar R, Shamet G, Mehta H, Alam N, Kaushal R, Chaturvedi O, Sharma N, Khaki B, Gupta D. 2016. Regeneration complexities of *Pinus gerardiana* in dry temperate forests of Indian Himalaya. Environ Sci Pollut Res 23: 7732-7743. DOI: 10.1007/s11356-015-6010-5

Kumar R, Shamet G, Mehta H, Alam N, Tomar J, Chaturvedi O, Khajuria N. 2014. Influence of gibberellic acid and temperature on seed germination in Chilgoza pine (*Pinus gerardiana* Wall.). Indian J Plant Physiol 19: 363-367. DOI: 10.1007/s40502-014-0119-2

Loya-Rebollar E, Sáenz-Romero C, Lindig-Cisneros R, Lobit P, Villegas-Moreno J, Sánchez-Vargas N. 2013. Clinal variation in *Pinus hartwegii* populations and its application for adaptation to climate change. Silvae genetica 62: 86-95. DOI: 10.1515/sg-2013-0011

Malik A, Shamet G, Ali M. 2008. Seed Stratification of *Pinus gerardiana* Wall.: Effect of stratification duration and temperature. Indian Forester 134: 1072-1078.

Meloni M, Perini D, Filigheddu R, Binelli G. 2006. Genetic variation in five Mediterranean populations of *Juniperus phoenicea* as revealed by inter-simple sequence repeat (ISSR) markers. Annals Bot 97: 299-304. DOI: 10.1093/aob/mcj024

Ordonez A, Gomez J, Vattuone M. 2006. Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. Food Chem 97: 452-458. DOI: 10.1016/j.foodchem.2005.05.024

Peltier R, Dauffy V. 2009. The Chilgoza of Kinnaur. Influence of the *Pinus gerardiana* edible seed market chain organization on forest regeneration in the Indian Himalayas. Fruits 64: 99-110. DOI: 10.1051/fruits/2009005

Saeed S, Ahmed A, Laghari S, Ali G, Begum S. 2020. Genetic and chemical variation of *Seriphidium quettense* endemic taxa of two mountainous regions of Quetta, Pakistan. Appl Ecol Environ Res 18: 2413-2423. DOI: 10.15666/aeer/1802_24132423

Saeed S, Ahmed A, Shah SH, Begum S, Zeeshan M, Khan W. 2018. Phytotoxic potential of wild medicinal plants from different

altitudinal gradients of Quetta Balochistan Pakistan on *Convolvulus arvensis* L. Pak J Weed Sci Res 24 (4): 323-334.
Saeed S, Barozai MYK, Ahmed A, Tareen RB, Ali S. 2017. Impact of ecological diversity on genetic and phytochemical variation injuniperus excelsa from high elevation zones of Quetta Valley, Pakistan. Pak J Bot 49: 201-206.
Sáenz-Romero C, Beaulieu J, Rehfeldt GE. 2011. Altitudinal genetic variation among *Pinus patula* populations from Oaxaca, Mexico, in growth chambers simulating global warming temperatures. Agrociencia 45: 399-411.
Sani SGAS, Chang PL, Zubair A, Carrasquilla-Garcia N, Cordeiro M, Penmetsa RV, Munis MFH, Nuzhdin SV, Cook DR, von Wettberg EJ. 2018. Genetic diversity, population structure, and genetic correlation with climatic variation in chickpea (*Cicer arietinum*) landraces from Pakistan. Plant Genome 11: 1. DOI: 10.3835/plantgenome2017.08.0067
Shalizi MN, Khurram S. 2016. Socio-economic importance of chilgoza pine forest of Afghanistan: A survey-based assessment. Asian J Sci Technol 7: 3556-3559.
Sharma V. 2005. Effect of artificial stratification of Chilgoza pine (*Pinus gerardiana*) seeds on its germination. Short Rotation Forestry for Industrial and Rural Development, ISTA, Nauni, Solan, India 266-270.
Sinha D, Singh J, Tandon P, Kakkar P. 2013. Genetic diversity of *Pinus roxburghii* sarg. collected from different Himalayan regions of India assessed by random amplified polymorphic DNA analysis. Toxicol Int 20 (3): 208-213. DOI: 10.4103/0971-6580.121667
Slinkard K, Singleton VL. 1977. Total phenol analysis: Automation and comparison with manual methods. Am J Enol Viticult 28: 49-55.
Thomas B, Macdonald S, Hicks M, Adams D, Hodgetts R. 1999. Effects of reforestation methods on genetic diversity of lodgepole pine: An assessment using microsatellite and randomly amplified polymorphic DNA markers. Theoretic Appl Genet 98: 793-801.