Research Article

Genetic Diversity of *Alnus nitida* Reported from Dir Lower, Khyber Pakhtunkhwa, Pakistan

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Abstract | Morphological characterization is important in determining the genetic variation of genotypes among different plant species and its knowledge help the breeders and farmers to select the best variety. *Alnus nitida* is one of the native and most important plants in District Dir Lower, Khyber Pakhtunkhwa, Pakistan, mostly used for medicinal purposes. In the present study, total 50 genotypes of *Alnus nitida* were collected from Dir lower and evaluated for morphological traits (leaf, petiole, nut, and catkin size). A significant level of variations was observed in the size of the leaf (10.22%), petiole (24.84%), catkin (9.19%), and nut (3.08%). There is a significant correlation between petiole size and leaf size, which is an important nutritional parameter that could be used successfully in future breeding programs. Based on cluster analysis all the genotypes were divided into two main lineages; lineage A which is further divided into 3 clusters (C1, C2, and C3), and lineage B, further divided into 2 clusters (C4 and C5). The principal component analysis estimated the total variations in the range of 51.43 to 100% with an Eigenvalue of 0.25. It was concluded from the results that *Alnus nitida* L. genotypes available in Pakistan have come from a narrow gene pool and such types of variations can be exploited to develop new varieties with desirable traits.

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Introduction

Alnus nitida belongs to the family Betulaceae, is a deciduous tree. The species is monoecious, flowers are either male or female. *Alnus nitida* (Spach) Endl is commonly known as Seril in Kashmir and Sharol in Punjabi, while in Pashto it is called Geiray. Its vernacular name is alder. It is a deciduous woody tree about 20 meters tall. It is native to the Himalayas and found in Kashmir to Western Nepal at an elevation of 1000 to 2700 m (Mandak *et al*., 2016). Young shoots are velvety and pubescent, becoming glabrous when old. Leaves are ovate to elliptic-ovate, petiole 1-4 cm long, glabrous to pubescent. Male flowers are borne in catkins, up to 19cm long. Female flowers arise in erect mostly solitary wood cones 3-3.57 cm x 1.28 cm, bract broadly ovate. Nut sizes are 2.5-4.0 mm long, fringed by the thin arrow and more or less leathery wings. *Alnus nitida* species comprised diarylheptanoids which is a group of natural compounds containing 1, 77-diphenylheptane skeleton and have diverse remedial effects such as anti-inflammatory,

antitumor, and antioxidant (Lv and She, 2011).

It can grow in semi-shady places and prefers dry moist or wet soil (Tung *et al*., 2010). The species is widely distributed and is most common along with the river's banks. These species occur in the temperate part of the Himalayas (Afghanistan, Pakistan, and Kashmir). It usually occurs at the elevation of (between 1000 and 3500 m). These species occur as a large tree reaching 20m in height or taller. A symbiotic relationship has been reported between this species and certain soil microorganisms. As a result, nodules are formed on the roots of the plants for nitrogen fixation. Seed can be sown in the spring. The seedling can either be planted in the autumn or winter. *Alnus nitida* have ethnobotanical and medicinal importance (Ozcan *et al*., 2009). A decoction of the bark is applied to treat swelling and body pain (Chung *et al*., 1998). The bark is also used in some places for tanning purposes. In oriental medicines, the plant has been used for the treatment of diarrhea, common fever, hemorrhages, and burn injuries (Sati *et al*., 2011). Besides, potential antioxidant, anti-inflammatory, anticancer and hepatoprotective activities have also been reported for this plant (Middleton and Kandaswami, 1994).

To find the ecological range of the plant's species, the ecological distribution of the plant's species within the specific habitat of a particular area is very important. It determines the exact location, altitude, latitude, longitude of the selected plant's species (Dobremez *et al*., 2009). It is a sort of documentation of the plant's species within a specific habitat. It will tell us about the altitudinal, longitudinal values of the plants. GPS will be used for this purpose and it will tell us about the altitudinal range of the plants from sea level. Morphological Characterization is either based on phenotypic observations or quantification of the quantitative traits (Severin *et al*., 2019). The phenotypic or quantitative traits include leaf size, petiole size, catkin size, and nut size (Frary *et al*., 2019). It is mainly used to check the level of genetic diversity and variability in the genotypes of *Alnus nitida* (Khan *et al*., 2020).

Throughout the world, *Alnus nitida* is the most popular plant having natural antioxidants and has attracted much interest because of its health benefits. This is the first study for documentation and evaluation of genetic diversity through morphological characterization of *Alnus nitida* in Dir lower Khyber Pakhtun Khwa, Pakistan. Keeping in view the present study was conducted to determine I. Exploration and documentation of *Alnus nitida* genotypes collected from Dir Lower II. To evaluate genetic diversity through agro-morphological characterization in the collected genotypes of *Alnus nitida.*

Materials and Methods

Different exploratory trips were arranged to various locations of District Dir Lower, Khyber Pakhtunkhwa, Pakistan, during 2018-2020. A total of 50 locations were identified and tagged for data collection. Flora of Pakistan was used for the identification of plant species. The plant samples were identified by Plant taxonomist, Dr. Ali Hazrat, Associate professor Department of Botany University of Malakand. Global position system **(**GPS) was used for ecological distribution and different habitats were documented for the studying biogeographical distribution of the species to identify the climatic suitable zone of the selected plant species in Dir lower.

Morphological characterization of Alnus nitida

Morphological Characterization (Schrader and Graves, 2004) was based on phenotypic observations or quantification of the quantitative traits. The phenotypic quantitative traits were leaf size, petiole size, catkin size, and nut size. It was mainly used to check the level of diversity (Mejnartowicz, 2008) and variability (Mingeot *et al*., 2016) among the genotypes of *Alnus nitida*.

Data analysis

For the evaluation of genetic diversity, data were recorded from five plants samples, and mean values of each genotype were used for statistical data analysis. Descriptive statistics (Mean, Maximum, Minimum, and Coefficient of variance) were calculated using Microsoft Excel. The mean data of all the studied parameters were subjected to cluster analysis to visualize the cluster dendrogram through principal component analysis using PC ORD version 5.0, and Statistica version 7. Pearson correlation coefficients were assessed using SPSS version 22.

Results and Discussion

In the present study, 50 genotypes of *Alnus nitida* were collected from Dir lower and evaluated for understanding their relationships and diversity through

agro-morphological traits. Our overall results revealed significant variation among the genotypes for most of the agro morphological traits.

Ecological distribution of Alnus nitida reported from District Dir Lower, Khyber Pakhtunkhwa

The latitude of *Alnus nitida* in 50 localities of Dir lower was studied. According to ecological distribution, all of the 50 localities from Toormang to Kasai-were found at the latitude of 34°5239'.95"N It means 100% of the genotypes of *Alnus nitida* were present at the latitude of 34°5239'.95"N in Dir Lower. During the present research work, 18 (36%) locations were found at a longitude of 71°E, whereas 32 (64%) were recorded with the range of 72°E. The elevation range was recorded with 2810 ft and 5812ft where the lowest location was Hajiabad while the highest location was Razagram. The range of highest location shows that 4% of genotypes were found at the highest range 16% were found at normal range while the remaining 80% were recorded at the lowest range (Table 1).

Table 1: *GPS Data scored during 2018-2020 reported from District Dir Lower, Khyber Pakhtunkhwa.*

S.No	Locality Name	Latitude	Longitude	Altitude/Elevation (in feet)
1	Toormang	34°5239'.95"N	72°02'15.52"E	3280
2	Qaziabad	34°59'43.35"N	72°55'26.41"E	3250
3	Shaheedabad	34°55'62.41"N	72°56'55.40"E	3300
4	Booray	34°58'83.40"N	72°58'45.32"E	3328
5	Gopalam	34°06'44.48"N	72°56'35.26"E	2900
6	Mula patay	34°08'34.56"N	72°59'60.27"E	3866
7	Kandawona	34°51'36.28"N	72°59'46.19"E	3678
8	Dulai kandaw	34°52'38.14"N	72°46'40.31"E	3910
9	Guzano banda	34°07'60.30"N	72°51'26.15"E	4500
10	Koza banda	34°06'34.50"N	72°57'28.45"E	4419
11	Haideray	34°56'68.22"N	72°55'60.53"E	4800
12	Sia gawnai	34°05'71.18"N	72°54'46.58"E	4400
13	Seer	34°05'69.44"N	72°52'56.20"E	3214
14	Mangoo	34°04'29.18" N	72°57'14.18"E	4600
15	Jabai	34°05'41.33"N	72°46'60.11"E	3418
16	Ghuz patai	34°08'50.27"N	72°06'60.34"E	3696
17	Patmangai	34°59'20.25"N	72°08'19.30"E	3995
18	Shalkany	34°53'46.12"N	72°59'44.17"E	3470
19	Adookay	34°59'48.59"N	72°57'53.15"E	3850
20	Razagram	34°57'30.20"N	72°59'40.16"E	5812
21	Manokas	34°57'83.09"N	71°58'11.18"E	4168
22	Shalfalam	34°56'70.11"N	71°53'34.23"E	4000
23	Shikawlai	34°55'50.38"N	71°59'19.41"E	3160
24	Aikdaray	34° O9'53.22"N	72°56'29.50"E	4590
25	Luqman banda	34°49'22.46"N	72°55'09.33"E	5500
26	Bagh	34°46'46.18"N	72°58'36.46"E	3588
27	Markhano	34°44'41.31"N	72°58'60.16"E	4675
28	Khall	34°58'40.43'N	71°58'56.35"E	2862
29	Kandaro	34°04'30.51"N	71°28'34.12E	2900
30	Barkalay	34°57'27.45"N	71°56'53.19"E	2886
31	Rabat	34°52'27.56"N	71°57'50.18"E	2910
32	Danwaa	34°56'48.28"N	71°59'31.15"E	2812
33	Munjai	34°57'28.44"N	71°57'43.12"E	2852
34	Timergara	34°54'76.42"N	71°59'33.22"E	2812
35	Hajiabad	34°55'29.17"N	71°55'37.54"E	2810
36	Balambat	34°52'58.34"N	71°03'56.58"E	2815
37	Kotto	34°54'56.42"N	71°55'49.19"E	2900
38	Sacha kalay	34°42'36.12"N	71°55'46.25"E	3018
39	Mandesh	34°28'45.26"N	72°53'50.39"E	2844
40	Malak abad	34°26'36.54"N	72°58'50.55"E	2966
41	Dadamo kas	34°08'48.50"N	72°55'52.38"E	3280
42	Mian banda	34°55'54.49"N	71°55'36.48"E	2816
43	Mansoor banda	34°09'56.38"N	72°58'28018"E	2888
44	Pambazaro	34°46'44.17"N	71°58'35.29" E	3619
45	Namseer	34°56'46.38"N	71°57'40.28"E	2877
46	Kamar tall	34°59'46.28"N	71°57'50.57"E	3416
47	Watango	34°56'53.60"N	72°58'45.18"E	3633
48	Watangay	34°55'16.32"N	72°56'18.55"E	3692
49	Khadang	34°57'38.55"N	72°58'22.40"E	3118
50	Kasai	34°56'33.15"N	72°59'34.44"E	3144

Diversity in Alnus nitida genotypes through morphological traits

During the present study, 4 important morphological traits of *Alnus nitida* were studied for the estimation of genetic diversity and a significant variation was found for most of the traits in the collected 50 genotypes. The leaf size was ranged from 10 to 13 cm with a mean value of 11.94, the standard deviation of 1.22 and the % of the variation was 10.22. The petiole size ranged from 2 cm to 4 cm with the mean value of 3.26 cm, the standard deviation of 0.81, and the %variation was 24.84, followed by the catkin size range from 14 cm to 18cm with the mean value of 16.20, the standard deviation of 1.49, and% of the variation was 9.19. Similarly, the nut size ranged from 2.50 mm to 2.70 mm with the mean values of 2.59

cm, the standard deviation of 0 .08, and % coefficient of variation was 3.08% (Table 2 and 3).

Table 2: *Descriptive statistics of 50 Alnus nitida using 4 morphological traits reported from District Dir Lower, Khyber Pakhtunkhwa.*

Traits	Range	Minimum	Maximum	Mean	Std. Deviation	%Variation
Leaf size (cm)	3.00	10.00	13.00	11.94	1.22	10.22
Petiole size (cm)	2.00	2.00	4.00	3.26	0.81	24.84
Catkin size (cm)	4.00	14.00	18.00	16.20	1.49	9.19
Nut size (cm)	0.20	2.50	2.70	2.59	0.08	3.08

Table 3: *Mean value of four quantitative traits of 50 genotypes of Alnus nitida collected from Dir Lower, Khyber Pakhtunkhwa, Pakistan.*

S/No	Locality Name	Leaf size cm	Petiole size cm	Catkin size cm	Nut size mm
1	Toormang	13	4	18	2.5
2	Qaziabad	13	4	17	2.6
3	Shaheedabad	12	3	16	2.7
4	Booray	10	2	18	2.5
5	Gopalam	13	4	15	2.7
6	Mulapatay	12	3	16	2.6
7	Kandawona	10	2	17	2.6
	Dulaikandaw	11	3	18	2.5
	Guzanobanda	13	4	14	2.7
	Kozabanda	13	4	14	2.6
	Haideray	12	3	17	2.5
	Siagawnai	10	2	16	2.6
	Seer	10	2	18	2.7
	Mangoo	13	4	17	2.6
	Jabai	10	2	14	2.5
	Ghuzpatai	11	3	15	2.7
	Patmangai	13	4	18	2.6
	Shalkany	13	4	16	2.5
	Adookay	10	2	17	2.6
	Razagram	13	4	14	2.5
	Manokas	13	4	14	2.7
	Shalfalam	12	3	15	2.5
	Shikawlai	13	4	14	2.6
	Aikdaray	10	2	15	2.7
	Luqmanbanda	13	4	14	2.5
	Bagh	11	3	17	2.6
	Markhano	10	2	15	2.7
	Khall	12	3	18	2.5
	Kandaro	10	2	17	2.5
	Barkalay	13	4	16	2.7
	Rabat	13	4	18	2.6
	Danwaa	12	3	15	2.5
	Munjai	13	4	16	2.7
	Timergara	12	3	15	2.7
	Hajiabad	10	2	18	2.5
	Balambat	13	4	17	2.6
	Kotto	13	4	18	2.7
	Sachakalay	11	3	17	2.5
	Mandesh	13	4	14	2.7
	Malakabad	10	2	15	2.6
	Dadamokas	13	4	18	2.5
	Mianbanda	12	3	16	2.7
	Mansoorbanda	13	4	17	2.7
	Pambazaro	13	4	18	2.6
	Namseer	11	3	17	2.5
	Kamar tall	13	4	18	2.7
	Watango	12	3	14	2.6
	Watangay	13	4	17	2.5
	Khadang	12	3	18	2.5
	Kasai	13	4	14	2.7

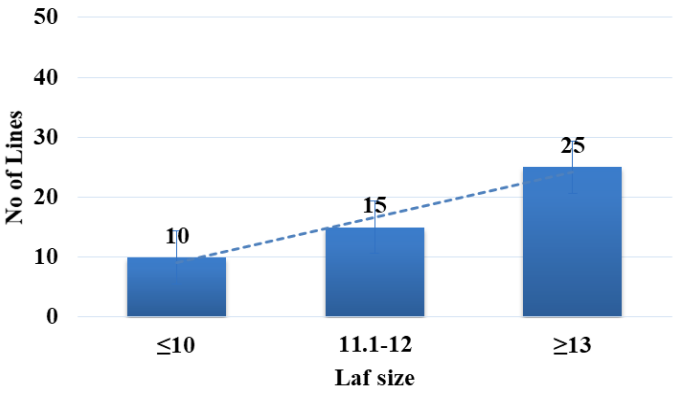

Figure 1: *Frequency distribution of leaf Size in 50 Alnus nitida genotypes reported from Dir lower.*

Leaf size of 50 Alnus nitida genotypes reported from Dir lower

During the present study, significant variation was found for leaf size. The frequency distribution was divided into 3 categories. The 1st group consists of 24 genotypes with the range of ≤10 cm and total variation of 48%, followed by 12 genotypes with the range of 11.1 to 12 cm with the variation of 20%, and the 3rd group consists of 25 genotypes with a range of ≥13 cm with the differences of 22% (Figure 1).

Petiole size of 50 Alnus nitida genotypes reported from Dir lower

The petiole size of *Alnus nitida* in 50 localities of Dir

lower was recorded and a significant variation was recorded. The frequency distribution was divided into 3 groups where the 1st consist of 9 genotypes with the range of ≤2 cm followed by 31 genotypes with the range of 3.1 to 4 cm and the last group consisted of 10 genotypes with the range of ≥5 cm respectively (Figure 2).

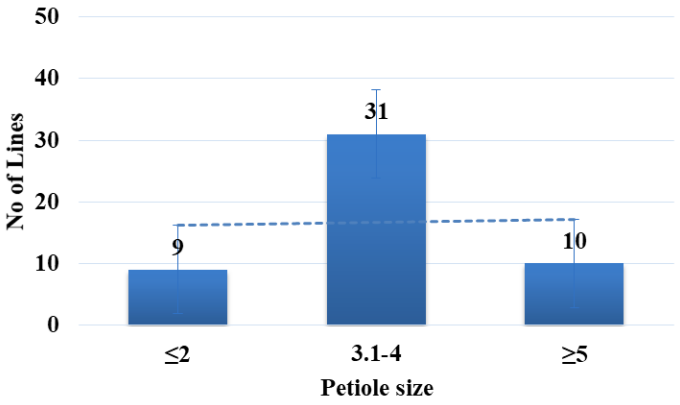

Figure 2: *Frequency distribution of petiole Size in 50 Alnus nitida genotypes reported from Dir lower.*

Catkin size of Alnus nitida of 50 Alnus nitida genotypes reported from Dir lower

The catkin size of *Alnus nitida* in 50 locations of Dir lower was studied. The frequency distribution of catkin size was divided into 5 categories. The 1st group consists of 10 genotypes with the range of ≤14 cm, followed by 15 genotypes with the range of 15.1 to 16 cm whereas 24 genotypes with the range of 17 to 18 cm while the 4th group consist of 1 genotype with the range of ≥19 cm. variations (Figure 3).

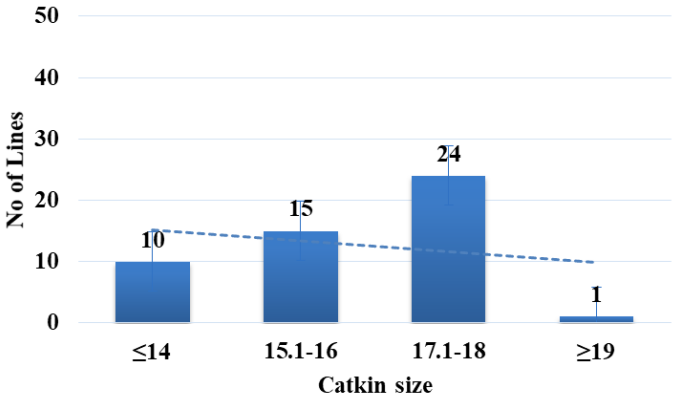

Figure 3: *Frequency distribution of catkin Size in 50 Alnus nitida genotypes reported from Dir lower.*

Nut size of 50 Alnus nitida genotypes reported from Dir lower

During the present study, significant diversity was recorded for nut size in the studied genotypes and all the genotypes were divided into 4 groups. The 1st group consisted of 14 genotypes with the range of ≤2.5 cm, followed by 25 genotypes with the range of 2.6 to 2.7 cm, 5 genotypes were recorded with the range of 2.8 to 2.9 cm, and the last group consisted of 6 genotypes with the range of ≥3 cm (Figure 4).

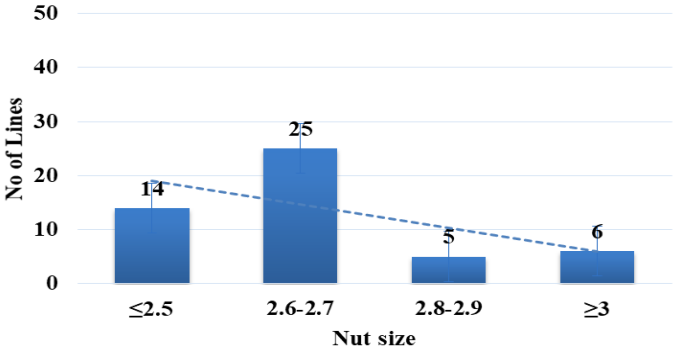

Figure 4: *Frequency distribution of nut Size in 50 Alnus nitida genotypes reported from Dir lower Khyber Pakhtunkhwa, Pakistan.*

Cluster analysis of four phenotypic traits of Alnus nitida collected from Dir Lower, Khyber Pakhtunkhwa, Pakistan

During the present study of *Alnus nitida* cluster analysis was performed using Ward's method, and all the genotypes were divided into 2 main lineages (1 and 2) at a distance of 25% where lineage 1 (L1) were further subdivided into 3 sub-cluster (C1, C2, and C3), while lineage 2 (L2) were divided into 2 sub-cluster (C4 and C5) at a distance of 75%. Each group member shows fewer variations to one another but there is large diversity when comparing with the other group members. The genotypes from Toormang and Hajiabad were found the most diverse and were at the extreme periphery of the dendrogram. The 1st group consists of 10 genotypes, the 2nd group consisting of 8 genotypes, and the 3rd consists of 11 genotypes from different locations. The 4th sub-cluster comprises 9 genotypes whereas the 5th sub-cluster consists of 10 genotypes (Figure 5).

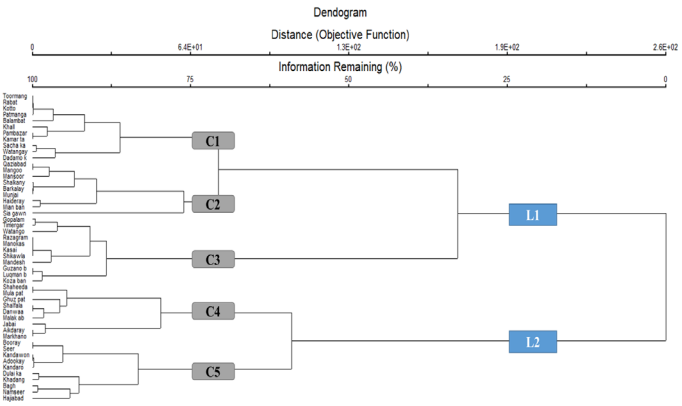

Figure 5: *Cluster dendrogram of 50 Alnus nitida genotypes based on 4 morphological traits collected from Dir Lower, Khyber Pakhtunkhwa, Pakistan.*

Principal component analysis (PCA)

Principal component analysis was performed using 4 morphological traits with the total variation of 100% Eigenvalue of 0.25. The first PC accounted for 51.43% out of the total variations 100%. The variations with the highest values are associated with the nut size (0.24) were found positive weight while on the other side leaf size (0.67 and petiole size (0.67 were also positive and catkin size (-0.17) was found negatively weight. In PC2 the total variation was 81.24% and the contribution of catkin size was 0.70 followed by petiole size 0.22 and leaf size 0.20 was found positively weight while nut size -0.64 was negatively weighed. In PC3 the total variation was 99.38% leaf size 0.05, and petiole size 0.02 were found positive while catkin size -0.68 and nut size -0.72 were found negative. Similarly, in PC4 the total variation was 100% while leaf size 0.70, nut size 0.06, and catkin size0.02had positive weight while petiole size -0.70 was found negative weight (Table 4).

Table 4: *PCA-Principal component analysis of 50 Alnus nitida genotypes using 4 quantitative traits reported from Dir Lower, Khyber Pakhtunkhwa.*

AXIS	PC1	PC2	PC3	PC4
Eigenvalue	2.05	1.19	0.72	0.02
% of Variance	51.43	29.81	18.13	0.61
Cum. % of Var.	51.43	81.24	99.38	100
Eigenvalue	2.08	1.08	0.58	0.25
Traits	**Eigenvector**			
Leaf size	0.67	0.20	0.05	0.70
Petiole	0.67	0.22	0.02	-0.70
Catkins	-0.17	0.70	-0.68	0.02
Nut size	0.24	-0.64	-0.72	0.06

Correlation analysis of 4 morphological traits of Alnus nitida reported from Dir lower

During the current study of *Alnus nitida* four morphological traits of 50 genotypes of *Alnus nitida* were evaluated (Lance *et al.*, 2009) to find out their correlation. During the present study petiole size was found strongly significant (0.97**) with leaf size, whereas catkin size, was found negative correlate with leaf size (-0.10), nut size was found significantly correlated with leaf size, and petiole size with the value of (0.15*), while found negatively (-0.27) correlated with catkin size (Table 5).

Table 5: *Correlations among the size of leaf, petiole, catkin, and nut of Alnus nitida in Dir Lower, Khyber Pakhtunkhwa.*

Traits	Leaf size	Petiole size	Catkin size	Nut size
Petiole size	0.97**			
Catkin size	- 0.10	- 0.07		
Nut size	0.15	0.15	- 0.27	
**. Correlation is significant at the 0.01 level.				
*. Correlation is significant at the 0.05 level.				

Conclusions and Recommendations

Ecological distribution is a very important step for the finding of the plant's species within the specific habitat of a particular area. The ecological distribution of *Alnus nitida* was studied for the first time in Dir lower. During the present study, 50 genotypes were collected from different regions and studied for morphological characterizations. The overall result shows significant variation for most of the traits. Of the total, 18 (36%) locations were found at a longitude of 71°E, whereas 32 (64%) were recorded with the range of 72°E. The elevation range was recorded with 2810 ft 5812ft. The range of highest location shows that 4% genotypes were found at the highest range 16% were found at normal range while the remaining 80% were recorded at the lowest range. Our results are in agreement with that of Mingeot *et al.* (2016) and Gu *et al.* (2009) who reported similar results in the genotypes of *Alnus glutinosa.*

A significant variation was found for 4 morphological traits where the variation for leaf size was 10.22%, petiole size 24.48%, catkin size 9.19%, and nut size with 3.08%. Our results are comparable with that of Mandak (2016) who studied genetic diversity in different genotypes *Alnus glutinosa* and reported a high level of genetic variations. Chen and Cheng (2008) reported similar results and found genetic variations in the fruit, seeds, and nut of *Alnus cremastogyne*. Our results agreed with the findings of Huh (1999) who studied the genetic diversity and population structure of Korean Alder *Alnus japonica* and found high-level genetic diversity. Genetic diversity provides the opportunity to plant breeders to select the potential genotypes for a specific area (Cubry *et al.*, 2015). The present study revealed significant diversity in the selected area and may be helpful to breeders for the selection of potential genotypes, the findings of our study may potentially assist in the genetic im-

provement of *Alnus nitida* genotypes. Our results also showed that *Alnus nitida* have considerable genetic variations Morphological characterization is the most important step toward genetic diversity but morphological are influenced by the environment. For future breeding, it is not sufficient for the selection of potential genotypes so it is recommended that in the future biochemical and molecular markers may be used.

Novelty Statement

This was the first study to analyze the genetic diversity based on morphological traits of the genotypes of *Alnus nitida* in the Dir Lower area. Taken together, the findings of our study may assist in the genetic improvement for plant breeders in the future of *Alnus nitida* genotypes

Author's Contribution

Javed Khan, Abdul Majid and Ali Hazrat: Collected result and did fieldwork, objective and title configuration
Nausheen Nazir, Muhammad Zahoor and Mohammad Ihsan: Result calibration with software's.
Azhar Hussain Shah: Discussion calibration with result.
Muhammad Ajmal Khan: Helped in the collection of data from the field.
Muhammad Yahya: References designing according to the journal standard.
Mohammad Nisar: Overall compilation of the paper.

Conflict of interest
The authors have shown no conflict of interest.

Reference

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