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MORPHOGENETIC CHARACTERIZATION OF GENUS *CRATAEGUS* FROM MUZAFFARABAD DIVISION, AZAD JAMMU AND KASHMIR PAKISTAN

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Abstract

Crataegus is highly medicinal plant and a genus of the family Rosaceae, globally distributed in the temperate zones of the northern hemisphere. Diversity, pharmacological aspects, and medical application of *Crataegus* species from India, Iran, and China have been worked out. But there is a notable knowledge gap in information on the genus *Crataegus* from the temperate Western Himalayan region of Pakistan. To have a comprehensive diversity look, there is a need for morphogenetic characterization, diversity assessment and preservation of the genus in the region which is the main objective of study. For morphogenetic characterization of genus *Crataegus* from division Muzaffarabad, Azad Jammu and Kashmir (AJK), characterization was done by field surveys during 2019-22. About 64 *Crataegus* accessions were assessed by using 8 morphological markers and 15 SSR based DNA markers. Data was analyzed by cluster and PCA and PIC analysis. Five major clusters emerged from the amplification morphological markers and six clusters based on SSR markers showing clear difference of markers choice. The current study also identified that the *Crataegus* genus is disappearing from the area because of anthropogenic impact, including deforestation, urbanization, agricultural practices, and unawareness about its importance. Further SSR based polymorphism is not sufficient as seen in PCR results and low PIC values indicating that deforestation and anthropogenic activities contribute to lowering the polymorphism. In conclusion the *Crataegus* populations in study areas are facing anthropogenic pressure and disappearing by overexploitation.

Key words: Genetic variability; Morphological diversity; Molecular markers; Cluster analysis; *Crataegus songarica*; *Crataegus clerkei*

Introduction

Crataegus is a genus of family Rosaceae and subfamily Maloideae, commonly known as hawthorn, having the ability to interbreed freely which do not form large trees and dominant canopies in a forest. Some species may attain a height of 12 m, while some are shrubby (Huang *et al.,* 2016). Several hundred species are included in the genus *Crataegus* and found across temperate zones in the Northern Hemisphere (Fichtner & Wissemann, 2021). *Crataegus* species have widespread distribution in the northern hemisphere's temperate zones, with around two hundred eighty species, and are native to Central Asia, Mediterranean region, Europe, and North Africa (Arya & Thakur, 2012). Traditional uses of *Crataegus* species include treatment for cardiovascular disorders such as peripheral heart diseases, angina, hypertension, hyperlipidemia, congestive heart failure, and diabetes mellitus (Abdul-Kareem *et al.,* 2009; Shatoor *et al.,* 2012).

Crataegus species have been used as food, medication, ornamentation, and gardening. Due to its bitters taste and potential medicinal properties, hawthorn fruit has garnered attention and is used in candies, confits, and other products, with extraordinary economic worth. Hawthorn is believed to have cardiovascular benefits, including improving heart health, regulating blood pressure, and reducing cholesterol levels and also aid in digestion (Chinese Pharmacopoeia Commission 2020). Medicinal investigations also demonstrate the ability of hawthorn to improve blood flow, antioxidant, hepatoprotective, antiaging, antihyperlipidemic antibacterial, anticancer, insomnia, asthma, gall bladder diseases, and diarrhea (Jurikova *et al.,* 2012; Dong *et al.,* 2017; Jennifer & Paula, 2012). Diversity, pharmacological aspects, and medical application of *Crataegus* species from India, Iran, and China have been worked out (Yusuf & Mericli, 2016; Kumar *et al.,* 2012 Verma *et al.,* 2007; Nabavi *et al.,* 2015).

DNA molecular markers are beneficial because of their application in molecular breeding of plants; these are phenotypically unbiased, polymorphic, codominant, reproducible, genome wide, usable, period and phase independent (Hoshino *et al.,* 2012). The knowledge of available germplasm genetic diversity is necessary in material selection for cultivation. On the basis of high reproducibility, high genome coverage, abundance, and co-dominance, SSRs are also called microsatellites, and these are known to be the most popular genetic markers (El-Esawi *et al.,* 2016). Microsatellites have been effectively used to examine the molecular diversity of plants (Nawaz *et al.,* 2017).

Various genetic markers have been used to support plant breeders for plant selection, comprising desired characters. For the use of genetic resources in the conservation of biodiversity and breeding of a population, its genetic characterization is essential. So, knowledge of available germplasm genetic diversity is necessary in material selection for cultivation. Specialized tools, such as DNA markers, can be applied to study the genetic diversity (Kapila *et al.,* 2008).

Understanding genetic diversity is crucial for the existence of *Crataegus* species in a variety of environments

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and for the development of their germplasm. The data in this study are valuable for enhancing the genetic diversity of *Crataegus* accessions in Division Muzaffarabad AJK. To guide the conservation of germplasm while confirming that the significant genetic diversity is conserved within the research region, the real application requires the characterization of molecular differences present in *Crataegus* accessions. SSR markers being codominance, automation suitability, high polymorphism, speed, and dependability were selected. This is the first in-depth investigation employing the potent microsatellite approach to evaluate genetic diversity and connections in Division Muzaffarabad Crataegus species. The 15 SSR primers used in this study were polymorphic and helpful for separating the accessions under investigation. The present study is designed to investigate the morphological genetic characterization and distribution of the genus *Crataegus* from division Muzaffarabad AJK.

Materials and Methods

Collection sites: Azad Jammu and Kashmir is situated around 34.250 N and 74.040 E. In general, the area experiences temperate, sub-temperate, and alpine climates. Neelum district has an alpine to dry subtropical climate with snowy winters and hot, dry summers. Muzaffarabad and Hattian Bala have an alpine to sub-tropical climate, with snow above 3500 meters, precipitation at lower elevations throughout winter, and a rainy season in July and August. (Khan *et al.,* 2018). The selected area for study lies in the western Himalayas, close to occupied Kashmir, and has subtropical to temperate (dry and wet temperate) and alpine vegetation (Anon., 2008). *Crataegus* species was collected from Division Muzaffarabad, AJK. Several field surveys were conducted in areas where species of the genus *Crataegus* were present during the year 2019–22 *Crataegus* samples (64) were collected and were identified by comparing them to herbarium specimens kept in herbarium Department of Botany, University of Azad Jammu and Kashmir and using the flora of Pakistan, the Flowers of the Himalaya, the Flowers of India, and the e Flora of China (Ali & Qaisar, 1980-1989; Nasir & Ali, 1970-1979). After identification, plant samples were dried, pressed, and mounted using standardized herbarium methods on herbarium sheets (Alexiades, 1996).

Morphological trait analysis: Morphological traits of *Crataegus* samples were evaluated using quantitative measurements. Wild hawthorn leaf and fruit samples were obtained from several geographical locations in Division Muzaffarabad, AJK. Fruit samples were randomly selected at each accession ripening date from trees. Fruit weight (g), fruit length (cm), and the seed number per fruit, among other morphological variables, were noted (Anon., 1980). The measurements were conducted on 30 randomly chosen samples using a vernier caliper for length, width, and an electronic balance with an accuracy of 0.01g for weight measurements. These characteristics were assessed and averaged across each accession.

Genetic trait analysis: Genetic characterization was carried out in the National Agricultural Research Center, Islamabad. Genomic DNA was extracted using the CTAB (Cetyltrimethylammonium Bromide) method with modification using 65ºC temperature for 1.5 hours (Verma *et al.,* 2007). Fifteen SSRs primers were used for PCR amplification. The primers are listed along with their sequences. In a reaction volume of 25µl, amplification was carried out under initial denaturation at 65ºC for 1 mint followed by cycling of melting temperature of 94ºC for 1 mint, annealing primers in range of 50-60°C (calculating from melting temperature of each primer se), extension at 72ºC for 1 mint and a final extension for 5 mints at 72ºC. The reaction mixture was given a brief spin and aliquoted into 0.2ml PCR tubes. DNA was added at the last, and the PCR was carried out in a thermal cycler. The final PCR amplified products were run an 1.5% gel and visualized under UV gel doc imaging system after staining with ethidium bromide for 20 mints.

Statistical analysis: Morphological data were analyzed using the computer packages SPSS version 23 and Past. Using software NTSYS-pc version 2.1, the amplified bands produced by SSR PCR amplification were scored according to whether or not bands were present for each primer (1) or absent (0). These scores were then utilized to compute a genetic similarity matrix using the simple matching coefficient. Using the "Unweighted Pair Group Method Using Arithmetic Means" (UPGMA) algorithm, cluster analysis was carried out on both morphological and molecular data. NTSYS-pc software was then used to build and plot dendrograms showing variety similarity. The degree of association between the original similarity matrix and the tree matrix in both the morphological and molecular analyses was determined by calculating the cophenetic correlation, while PIC value of molecular markers was calculated by Power Marker software.

Results

The present study was conducted with the objective of finding out the molecular and morphological diversity to investigate the relationship within and among the *Crataegus* species from division Muzaffarabad, AJK. Molecular as well as morphological characterization of *Crataegus* species by using molecular and morphological markers. Based on numerical characterization, dendrogram was constructed which shows maximum morphological similarities among sixty-four samples of *Crataegus. Sixty-four* samples of *Crataegus* were classified into 4 main clusters (A, B, C, D and E). Cluster A contained three specimens, cluster B consisted of 23, cluster C consisted of 12 species, cluster D consisted of 23 species, and cluster E consisted of 3. The data was analyzed on the similarity level.

These clusters were dissimilar from each other. The dendrogram successfully characterized the morphological diversity among *Crataegus* accessions. Outcrossing among the cultivars, which is a significant factor in diversity variation, showed the scattering of *Crataegus* accessions into various clusters and sub-clusters.

On the molecular profile, the amplification profile was produced by fifteen SSRs (SSR1, SSR2, SSR3, SSR4, SSR5, SSR6, SSR7, SSR8, SSR9, SSR10, SSR11, SSR12, SSR13, SSR14 and SSR15) primers (Fig. 1). The difference coefficient average was observed in 64 genotypes of *Crataegus* based on the data of SSR primers, using NTSYS software was used to make a dendrogram.

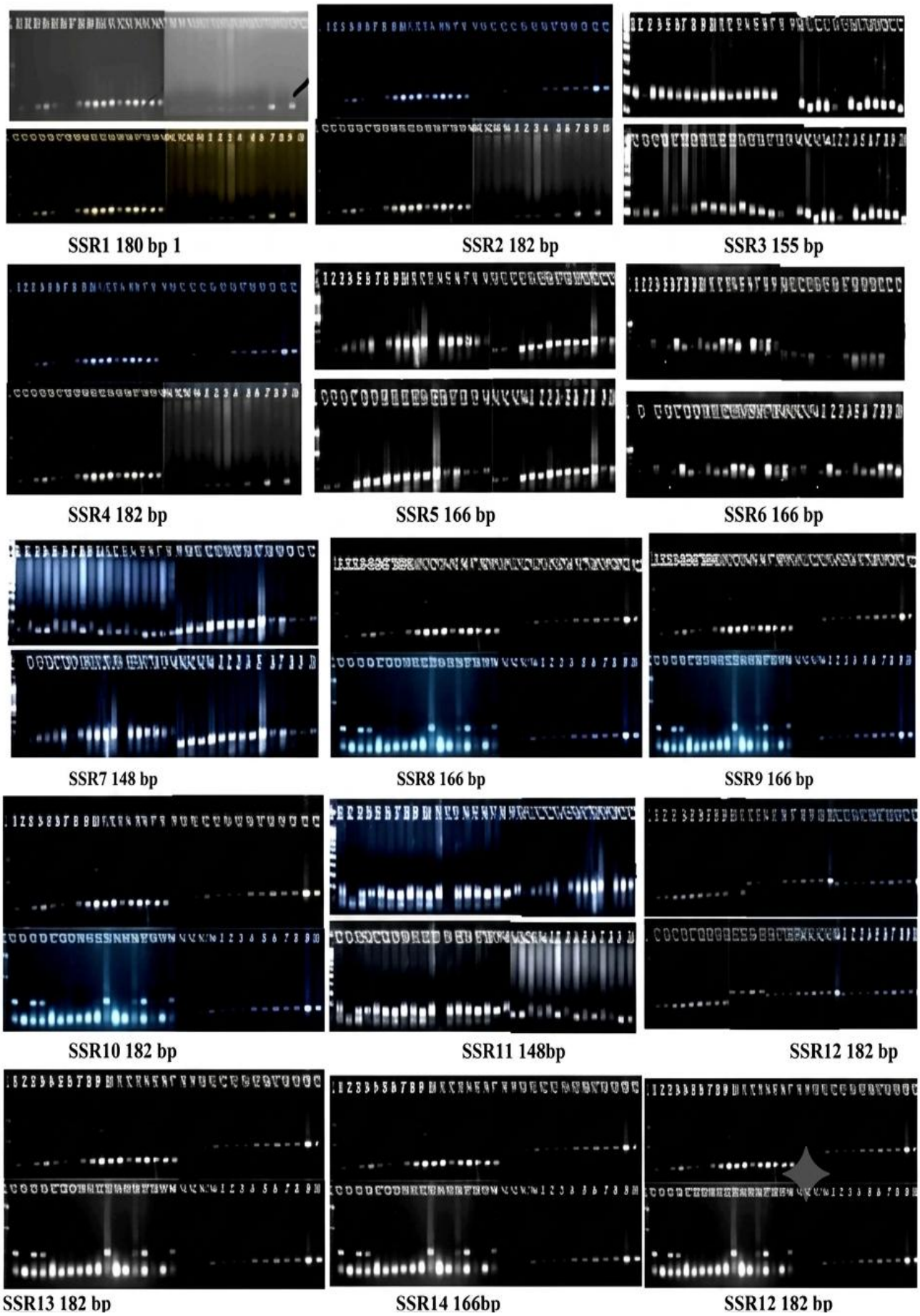

Fig. 1. Amplification of representative SSR primers from DNA isolated from 64 different collected leaf samples of *Crataegus* Lane 1: Ladder (1kb), Lane B1 - Lane N22 DNA sample. In second rows Lane 1: Ladder, Lane N23 - Lane J10 DNA sample.

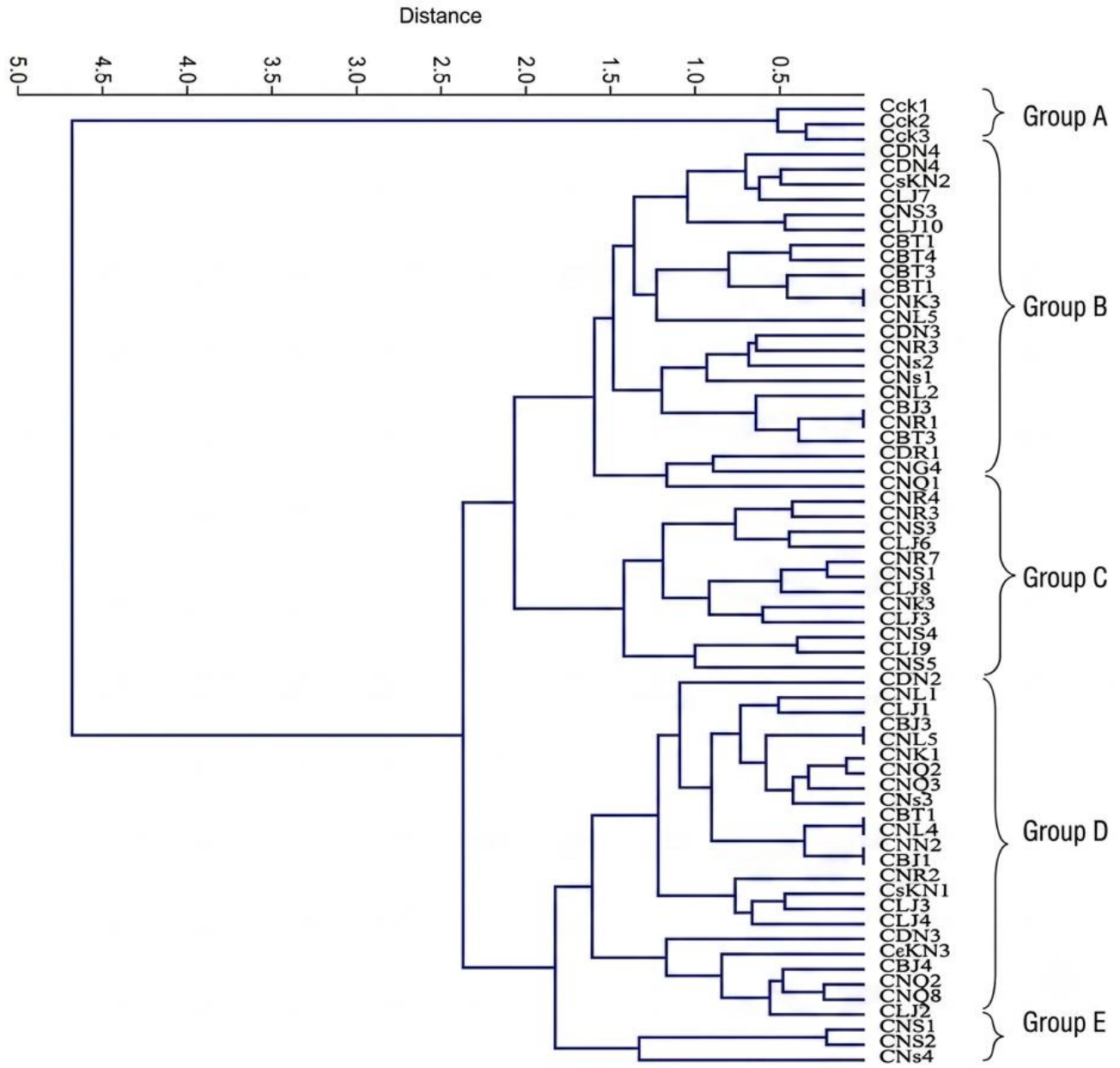

Fig. 2. Dendrogram revealing Morphological variations among sixty-four *Crataegus* accessions of Division Muzaffarabad and cluster analysis. 8 morphological variations were used as markers for this study.

Analysis of morphological traits

Cluster analysis: The data was examined using a cluster analysis based on the degree of similarity. Clustering algorithm analysis grouped 64 accessions using 8 morphological markers into five clusters (A, B, C, D and E) (Fig. 2). These clusters were dissimilar from each other. The dendrogram successfully characterized the morphological diversity among *Crataegus* accessions. Outcrossing among the cultivars, which is a significant factor in diversity variation, showed the scattering of *Crataegus* accessions into five clusters and then sub-clusters.

Cluster A consists of three species of *C. clerkei* collected from Neelum Kail at an altitude 2085-2096m. Their leaf size was the largest among all accessions, ranging from from7.58-.98 cm in length and 4.72-4.93cm in width, fruit length and width 2.14-2.43cm and 1.67-1.98cm respectively. The average fruit was 1.87g with 5 pyrenes. All accessions were without thorns. In cluster B 23 accessions (36%) are grouped together, belonging *C. songarica*. Cluster B comprises accessions of *C. songarica* collected from Neelum (Dwarian (CDN), Sharda (CNS), Reshna (CNR), Lawat (CNL), Qadir Abad (CNQ) and Surgan (CNs)) and Muzaffarabad (Bheri Jabbar (CBJ), Bheri Takkia (CBT), two from Neelum Qadirabad and one from Dwarian. These sites are suitable for the growth and regeneration *C. songarica*, which bear considerable sizes of leaves and fruits. This is may be due to fewer environmental differences between these two locations. Leaf size of these accessions ranged from 5.19-6.76cm, 3.96-5.58cm in length and width, respectively, while fruit length and width were 1.06-2.14cm and 1.02-1.56cm. All fruit were found with 2 pyrenes and the recorded weight was 0.79-1.63g. Cluster C comprised of 12 accessions (19%) of *C. songarica* collected from Neelum Reshna (3), Sharda (4), Keran (1) and Jehlum valley Leepa (4). Leaf size of these accessions ranged from 5.49-6.87cm, 4.87-6.08cm in length and width,

respectively, while fruit length and width were 1.1.4-2.01cm and 1.11-1.59cm. All fruit were found with 2 pyrenes and the recorded weight was 1.16-1.61g.

Cluster D consists of 23 accessions of *C. songarica* (36%). It is subdivided into DI and DII. Sub-cluster DI comprised of 17 accessions, 10 from Neelum (Dawarian (1), Lawat (3), Kail (3), Qadirabad (2) and Reshna (1)) 3 from Muzaffarabad (Bheri Jabbar (2) and Bheri Takkia (1) and 5 from Jehlum Valley (Leepa (5)). Sub cluster DII comprised of 6 accessions (9%) of *C. songarica* 4 from Neelum (Qadirabad (2), Dawarian (1), Kail (1)), 1 from Muzaffarabad (Bhri Jabbar), and 1 from Jehlum valley (Leepa). Accessions of this group have a leaf size of 4.05-5.28 cm in length, 3.14-3.77cm in width, fruit size 1.06-1.56 cm in length, 1.01-1.82cm in width, and 0.38-1.42g in weight was recorded. Cluster E comprised of 3 accessions (4%) of *C. songarica* from Neelum (Keran (2), Surgan (1). Their leaf length 4.14-4.90cm, width 3.1-3.8cm, fruit length 1.04-1.78 cm, width 1.13-1.18cm, and weight was 0.99-145g. The morphological marker showed that 64 accessions are divided into 5 groups based on their 8 morphological variations which is different from DNA based SSR markers-based clustering.

Principle component analysis: In the current study, PCA determined the similarity of acquired information. Morphological variation among *Crataegus* accessions using morphological markers. The pattern of morphological character variation among the accessions was described by PCA. A PCA bi-plot showed the similarities and differences between 64 *Crataegus* accessions. In PC1, with an eigenvalue of 1.56629 accounted for 52.018% of the total variability, followed by PC2 with an eigenvalue 0.839303 accounted for 27.874% of the total variation observed among the 64 *Crataegus* accessions. PC3 and PC4 had eigenvalue 0.2887465 and 0.101367, with total variability of 9.5778% and 3.3665%. PC5, PC6, PC7 and PC8 on the other hand, had eigenvalues of 0.0887456, 0.0741487, 0.0392276, and 0.0135736, respectively, and accounted for 2.9473%, 2.4626%, 1.3028% and 0.45079% of the total variability. The ordination of the characters on the upper two projections showed that certain variables had high correlations with one another, as evidenced by the overlapping patterns of variables. The right and left quadrants of the plot show the variables responsible for the majority of the variation. When two variables are on the same PC and very close to one another, it indicates that they have positive correlations and that an increase in one will result in an increase in the other. When two variables are on opposite ends of the PC, however, they have negative correlations, and an increase in one will result in a decrease in the other. As opposed to the upper two quadrates, the lower two quadrates of the projections contain a compact group of *C. songarica*. In the PCA diagram, each *C. clerkei* species was visible separately. The species inhabited an altitudinal range >2000 meters. It showed variations from other accessions in all morphological aspects. The greatest leaf and fruit length and width were recorded. The number of stones was five, and all the trees were without thorns. The configuration of the sixty-four *Crataegus* accessions belonging to two different species along the first two principal component axes is shown in Fig. 4. CCK 1, 2 and 3 morphological variations are not involved in clustering and discriminating of 64 accessions. While thorns, leaf length, width and pyrene number appeared as distinct morphological markers and behaved independent of others in clustering (Fig. 3).

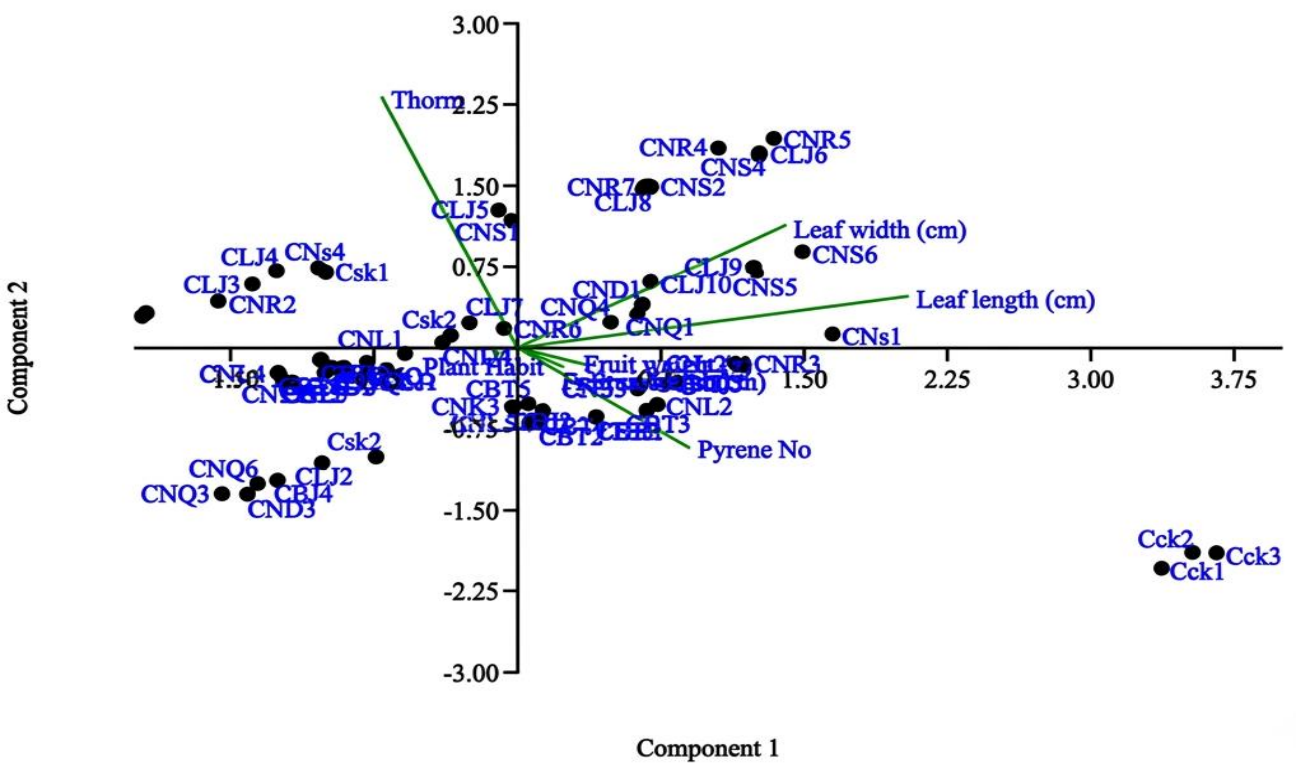

Fig. 3. Principle Component Analysis (PCA) describing the variations by using morphological markers among sixty four *Crataegus* accessions of Division Muzaffarabad.

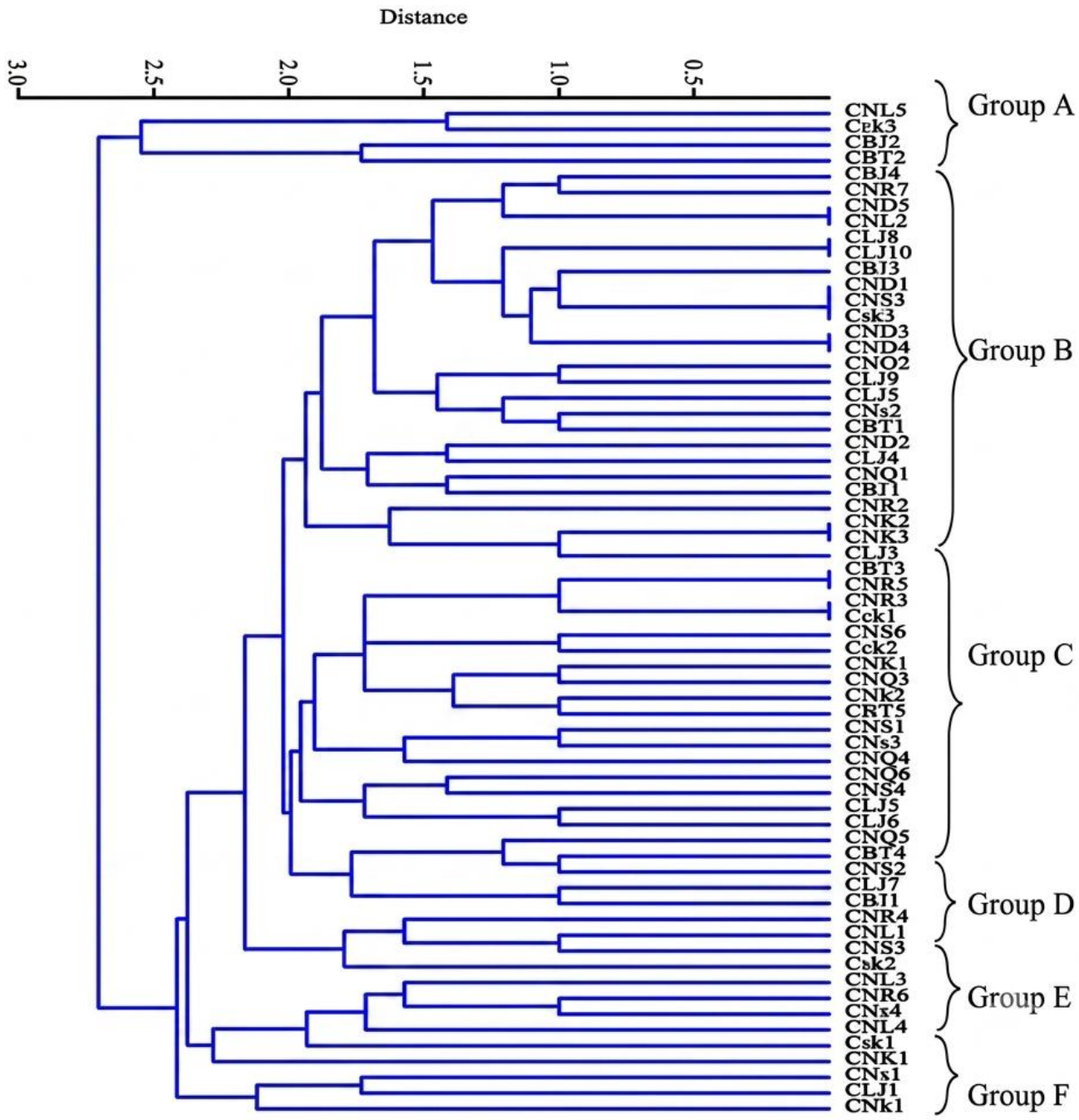

Fig. 4. Dendrogram revealing genetic variation among sixty four *Crataegus* accessions of Division Muzaffarabad based on fifteen SSR based molecular markers and cluster analysis (Wards).

Genetic characterization: On the molecular profile, the amplification profile was produced by fifteen SSRs (SSR1, SSR2, SSR3, SSR4, SSR5, SSR6, SSR7, SSR8, SSR9, SSR10, SSR11, SSR12, SSR13, SSR14 and SSR15) primers. The PCR amplified SSR of sixty-four accessions is shown in figure 1. Amplified SSR bands did not show polymorphic multiple bands (Fig. 1) showing that loss of polymorphism based on 15 SSR markers. The SSR markers showed that 64 accessions are divided into 6 groups based on their 15 DNA based repeats from SSR markers-based clustering.

Cluster analysis: The UPGMA clustering algorithm from SSR markers analysis grouped the 64 accessions into six main groups (Fig. 4).

Group A: The first group consists of 4 accessions, which are further divided into two subgroups. The first subgroup consists of CNL5 and Cck3. The second subgroup consists of CBJ2 and CBT2.

Group B: Second group have 25 accessions (CBJ4, CNR7, CND5, CNL2, CLJ8, CLJ10, CBJ5, CND1, CNS3, CsK3, CND3, CND4, CNQ2, CLJ9, CLJ5, CNs2, CBT1, CND2, CLJ4, CNQ1, and CBJ3, CNR2, CNK2, CNK3 and CLJ3).

Group C: The Third group comprises of 22 accessions (CBT3, CNR5, CNR3, CcK1, CNS6, CcK2, CNR1, CNQ3, CNK2, CBT5, CNS1, CNs3, CNQ4, CNQ6, CNS4, CLJ2 and CLJ6, CNQ5, CBT4, CNS2, CLJ7, and CBJ1).

Group D: The Fourth group consists of four accessions (CNR4, CNL1, CNS5 and CsK2).

Group E: Fifth group comprises of six accessions (CNL3, CNR6, CNs4, CNL4, Csk1 and CNK1, respectively.

According to the explanation of clustering provided above, most accessions were grouped together with respect to collection site. This may be because they have been reproducing continuously at that location for a number of years and because all of the local selections have genetic makeups that are largely similar. Although there is less use of germplasm for additional multiplication due to geographic differences.

Principal component analysis (PCA): The pattern of molecular marker variations among the accessions was characterized by PCA. A PCA bi-plot exhibited the similarities and differences among 64 *Crataegus* accessions. PC1 (SSR1), which accounted for 18.514% variability, had the eigenvalue of 0.439837 was followed by (SSR2), with eigenvalue 0.31627, accounting for 13.312% of the total variation observed among the 64 *Crataegus* accessions. PC3 (SSR3) had eigenvalue 0.275168 with a total variability of 11.582%. PC4 (SSR4), PC5 (SSR5), PC6 (SSR6), PC7 (SSR7), PC8 (SSR8), PC9 (SSR9) and PC10 on the other hand, had eigenvalues of 0.239728, 0.199103, 0.194039, 0.152545, 0.121754, 0.106963 and 0.100288 respectively and accounted for 10.091%, 8.3806%, 8.1675%, 6.421%, 5.1249%, 4.5023% and 4.2213% of the total variability. PC11 (SSR11), PC12 (SSR12), PC13 (SSR13), PC14 (SSR14), and PC15 (SSR15) had eigenvalues of 0.090961, 0.053898, 0.049212, 0.022725, and 0.013254, respectively, and accounted for 3.8287%, 2.2687%, 2.0714%, 0.95654% and 0.55787% of the total variability.

The left and right quadrants of the plot show the variables responsible for the majority of the variation. When two variables are on the same PC and very close to one another, it indicates that they have positive correlations and that an increase in one will result in an increase in the other. When two variables are at opposite ends of the PC, however, they have negative correlations, and a rise in one will result in a decrease in the other. On top of the quadrates unattached group of *C. songarica* accessions consisted of the samples collected from Neelum (Dawarian, Lawat, Reshna, Qadirabad, Keren), Jehlum valley (Leepa Trada Sharif, Leepa Khas), and Muzaffarabad (Bheri Jabbar), describing strong difference and polymorphism from topmost to lower quadrate within accessions. However, accessions from Neelum (Kail, Lower Dawarian, Surgan, Qadirabad, Reshna) Bheri Jabbar kinari and Leepa Nokot projected a comparatively compact group. The configuration of the sixty-four accessions belonging to two different species, laterally the two main principal constituent axes are presented in figure 5 in which SSR markers revealed genetic relationships among Cratagus samples. Various genotypes reveal genetic similarity with dispersed samples within population indicated variability showing advantage of SSR markers. SSR14, SSR10 and SSR15 markers contribute more strongly to the observed variation among genotypes as indicated by long vectors and direction is depictive of SSR markers influences in separation of samples. Samples lying in same quadrants showed more similarity and in different showed more genetic differences.

PIC value of SSR markers: The PIC value was calculated computer package using Power Marker. The maximum PIC value was recorded in SSR14, which is 49%. The minimum PIC value was recorded in SSR2, which is 14% and the average PIC value was 31%. Maximum allelic frequency was recorded in SSR13 98% and minimum allelic frequency was revealed by SSR14 45%. Average allelic frequency was 77 %. From the PCA it is clear that all SSR markers behaved differently in clustering the accessions. SSR14, SSR15, CNS5 and SSR10 showed a distinct efficiency as these four markers are clearly seen in axis with distant from all others (Fig. 5).

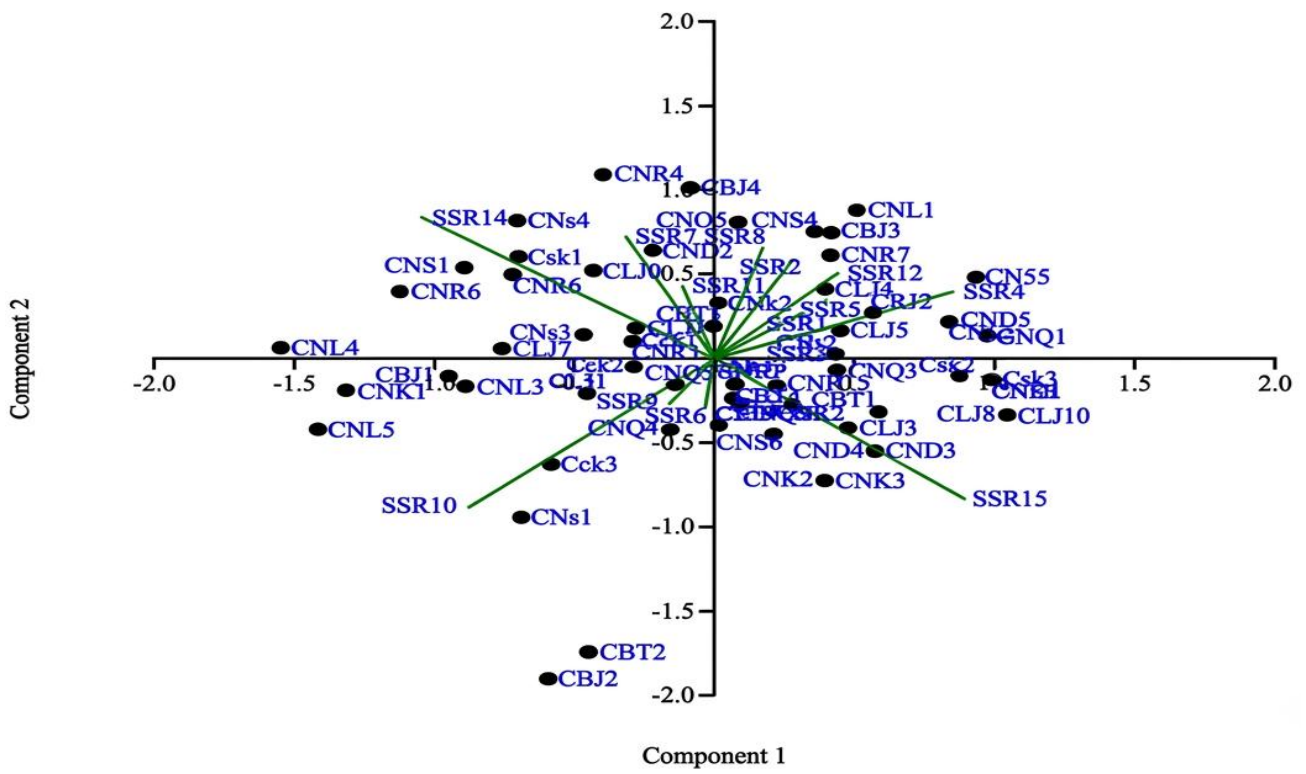

Fig. 5. Principal Component Analysis (PCA) describing the genetic variation among sixty four *Crataegus* accessions of Division Muzaffarabad based on fifteen SSR based molecular markers.

Discussion

Variable traits such as morphological traits are often used to characterize and categorize germplasm, which refers to the genetic material of organisms that can be used for breeding purposes. Selection of the right morphological characteristics in order to effectively conserve and utilize genetic resources (Giraldo *et al.,* 2010). Morphological and DNA markers are different as morphological variations are under influence of environmental factors while DNA markers are somewhat conserved. Moreover, low PIC values for SSR and morphological markers account for low polymorphism in studied populations (Zhao *et al*., 2023). One of the factors used to differentiate genotypes is leaf characteristics, and the results may vary based on the plant material used (Balasooriya *et al.,* 2009). Different hawthorn genotypes have been shown to have leaf length parameters that range from 20.4 mm to 130.3 mm, leaf width parameters that range from 13.3 mm to 45.7 mm, and petiole values that range from 6.2 mm to 20.0 mm (Erfani-Moghadam *et al.,* 2016). Several investigations have been carried out for the identification of fruit characters of different hawthorn species. According to reports, the fruit length in various genotypes ranges from 13.0 mm to 18.20 mm in Iran (Moghadam & Kheiralipour, 2015) and from 23.90 mm to 27.00 mm in Turkey (Dursun *et al.,* 2021). The fruit weight estimates were found to range from 0.65 g to 4.19 g in research (Yanar *et al.,* 2011). Our research on fruit traits generally revealed similarities and partial differences with findings in the literature. The usage of distinct material could be the cause of this.

Principal component and cluster analysis supportive approaches for screening accessions (Karimi *et al.,* 2009). PCA is a preffered for understanding the underlying structure of multivariate datasets and reducing their complexity while retaining essential information in statistics, machine learning, and data analysis as it identifies patterns and correlations among variables and summarizes this information in a smaller number of components. It allows for a reduction in the number of dimensions needed to represent the dataset while minimizing the loss of information. The population's overall variation is most heavily influenced by the first principal component, which is followed by subsequent components. PCA has been employed in germplasm with success and evaluation of various plants over a period of time as they demonstrate the relationship and correlation between variables under study (Maji & Shaibu, 2012; Hamza *et al.,* 2014). It enables the investigation of the connection between observation and variable data structure reformation. By eliminating intercorrelation between variables, PCA tends to decrease the aspects of multivariate data and makes it possible to recognize a multi-dimensional association plotted on two or three main axes (Nwangburuka *et al.,* 2011). The relatedness of the information obtained was assessed in the current study using PCA.

The criteria verified by Guei *et al.,* (2005), recommended that the characters associated with the first three principal components are more helpful in differentiating the accessions and are frequently the most essential in reflecting the dissimilarity patterns among accessions. Therefore, rather than focusing on all the characters under study, it is helpful for the development of genetically significant traits that contribute more to variability. It helps genetically enhancing PCA can be used as an essential tool to speed up the breeding program because it extracts all the crucial components and highlights how they contribute to overall variability (Das *et al.,* 2017).

A population's genetic variance is scaled using a locus's PIC value. The locus is thought to be highly polymorphic if the PIC value is more than 0.5. (Vaiman *et al.,* 1994) as Khiari *et al.,* (2015) found higher PIC value of the current study and that of Emami *et al.,* (2018). The PIC value calculated in the current study ranges from 0.03 to 0.49, while 0.60 and 0.89 have been reported by Emami *et al.,* (2018) and Khiari *et al.,* (2015) which reflects the low level of genetic variation in present study. UPGMA data analysis and the highest number of alleles can be used to identify genotypes. The highest allelic frequency was recorded only from SSR13, and the highest PIC value was only from SSR14 which can be used for the investigation of *Crataegus* genotypes. Using RAPD, studies on the genetic diversity of the *C. monogyna* population indicated a significant amount of diversity in northern Italy (Ferrazzzini *et al.,* 2008) which showed that population structure might be different and secondly RAPD is small length marker and can vary quickly in genome.

On the other hand, using chloroplast DNA markers, an insignificant amount of genetic diversity was discovered for *C. monogyna* and *C. laevigata* (Fineschi *et al.,* 2005) due to high conserved nature of chloroplast genome. The loss of genetic diversity as shown in present by lack of polymorphic bands of 15 SSR markers. Deforestation and other human activities are continuously fermenting its populations leading to the hinderance of cross pollination among populations and ultimately enhancing inbreeding and low polymorphism (Hantao *et al.*, 2017). In order to demonstrate the significant genetic diversity, Beigmohamadi *et al.,* (2011) and Sheng *et al.,* (2017) observed 93.37% and 98.35% polymorphism among the genotypes of *C. pontica* and *C. songarica,* respectively which could be due to marker choice and population's structure (Guney *et al.,* 2018).

During the current study, *Crataegus* accessions collected from division Muzaffarabad using morphological traits of leaves, fruit, seeds, thorns, and also the plant habit, whether it was shrub or tree, and 15 SSR DNA molecular data. Data sets were analyzed by multivariate and univariate methods. Both analyses divided the accession into groups. Dendrogram of morphological traits clustered the *C. clerkei* accessions separate from that of *C. songarica,* while these accessions were clustered among accessions of *C. songarica* by molecular data. One accession of *C. celrkei* was grouped with accessions of *C. songarica* collected from Lawat (Neelum) and Bheri Muzaffarabad; others were clustered with collections from Reshna Neelum. *C. songarica* and *C. clerkei* species are threatened from the area as a result of human influence in terms of deforestation, urbanization, agricultural practices, and unawareness about its importance. SSR based polymorphism has been losing and low PIC values demonstrating that deforestation and anthropogenic activities contribute to lowering the polymorphism. It is vital to initiate breeding program by using various markers (DNA and morphological) are needed to get a inclusive

appearance of *Crataegus* diversity, population structure, conservation status and restoration. It is necessary to restore diversity by storing seeds and other plant materials for research, breeding and conservation. Further, habitat restoration with protection as well as management of natural population can be ranked to save it in natural habitat. Such joint approach can aid in preservation of genetic resource and future longstanding sustainable utilization of Crataegus.

Conflict of Interest: Authors declare that there is no conflict of interest.

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