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High Genetic Diversity in the Himalayan Common Bean (*Phaseolus vulgaris*) Germplasm with Divergence from Its Center of Origin in the Mesoamerica and Andes

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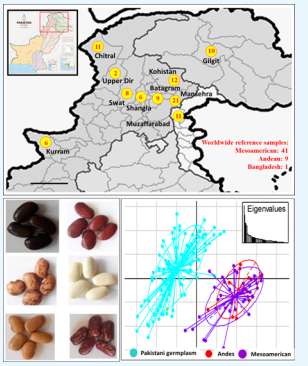
ABSTRACT: The common bean is found in the Himalayan region of Pakistan with substantial morphological variability. Genetic diversity within any crop species is a precursor for genetic improvement; however, little is known about common bean genetic diversity in this region. We explored the genetic diversity in the common bean from the Himalayan region (Khyber Pakhtunkhwa, Gilgit−Baltistan, Kashmir) of Pakistan. Microsatellite genotyping was carried out for 147 samples with 40 simple sequence repeat (SSR) markers. The results revealed a clear divergence of the Pakistani population from the primary gene pool (with F_{ST} values of 0.2 with Andes and 0.27 with Mesoamerica). However, within the Himalayan germplasm, no clear evidence of spatial structure was observed (with the maximum F_{ST} values of only 0.025), probably due to the dispersal of seeds by human activity within the region. This was further elucidated by the discriminant analyses of principal components. Considering the diversity parameters, high genotypic diversity was observed for the indigenous lines (0.990), comparable to the primary gene pool (0.976 for Mesoamerica and 0.976 for Andes populations). A high genotypic diversity was observed within the Himalayan population (ranging from 0.500 for Upper Dir to 0.952 for Mansehra). Gene diversity across loci varied between 0.28 for Chitral to 0.38 for Kurram. Our results suggested a divergent and independent evolution of the Himalayan population, which might have led to the diversification of the common bean germplasm in the region postintroduction into the region. The diversity observed could also be exploited in future breeding programs for the development and introduction of climate-resilient varieties.



INTRODUCTION

Genetic diversity in crop germplasm is a prerequisite for any crop improvement program aiming to attain food security. Crop plants have the maximum diversity in areas where it is evolved with many local types and wild relatives.[1−3] However, the level of diversity could also be higher in regions where a crop was introduced out of its center of diversity but cultivated over a large and diverse area and could potentially represent the secondary center of diversity. Deciphering the diversity and population structure of such crops is a crucial domain of plant breeding and genetics. One such crop is the common bean, introduced into many parts of the world out of its center of origin in Mesoamerica and Andes, including the northern part of Pakistan.

The common bean (*Phaseolus vulgaris* L.) is one of the most important legumes grown as a vegetable for both fresh pods as well as a pulse for its seed in Pakistan and around the world.[4] Among other legumes, the common bean is produced for direct human consumption with high commercial value.[5,6] There are about 80 cultivated and wild species in the genus *Phaseolus*, of which *P. vulgaris* is the most cultivated species.[7,8] The genome of this species is 580 Mbp in size and is a true diploid species, $2n = 2x = 22$.[9] Myanmar, India, Brazil, and China are among the largest producers of dry beans.[10] Although the crop is grown over a substantial area of arable land in Pakistan, a large gap exists in common bean production and consumption primarily due to the lack of high-yielding varieties. In fact, no variety was registered until 2021, when the first ever bean variety, "Himalaya-1", was approved for cultivation in the northern part of Pakistan. Productivity could be enhanced through the development of more of such varieties through exploration of broad genetic diversity and identification of high-yielding germplasm,[11] which could be obtained through information about its diversity in relation to the crops' center of domestication.

The common bean is considered to be domesticated in the central Americas almost 7000 years ago with two main regions of domestication in Mesoamerica and Andes.[12−14] However, the evolution of the Andean gene pool from the Mesoamerican germplasm is suggested by several recent molecular stud-

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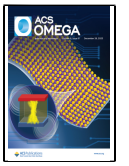

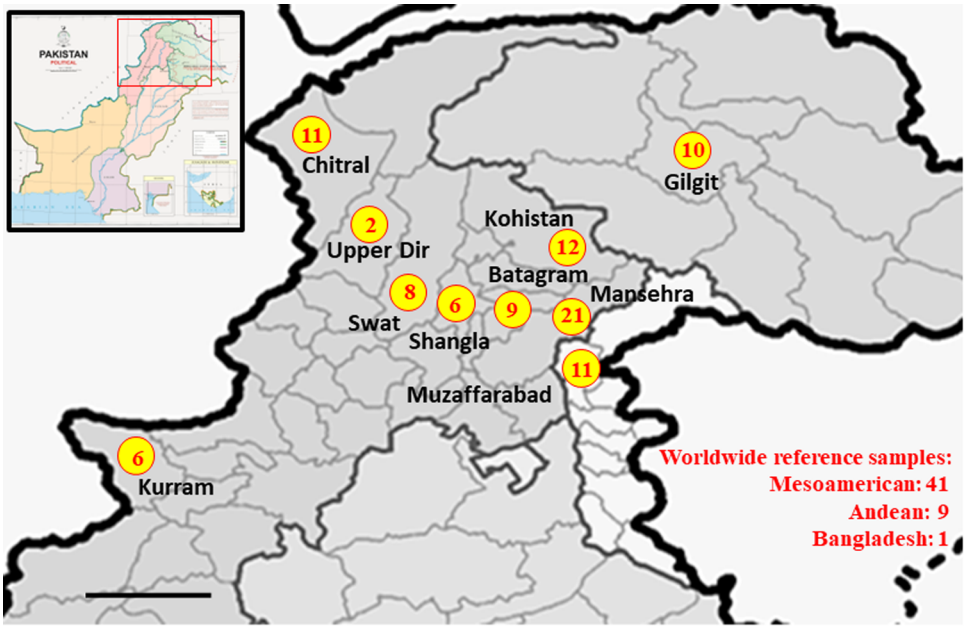

Figure 1. Map showing different districts in the Himalayan region of Pakistan from where common beans germplasm was collected for assessing diversity and divergence. The sample size is mentioned in the circle, and the worldwide reference samples are also mentioned in the right bottom.

ies,[15−18] which shows that the Mesoamerican gene pool is an important source of genetic diversity.[19] High genetic diversity has also been reported in African, Asian, and European germplasm, suggesting the potential existence of other centers of genetic diversity in these continents.[20−24]

In Pakistan, pulses like common bean, pea, cowpea, and lentils are grown over a large area due to the favorable climatic conditions. The common bean is particularly grown in the Himalayan region,[4] where subsistence farming is the main source of food security for resource-poor farmers.[25] Himalayan region has been shown to be one of the biodiversity hot spots for many plants and microbial species.[26,27] Studies have been made on the Himalayan common bean populations in India;[1−3] however, little is known about the population genetic structure of common bean in various valleys of the Himalayan region of Pakistan and the within-population subdivision. Thus, it would be interesting to assess the divergence of this Himalayan common bean population in relation to the germplasm available in the center of domestication. Characterization of this gene pool and its protection and conservation are also crucial for subsequent utilization for crop genetic improvement.

High genetic diversity in any crop species could provide desirable variation for adaptation to a range of environmental conditions and thus enable genetic improvement in the current scenario of climate change (drought, heat, salinity, and nutrient stresses).[28] Selection of desirable superior genotypes relies on the presence of genetic diversity in the germplasm for genetic improvement. Genetic diversity studies are therefore important for the genetic improvement program.[29−31] Breeding programs aimed at crop genetic improvement as well as germplasm management/maintenance/protection and conservation also depend on the availability of information on genetic diversity in indigenous common bean germplasm.[32]

Traditionally, the characterization of germplasm was done on morphological and agronomic traits that were of great interest to breeding programs.[4,33−35] This characterization is required not only for breeding and genetic improvement purposes but also for understanding domestication.[36−39] More reliable results regarding the variation and characterization of races and cultivars within a species can be obtained using morphological, molecular, and biochemical characteristics.[40−43] Molecular markers like microsatellite markers or simple sequence repeats (SSRs)[39,40,42,44,45] were helpful for the evaluation of common bean landraces, especially in areas where specialized circumstances and resources are limited for sequencing.[42] Despite numerous studies on the assessment of diversity in international collections, no work was done to molecularly genotype the Himalayan common bean germplasm and conduct population genetic analyses.

Considering the lack of any information regarding the level of diversity and population structure of the common bean landraces cultivated in the Himalayan region of Pakistan, the study was conducted to investigate the genetic diversity of Himalayan common beans based on molecular genotyping. The specific objectives were (i) to assess the genetic diversity and divergence in various *P. vulgaris* populations from the Himalayan region of Pakistan and (ii) to assess the divergence of the Himalayan common bean population in relation to the reference germplasm available in the crop's center of domestication.

MATERIALS AND METHODS

Collection of Plant Material. Common bean collection consisting of a total of 147 accessions (96 Himalayan, 41 Mesoamerican, 9 Andean, and 1 genotype from Bangladesh) was explored for assessment of diversity and divergence. Of these, we procured Mesoamerican and Andean germplasm from Washington State University, the United States of America (Figure 1 and Table S1).[46−4748] However, indigenous accessions were collected from the Himalaya region of Pakistan (different areas of Khyber Pakhtunkhwa, Gilgit−Baltistan, and Kashmir). In the Khyber Pakhtunkhwa accessions, the collection was mainly done from Mansehra, Swat, Chitral, Battagram, Kohistan, Kurram, Shangla, and Upper Dir (Figure 1). As mentioned earlier, none of the varieties was released in

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Pakistan before this study; therefore, these accessions could be considered as local landraces.

Genomic DNA Extraction. DNA was extracted from sprouting obtained by growing all of the collected accessions in pots under controlled conditions (greenhouse conditions). Second trifoliate leaves of 15-day-old seedlings were collected and stored at −80 °C. Two hundred milligrams of leaf tissue was used to extract the total genomic DNA following a modified cetyltrimethylammonium bromide (CTAB) procedure.[49,50] The concentration of the extracted DNA was determined using a Nanodrop spectrophotometer (Thermo Scientific USA) and then diluted to a 25 ng/μL final concentration.

Molecular Genotyping and PCR Amplification. Molecular genotyping was performed using 40 SSR primers. Polymerase chain reaction (PCR) was performed after DNA extraction and quantification for amplification using Applied Biosystems Thermocycler (Veriti 96 wells). The volume of each PCR was 20 μL consisting of 2.4 μL of $MgCl_2$, 0.4 μL of dNTPs, 0.2 μL of taq DNA polymerase, 2 μL of taq buffer, 1 μL of forward, 1 μL of reverse primers of SSR markers, 1 μL of DNA template, and 13 μL of ddH_2O. The PCR products were resolved on 2% Agarose gel and visualized using a UV Trans illuminator. The alleles were first compared with the DNA ladder of known band size (using a 100 bp ladder) and then scored accordingly.

Population Genetic Analysis of the SSR Data. The data of the SSR primers was compiled in MS Excel as input files (as per the required format of various software) to be used for subsequent population genetic analysis.[26] To find multilocus genotypes (MLGs) and assess the suitability of SSR markers, the data were analyzed with the help of R software using the POPPR package.[51] Similarly, to measure the population subdivision, F_{ST} estimation was done using the GENETIX program. Principal component analysis (PCA) and principal coordinate analysis (PCoA) were adopted using the ADEGENET package to compute various clusters in populations as well as genotypes in each of these cluster groups.[51] In addition to this, to find genetic clusters in the population and the genotypes in these clusters, the ADEGENT package was further adopted for the discriminant analysis of principal components (DAPCs).[51] The phylogenetic tree was constructed using the POPULATION software[52] on the basis of the Nei genetic distance.[53] Finally, using the POPPR package of R software, we also calculated the genotypic diversity, genetic diversity, allele richness, and private alleles.[54]

All of these analyses were carried out by comparing the Himalayan population with the Mesoamerican and Andean germplasm of the common bean (the two primary gene pools) and within the Himalayan populations.

RESULTS

Variability of the Microsatellite Markers Observed in the Common Bean Genotypes. All of the loci amplified were found to be polymorphic when the overall 147 samples were considered (Table 1). We found a range of 0.04 (BM157) to 0.50 (BM141, BM156, BMb654, PVBr93, X04660, X57022) for gene diversity across loci, whereas the evenness across loci varied between 0.40 (BM157) and 1.00 (BM141, BM156, BMb654). When the number of MLGs detected was plotted against the number of loci, we found that the markers tested in this study were adequate to capture the maximum diversity in the population (Figure S1). Markers' ability to capture multilocus genotypes increased with the increase in the number of loci. We already detected the maximum number of MLGs (147) by 20 loci, reaching the maximum distribution of around 147 MLGs at 40 loci (Figure S1, Supporting Information).

Table 1. Number of Alleles, Simpson's Diversity Index, Gene Diversity, and Evenness Index Calculated for 40 SSR Loci in 147 Common Bean Genotypes Sampled from Himalayan, Mesoamerican, and Andean Regions

locus	Simpson's index (1-D)	gene diversity	evenness
BM139	0.33	0.34	0.74
BM141	0.50	0.50	1.00
BM142	0.46	0.47	0.93
BM150	0.37	0.37	0.79
BM151	0.44	0.44	0.89
BM154	0.44	0.44	0.88
BM155	0.48	0.48	0.96
BM156	0.50	0.50	1.00
BM157	0.04	0.04	0.40
BM158	0.46	0.46	0.93
BM159	0.38	0.38	0.80
BM181	0.08	0.08	0.46
BM185	0.44	0.44	0.89
BM189	0.40	0.40	0.82
BM199	0.11	0.12	0.50
BM212	0.47	0.48	0.95
BMb152	0.44	0.44	0.89
BMb654	0.50	0.50	1.00
bmd10	0.48	0.48	0.96
BMd53	0.19	0.20	0.59
BMd54	0.34	0.34	0.75
M7589	0.14	0.14	0.53
Pvag001	0.16	0.16	0.55
PVBr93	0.49	0.50	0.98
Pvbr185	0.21	0.22	0.61
Pvbr213	0.36	0.36	0.77
Pvctt001	0.46	0.47	0.93
Pvgccacc001	0.36	0.37	0.78
pvm097	0.27	0.28	0.67
X59469	0.16	0.16	0.55
X61293	0.36	0.37	0.78
X74919	0.47	0.47	0.94
X96999	0.40	0.40	0.83
U77935	0.31	0.31	0.71
X04660	0.50	0.50	0.99
X57022	0.50	0.50	0.99
X80051	0.25	0.26	0.65
Cc4	0.40	0.40	0.83
Cc5	0.34	0.34	0.75
Cc7	0.38	0.39	0.81
Mean	**0.36**	**0.36**	**0.79**

Diversity and Divergence within the Himalayan Region. The level of diversity was high across all locations within the Himalayan common bean germplasm, as observed through genotypic diversity, its evenness, gene diversity, and index of association (Table 2). All of the accessions were a distinct multilocus genotype, and none was clonal to another accession. High genotypic diversity was observed within various germplasm collections representing the Himalayan region of Pakistan, with the maximum recorded for Mansehra (0.952) and higher than 0.90 at five locations, while more than

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Table 2. Diversity Parameters within the Himalayan Common Bean (*Phaseolus vulgaris* L.) Populations

population	sample size	no. of different multilocus genotypes	Simpson's diversity index	evenness index	gene diversity	standardized index of association (rbarD)
Battagram	9	9	0.889	1	0.356	0.015
Chitral	11	11	0.909	1	0.285	0.022
Gilgit	10	10	0.900	1	0.391	0.040
Kashmir	11	11	0.909	1	0.347	0.025
Kohistan	12	12	0.917	1	0.338	0.013
Kurram	6	6	0.833	1	0.380	0.005
Mansehra	21	21	0.952	1	0.338	0.013
Shangla	6	6	0.833	1	0.363	0.058
Swat	8	8	0.875	1	0.304	0.056
Upper Dir	2	2	0.500	1	0.375	NA
overall population	**96**	**96**	**0.990**	**1**	**0.342**	**0.020**

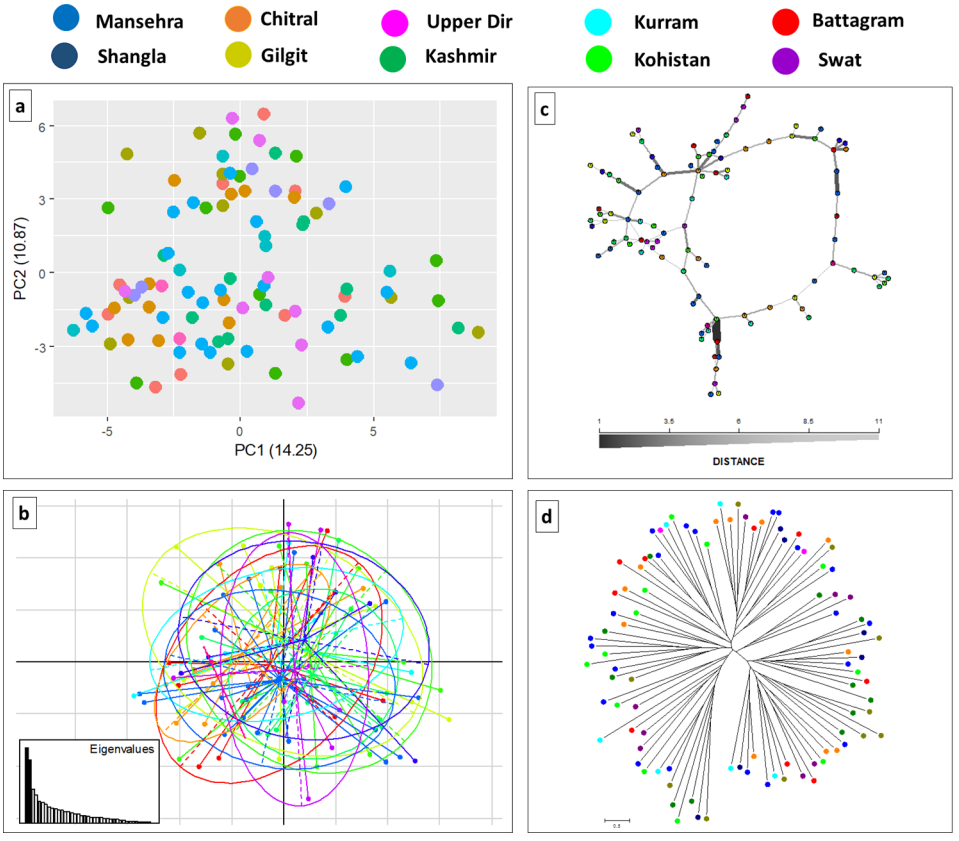

Figure 2. Divergence of indigenous common bean germplasm from Pakistan with the reference gene pool from the primary center of origin in the Mesoamerican and Andes regions. Principal component analysis (A), principal coordinate analysis (B), network analyses (C), and phylogenetic tree (D) applied to their molecular genotypic profile.

0.80 at four locations. The only district with relatively low diversity was Upper Dir (0.500), a location with a small sample size. The overall genotypic diversity observed within the Himalayan region was 0.990. Gene diversity in the overall population within the Himalaya region was 0.342, with a higher diversity of 0.391 observed in Gilgit, followed by 0.380 in Kurram, while the lowest value of 0.285 was observed in Chitral.

Analyses of the population subdivision within the Himalayan region revealed the lack of any divergence among the different locations. Principal component analysis showed that the two principal components accounted for 14.25 and 10.87% of the variation, respectively, and cumulatively explained 25.12% of the total variation. The distribution and subdivision were based on their genetic relatedness (Figure 2a). The principal coordinate analysis revealed an overlapping population within samples collected from the Himalayan region (Figure 2b). Neighbor-joining analysis and network analysis further demonstrated weak population subdivisions within the Himalayan region (Figure 2c,d).

Diversity and Divergence of Himalayan Population in Comparison with the Primary Gene Pool. The gene diversity observed in the Himalayan germplasm was 0.342, while it was 0.243 in the Andean and 0.245 in the Mesoamerican germplasm. Similarly, Simpson's index revealed high genotypic diversity in the Himalayan populations (0.990) as in the Mesoamerican (0.976) and Andean (0.889) populations (Table 3). All of the genotypes were represented

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ACS Omega 2023, 8, 48787−48797

Table 3. Diversity Parameters in Himalayan Common Bean (*Phaseolus vulgaris* L.) Populations along with Exotic and Reference Mesoamerican and Andes Gene Pools

diversity parameters	Himalaya	Mesoamerican	Andes	exotic	overall population
sample size	96	41	9	1	147
number of different MLGs	96	41	9	1	147
Simpson's index	0.990	0.976	0.889	0	0.993
evenness index	1	1	1		1
gene diversity	0.342	0.245	0.243		0.362
standardized index of association (rDbar)	0.020	0.045	0.022		0.029

by distinct MLG, and no clones were observed. However, the sample size and genotyping strategy might have influenced the estimation for the lines from the center of origin, as those are expected to be of much higher diversity.

A clear divergence of the Himalayan germplasm of the common bean was observed in comparison with reference to the Mesoamerican and Andean populations (primary gene pool) as revealed by different population genetic analyses, i.e., PCA, PCoA, NJ tree, and network analyses (Figures 3 and S2). There was a subdivision of the germplasm into two major groups: one group consisted of the Himalayan population, while the other group contained the primary gene pool, i.e., the Mesoamerican and Andean populations. The only exotic line from Bangladesh was grouped with the Himalayan germplasm of Pakistan. In the case of PCA, a total of 16.45 and 9.92% (26.37% of the total variation) of the variation were controlled by the two principal components, respectively. Of these two components, PC1 showed a high impact, which accounted for 16.45% of the total variability. Further confirmation of the population subdivision was obtained through another test called the "principal coordinate analysis" (PCoA), where the Himalayan population could be seen to be clearly divergent from the primary gene pool (Figure 3b). The relationship was also confirmed by the neighbor-joining tree and network analyses, where the Mesoamerican and Andean populations were relatively closer to each other, while these were distant from the Himalayan gene pool (Figure 3c,d). This analysis elucidated a clear divergence among the Himalayan,

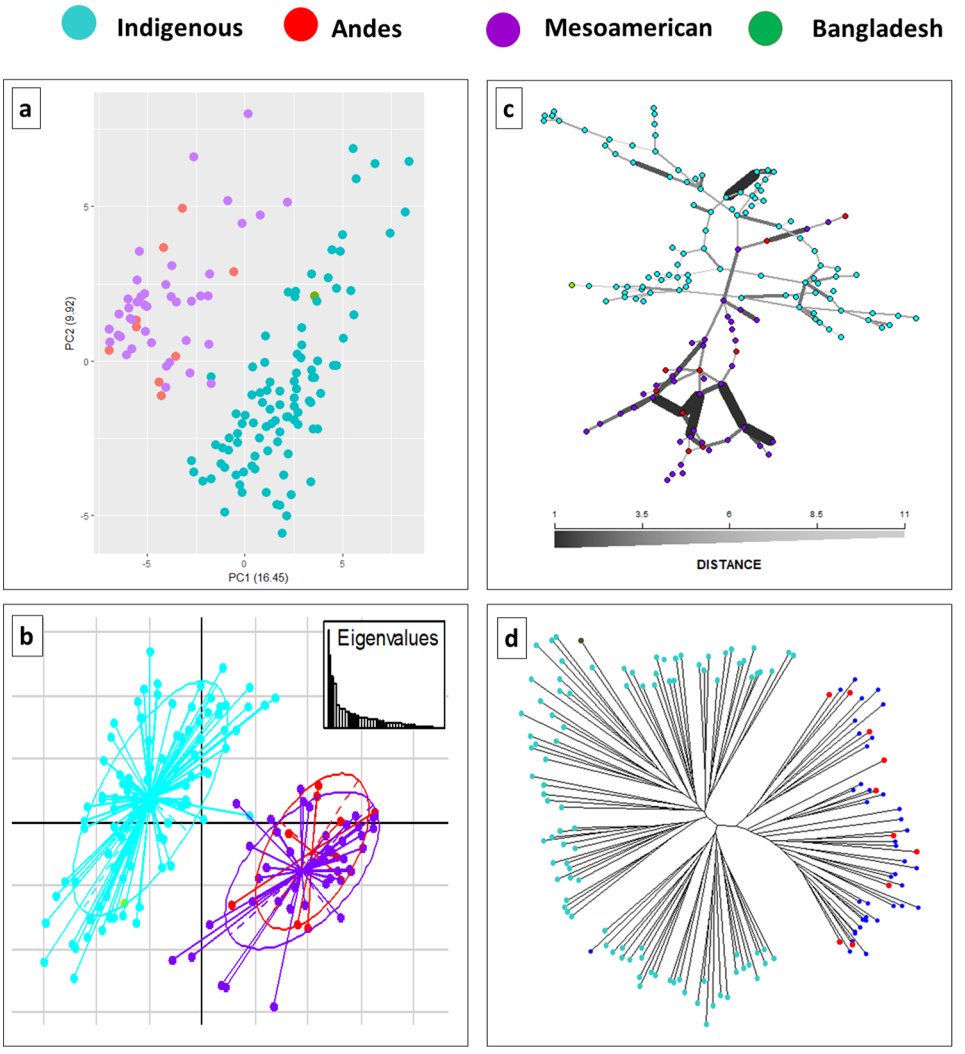

Figure 3. Within Himalayan germplasm divergence among common bean populations collected from various locations of the Himalayan region of Pakistan, based on principal component analysis (A), principal coordinate analysis (B), network analyses (C), and phylogenetic tree (D) applied to their molecular genotypic profile.

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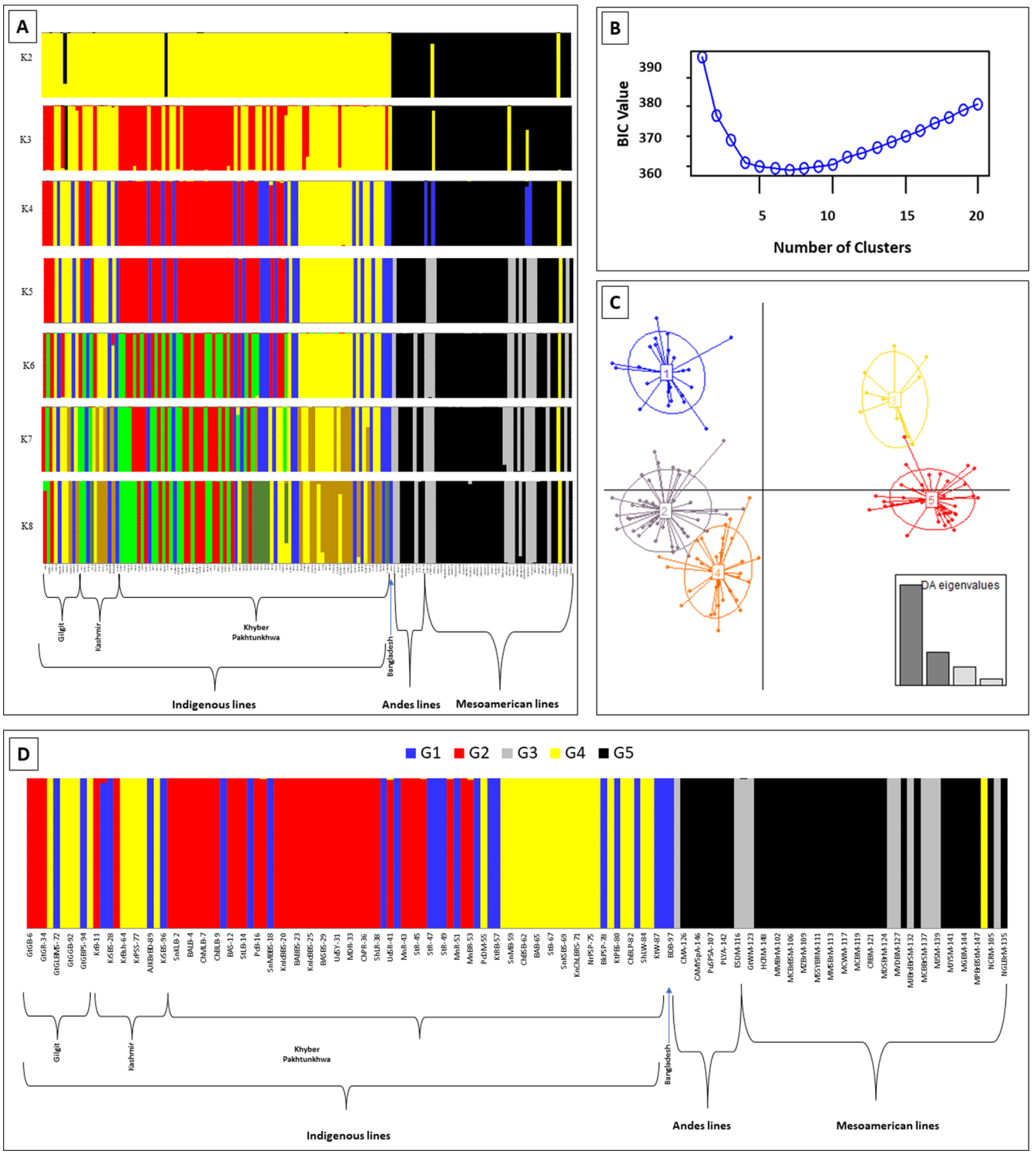

Figure 4. Discriminant analyses of principal components (DAPCs) showing the grouping of common bean germplasm collected from various locations of the Himalayan region of Pakistan along with representative lines from the center of diversity in Mesoamerica and Andes. (A) Clustering of germplasm into different genetic groups while considering K ranging from 2 to 8. (B) Bayesian information criteria as evolved with different K levels. (C) Scatter plot while considering only three genetic clusters. (D) Assignment of common bean germplasm at $K = 3$.

Mesoamerican, and Andean populations (Figure S2, Supporting Information).

The pairwise strong F_{ST} values for Himalayan, Mesoamerican, and Andean groups further confirmed the population subdivision. Pairwise F_{ST} values were 0.253 for Himalayan and Andean, 0.940 for Andean and Mesoamerican, and 0.271 for Himalayan and Mesoamerican.

Grouping of the Tested Germplasm into Various Groups. The existence of different genetic groups and the assignment of the germplasm into these groups were examined using discriminant analyses of principal components (DAPCs), considering K (number of groups) ranging from 2 to 8. The Himalayan gene pool clearly diverged from the primary gene pool, right at $K = 2$ (Figure 4a). Further increasing the K

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enabled identification of groups within the Himalayan and/or primary gene pool, although this is not explained by the geographical location. The Bayesian information criteria (BIC) suggested the existence of at least five genetic groups (Figure 4b). At $K = 5$, all of the identified groups were divergent from each other, as shown in the scatter plot (Figure 4c). Considering these five genetic groups, three groups (G1, G2, and G4) were specific to the Himalayan gene pool, while G3 and G5 were specific to the Mesoamerican and Andean reference gene pools (Figure 4d). Overall, the analyses also confirmed the clear divergence of the Himalayan gene pool with the primary gene pool and a lack of spatial structure within the Himalayan gene pool.

DISCUSSION

An analysis of genetic diversity is important for identifying the nature and magnitude of variability for the efficient management and conservation of germplasm as well as practical plant breeding.[55−59] Our results revealed a high diversity within the Pakistani Himalayan common beans genotypes with clear divergence from the reference primary gene pools of common beans, i.e., Mesoamerican and Andean. It can be helpful for detecting divergence among gene pools for reliable scoring and selection of individuals for breeding programs.

The diversity analyses revealed a high genotypic diversity within the Himalayan region of Pakistan, at the same level as observed for the reference germplasm from the primary center of origin. Previously, it was reported that major genetic diversity based on morphological traits exists for common beans in the Himalayan region of India.[60,61] A high level of genetic diversity was also found in the Calabrian germplasm of Italy, a region also outside the primary center of origin.[62] This enhanced genetic diversity may be due to a wide geographic distribution with different climatic conditions, thus exerting a varying recurrent selection through the farmers for different agronomic and quality traits. Thus, a high diversity was evident in the Himalayan region, along with a clear divergence. Interestingly, the high diversity was observed across all of the sublocations within the Himalayan region, except Upper Dir. The presence of high diversity in the population provides a capability to adapt to changing environments and also provides a base for further genetic improvement.[63] Populations containing more variations in alleles could be best suited to the current scenario of climate change and thus develop adaptive traits. This genetic divergence is suggested to be useful in the selection of diverse parents for hybridization.[59,62,64] Population subdivision within the Himalayan population was overlapping, showing low differentiation across locations, suggesting a frequent gene flow between these geographical locations, most probably due to human activities that carry the material from one location to the other.

A comparison of the level of diversity in the Himalayan gene pool with the reference gene pool revealed almost equal levels of high diversity as observed for the Andean and Mesoamerican primary gene pools. The relatively higher value for the Himalayan population could result from the relatively narrow diversity of the selected reference germplasm. The sample size and genotyping strategy might have influenced the estimation of diversity for the Mesoamerican and Andean germplasm, as a limited sample size was used. A high sample size coupled with more robust genotyping based on genome sequencing would enable a better resolution of these conclusions. A similar range of genetic diversity was reported by Cabral et al. and Perseguini et al.[31,65] The genetic diversity in the Mesoamerican population was slightly higher than that in the Andean population. Kwak and Gepts also reported comparatively higher genetic diversity in the Mesoamerican group than in the Andean group. The Andean group showed less gene diversity as compared to the Mesoamerican as well as Himalayan populations.[9] Rossi et al. suggested a reduction in the diversity of the Andean gene pool in their study of the population structure of wild and domesticated populations of common beans.[66] Similarly, Kelly described less genetic diversity in the Andean gene pool based on DNA analysis.[67] Bitocchi et al. also reported a strong bottleneck in the Andean gene pool and less genetic diversity than that in the Mesoamerican gene pool.[19]

The extent of divergence between different groups was clearly evident, as revealed by different population subdivision analyses. Only two accessions occurred outside of their groups, i.e., Mexican genotypes belonging to the Mesoamerican gene pool were placed near the Himalayan group, while an accession from Gilgit of the Himalayan group occurred near the Mesoamerican group. Similar exceptions were also reported by Blair et al. in their study.[68] These may be due to labeling error but it could be confirmed by further studies. The Himalayan gene pool consisted of genotypes collected mainly from Khyber Pakhtunkhwa, Gilgit, and Kashmir areas of the Himalayan region and the exotic accession of Bangladesh. It was notable in our study that the Himalayan population and exotic accession were closely related to each other based on the results of the SSR data. This showed gene flow from Pakistan to Bangladesh and India at some point in time. The Pakistan structure also revealed a lack of structure due to locations and potential adaptation to the abiotic conditions at these locations, unlike those observed for wild relatives.[69]

The existence of at least five genetic groups identified through the DAPCs could be useful for the selection of parents for potential crosses.[59] Crossing distant and diverse parents enables the generation of segregating progenies with more variation and thus could be useful to identify lines with desirable characteristics.[58,63] Crosses could be made using different lines from both indigenous Himalayan and primary gene pools. Nonetheless, the characterization of these lines and acquisition of their genotypic data could be useful for developing varieties from the available germplasm, directly using "selection from indigenous variation" as a breeding method.

Although the current data set and analyses are enough to draw the above-described conclusions, the use of genomic tools, along with the utilization of more powerful algorithms like the approximate Bayesian computation (ABC) analyses, will further elaborate the evolutionary history of this population. The use of SNPs and genomic tools are indeed more powerful[22,68,70] and could enable us to explore further the evolutionary history of the common bean germplasm of Pakistan. This will further benefit if even more samples from the center of origin are included, representing various races, ecotypes, or lineages within the germplasm of the Andean and Mesoamerican regions.

Considering the overall results of diversity and divergence, the Himalayan gene pool was found to be novel, and it can be termed as the Asian or Himalayan group based on the geographic origin of this mountain zone, which can easily be distinguished from the Mesoamerican and Andean gene pools. The Himalayan region may be suggested as a secondary center of diversity. Previously, the Himalayan region was reported to

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be an integral part of the secondary diversity of common beans on the basis of the characterization of germplasm conserved in the Indian gene bank through morphological, phenological, and agricultural traits.[57] Our results are supported by the findings of the Gepts and Debouck study in which the Himalayan regions were considered to be the potential secondary center of diversity.[13] However, the hypothesis of secondary domestication could not be advocated here, as nothing is known about the existence of wild relatives in the region.[19] The divergence of the Pakistani common bean germplasm from the germplasm from the center of origin could be explained by the local diversification of the Himalayan population along with the fact that the study is based on microsatellite markers, which are known for rapid mutation and best suited for studying the contemporary evolution.[78,79] Further collaborative studies using sequencing techniques and extended samples (of both common beans and any available wild relatives) from India, Nepal, and Bangladesh would be required for the exploitation of diversity in the Himalayan region.

The work done in the neighboring Himalayan region (India, for example) has also shown high diversity in the local common bean germplasm.[36−39] The germplasm has been characterized using different genotyping techniques, all of which have resulted in the identification of high diversity. Apart from neutral diversity, these studies have also elucidated the genetic architecture of different economically important traits.[36,37] Studies based on comparison of the germplasm from the region across different national groups of researchers along with some international core set must be useful for both genetic improvement as well as understanding the patterns of domestication in this important crop.[36−39]

Within Pakistan, further sampling would be useful in less explored locations like those in Baluchistan, Upper Chitral, Hunza, Skardu, Nagar, Astor, Kohistan, and tribal districts to unlock further the cryptic diversity in common bean germplasm of Pakistan, as suggested earlier.[71] This should be analyzed in the context of adaptive evolution.[71−73] This diverse gene pool should be used in further prebreeding programs to create new variations, identify genetic linkage groups, conduct genomic association studies, speed breeding, gene editing, recurrent backcrossing, interspecific schemes, and develop improved varieties.[74−77]

CONCLUSIONS

The study is one of the first to explore the indigenous common bean germplasm from the Himalayan region of Pakistan. It was found that this Himalayan common bean gene pool was highly diverse and clearly divergent from the reference primary gene pool. This high diversity was found across all of the locations, which lacked any spatial structure. The Himalayan population could thus be considered a distinct group, which diversified after the introduction into the region. This information about population structure enabled us to reflect on the diversification and differentiation of the common beans in the Himalayan region after its introduction from the Mesoamerican and Andean gene pools. In the Pakistani context, our results must be helpful in conservation, protection, management of germplasm, and further utilization in breeding programs for crop improvement.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c05150.

The manuscript contains the Supporting Information (available online) as listed below: details of the lines of *Phaseolus vulgaris*, representing the reference lines, used in comparison with Pakistani germplasm (Table S1); number of MLGs detected against the number of loci resampled with the POPPR package of R software (Figure S1); distribution of common bean germplasm from different locations of the Himalayan region of Pakistan with reference to primary gene pool of Mesoamerican and Andean gene pool based applied on their molecular genotyping profile (Figure S2) (PDF)

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Author Contributions

I.N., G.M.A., and S.A. designed the study. I.N., T.Z., B.S.Z., M.U.R., and A.J. conducted the sample collection and molecular genotyping. M.U.R. and S.A. conducted the population genetic analyses. I.N. and S.A. wrote the manuscript. I.N., G.M.A., and S.A. provided resources to conduct the study.

Notes

The authors declare no competing financial interest.

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