

Characterization and population structure assessment of Indian Common bean using Microsatellite Markers

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Research Article

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Abstract

The Common bean (*Phaseolus vulgaris* L.) is an important crop of family Fabaceae which is used as a potential source of proteins, fibres and minerals across the globe. The Indian Himalayan region harbours plentiful varieties of common bean, but it is nearly unexplored till date. The present study was attempted to assess genetic diversity and population structure of 119 common bean samples belonging to 20 diverse accessions gathered from Uttarakhand, India using newly designed chloroplast microsatellite (SSR) markers. A total of 218 polymorphic alleles were identified for 8 SSR loci. Mean number of alleles per locus ($N_a = 1.55$), effective allele number ($N_e = 1.370$), Shannon information index ($I = 0.313$), expected heterozygosity ($H_e = 0.213$) and average polymorphic loci (10.9) were estimated based on Cp-SSR data. Maximum genetic diversity (H_e) was recorded in the Jhalla accession where it was found minimum in the accession collected from Supi. Bayesian-based STRUCTURE evaluation using SSR based information showed that 20 bean accessions were genetically separated into two main clusters. Also, the genetic distance-based cluster analysis separated 20 common bean accessions using these markers into 2 major clusters corresponding Mesoamerican and Andean. These Cp-SSR markers also demonstrated transferability among Fabaceae members like *Vigna radiata*, *Macrotyloma uniflorum*, *Glycine max*, *Vigna mungo* in this study. The findings from the study can be used to select better accessions of the bean for production, conservation, and future breeding programs. Likewise, markers displaying transferability can be used for monitoring the genetic heterogeneity of other members of the family Fabaceae.

Introduction

Phaseolus is a large genus of the family Fabaceae comprises about eighty species in the world (Ulloa Ulloa et al. 2018; Chacón-Sánchez et al. 2021). Five species namely, *Phaseolus vulgaris* L. (Common bean), *Phaseolus lunatus* L. (Lima bean), *Phaseolus acutifolius* A. Gray (Tepary bean), *Phaseolus coccineus* L. (Runner bean) and *Phaseolus dumosus* or *Phaseolus polyanthus* Greenman (Year bean) are the dominant species of this family (Mina-vargas et al. 2016; Nadeem et al. 2018). The common bean is most important species of this genus believed to be domesticated approximately 8,000 years ago in Central America as its geographical origin (Chacón-Sánchez et al. 2021). After domestication, the species was introduced in other parts of the world by the Portuguese traders. Two major diverse gene pools of common bean have been reported- Mesoamerican and Andean (Angioi et al. 2010). The Mesoamerican gene pool has been distributed from Mexico throughout the Central America, into Columbia and Venezuela, while the Andean gene pool has its distribution throughout the southern Peru, Chile, Bolivia and Argentina (Rana et al. 2015; Chaudhary et al. 2017). In India, common bean was dispersed mainly by Portuguese, English, Dutch, and French traders in the beginning of the 16th century via the Red and Arabian Seas and Chinese through the Hindustan Silk Route (Rana et al. 2015). Since then, the species has undergone an adaptive evolutionary process for approximately 400 years in these areas (Westphal 1974; Chaudhary et al. 2017). The morphological, biochemical, and molecular evaluation of common bean revealed that the Mesoamerican originated wild community differs from the Andean (Desiderio et al. 2013). These two original gene pools are morphologically diverse from each other, in seed size and some other variations (Angioi et al. 2010). Another differentiating factor is the S or T type of phaseolin protein found in the seeds of common bean (Chaudhary et al. 2017).

Common bean (also known as kidney bean or Rajmash) is one of the most ancient legumes cultivated over a 90% growing area globally for its edible seeds as dry and green beans (Celmeli et al. 2018). The global annual production of bean grain is about 31 million tons and Brazil is the leading producer of common bean, followed by India, Myanmar, China, United States and Mexico (FAOSTAT 2020; Delfini et al. 2021). In India, Uttarakhand produces 40.22 tonnes of common bean and acquires 16th position at national level [National Horticulture Board (NHB), 2017–2018]. Immature pods of this species, known as snap beans, are eaten as vegetable while the straw from the plant is utilised as fodder. Common bean is consumed worldwide as a major reservoir of protein, dietary fibres and microelements (Broughton et

al. 2003; Głowacka et al. 2019). It is highly proteinaceous (14–33%) and consumed preferentially as a vegetarian protein reserve (Venkidasamy et al. 2019; Flores-sosa et al. 2020). In addition, common bean is a prosperous resource of iron, zinc, folic acid, potassium, phosphorous, magnesium, manganese, selenium etc (Broughton et al. 2003; Petry et al. 2015). Besides this, diverse essential amino acids like phenylalanine + tyrosine (53–105 mg/g), lysine (10–104 mg/g) and leucine (14–92 mg/g) have been recorded from common bean. Moreover, the species is deficient in the sulphur containing amino acids: methionine + cysteine (Moraes et al. 1971; Flores-sosa et al. 2020).

Generally, common bean is a self-fertilizing crop grown in tropical, semi-tropical, and temperate regions of the world (Wang et al. 2012; Gupta et al. 2019). In India, common bean has been cultivated in the plains of Uttar Pradesh, Andhra Pradesh and Maharashtra during autumn, and in hilly areas such as Uttarakhand, it is grown as a kharif crop during summer and winter (Sharma et al. 2014). Many well-known landraces named Auli, Harshil and Munsiyari have been cultivated in the high mountain regions of Uttarakhand which are morphologically distinct and possess remarkable adaptability to local environmental settings (Rana et al. 2015). Although the genetic characterization of a few local accessions/varieties of common bean gathered from Pantanagar (Uttarakhand) have been investigated using microsatellite markers (Kumar et al. 2013), local accessions of the species from the entire Uttarakhand have not been evaluated systematically and scientifically till date. Therefore, the present study is attempted (i) to design and validate new chloroplast markers, (ii) to measure the magnitude of genetic polymorphism, diversity, and population composition among common bean accessions, and (iii) to investigate the cross transferability among the selected members of the genus.

Material And Methods

Plant materials

Altogether, 119 genotypes of twenty diverse common bean accessions were gathered from local farmers' fields in Uttarakhand, India and subjected to further study (Table 1; Fig. 1). Local pulses such as *Vigna radiata* (Mung bean), *Macrotyloma uniflorum* (Gahat), *Glycine max* (Soybean), and *Vigna mungo* (Black gram) were also collected from the farmers of Jakholi, Rudraprayag, Uttarakhand (Longitude- 78°53'33.57" °E; Latitude- 30°23'33.02 °N; Altitude 1792) and used for further study. Common bean seeds collected from various locations were brought into the laboratory and sown in a seedling tray. After 33 days of seed sowing [seed sowed in off season (December)], the fresh young juvenile leaves were harvested, washed, and used for further experimentation.

Chloroplast microsatellites (cpSSR) marker designing

The complete common bean chloroplast genome (Guo et al. 2007) was retrieved from NCBI (National Center for Biotechnology Information) and the MISA (Microsatellite Search Module) a web based server was implemented for the identification of SSRs (Thiel et al. 2003). The minimum length threshold condition applied to investigate SSRs was dinucleotide (6 bp repeats), trinucleotide (4 bp repeats), and tetranucleotide (3 bp repeats), while mononucleotides (5 or 6 nucleotide repeats) were debarred from the evaluation. Ideal and compound SSRs were determined by means of the MISA pipeline. The repeat sequences were interrupted by non-repeat sequences (100 bp) in the case of compound SSRs. The Batch Primer-3 version 1.0 programme developed by You et al. (2008) was accustomed to design primers flanking repeated regions of chloroplast SSR loci in the chloroplast genome (You et al. 2008). The cpSSR primers were developed using parameters as follows: (i) primer length ranged from 18 to 23 bp; (ii) product size ranged from 100–300 bp; and (iii) an optimum GC content of 50% with a range of 40%–70% (Table 2). The identified chloroplast-based markers were designated as PV_cpSSR (*Phaseolus vulgaris* chloroplast simple sequence repeats) primers.

DNA isolation and cpSSR marker amplification

The fresh, young, juvenile leaves at the apex of the plant were used for genomic DNA extraction using the CTAB (Cetyl tri-methyl ammonium bromide) buffer assay with slight alterations (Jugran et al. 2013a & b). The quality of extracted DNA samples was examined by electrophoresing on an agarose gel (1%) prepared in 0.5 X TBE (Tris-Cl, Boric acid, EDTA) buffer. DNA fragments were visualised under the gel documentation system (I Gene Labserve, India) using a standard DNA ladder. DNA of common bean was amplified through PCR reaction using 20 µL of reaction mixture having 1µL template DNA, 10mM DNTPs, 10 picomole of each forward and reverse primer, 1U *Taq* polymerase, 10X PCR buffer and 25mM MgCl₂ by following Sharma et al. (2019). All reactions were conducted in a thermocycler (Biometra, Germany) using the following reaction conditions: early denaturation for 2 min at 94 °C, 35 cycles of denaturation for 30 seconds at 94 °C, 1 min annealing at 55 °C, 2 min extension at 72 °C, and final extension at 72 °C for 5 min. PCR products (amplicon) were qualitatively analysed on a 3% agarose gel (Bio Rad, USA) and fragment size was estimated using the 100 bp DNA ladder (O' Gene Ruler, Hi Media) as a reference (Figure S1a). A total of fifteen newly designed cpSSR markers were utilised for DNA amplification. Of which only eight Cp-SSR markers showed polymorphism after initial screening and these eight primer pairs were used for final amplification of common bean samples. Likewise, the cross-transferability of cp-SSR markers among other Fabaceae members namely *Vigna radiata* (Mung bean), *Macrotyloma uniflorum* (Gahat), *Glycine max* (Soybean), and *Vigna mungo* (Black gram) were also evaluated using the same method (Fig. S1b).

Data analysis

Out of 15 primer pairs screened, only 8 primer pairs exhibited polymorphism and produced clear and reproducible fragments, and therefore used for final study. A total of 119 genotypes of 20 common bean accessions from Uttarakhand were subjected to detect polymorphism at genetic level using eight primer pairs. Markers that produced the same sized fragments throughout the common bean were designated as mono-morphic, while cpSSRs producing varied fragments were considered polymorphic. A genotype that does not cause any type of amplification under standard circumstances is considered a null allele. The polymorphic information content (PIC) of each marker was measured using the following formula mentioned in Anderson et al. (1993): $PIC = 1 - \sum p_{ij}^2$, where p_{ij} is the frequency of the patterns (j) for each cpSSR marker (i)

Markers showing polymorphism were utilised to assess various attributes related to variability in common bean. The SSR fragments were scored on the basis of the occurrence (1) or non-occurrence (0) in all the samples examined. The binary data of each sample from diverse accessions was investigated using POPGENE version 32 (Yeh et al. 1999). The matrix was used to determine polymorphic loci number (Np), polymorphic loci percent (Pp%), number of observed alleles (Na), effective alleles per locus (Ne), heterozygosity (He= Nei's gene diversity), Shannon's information index (I), gene flow estimation (Nm), and genetic differentiation (Gst) using the POPGENE program. The PHYLIP version 3.68 software was employed to create a dendrogram of studied accessions by first transforming binary data into PHYLIP format, followed by generating matrices using the GENEDIST program. Ultimately, the NEIGHBOR program, followed by the CONSENSE programme from PHYLIP was employed to create an unrooted phylogenetic tree (Felsenstein 1995). A principal coordinate analysis (PCoA) was executed to evaluate the genetic relationship between accessions based on Nei genetic distance using a covariance standardised PCoA method in GenAlEx 6.5 (Peakall and Smouse 2006, 2012). An analysis of molecular variance (AMOVA) was performed to partition hereditary differences among accessions using the above-mentioned program. Cross-transferability and fragment size produced with cpSSR markers were recorded for all studied species. The degree of mixing and population composition was estimated based on the Bayesian clustering algorithm by using STRUCTURE software version 2.3.4 (Pritchard et al. 2000). Subpopulations within common bean samples were measured by 3 independent interactions as K values from 1 to 10 initially with different interactions and a burning length period to remove additional load on the computer. Final analysis was performed at 100,000 interactions with a 300,000-burn period using 20 interactions of K1 to 5 by employing an admixture model throughout

the previously allocated population as a sampling location and with the frequency of linked alleles among populations. The numbers of best possible K groups were determined by estimating ΔK parameter using Structure Harvester program (Earl and von Holdt 2012) as projected by Evanno's method (Evanno et al. 2005).

Results

Data mining and cp-SSR marker development

Common bean chloroplast genome (NCBI Reference Sequence: NC_009259.1) was loaded from National Center for Biotechnology Information (NCBI) and used for mining of SSRs. Fifteen primers were developed which qualified different parameters during the process, and all the selected primers were synthesised and used for validation of amplification in 119 individuals from 20 common bean accessions. With the availability of complete genomic sequences of common bean and co-dominant property, ease of use, repeatability, and multi-allelic nature establish SSRs as the preferred marker of choice to measure genetic inconsistency (Kumar et al. 2006; Matondo et al. 2017).

Genetic variability of common bean

Eight Cp-SSR primer pairs were utilized for DNA amplification of 119 individuals of common bean. Average number of polymorphic loci was recorded 10.9 from 119 individuals of common bean. The study showed that marker PV_cpSSR3 was reasonably informative ($0.25 < PIC < 0.5$) while the remaining seven markers were highly informative in nature ($0.5 < PIC < 0.75$). The PIC value of these primers ranged from 0.428 (PV_cpSSR3) to 0.817 (PV_cpSSR12), with a mean of 0.615 (Table 2). Further, the allele size varies from 112 to 368 bp (Table 2). Highest (80%) polymorphic loci percent was detected in Harsil accession and lowest (40%) in Khati & Natwar accessions using Cp-SSR markers. The N_a ranged from 1.40 (Khati & Natwar accessions) to 1.80 (Harsil accession) with a mean of 1.55, N_e ranged from 1.229 (Supi accession) to 1.505 (Harsil accession) with a mean value of 1.370, I varied from 0.223 (Supi accession) to 0.431 (Harsil accession). Heterozygosity (H_e) was recorded at its maximum (0.292) in the Jhalla accession while it was observed at its lowest (0.145) in the Supi accession (Table 3).

Genetic differentiation and gene flow

Total genetic variation was partitioned by subjecting the common bean samples to evaluate the molecular variance (AMOVA) by estimating the variance among populations and within populations. The high within population variation (99%) was recorded as compared to the among (1%) population variation (Table 4). These findings were somewhat complemented by the G_{ST} (0.264), which also demonstrated high within population diversity (73.6%) in the studied accessions. The gene flow level was observed to be 1.396 among common bean accessions under the study.

Genetic relationships and population composition

The pair-wise genetic dissimilarity between accessions of common bean was evaluated to measure the relationship between accessions using Nei's approach (Nei's 1978). A maximum (0.1977) genetic distance was recorded between Jatoli and Harsil accessions, while it was minimum (0.0173) between Lata and Jumma accessions (Table S2). The dendrogram construction through the neighbor joining method separated all studied samples into two main groups: group A and B. Group A encompasses the sample gathered from Jhalla, while Group B contains the remaining 19 accessions. Group B was further separated into two sub-clusters, BI and BII (Fig.3). To estimate the genetic structure, PCoA analysis showed distribution of 20 accessions in a three dimensions space constructed on genetic distances. The genetic variance percentage defined by all three PCoA coordinates in the study was 8.57%, 16.73%, and 24.44% (Fig. 4). In order to culminate the possible genetic populations, 119 genotypes were assessed by structure software using an admixture model. Evanno's ΔK statistics were utilised to select the ideal K value based on the increase

in possibility ratios between runs. The ideal sub-populations were found to be $K = 2$ (Fig.2). Based on $K = 2$ groups, population structure investigation exhibited that all common bean samples were distributed into 2 major groups. The relationship among populations in the two components is similar to the clustering pattern obtained in the study. Population structure of common bean relies on binary data obtained using cpSSR markers, was investigated by Pritchard et al. (2000) method and demonstrated that the log likelihood approximations increased regularly as K increased and started to decrease when $K = 2$ (Figure 2 a). A mean log likelihood plot is prepared by placing values over 10 runs for K values ranging from 1 to 5. The optimum K value was 2 as assessed by the ΔK statistic STRUCTURE based on employed markers (Figure 2 b). It was observed that the best possible subgroup number was reasonably low as compared to the total studied accessions, exhibited extensive gene flow levels, either presently or historically. At probability threshold (Q) of 0.60 using cpSSR marker's structure analysis, generally the samples were visibly isolated to a definite cluster. Of which, 39 individuals (32.773%) were dispersed among cluster-1 and 76 individuals (63.866%) were found in cluster-2. No clear pattern of allocation of the individuals of the studied accessions was detected in Cluster-1 and Cluster-2, depending on the threshold of 60% in structure analysis.

Taxon analysis and marker transferability

Four commonly growing pulse species namely, *Glycine max*, *Macrotyloma uniflorum*, *Vigna mungo*, and *Vigna radiata* along with common bean samples were evaluated for genetic variability and transferability using cpSSR markers designed for the aforementioned study. In sum, eight markers displayed polymorphism in the samples and the existence of transferability in the studied samples (Fig. S1).

Discussion

Identification of genotypic variations and their underlying structure is imperative for future breeding programs and conservation of plant genetic resources (Savic et al. 2020). Therefore, the development of molecular tools for characterizing genetic variability is indispensable as it significantly enhanced the effectiveness of utilization of accessible genetic resources. Despite of the diverse classes of currently available markers, knowledge of their genetic informative potential is critical to exploit the germplasm diversity. SSRs are used to examine the genetic diversity and linkage mapping, among other aspects, has been widely explained in the literature (Angioi et al 2008; Vidak et al. 2017). For the common bean, the present work is the first to assess the genetic diversity and structure of common bean from Uttarakhand (India) using SSR markers. They were found to be reasonably effective in assessing the genetic diversity of common bean genotypes. In Indian Himalayan Region common bean is one of the extensively cultivated legume crops. Prerequisite information about the genetic multiplicity of common bean is essential to achieve effective breeding programs. Among molecular markers, SSR marker is considered accurate and reliable tool to characterize genetic variability among legume crops. The cp-SSR markers used to assess 119 individuals from 20 accessions in this study amplified a total of 218 polymorphic alleles ranging from 8 (Khatri and Natwar) to 16 (Harsil) polymorphic alleles per loci with a mean of 10.9. These findings are in agreement with the previous study in which 138 genotypes from Jammu and Kashmir and one variety from VPKAS, Almora, India was investigated (Mahajan et al. 2016). The study of Valentini et al. (2018), reported a mean of 4 alleles per SSR locus when studying the 109 accessions from Brazil, whereas genetic assessment of 102 genotypes from Jammu, Kashmir and Ladakh, India, Bashir et al. (2020) demonstrated the incidence of 30 alleles per locus of SSR. The reason for a smaller number of alleles as compared to Bashir et al. (2020) might be due to the use of chloroplast SSRs in our study, as genomic SSRs can resolve within gene pool variation.

In present study, the percentage of polymorphic loci ranged from 40 to 80% for different SSR primers with a mean of 54.5% which is approximately parallel to (66.7%) the study of Hegay et al. 2012. Percentage of polymorphic loci may

vary from 0 (Hegay et al. 2012) to 100% (Asfaw et al. 2009). Furthermore, Pp% was found to be 83.33% in an ISSR (Inter Simple Sequence Repeats) marker-based study involving 28 accessions from Jammu and Kashmir, India (Dar et al. 2016). Current study showed average gene diversity (expected heterozygosity) as 0.213, which is nearly similar to other studies on common bean (Mercati et al. 2013; Pratap et al. 2016; Mahajan et al. 2016; Matondo et al. 2018). However, expected heterozygosity was observed lower as compared to the investigation of Bilir et al. (2019) in 102 genotypes from Turkey and study of Mir et al. (2021) in 96 genotypes from Jammu and Kashmir (India). Higher heterozygosity in Bilir et al. (2019) and Mir et al. (2021) is because of the abundant sampling of common bean genotypes. PIC is the distinguishing ability of a particular marker, predominantly based on alleles per locus and allele frequency in studied germplasm (Mercati et al. 2013; Suvan et al. 2019). As stated by Bashir et al. (2020), SSR-based PIC values can be exploited to detect the capability of the marker to detect genetic multiplicity. The PIC value was found to be 0.615 which was in order with the value (0.634) reported by Mahajan et al. 2016 in Indian germplasm and Matondo et al. (2017) in DR- Congo germplasm. It was found lesser than the earlier studies of researchers from Mizoram, India (Dutta et al. 2015) and Jammu & Kashmir and Ladakh, India (Bashir et al. 2020). The lower PIC values are recorded from closely associated genotypes and higher values for genetically distant genotypes. On the other hand, the PIC in the present study was found to be higher as compared to six traditional common bean varieties studied in Himachal Pradesh, India (Sharma et al. 2014) and 135 genotypes studied from northern India (Gupta et al. 2020). Based on the studies of Bashir et al. (2020), the high level of polymorphism is due to huge diversity among genotypes and selection of highly polymorphic markers. In addition to support this, Sharma et al. (2014) stated that a marker with a PIC ranging from 0.3 to 0.8 is considered functional for the measurement of genetic differences in the population. The present research on common bean displayed a PIC range from 0.428 to 0.817, indicating that microsatellite markers are considerably useful and possess good discrimination capacity. Also, discussing about four pulse species assessed in recent study, it was (0.409) lesser than Suvan et al. (2019), who reported a PIC value of 0.60 for the SSR marker in black gram, 0.60 in mung bean (Pratap et al. 2015), higher than 0.199 in Soybean (Bisen et al. 2015) and more or less comparable to 0.50 in Gahat (Chahota et al. 2017). Data evidence from Pratap et al. (2016) also stated that a high fraction of SSR markers were transferred from common bean to mung bean that indicates the higher possibility of SSR transfer to other legumes. Previously, it was demonstrated that primer pairs devised for one species can be used for other species of the same genus along with different genera of the same family (Oliveira et al. 2006). This microsatellite attribute is known as transferability or cross-species amplification, which can be explored as a tool to measure the genetic variability of related species or genera. Subsequently, distinction observed in alleles per loci, heterozygosity and PIC may be attributed to the germplasm size and versatility, ecogeographical locations of gathered germplasm and number of polymorphic markers used in the study.

The amount of molecular variance in this study showed that within population holds large level of genetic variations (99%) as compared to among population (1%). These findings are relatively comparable to the study from Mexico, Gill langarica et al. (2011), where 93.8% variance was discovered within populations and 0.87% and 5.32% variance among populations within groups and among groups respectively. Likewise, the study from Ethiopia and Kenya displayed 66% variance within gene pool and 34% among gene pools (Asfaw et al. 2009). Somewhat, Analogous outcomes were also recorded from Jammu and Kashmir (India), where high (75%) and low (25%) diversity detected among accessions (Dar et al. (2016). The comparative analysis indicates that topographical structure can be the accountable factor for constraining genetic discrimination among populations, which may lead to much superior genetic variation within populations (Gill langarica et al. 2011). Likewise, in this study, average genetic differentiation (G_{ST}) was found 0.264 which was less than 0.41 reported by Zhang et al. (2008). Similarly, gene flow (N_m) was measured to be 1.396 which was less than 2.6 and 3.927 in Rajmash accessions described by Zhang et al. (2008) and Asfaw et al. (2009), respectively. To estimate the evolution of a particular species, it is significant to divide populations according to the geographical location. The unweighted neighbor joining method categorized gathered germplasm into two groups.

Further, the PCoA coordinates displayed clear division of genotypes by accessions in the present study. Likewise, population architecture assessment based on Bayesian method exhibited the formation of two subpopulations ($K = 2$) in our study. The outcomes of the present study support the earlier report published by Chaudhary et al. (2017) which states that the Indian Himalayan region (north-western) common bean germplasm made up of two gene pools - Mesoamerican and Andean. Their phaseolin analysis showed two main types of phaseolin - S and T - type in the bean samples from Indian Himalayan region with the prominence of T-type (Andean) phaseolin in local bean landraces from Jammu and Kashmir. However, other previous studies have separated common bean population into 2–7 subpopulations based on population structure assessment Asfaw et al. 2009; Blair et al. 2012; Nemli et al. 2014; Valentini et al. 2018; Bashir et al. 2020). Maternal inheritance characteristics of cp-SSR markers make them able to observe changes in population composition in most angiosperms (Angioi et al. 2010). Therefore, they are broadly used in the analysis of population genetics, genetic diversity, and evolutionary studies of different plants (Pan et al. 2014). The cpSSR markers may perhaps contribute to distinctiveness, uniformity, and stability (DUS) characterization and plant varietal registration, linkage studies etc. (Cabral et al. 2011; Matondo et al. 2017). The genetic variations revealed by cpSSR markers in this study established their usefulness for studying other legume crops as well. Genetic diversity demonstrated by these markers might be employed in advanced breeding programmes in India for better-quality germplasm selection of common bean.

Conclusion

Information on the distribution of wild and cultivated germplasm of a species is essentially needed to estimate its genetic multiplicity. In the present study, designed SSR markers from the chloroplast portion of the species have established a considerable number of variations among common bean accessions from Uttarakhand. The significant amount of polymorphism recorded among studied samples could be further extended for the identification and conservation studies of local accessions. The present investigation revealed that chloroplast SSRs with higher polymorphisms can be employed for genome wide association mapping in near future. The marker set provides a useful resource for accession detection, evolutionary studies, and assists in molecular breeding of significant common bean accessions. Thus, genetic resources developed through this study could be utilised by breeders for the large-scale screening and DNA fingerprinting of common bean accessions and other Fabaceae members for conservation and genotypic improvement.

Declarations

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Declarations

All authors have shown that there are no competing financial gains that may appear to persuade the work reported in this MS.

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Conflicts of interest

All authors declare no conflicts of interest in publishing this MS.

Availability of data and material

All data linked with this manuscript is available within this Manuscript.

Code availability NA

Author contribution

AKJ conceptualized the study. YB, HS and AKJ standardized methodology and conducted the experiments. YB and AKJ performed data analysis. AKJ contributed in management of resources and funds. All authors contributed in Writing - Review & Editing of the manuscript.

Ethics approval

This MS comprises the original work of the authors' which has not been previously published elsewhere. This article is not currently considered in other publications.

Consent to participate

NA

Consent for publication

All the authors are informed and agreed to submit this manuscript to the GRCE Journal for publication.

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Tables

Table 1: Common bean accessions collected from diverse locations of Uttarakhand

S. No.	Accession Number	Collection Site	District	Latitude (N)	Longitude (E)	Altitude (m)	No of samples
1	AKJ/KK/PS/Harshil/18	Harsil	Uttarkashi	31°02'18"	78°44'14"	2498	8
2	AKJ/KR/Jatoli/38	Jatoli	Bageshwar	30°06'56"	79°55'42"	2056	7
3	AKJ/AR/KK/Dargi/15	Dargi	Tehri	30°19'06"	78°24'39"	1700	5
4	AKJ/YB/PS/Parvada/47	Parvada	Nainital	29°25'29"	79°39'01"	2046	5
5	AKJ/KR/Jaikuni/36	Jakuni	Bageshwar	30°08'35"	79°55'22"	2402	6
6	AKJ/RA/Bheeda/31	Bheeda	Pauri	30°01'20"	79°02'52"	1800	7
7	AKJ/KK/AR/Jakhol/26	Jakhol	Uttarkashi	31°06'52"	78°15'01"	2200	7
8	AKJ/KK/AR/Sankari/25	Sankari	Uttarkashi	31°04'40"	78°11'03"	1850	6
9	AKJ/YB/Khati/37	Khati	Bageshwar	30°06'44"	79°56'24"	2245	5
10	AKJ/KK/PD/Jhalla/23	Jhalla	Uttarkashi	31°01'34"	78°42'52"	1158	6
11	AKJ/YB/PS/Chanpatta/41	Chanpatta	Pithoragarh	29°48'46"	80°14'50"	1727	6
12	AKJ/AR/PD/Tolma/04	Tolma	Chamoli	30°31'24"	79°45'02"	2655	6
13	AKJ/YB/KR/Bona/39	Bona	Pithoragarh	30°03'57"	80°22'40"	2134	6
14	AKJ/KK/AR/Natwar/024	Natwar	Uttarkashi	31°03'53"	78°06'19"	1158	5
15	AKJ/AR/KK/Raithal/021	Raithal	Uttarkashi	30°49'01"	79°36'13"	2140	6
16	AKJ/KK/PS/Haltari/42	Haltari	Uttarkashi	31°03'31"	78°09'00"	2416	6
17	AKJ/YB/PS/Supi/43	Supi	Nainital	29°26'45"	79°39'01"	520	5
18	AKJ/YB/PS/Umagarh/45	Umagarh	Nainital	29°25'50"	79°33'08"	1895	5
19	AKJ/RA/PD/Lata/06	Lata	Chamoli	30°29'40"	79°42'50"	2370	6
20	AKJ/RA/PD/Jumma/01	Jumma	Chamoli	30°36'18"	79°48'23"	2451	6

Table 2: Chloroplast Single Sequence Repeat (cp-SSR) markers applied for genetic diversity evaluation of various accessions of Common beans

Locus	Seq ID	Primer Sequences (5'- 3')	Position	Repeat Motif	Size Range (bp)	Length/Tm	PIC
1	PV_cpSSR3F	ATGGTGATTGCACAGTCC	psbM-petN IGS	(AT)6	112-208	18 Tm ₄₈	0.428
	PV_cpSSR3R	GAAGAAATGGATTCTACTCC				21 Tm ₅₀	
2	PV_cpSSR5F	CCACATATCTATTGTGGC CA	atpl-atpH IGS	(ATCT)3	249-368	21 Tm ₅₀	0.590
	PV_cpSSR5R	CCCATATGGATACAATCAAGG				21 Tm ₅₀	
3	PV_cpSSR7F	AGTTCCGCCTATTTATCAAC	petA-psbJ IGS	(AT)6	150-256	20 Tm ₄₈	0.524
	PV_cpSSR7R	GGA CTCTAGGAAAGGACAAAG				21 Tm ₅₂	
4	PV_cpSSR8F	CGAACTGAACTAAGACCGTTT	trnW-CCA IGS	(AT)9	150-200	21 Tm ₅₀	0.665
	PV_cpSSR8R	CCGTATTCTATGAGATGAGCA				21 Tm ₅₀	
5	PV_cpSSR11F	AATCCCCTTTTCTTACCAAG	ycf1-ndhF	(TATT)3	120-235	20 Tm ₄₈	0.803
	PV_cpSSR11R	GGGCGAATATCTTCGTATATC				21 Tm ₅₀	
6	PV_cpSSR12F	CTCGGTGCATAGAATTTACAC	ndhG-ndhI IGS	(TA)7	195-235	20 Tm ₅₀	0.817
	PV_cpSSR12R	GGGTCGTTTACCAGTATCAGT				21 Tm ₅₂	
7	PV_cpSSR13F	GGGAAAAACAACCACTTCTA	ndhA	(TA)6	300	20 Tm ₄₈	0.567
	PV_cpSSR13R	TTTGCTATACGGTTCTCCTT				20 Tm ₄₈	
8	PV_cpSSR14F	CGCAAACATGATTCAAATGG	psaC IGS-	(TTGA)3	285	20	0.529

ndhE		T _{m48}
PV_cpSSR14R	ACCGGCTATTGTTTCCTCAAT	21
		T _{m50}
		Mean
		0.615

T_m= Melting temperature; IGS = intergenic spacer; PIC= polymorphism information content

Table 3: Characteristics of 15 chloroplast microsatellite markers in 20 accessions of common beans

S. No.	Accession Number	No of polymorphic loci	Polymorphic loci (%)	Na	Ne	He	I
1	AKJ/KK/PS/Harshil/18	16	80	1.80	1.505	0.290	0.431
2	AKJ/KR/Jatoli/38	13	65	1.65	1.454	0.261	0.382
3	AKJ/AR/KK/Dargi/15	11	55	1.55	1.413	0.229	0.332
4	AKJ/YB/PS/Parvada/47	13	65	1.65	1.430	0.246	0.364
5	AKJ/KR/Jaikuni/36	9	45	1.45	1.287	0.171	0.254
6	AKJ/RA/Bheeda/31	11	55	1.55	1.422	0.234	0.338
7	AKJ/KK/AR/Jakhol/26	10	50	1.50	1.363	0.203	0.295
8	AKJ/KK/AR/Sankari/25	11	55	1.55	1.367	0.214	0.316
9	AKJ/YB/Khati/37	8	40	1.40	1.283	0.161	0.235
10	AKJ/KK/PD/Jhalla/23	15	75	1.75	1.510	0.292	0.429
11	AKJ/YB/PS/Chanpatta/41	11	55	1.55	1.384	0.222	0.325
12	AKJ/AR/PD/Tolma/04	11	55	1.55	1.344	0.200	0.298
13	AKJ/YB/KR/Bona/39	10	50	1.50	1.332	0.193	0.285
14	AKJ/KK/AR/Natwar/024	8	40	1.40	1.303	0.170	0.246
15	AKJ/AR/KK/Raithal/021	12	60	1.60	1.446	0.252	0.365
16	AKJ/KK/PS/Haltari/42	12	60	1.60	1.391	0.231	0.342
17	AKJ/YB/PS/Supi/43	9	45	1.45	1.229	0.145	0.223
18	AKJ/YB/PS/Umagarh/45	10	50	1.50	1.335	0.198	0.292
19	AKJ/RA/PD/Lata/06	9	45	1.45	1.298	0.174	0.258
20	AKJ/RA/PD/Jumma/01	9	45	1.45	1.301	0.177	0.261
Mean		10.9	54.5	1.55	1.370	0.213	0.313

Na= observed number of alleles, Ne = effective number of alleles; He = gene diversity; I = Shannon's information index

Table 4: Molecular variance analysis (AMOVA) using Gene Alex 6.5 software

Source	df	Sum of Square	Estimated Variance	% Variance
Among Pops	19	98.045	0.031	1%
Within Pops	99	492.644	4.976	99%
Total	118	590.689	5.007	100%

Figures



Figure 1

Seeds of twenty different accessions of common bean collected from Uttarakhand to study genetic variation

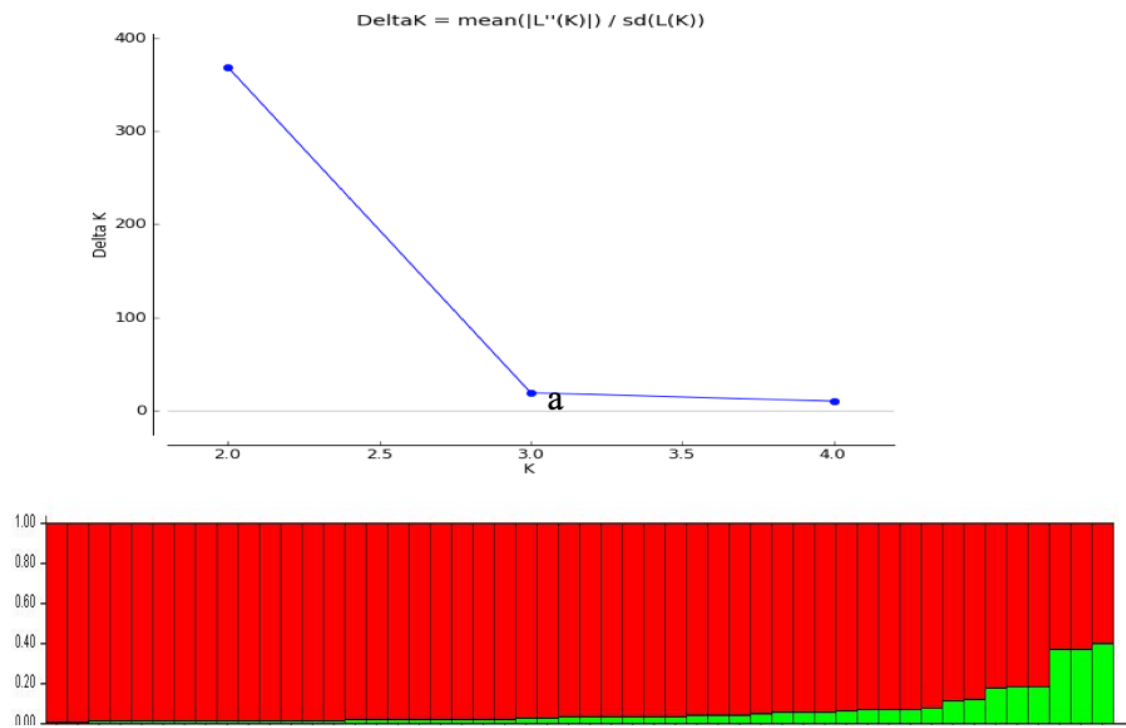


Figure 2

(a) Estimation of most possible number of subpopulations based on the delta K value determined using the program Structure Harvester; **(b)** Population structure of 119 individuals of 20 common bean accessions concluded from Cp-SSR markers. Each individual is represented by a vertically color-coded segment indicating the ideal fraction to the K value= 2

Fig. 3

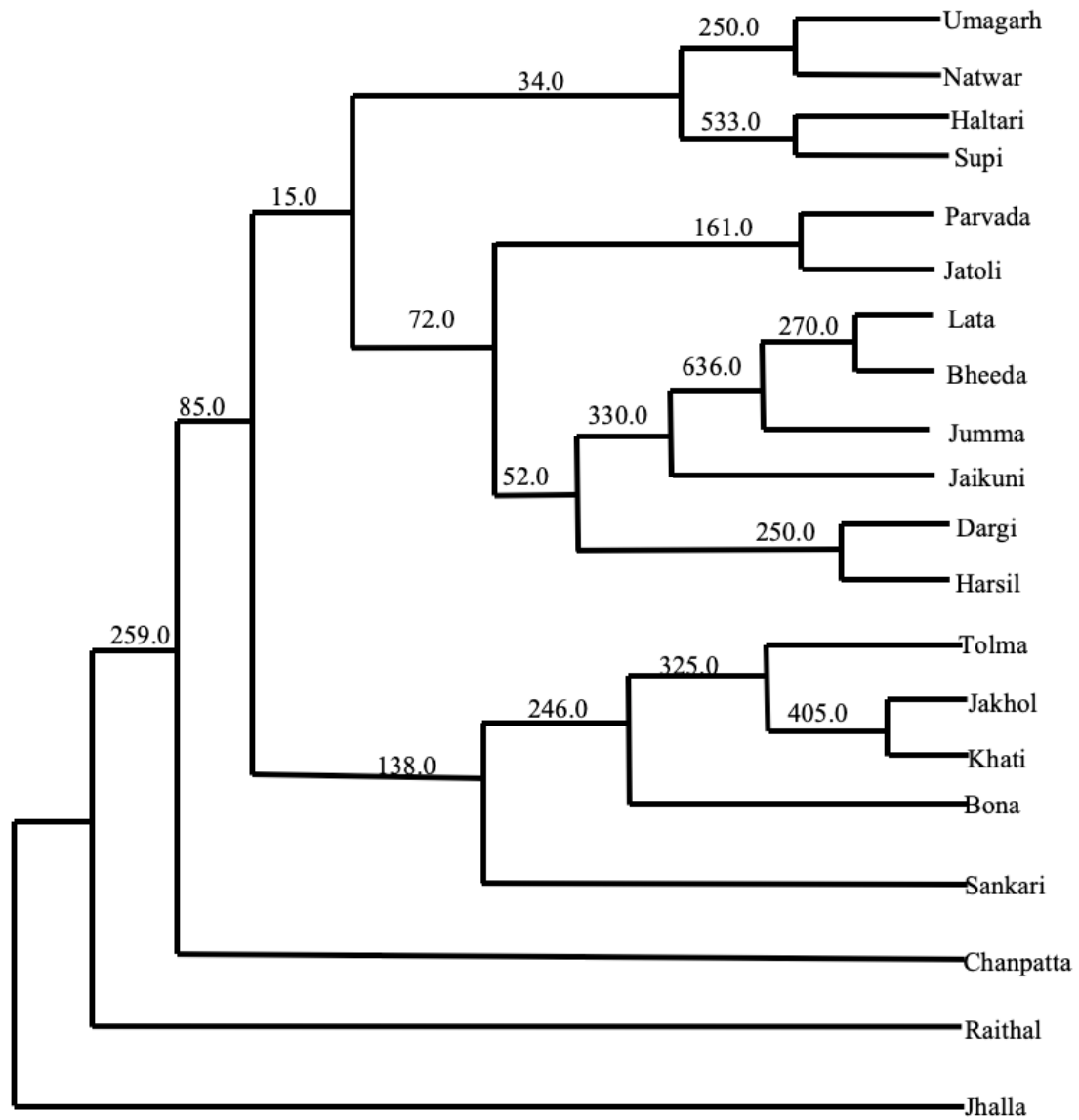


Figure 3

Distribution of 20 common bean accessions from Uttarakhand based on selected SSR loci recorded by the neighbor joining method using PHYLIP Program

Fig. 4

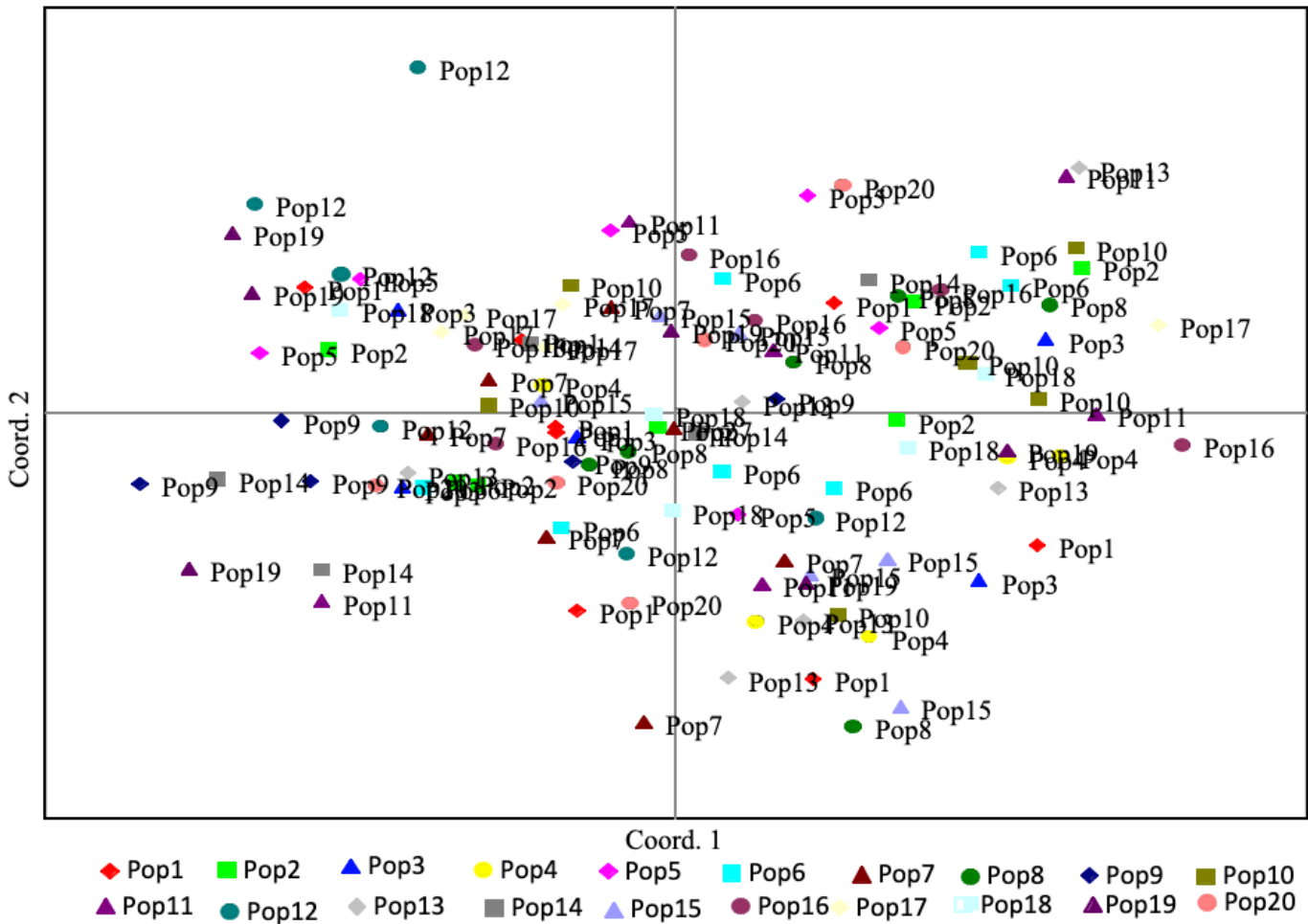


Figure 4

PCoA plot of common bean accessions based on cp-SSR marker data. Coordinate 1 and coordinate 2 comprised about 8.57 and 16.73% variations respectively

Supplementary Files

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