GENETIC DIVERSITY ANALYSIS OF COMMON BEAN GERMPLASM IN SRI LANKA

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Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the most widely cultivated legumes grown for its dietary fiber and protein. It is very popular in hilly areas of Sri Lanka. So far eleven bean varieties have been released by the Department of Agriculture (DOA). However, the average national yield of the crop is lower than international average yield [1] because its full genetic potential could not be effectively utilized. Therefore, it is important to genetically characterize this local germplasm to profile useful information on the level of polymorphism and to determine genes of agronomic importance that can potentially be integrated into future bean breeding programs. Although within the availability of many released bean varieties, adoption rates by the farmers remain low due to several drawbacks in recommended varieties including their susceptibility to diseases. Therefore, bean breeding program focuses on consumer and farmer preferences and sets breeding objectives accordingly. Such local germplasms constitute valuable genetic resources that could be commercially exploited [2]. The present investigation is aimed at assessing the genetic distance of most popular farmer selections and Department of Agriculture (DOA) recommended common bean materials, based on morphological and SSR markers. The information of this study will be benefited for the identification and selection of suitable germplasm for improvement of bean in Sri Lanka.

Materials and Methods

Experimental population

Thirteen genotypes, comprising of five varieties recommended by the DOA (*Lanka butter*, *KWG*, *Bandarawela Green*, *Balangoda Nil* and *Kappetipola Nil*) and eight farmer selections (*Mandaramnuwara Kalu; MNK, Mandaramnuwara Sudu; MNS, Mandaramnuwara Sudu selected; MNSS, Mandaramnuwara Kalu; MNKa, Bandarawela Kalu; BWK, Bandarawela Kaha; BWKa, Galpalama Kalu; GPK, Galpalama Kaha; GPKa*) were evaluated. All thirteen genotypes were obtained from the breeder farms in Bandarawela region, Central province.

Morphological Characterization

The field study was carried out at the plant house of Horticultural Crop Research and Development Institute (HORDI), Peradeniya during the 2015/2016 growing season. Thirteen pots were used with three replicates per each pot. Thirty different qualitative traits were characterized according to IBPGR *Phaseolus vulgaris* L. Descriptor [3].

Molecular Characterization

Three seeds per genotype were singly planted in a pot in the green house at HORDI. Genomic DNA was extracted from young leaves using the CTAB procedure. PCR were performed in a total volume of 15µL. Thirty different SSR primers were used [4, 5].The amplified products were electrophoresed on 1.5% agarose gels and visualized under UV.

Data Analysis

Morphological Data

The Minitab (2006) statistical software was used for cluster analysis and principal component analysis (PCA)

Molecular Data

Reproducible SSR bands data were analyzed using the Popgene programme version 1.31 [5]. Relationship among genotypes was estimated based on Nei's genetic distance using UPGMA clustering method.

Results and Discussion

Morphological Characterization

Five principal components accounted for 86.40% of the total variability (Data not shown). It was suggested that traits such as seed gloss, seed uniformity, and growth habit may be important for genetic improvement and consumer preference, but are less relevant to characterize the genetic diversity. In addition, the farmer selections except *BWKa* and *GPK* and all the DOA recommended varieties except Lanka *butter* and *Bandarawela Green* showed reduced pigmentation in flower color. Moreover, this study revealed that specific morphological traits (*i.e*. flower color, 50% flowering, seed coat lighter color, seed coat darker color and petiole color) can be used for the selection of common bean genotypes for crop improvement programs.

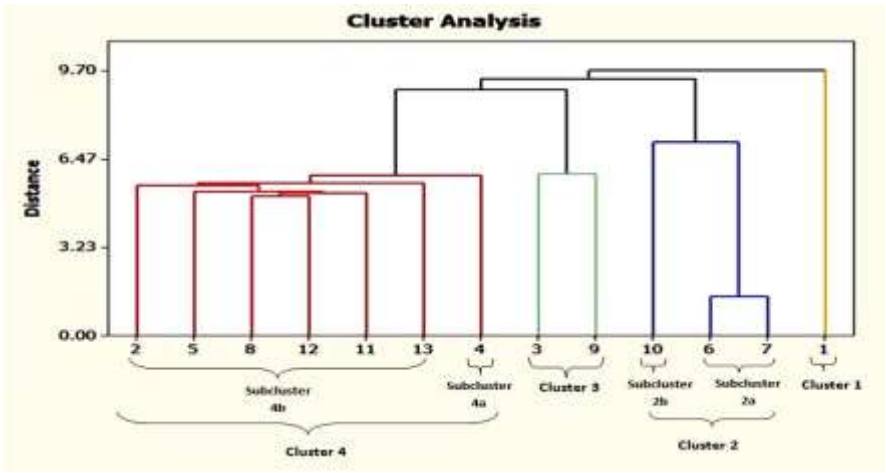

Figure 1. Cluster analysis based on morphological relationship among common bean (1, *Lanka butter*; 2, *KWG*; 3,*Bandarawela Green*; 4, *Balangoda Nil*; 5, *Keppetipola Nil*; 6, *MNK*; 7, *MNKa* ; 8, *MNS*; 9, *BWKa*; 10, *BWK*; 11, *GPKa*; 12, *GPK*; 13, *MNSS*) germplasm in up country region of Sri Lanka drawn from single linkage Euclidean genetic distance

The multivariate hierarchical clustering procedure resulted genetic dissimilarity value ranged from 0 - 12.21 (Figure 1). All thirteen genotypes were grouped into four major clusters. This study indicated that individuals possessing similar characters were clustered together and exhibited higher homogeneity, presumably because they have been collected from similar locations with similar climate and soil type and there has been seed-exchange instances between farmers of closed regions. In addition, most of the farmer selection genotypes clustered with DOA recommended varieties. This could be due to the Introgression between domesticated or farmer selection populations and DOA recommended varieties and it may appreciably modify the organization of the domesticated gene pool in Sri Lanka. Nevertheless '*Lanka butter'* forming a separate cluster from all the samples may indicates that this variety has been marked limited or no introgression within the population.

It is inferred from the present investigation that hybridization involving the inter-cluster representatives would be more useful in common bean breeding programmes to determine the varieties for higher farmer and consumer preference. The genotypes *MNK*, *MNKa* and *BWK*, separated from the rest of the genotypes and formed the cluster 2. These are the most diverse genotypes among the accessions.

Molecular Characterization

Sixteen primer pairs: BM200, BMd15, BM114, BM141, BM143, BM153, BM160, BMd9, BMd053, PVAG-004, PVBR-14, BM137, BM188, GATS-91, BM187 and BMd27 showed diversity in common bean(Figure 2)[4, 5].

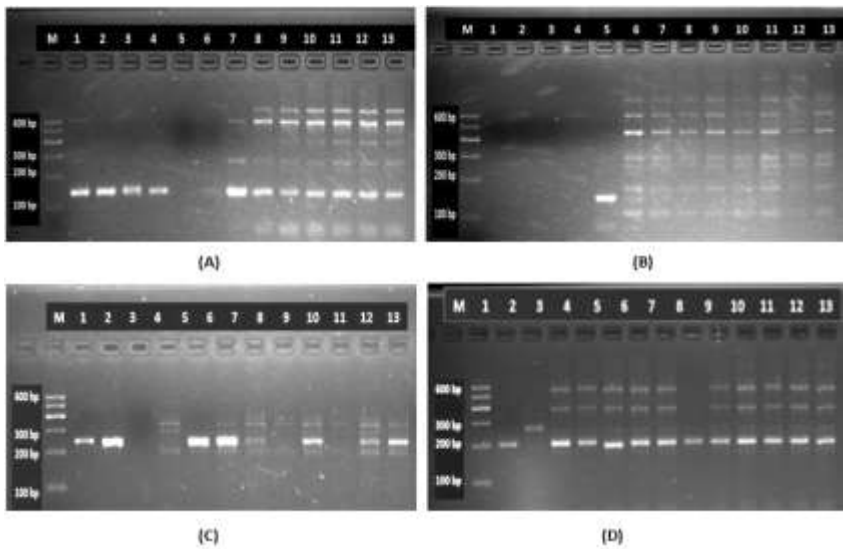

Figure 2. Amplification products of Primer BM183 (A), BMd9 (B), BM114 (C) and primer BMd27 (D) detecting different alleles in thirteen common bean samples; Lane M, 600bp DNA ladder; Lane 1, *Lanka butter*; Lane2, *KWG*; Lane 3, *Bandarawela* Green; Lane 4, *Balangoda Nil*; Lane 5, *Keppetipola Nil*; Lane 6, *MNK*; Lane 7, *MNKa*; Lane 8, *MNS*; Lane 9, *BWKa*; Lane 10, *BWK*; Lane 11, *GPKa*; Lane 12, *GPK*; Lane 13, *MNSS*

Alleles per locus was 1.3 and this was a relatively fair amplification given that the primers had been pre-tested [4].The equivalent amplification in this study could be attributed to use of primers that were specifically designed from common bean species. Pairwise genetic distance, estimated among thirteen genotypes ranged from 0.0150 to 0.5397. Based on dissimilarity matrix; *'Keppetipola Nil'* and *'Lanka butter';* two DOA recommended varieties were the most genetically dissimilar ones showing 53.97% genetic distance. On the other hand, two farmer selections (*MNSS* and *GPKa)* were the most genetically similar ones showing 1.5% genetic distance. Common bean varieties could be distinguished at the genetic distance of 0.1673, according to the genetic distance analysis of DOA recommended varieties.

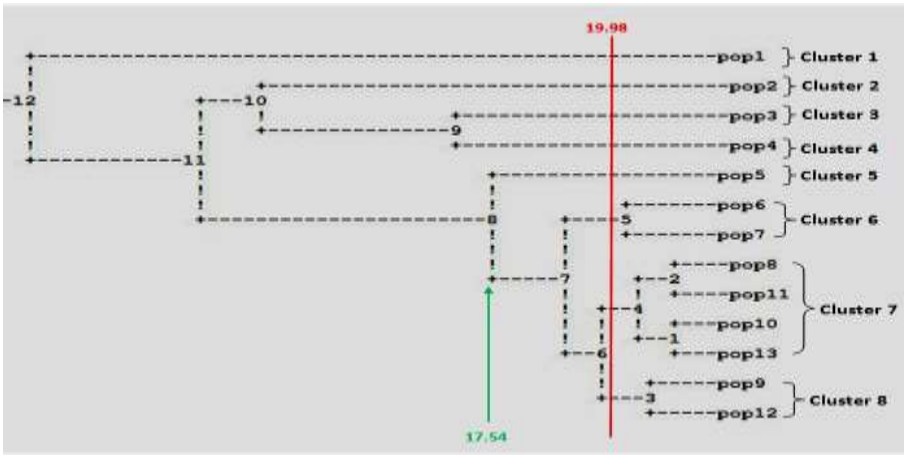

Figure 3. Cluster analysis based on molecular characterization among common bean (pop1, *Lanka butter*; pop2, *KW*G; pop3, *Bandarawela Green*; pop4, *Balangoda Nil*; pop5, *Keppetipola Nil*; pop6, *MNK*; pop7, *MNKa*; pop8, *MNS*; pop9, *BWKa*; pop10, *BWK*; pop11, *GPKa*; pop12, *GPK*; pop13, *MNSS*) germplasm in up country region of Sri Lanka drawn from Nei's genetic distance

Cluster analysis of SSR data revealed eight major clusters at 19.98 (Figure 3). All the DOA recommended varieties formed separate clusters. *'Keppetipola Nil'* formed a cluster, closer to all farmer selections whereas *'Lanka butter'* formed a cluster far away from the rest of the genotypes. Three separate clusters (6, 7 and 8) formed by farmer selections.

Though the entire farmer selections came together with '*Keppetipola Nil'* for 17.25 of Nei's genetic distances all separated from *'Keppetipola Nil'* at 17.85 distances. *MNK* was the most closed farmer selection to *'Keppetipola Nil'* whereas *GPK* was the most distant one.Cluster analysis of both morphological and molecular data revealed that *'Lanka butter'* was the most dissimilar to all the other samples that were tested. *MNK* and *MNKa* morphologically and at molecular level consisted in same cluster in addition to *MNS* and *MNKa*. Three separate clusters of farmer selections (*MNK* and *MNKa*; *MNS*, *GPKa*, *BWK* and *MNS*; *BWKa* and *GPK*) had genetic distance higher than genetic distance of 0.1673. Therefore, it has potential to

incorporate these three separate clusters of farmer selections as three different lines in future breeding and crop improvement programmes.

Conclusions and Recommendations

Flower color, 50% flowering and seed coat color were the most significantly attributed morphological components for genetic variation. SSR profiling technique provided useful information of the informative primers (BM200, BMd15, BM114, BM141, BM143, BM153, BM160, BMd9, BMd053, PVAG-004, PVBR-14, BM137, BM188, GATS-91, BM187 and BMd27) which will be useful in genetic analysis of bean accessions in germplasm holdings. Both molecular and morphological data sets were equally effective to quantify and organize the genetic diversity of common bean germplasm in Sri Lanka. Three separate clusters of farmer selections (*MNK* and *MNKa*; *MNS*, *GPKa*, *BWK* and *MNS*; *BWKa* and *GPK*) could be developed as three different lines to use in future breeding programmes due to their higher genetic divergence from the tested DOA recommended varieties.

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