Genetic diversity and conservation of common bean (*Phaseolus vulgaris* L., Fabaceae) landraces in Nilgiris

Franklin Charles Jose[1,*], M. M. Sudheer Mohammed[2], George Thomas[3], George Varghese[3], N. Selvaraj[4] and M. Dorai[1]

[1]Department of Plant Biology and Biotechnology, Government Arts College, Stone House Hill P.O., Udhagamandalam 643 002, India
[2]Department of Plant Biology and Biotechnology, Government Arts College (Autonomous), Coimbatore 641 018, India
[3]Department of Plant Molecular Biology, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram 695 014, India
[4]Horticultural Research Station, Tamil Nadu Agricultural University, Centenary Rose Park, Udhagamandalam 643 001, India

Genetic diversity was studied among 20 common bean (*Phaseolus vulgaris* L.) landraces collected from different traditional farming villages of Nilgiris District, Tamil Nadu, India, with Random Amplified Polymorphic DNA (RAPD) markers. Evaluation of genetic diversity is essential for conservation, management and to trace the hybrids. Thirteen RAPD primers were selected from an initial screening with 72 primers. The PCR product revealed 102 bands, out of which 63 were found to be polymorphic (63.5%). Jaccard's pair-wise similarity coefficient (0.50 to 0.95) indicating an intra-specific genetic variation prevails in landraces of common bean in the Nilgiris biosphere reserve. No two accessions had a similarity of one or a distance of zero, showing that there were no duplicate entries. A dendrogram of the relationship of accessions constructed based on Jaccard's coefficients of 102 RAPD markers using the average distance method (UPGMA), separated the accessions into two major clusters, A and B, with Mesoamerican and Andean gene pools respectively. Principal coordinate analysis of the same dataset revealed similar results as those of the dendrogram, with the first two components accounting for 61.8% of the total variation. Among the 20 landraces, seven were Mesoamerican origin, 11 Andean origin, and two were possible recombinants between the two gene pools. A correlation was observed between RAPD dendrogram clustering and seed weight. The common bean population of the Nilgiris is highly diverse and the Nilgiris can be considered as a secondary centre of genetic diversity of common bean. A better knowledge of genetic aspects of common bean will help in genetic improvement and conservation programmes for its endangered landraces in the Nilgiris.

Keywords: Genetic variation, germplasm conservation, multivariate analysis, *Phaseolus vulgaris*, RAPD markers.

THE common bean (*Phaseolus vulgaris* L.) was introduced from the Americas into the Nilgiris approximately 400 years ago. It is the most important legume worldwide for direct human consumption and has the broadest range of genetic resources[1]. The Blue Mountains or Nilgiri hills in Tamil Nadu, India, lies between 11°12′N and 11°43′N, 76°14′E and 77°1′E. The region is a veritable paradise because of the rich and diversified geographical conditions, flora and fauna which have played a pioneering role in the introduction and cultivation of plants since colonial times[2]. This region is highly species-rich and is considered one of the 25 biodiversity hotspots of the world[3].

The importance of common bean landraces in Nilgiris agriculture cannot be neglected. Most of these varieties are resistant to anthracnose, bean common mosaic virus and rust, can be grown even if there is scarcity of water. Most of the landraces are tall plants, and produce pods in long periods than the short duration commercial cultivars. The seeds of these landraces are used as sowing material by the traditional small-scale farmers, who are conserving these landraces for centuries. The immense genetic diversity of landraces of crops is the most directly useful and economically valuable part of biodiversity. Unlike high-yielding varieties, the landraces maintained by farmers are endowed with tremendous genetic variability, as they are not subjected to subtle selection over a long period.

Because of the limitations of morphological and biochemical markers, efforts are being directed to use molecular markers for characterizing germplasm diversity. Molecular markers have demonstrated a potential to detect genetic diversity and to aid in the management of plant genetic resources[4,5]. In contrast to morphological traits, molecular markers can reveal differences among genotypes at the DNA level, providing a more direct, reliable and efficient tool for germplasm characterization, conservation and management. Several types of molecular markers are available today, including those based on restriction fragment length polymorphism (RFLP)[6], random amplified polymorphic DNA (RAPD)[7,8], amplified fragment length polymorphism (AFLP)[9] and simple-sequence repeats (SSRs)[10].

*For correspondence. (e-mail: fcjooty@yahoo.com)

In the present study, we have used RAPD method of DNA fingerprinting, which is widely used in conservation biology because of quick results, cost-effectiveness and reproducibility. The PCR-based RAPD approach using arbitrary primers requires only nanogram quantities of template DNA, no radioactive probes, and is relatively simple compared to other techniques[11]. RAPD is a useful predictive tool to identify areas of maximum diversity and may be used to estimate levels of genetic variability in natural populations. Morphological, biochemical and molecular analyses have been suggested for the evaluation of genetic diversity in common bean from America[12–16], Galicia[17], Central Himalaya[18], Italy[19] and China[20].

So far, no systematic studies have been conducted to assess the morphological and genetic variation in the Nilgiris common bean landraces, because of unavailability in outside markets. Tribal agricultural farmers mainly use these beans to prepare traditional food items for their own use. The genetic discrimination of an individual is an important step in investigating the population biology of any species and a major contribution that conservation geneticists can make for evaluating population viability. On the basis of the above, the present study was undertaken to investigate genetic diversity and origin of *P. vulgaris* L. landraces cultivated by tribal agricultural farmers in the Nilgiris.

Materials and methods

Sample collection

The present study included 20 landraces of common bean collected from different villages of the Nilgiris. The villages from where the seeds were collected, its common name, phesolin type, growth habit, 100 seed weight and altitude of the region above mean sea level were recorded (Table 1). Two landraces (LR2 and LR9) were obtained from the Regional Horticultural Research Station (RHRS) of Tamil Nadu Agricultural University (TNAU). The genotypes were selected for genetic analysis after thorough morphological evaluation in the farmer's field, according to the IBPGR descriptor list for *P. vulgaris* L.[21]. Plant height, seed coat colour and seed weight (expressed in g/100 seeds) were used as the primary indicators of morphological variation because of the marked difference in these characters between different landraces. Twenty-five seeds from each landrace were collected and germinated under laboratory conditions. The healthy, young leaves collected from these samples were used for RAPD analysis.

DNA isolation and primer screening

DNA was extracted from young, tender leaves collected separately from each individual and stored at –70°C in sealed polythene bags. Genomic DNA was isolated from 100 mg of the tender leaf tissues using Gen Elute Plant Genomic DNA Purification Kit (Sigma) following the manufacturer's instructions. About 2 µl of genomic DNA isolated from 100 mg of leaf tissue was subjected to electrophoresis on a 0.8% agarose gel containing 500 ng/µl of ethidium bromide. After electrophoresis, the gel was viewed over a UV transilluminator and the quality and quantity of DNA was assessed using undigested λ DNA as control. Only extracts without RNA smears on agarose gel and with UV-light absorption ratios (A260/A280) between 1.8 and 2.0 were used. The genomic DNA was diluted to 4 ng/µl and stored at 4°C as working solution, while the stock DNA (undiluted) was stored at –20°C in aliquots. Initial screening was done with 72 primers (Operon Technologies Inc., CA, USA) using DNA from five randomly selected landraces. PCR–RAPD analysis was repeated at least twice and only primers producing strong and reproducible bands were used in the final analysis of all the 20 landraces.

Polymerase chain reaction

Polymerase chain reaction (PCR) was carried out in a 20 µl reaction volume containing 28 ng of genomic DNA, 1 unit of *Taq* DNA polymerase (Bangalore Genei), 4 µl primer, 0.2 mM dNTPs, 10 mM Tris-HCl (pH 9.0), 1.5 mM $MgCl_2$ and 50 mM KCl. Amplification was carried out in a thermocycler (Eppendorf) with an initial strand separation at 94°C for 4 min, followed by 40 cycles of 1 min at 94°C, 1 min at 37°C and 1.5 min at 72°C. After 40 cycles, there was a final extension step of 5 min at 72°C. Amplification products were resolved on 1.2% agarose gels in 1% TAE buffer at 70 V. The gels were photographed using a gel documentation system (SynGene). Size of the amplified products was compared to λ DNA/*Eco*RI double-digest marker (Bangalore Genei).

Data analysis

Each amplified RAPD marker was treated as a unit character and was scored as present (1) or absent (0) for each sample. Ambiguous bands that could not be clearly distinguished were not scored. The percentage of polymorphism was calculated as the proportion of polymorphic bands over the total number of bands. The 1/0 matrix was prepared for all fragments scored and the data were used to generate genetic similarity (GS) based on Jaccard's coefficient of similarity as follows: $GS(ij) = a/(a + b + c)$, where $GS(ij)$ is the measure of genetic similarity between individuals i and j, a is the number of polymorphic bands that are shared by i and j, b is the number of bands present in i and absent in j, and c is the number of bands present in j and absent in i. Jaccard's coefficients were used to construct a dendrogram using UPGMA. The Jaccard's

Table 1. *Phaseolus vulgaris* L. landraces in the Nilgiris

Accession no.	Market class	Village/place of collection	Altitude m amsl	Growth habit (I, III, IV)	Seed weight (g/100 seeds)
LR1	Cranberry	Nanjanadu	2376	I	39.9
LR2	Black turtle	RHRS, TNAU*	2376	I	16.5
LR3	Cranberry big	Adigaratti	2261	I	53.0
LR4	Pale brown kidney	Tumanatti	1920	I	36.7
LR5	White kidney	Nanjanadu	2376	I	32.8
LR6	Red kidney	Ozahatti	2261	I	46.0
LR7	Black bean	Nanjanadu	2376	IV	32.3
LR8	Great northern	Ebanad	1930	III	34.3
LR9	White eyed bean	RHRS, TNAU*	2376	IV	23.7
LR10	Greenish striped kidney	Nundala	2180	IV	67.2
LR11	Ash bean	Nanjanadu	2376	IV	66.9
LR12	Large pinto	Tuneri	2066	IV	66.0
LR13	Pinto bean	Muttinadu	2200	IV	66.1
LR14	Pink bean	Sholur	2277	IV	31.8
LR15	Black striped	Wellington	2041	IV	68.2
LR16	White round	Bikkatti	2532	IV	69.4
LR17	Dark brown	Nanjanadu	2376	I	30.8
LR18	Brown kidney	Kokkal	2270	IV	63.5
LR19	Dark brown	Ebanad	2060	IV	36.6
LR20	Small kidney	Muttinadu	2200	III	23.5

*Regional Horticultural Research Station, Tamil Nadu Agricultural University, Udhagamandalam.

similarity matrix was then used as the basis for ordination by principal coordinates analysis (PCoA), which was performed to show the distribution of the genotypes in a scatter plot using the Multivariate Statistical Package version 3 software.

Results

DNA fingerprinting

DNA extracted from 20 common bean landraces was examined for the PCR–RAPD patterns. Out of the 72 primers screened, 13 were selected based on robustness of amplification, reproducibility, scorability of banding patterns and were used for diversity analysis in all landraces. The 13 selected decamer oligonucleotide primers (Table 2) generated 102 amplification products, out of which 63 bands (63.5%) were polymorphic. The number of bands per primer ranged from 4 (OPE19) to 13 (OPJ9), with an average of 7.8 bands per primer. The range of polymorphic bands per primer was 3 (OPA1, OPA4 and OPF16) to 7 (OPE6, OPJ9 and OPJ16), with a mean of 4.8 polymeric bands per primer (Table 2). The representative RAPD patterns generated by primers OPE6, OPA4 and OPE19 for 20 landraces are given in Figure 1. These three primers are efficient in discriminating landraces into Andean and Mesoamerican races.

Genetic similarity, cluster analysis and PCoA

The pair-wise Jaccard's coefficients genetic similarity matrix was prepared based on RAPD data. The genetic similarity coefficients among the common bean landraces varied from 0.50 (between varieties LR3 and LR9) to 0.95 (between varieties LR1 and LR16; Table 3). Cluster analysis was performed on RAPD data using UPGMA, which showed overall genetic relationships among the landraces of common bean (Figure 2). PCoA was carried out in order to determine the genetic relationships among the landraces. The landraces were plotted on principal coordinates 1 and 2, accounting for 53.0 and 8.8% of the variation respectively, and together explaining 61.8% of the total variation (Figure 3). UPGMA clustering and PCoA of RAPD data indicated that the landraces of *P. vulgaris* population comprise two major groups, clusters M and A (Figure 2). The similarity coefficients ranged from 0.50 to 0.95, indicating that the two clusters did not show 100% similarity.

Mesoamerican, Andean and hybrid gene pools

Cluster 'M' comprised of seven landraces (LR2, LR7, LR8, LR9, LR14, LR19 and LR20) which are of Mesoamerican origin. Cluster 'A' comprised of 11 landraces (LR1, LR16, LR12, LR13, LR18, LR3, LR11, LR10, LR4, LR15 and LR6) which are of Andean origin. Cluster 'H' consists of two landraces (LR5 and LR17) which are hybrids. Maximum similarities were observed between LR8 and LR9, with a similarity value of 0.89. LR20 and LR19 formed a separate cluster within cluster M, with a similarity value of 0.81. LR2 formed an operational taxonomic unit in cluster M. The highest similarity (0.95) was observed among LR1, LR16, LR12 and LR13. Cluster analysis and PCoA of the similarity indices (Figures 2 and 3)

Table 2. Primers with their sequences used for RAPD analysis of *Phaseolus vulgaris* L. landraces in the Nilgiris. Total number of bands, polymorphic bands, and percentage of polymorphism yielded by each primer are also listed

Primer code	Primer sequence (5′ → 3′)	Total no. of bands	No. of polymorphic bands	Percentage of polymorphism
OPA1	CAGGCCCTTC	7	3	42.8
OPA4	AATCGGGCTG	7	3	42.8
OPE1	CCCAAGGTCC	8	4	50.0
OPE6	AAGACCCCTC	10	7	70.0
OPE12	TTATCGCCCC	8	5	62.5
OPE19	ACGGCGTATG	4	4	100
OPF6	GGGAATTCGG	10	6	60
OPF16	GGAGTACTGG	5	3	60
OPJ9	TGAGCCTCAC	13	7	53.8
OPJ16	CTGCTTAGGG	8	7	87.5
OPAC12	GGCGAGTGTG	6	5	83.3
OPAO8	ACTGGCTCTC	9	5	55.5
OPAR1	CCATTCCGAG	7	4	57.1
Total		102	63	63.5
Mean per primer		7.8	4.8	

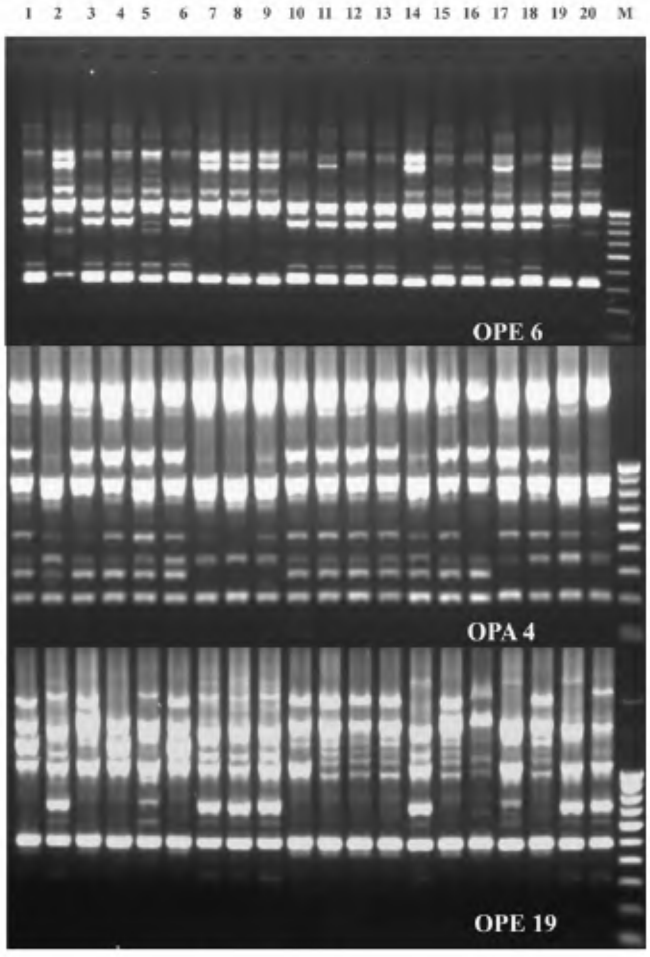

Figure 1. RAPD profile of 20 landraces of *Phaseolus vulgaris* L. produced using the random decamer primers OPE6, OPA4 and OPE19. M, 100 bp DNA ladder. Lane numbers correspond to the landtraces LR1 to LR20 given in Table 1.

support the above results. In the RAPD dendrogram (Figure 2) two landraces, LR5 and LR17, formed a separate group in the Andean gene pool, very closely associated with the Mesoamerican gene pool. This may be a natural hybrid gene pool between two major gene pools[22,23]. In PCoA, the hybrid landraces (LR5 and LR17) formed a distinct group different from the Mesoamerican and Andean gene pools (Figure 3).

RAPD clustering and seed weight

A correlation was observed between seed weight, RAPD clustering and PCoA of RAPD data. The average 100 seed weight of Mesoamerican landraces ranged from 16.5 to 36.6 g and that of the Andean gene pool ranged from 36.7 to 69.4 g. Therefore, there was a fine separation of the major gene pools with regard to seed weight, without any overlapping between them. The hybrid gene pool comprising two landraces, LR5 and LR17, with 100 seed weight (32.8 and 30.8 g respectively) and phaseolin types ('S' and 'T' respectively) was dwarf plants with kidney-shaped seeds. Similarly, LR2, the landrace with the lowest seed weight formed a distinct taxonomical unit in the Mesoamerican group. Thus, a correlation was observed between RAPD clustering and seed weight in common bean landraces. This indicates that RAPD markers are well suited to determine the genetic diversity and differentiation present in common bean landraces in the Nilgiris.

Discussion

The present study addresses the utility of RAPD markers in revealing genetic relationships at the molecular level among landraces of common bean in the Nilgiris. Sources of polymorphism in the RAPD assay may be due to base change within the priming site sequence, deletions of

Table 3. Similarity matrix for Jaccard's coefficients for 20 landraces (LR1-20) of *Phaseolus vulgaris* based on 102 bands obtained with 13 RAPD primers

	LR1	LR2	LR3	LR4	LR5	LR6	LR7	LR8	LR9	LR10	LR11	LR12	LR13	LR14	LR15	LR16	LR17	LR18	LR19	LR20
LR1	1.00																			
LR2	0.55	1.00																		
LR3	0.89	0.56	1.00																	
LR4	0.90	0.56	0.84	1.00																
LR5	0.71	0.70	0.68	0.70	1.00															
LR6	0.90	0.58	0.89	0.88	0.73	1.00														
LR7	0.53	0.81	0.54	0.53	0.65	0.53	1.00													
LR8	0.54	0.80	0.56	0.53	0.64	0.52	0.90	1.00												
LR9	0.53	0.83	0.50	0.52	0.63	0.53	0.88	0.91	1.00											
LR10	0.84	0.57	0.90	0.84	0.71	0.89	0.56	0.55	0.53	1.00										
LR11	0.88	0.57	0.91	0.83	0.69	0.88	0.55	0.58	0.53	0.89	1.00									
LR12	0.87	0.58	0.86	0.82	0.65	0.90	0.54	0.52	0.52	0.86	0.86	1.00								
LR13	0.92	0.56	0.89	0.87	0.69	0.93	0.54	0.54	0.53	0.89	0.88	0.95	1.00							
LR14	0.58	0.82	0.55	0.57	0.67	0.57	0.85	0.88	0.89	0.57	0.59	0.55	0.57	1.00						
LR15	0.84	0.57	0.83	0.77	0.64	0.86	0.51	0.52	0.53	0.80	0.84	0.88	0.84	0.55	1.00					
LR16	0.95	0.53	0.91	0.87	0.68	0.90	0.52	0.55	0.51	0.86	0.88	0.87	0.92	0.55	0.84	1.00				
LR17	0.76	0.68	0.73	0.73	0.79	0.74	0.66	0.68	0.66	0.71	0.74	0.68	0.70	0.68	0.67	0.74	1.00			
LR18	0.89	0.56	0.86	0.82	0.68	0.87	0.57	0.58	0.58	0.85	0.85	0.86	0.91	0.58	0.83	0.89	0.75	1.00		
LR19	0.60	0.79	0.57	0.59	0.72	0.58	0.77	0.82	0.81	0.56	0.60	0.54	0.55	0.80	0.56	0.57	0.75	0.59	1.00	
LR20	0.54	0.80	0.54	0.52	0.65	0.53	0.78	0.79	0.80	0.53	0.54	0.52	0.51	0.77	0.53	0.55	0.68	0.55	0.82	1.00

priming site, insertions that render priming sites too distant to support amplification, and deletions or insertions that change the size of the DNA fragments which act to prevent its amplification[8]. RAPD markers were able to distinguish groups within both the Andean and Mesoamerican gene pools[14,15]. The polymorphism revealed by RAPD has been problematic due to their dominance. As heterozygotes are not normally detectable, the results are not readily usable for computing Hardy–Weinberg equilibrium or Nei's standard genetic distance[24]. Therefore in this study RAPD polymorphisms were analysed with a phonetic distance measure (Jaccard's coefficient) from which a dendrogram was constructed (Figure 2), providing an indication of the diversity present within the landraces of common bean.

Mesoamerican group

This group is represented by 35% of the total population, with seven landraces (LR2, LR7, LR8, LR9, LR14, LR19 and LR20) in cluster M (Figure 2). In this group, one dwarf (LR2), two tall non-woody (LR20 and LR8) and four tall woody types (LR19, LR14, LR9 and LR7) were described. The average weight of 100 seeds ranged from 16.5 to 36.6 g. As suggested by Evans[25,26], the Mesoamerican gene pool has both small (<25 g per 100 seed weight) and medium (25–40 g per 100 seed weight) seeds. The lowest seed weight (16.5 g per 100 seeds) was observed in LR2. Zizumbo-Villarreal *et al.*[27] reported that the average weedy seed weight ranged from 19 to 21 g/100 seeds and that of wild seeds ranged from 4 to 7 g/100 seeds. Moreover, this variety is less common in cultivation and least preferred by farmers due to its small black seeds. In the dendrogram cluster (Figure 2), this formed an operational taxonomic unit very distant from other Mesoamerican races, probably due to its unique agro-morphological characteristics and the presence of some rare alleles. Such RAPD cluster with only one accession in common bean landraces is common[14,15,18]. DNA analysis with RAPD markers confirmed the existence of these three races among the climbing beans of Mesoamerican origin in Guatemala and neighbouring countries[15].

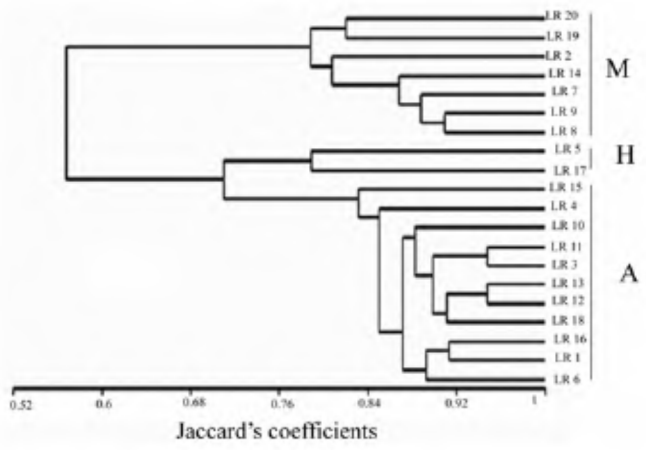

Figure 2. Genetic similarity dendrogram based on 102 RAPD markers in 20 common bean landtraces (LR1–LR20). M, Mesoamerican; H, Hybrid; A, Andean.

As evident from the dendrogram (Figure 2), the Mesoamerican landraces in the Nilgiris are highly diverse with a similarity coefficient lower compared to their Andean counterpart. It has been calculated that more than 60% of the world production derives from domesticates of Mesoamerican origin[15]. Nevertheless, in the Nilgiris it represents only 35% of the total population and is used as both snap and dry beans. One reason might be the preference of farmers for the large-seeded Andean beans, at least for dry beans, over the small-seeded Mesoamerican beans. As a result, the distribution and use of Mesoamerican beans are less in the Nilgiris compared to the Andean races. Moreover, in recent years breeding activities have focused on only a few highly priced, large-seeded market classes that could have contributed to a reduction in genetic diversity within the small-seeded, less desirable cultivars. Regarding the landraces of common bean in the Nilgiris, it is not true that farmers always select varieties with big seeds; instead, they select plants for their special requirements. For example, LR20 is small-seeded and widely grown for its unique uses in certain recipes and good market value. In spite of these hurdles, the Mesoamerican germplasm in the Nilgiris is genetically more diverse than the Andean race, which is evident from the distribution of its landraces in the RAPD dendrogram (Figure 2).

Andean group

This group consists of landraces in cluster B. This cluster is constituted by 11 landraces (55% of the total), out of which four are dwarf and seven are tall (Figure 2 and Table 1). In this cluster, two dwarf varieties (LR5 and LR17) formed a sub-cluster with a similarity value of 0.79 (Figure 2) and are considered to be hybrids between the Mesoamerican and Andean gene pools. The Andean race is characterized by large seeds, >40 g/100 seed weight[24,25] and T type phaseolin[28]. The average 100 seed weight in Andean race ranged from 36.7 to 69.4 g (Table 1). The T type phaseolin was significantly correlated with higher seed weight, increased overall seed protein and an increase in the percentage of phaseolin compared to S type phaseolin observed in Italian landraces[29]. A correlation between plant height and seed weight in the Nilgiris Andean gene pool was evident. The four dwarf plants had seed weight <60.0 g, while all the tall landraces had >60.0 g (Table 1).

In the Nilgiris, the majority of common bean under cultivation was from the Andean gene pool. The same

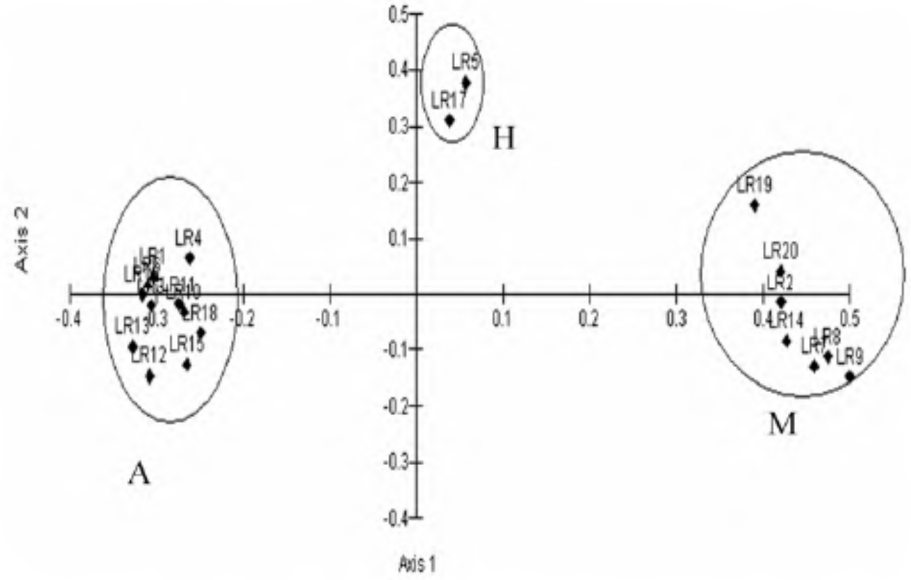

Figure 3. Principal coordinate analysis of 20 landraces of common bean (LR1–LR20) based on Jaccard's similarity coefficients.

phenomenon was noticed in other common bean-growing regions such as China[20], Italy[29] and Argentina[30]. The high preference for Andean landraces may be due to some desirable traits such as taste, growth habit, seed size and colour. In the Nilgiris, LR4 is the major snap bean and LR11 is the most preferred and highly priced dry bean. This tall bean with ash coloured seed is widely cultivated in almost all the villages. Tribal farmers prepare a special dish out of these beans during special functions and festivals. However, RAPD analysis revealed that this landrace is closely associated with LR3 (Figure 2), a small-seeded dwarf plant commonly used as dry beans.

The genetic diversity of the Andean race is a matter of controversy. Many researchers are of the opinion that in spite of vast diversity in plant and grain morphology, generally Andean landraces of common bean proved to have a very narrow genetic base. Beebe *et al.*[31] revealed a narrow genetic base for the Andean race based on AFLP analysis and the need for widening the genetic basis of cultivated Andean landraces was suggested[13,32,33]. Our findings in the Nilgiri beans throw light upon the fact that the Andean races are genetically less diverse than the Mesoamerican gene landraces (Figures 1–3). However, the hybrid gene pool (LR5 and LR17) contributes significantly to the genetic diversity of the Andean beans. Contrary to these findings, in a study in Argentina[30], very high diversity in four complex primitive races of Andean beans was observed. In China, one of the secondary centres of common bean diversity, Xiaoyan *et al.*[20] found that the level of diversity for Chinese landraces of Andean origin was higher than that of Chinese landraces of Mesoamerican origin due to the presence of more infrequent alleles.

In this context, broadening the genetic base of the Andean landraces must be considered. Previous studies revealed that the Andean cultivars are more difficult to improve[34]. The introgression of additional genetic diversity into the Andean domesticated gene pool may acquire added importance in the light of genetic bottlenecks induced by domestication in common bean[32]. One solution to this problem is the use of natural inter-gene pool hybrids (LR5 and LR17) for breeding purposes.

Inter-gene pool hybrids

Landraces that possess phaseolin typical of one gene pool and many morphological and allozyme traits of the other gene pool are classified as inter-gene pool recombinants[22]. Two accessions, LR5 and LR17, formed a separate group in PCoA (Figure 3). Both have 100 seed weight 32.8 g and 30.8 g respectively, a characteristic feature of the Mesoamerican race. The lack of correlation between phaseolin type and RAPD data in these two landraces might be due to a genetic recombination between the Andean and the Mesoamerican gene pools. It represents inter-gene pool introgression that occurred naturally and selected by farmers in the Nilgiris. The occurrence of recombinants in the Mesoamerican gene pool was revealed[27] and an introgression of genes from the Andean race was observed in Mexican landraces when analysed with AFLP markers[15,23]. Rodino *et al.*[22,35] observed inter-gene pool recombinants in a core collection of common bean landraces from the Iberian peninsula, and evaluated them using morphological, agronomic and biochemical markers. Xiaoyan *et al.*[20] identified Mesoamerican introgression in Chinese common bean landraces when assessed with SSR markers.

Common bean is generally considered an autogamous species, but out-crossing rates as high as 60–70% have been observed[36]. Francisco *et al.*[37] reported exceptionally high rates of out-crossing in some common bean cultivars in California. In addition, it seems that even the lowest

rates of out-crossing reported are sufficient to generate broad variability over hundreds or thousands of years[33]. The inter-gene pool hybrids in the Nilgiris could have been the result of a high frequency of pollinating insects such as honeybees, short distances between plants and co-cultivation of landraces from different gene pools[27]. Cultivation of a mixture of Andean and Middle American beans in close proximity in home gardens combined with occasional out-crossing may have further facilitated introgression between the two gene pools. On the other hand, the genetic variation observed in these landraces might have resulted during the long cultivation history of the species (500 years), as an adaptation to the local agro-climatic conditions. Once these adaptive variations are fixed in the genotypes, subsequently they could have been passed onto the next generation. In the end, these could have resulted in locally adapted genotypes.

We cannot neglect the significance of this hybrid gene pool in common bean improvement in the Nilgiris. The genetically narrow Andean races can improve by crossing with Mesoamerican germplasm. Nevertheless, such crosses often produce poor progeny due to hybrid weakness[38,39]. In this context, one alternative is to use landraces that display the introgression of Mesoamerican genes, such as those forming a sub-cluster in the Andean gene pool (Figures 2 and 3). These inter-gene pool recombinants may be of interest to breeders and geneticists because they could constitute bridging germplasm that may aid in the transfer of useful genes between the two gene pools. Thus, these accessions may merit further studies. These two landraces are dwarf, with small seeds most preferred by farmers for their white seeds and snap beans respectively. Therefore, the Nilgiris' common bean germplasm is more complex and contains additional diversity that remains to be explored for genetic and breeding purposes.

RAPD and seed weight

Our study shows a correlation between RAPD clustering and 100 seed weight (Table 1 and Figure 2). The landraces varied in terms of seed size from a high of 69.4 g per 100 seed to a low of 16.5 g per 100 seed. A correlation between RAPD banding pattern and seed size was observed in Italian common bean landraces[29]. However, no correlation was observed between RAPD branching pattern and morphological data for landraces of common bean collected from Central Himalaya[18]. In our study, with regard to seed weight, no overlapping was observed between the gene pools. Similarly, the small-seeded intergene pool hybrids (LR5 and LR17) with seed weight 32.8 g per 100 seed and 30.8 g per 100 seed formed a sub-cluster in the large-seeded Andean genepool. Duarte *et al.*[40] found a close association between phenology and genetic analysis, and suggested the loci that control molecular and morphological characteristics are closely associated.

Conclusion

These results indicate that the level of genetic variation has not eroded since the introduction of the common bean from the American centres of domestication into the Nilgiris. The level of polymorphism observed in the present study was moderately high, indicating a wide and diverse genetic base for the common bean landraces in the Nilgiris. The 63.5% RAPD polymorphic bands and 67% average genetic differentiation coefficient suggest that the *P. vulgaris* landraces maintain a higher intraspecific genetic diversity in the Nilgiris. There exist clear-cut signs of introgression between the two gene pools as indicated by the hybrids, which merit further investigation. The occurrence of both the gene pools in the Nilgiris is highly significant for further breeding strategies like interracial hybridization and production of ideal genotypes of common bean. Such inter-gene pool and interracial crosses will also facilitate broadening the genetic base of cultivars and maximizing gains from selection for plant type, adaptation, yield and resistance to high and low temperature. The predominance of large-seeded Andean germplasm in the Nilgiris is due to the preference given by farmers and consumers over the small and medium seeded Mesoamerican varieties. The present-day common bean germplasm in the Nilgiris might be the last remains of a vast introduction during colonial times. Moreover, the common bean landraces in the Nilgiris face serious threat of extinction with the introduction of short-duration commercial cultivars. A study of molecular profiles to obtain DNA fingerprints in order to establish the molecular identity of common bean in the Nilgiris for documenting the germplasm seems a worthwhile endeavour. Such DNA fingerprinting pattern would also help monitor genetic stability in the common bean. Our understanding of the genetic diversity of the *P. vulgaris* population can contribute valuable guidelines for conservation strategies and should therefore be an essential part of proper conservation management.

1. Singh, S. P., Improvement of small-seeded race Mesoamerican cultivars. In *Common Bean Improvement in the Twenty-First Century* (ed. Singh, S. P.), Kluwer, Dordrecht, 1999, pp. 225–274.
2. Hastings, K. B., The relationship between the Indian Botanic Garden, Howrah and the Royal Botanic Gardens, Kew. *Econ. Bot. Bull. Bot. Surv. India*, 1988, **28**, 1–12.
3. Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B. and Kent, J., Biodiversity hotspots for conservation priorities. *Nature*, 2000, **403**, 853–858.
4. Virk, P. S., Newbury, H. J., Jackson, M. T. and Ford-Lloyd, B. V., Are mapped or anonymous markers more useful for assessing genetic diversity? *Theor. Appl. Genet.*, 2000, **100**, 607–613.
5. Song, Z. P., Xu, X., Wang, B., Chen, J. K. and Lu, B. R., Genetic diversity in the northernmost *Oryza rufipogon* populations estimated by SSR markers. *Theor. Appl. Genet.*, 2003, **107**, 1492–1499.
6. Botstein, D., White, R. L., Skolnick, M. and Davis, R. W., Construction of a genetic linkage map in man using restriction fragment length polymorphism. *Am. J. Hum. Genet.*, 1980, **32**, 314–331.

7. Welsh, J. and McClelland, M., Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Res.*, 1990, **18**, 7213–7218.
8. Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, S. V., DNA polymorphism amplified by arbitrary primers as useful genetic markers. *Nucleic Acids Res.*, 1990, **18**, 6531–6535.
9. Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T. and Hornes, M., AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res.*, 1995, **23**, 4407–4414.
10. Tautz, D., Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Res.*, 1989, **17**, 6463–6471.
11. Williams, J. G. K., Hanafey, M. K., Rafalski, J. A. and Tingey, S. V., Genetic analysis using random amplified polymorphic DNA markers. *Methods Enzymol.*, 1993, **218**, 704–740.
12. Singh, S. P., Nodari, R. and Gepts, P., Genetic diversity in cultivated common bean: I. Allozymes. *Crop Sci.*, 1991, **31**, 19–23.
13. Singh, S. P., Gutierrez, J. A., Molina, A., Urrea, C. and Gepts, P., Genetic diversity in cultivated common bean: II. Marker-based analysis of morphological and agronomic traits. *Crop Sci.*, 1991, **31**, 23–29.
14. Haley, S. D., Miklas, P. N., Afanador, L. and Kelly, J. D., Random amplified polymorphic DNA (RAPD) marker variability between and within gene pools of common bean. *J. Am. Soc. Hortic. Sci.*, 1994, **119**, 122–125.
15. Beebe, S., Skroch, P. W., Tohme, J., Duque, M. C., Pedraza, F. and Nienhuis, J., Structure of genetic diversity among common bean landraces of Middle American origin based on correspondence analysis of RAPD. *Crop Sci.*, 2000, **40**, 264–273.
16. Metais, I. C., Aubry, B., Hamon, R. J. and Peltier, V., Description and analysis of genetic diversity between commercial bean lines (*Phaseolus vulgaris* L.). *Theor. Appl. Genet.*, 2000, **101**, 1207–1214.
17. Escribano, M. R., Santalla, M., Casquero, P. A. and de Ron, A. M., Patterns of genetic diversity in landraces of common bean (*Phaseolus vulgaris* L.) from Galicia. *Plant Breed.*, 2006, **117**, 49–56.
18. Manoj, T., Singh, N. K., Meenal, R. and Narendra, K., RAPD markers in the analysis of genetic diversity among common bean germplasm from Central Himalaya. *Genet. Res. Crop Evol.*, 2005, **52**, 315–324.
19. Lioi, L., Piergiovanni, A. R., Pignone, D., Puglisi, S., Santantonio, M. and Sonnante, G., Genetic diversity of some surviving on-farm Italian common bean (*Phaseolus vulgaris* L.) landraces. *Plant Breed.*, 2005, **124**, 576–581.
20. Xiaoyan, Z., Matthew, W. B. and Shumin, W., Genetic diversity of Chinese common bean (*Phaseolus vulgaris* L.) landraces assessed with simple sequence repeat markers. *Theor. Appl. Genet.*, 2008, **117**, 629–640.
21. IBPGR, Descriptor list for *Phaseolus vulgaris* L. International Board for Plant Genetic Resources, Rome, 1982.
22. Rodino, A. P., Santalla, M., Gonzalez, A. V., Ron, A. M. De and Singh, S. P., Novel genetic variation in common bean from the Iberian Peninsula. *Crop Sci.*, 2006, **46**, 2540–2546.
23. Rosales-Serna, R., Hernandez-Delgado, S., Gonzalez-Paz, M., Acosta-Gallegos, J. A. and Mayek-Perez, N., Genetic relationships and diversity revealed by AFLP markers in Mexican common bean bred cultivars. *Crop Sci.*, 2005, **45**, 1951–1957.
24. Lynch, M. and Milligan, B. G., Analysis of population genetic structure with RAPD markers. *Mol. Ecol.*, 1994, **3**, 91–99.
25. Evans, A. M., Plant architecture and physiological efficiency in the field bean. In *Potentials of Field Bean and other Food Legumes in Latin America* (ed. Wall, D.), CIAT, Cali, Colombia, 1973, pp. 279–284.
26. Evans, A. M., Structure, variation, evolution, and classification in *Phaseolus*. In *Advances in Legume Science* (eds Summerfield, R. J. and Bunting, A. H.), Royal Botanic Gardens, Kew, UK, 1980, pp. 337–347.
27. Zizumbo-Villarreal, D., Colunga-Garcia-Marin, P., Payro de la Cruz, E., Delgado-Valerio, P. and Gepts, P., Population structure and evolutionary dynamics of wild–weedy–domesticated complexes of common bean in a Mesoamerican region. *Crop Sci.*, 2005, **45**, 1073–1083.
28. Gepts, P., Osborn, T. C., Rashka, K. and Bliss, F. A., Phaseolin protein variability in wild forms and landraces of the common bean (*Phaseolus vulgaris*): evidence for multiple centers of domestication. *Econ. Bot.*, 1986, **40**, 451–468.
29. Ilaria, M., Alessandra, B., Maurizio, M., Pietro, C. and Giovenni, D., Characterization of some Italian common bean (*Phaseolus vulgaris* L.) landraces by RAPD, semi-random and ISSR molecular markers. *Genet. Res. Crop Evol.*, 2007, **54**, 175–188.
30. Ron, A. M., De, Menéndez, M. C. and Santalla, M., Variation in primitive landraces of common bean (*Phaseolus vulgaris* L.) from Argentina. *Genet. Res. Crop Evol.*, 2004, **51**, 883–894.
31. Beebe, S., Rengifo, J., Gaitan, E., Duque, M. C. and Tohme, J., Diversity and origin of Andean landraces of common bean. *Crop Sci.*, 2001, **41**, 854–862.
32. Sonnante, G., Stocklom, T., Nodari, R. O., Becerra Velasquez, V. L. and Gepts, P., Evolution of genetic diversity during the domestication of common bean (*Phaseolus vulgaris* L.). *Theor. Appl. Genet.*, 1994, **89**, 629–635.
33. Beebe, S., Toro, O., Gonzalez, A. V., Chacon, M. I. and Debouck, D. G., Wild–weed–crop complexes of common bean (*Phaseolus vulgaris* L., Fabaceae) in the Andes of Peru and Colombia, and their implications for conservation and breeding. *Genet. Res. Crop Evol.*, 1997, **44**, 73–91.
34. White, J., Kornegay, J., Castillo, J., Molano, C. H., Cajiao, C. and Tejada, G., Effect of growth habit on yield of large-seeded bush cultivars of common bean. *Field Crops Res.*, 1992, **29**, 151–161.
35. Rodino, A. P., Santalla, M., Ron, A. M. De and Singh, S. P., A core collection of common bean from the Iberian peninsula. *Euphytica*, 2003, **131**, 165–175.
36. Wells, W. C., Isom, W. H. and Waines, J. G., Outcrossing rates of six common bean lines. *Crop Sci.*, 1988, **28**, 177–178.
37. Ibarra-Perez, F. J., Ehdaie, B. and Wainess, G. J., Estimation of out-crossing rate in common bean. *Crop Sci.*, 1997, **37**, 60–65.
38. Gepts, P. and Bliss, F. A., F1 hybrid weakness in the common bean: differential geographic origin suggests two gene pools in cultivated bean germplasm. *J. Hered.*, 1985, **76**, 447–450.
39. Singh, S. P., Selection for seed yield in Middle American versus Andean × Middle American interracial common bean populations. *Plant Breed.*, 1995, **114**, 269–271.
40. Duarte, J. M., Santos, J. B. and Melo, L. C., Genetic divergence among common bean cultivars from different races based on RAPD markers. *Genet. Mol. Biol.*, 1999, **22**, 419–426.

ACKNOWLEDGEMENTS. We are grateful to Paul Gepts, Roberto Bollini, Steve Beebe and Cesar Ocampo for their valuable suggestions, the traditional 'Badaga' farmers of Nilgiri District who are conserving and cultivating this germplasm for many centuries, and Dr M. Radhakrishna Pillai, Director, RGCB, Thiruvananthapuram, for providing necessary facilities to carry out RAPD analysis. This study was made possible through a grant from the South Eastern Regional Office (SERO), of the University Grants Commission, Hyderabad, in the form of Minor Research Project to F.C.J. We thank the reviewers for their in-depth review and valuable comments to improve this manuscript.

Received 10 December 2008; revised accepted 1 June 2009