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Examination of genetic diversity in common bean (*Phaseolus vulgaris* L.) using random amplified polymorphic DNA (RAPD) markers

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To study the pattern of genetic diversity in 45 genotypes of common bean, 19 RAPD primers were used. Of 253 bands produced, 236 bands (94.22%) were polymorphic in which maximum number (20 polymorphic bands) were observed in the profiles of the primer OPB-07. Highest PIC value (0.79) was observed for the primers OPG-14 and OPE-1, whereas lowest PIC value (0.34) was recorded for the primer OPA-11 with an average PIC value of 0.54. Similarly, highest effective multiplex ratio value (0.11) was scored for the primers OPA-04 and OPG-6 and the lowest value (0.05) was recorded for the primer OPB-07. Primer OPG-14 and OPE-1 exhibited the highest marker index value (0.078) and the lowest value (0.024) was recorded for OPA-11. Pair-wise genetic similarity coefficients of genotypes varied from 0.56 to 0.92 with an average of 0.70 amongst 45 genotypes. The maximum similarity coefficient (0.92) was noticed between G-9 and G-8. Cluster analysis separated 45 genotypes into seven major clusters which were further grouped into various sub-clusters. Among these seven clusters, the maximum number of genotypes (12) were recorded in cluster I, while cluster VII formed a mono-genotypic cluster.

Key words: Genetic diversity, polymorphism, cluster analysis, random amplified polymorphic DNA markers (RAPD), common bean.

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) belongs to the family *Leguminosae*, subfamily *Papilionoideae*, and tribe *Phaseoleae*. It is considered the most important grain legume for human consumption in the world. Beans are considered an excellent source of proteins and minerals, for example iron, magnesium, manganese, and to a lesser extent, zinc, copper, calcium, and group B vitamins. In addition, beans have high amounts of fiber and complex carbohydrates. Common bean is produced in a wide range of climatic conditions and is also cultivated as a source of nitrogen in crop rotations (Hillocks et al., 2006). In fact, it is a good genetic model because of its small genome and diploid in nature with 2n = 2x =22 (Santalla et al., 2010).

Analysis of genetic relationships in crop species is an important component of crop improvement programs, as

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Table 1. Base sequence of RAPD primers used for DNA fingerprinting.

S/N	Primer name	5' —— sequence → 3'
01	OPA-01	5'-CAGGCCCTTC-3
02	OPA-03	5'-AGTCAGCCAC-3'
03.	OPA-04	5'-AATCGGGCTG-3
04.	OPA-05	5'-AGGGGTCTTG-3'
05	OPA-07	5'-GAAACGGGTG-3'
06	OPA-09	5'-GGGTAACGCC-3'
07	OPA-10	5'-GTGATCGCAG-3'
08	OPA-11	5'-CAATCGCCGT-3'
09	OPA–17	5'-CACCGCTTGT-3
10	OPB-07	5'-GGTGACGCAG-3'
11	OPB-10	5'-CTGCTGGGAC-3'
12	OPC-02	5'-GTGAGGCGTC-3'
13	OPC-08	5'-TGGACCGGTG-3'
14	OPD-07	5'-TTGGCACGGG-3'
15	OPG–06	5'-GTGCCTAACC-3'
16	OPG–14	5'GGATGAGACC-3'
17	OPE-01	5'CCCAAGGTCC-3'
18	OPE-06	5'AAGACCCCTC-3'
19	OPE-12	5'TTATCGCCCC-3'

it serves to provide information about genetic diversity, and is a platform for stratified sampling of breeding populations. Traditionally, diversity is assessed by measuring variation in phenotypic traits such as flower color, growth habit or quantitative agronomic traits like yield potential, stress tolerance, etc., which are of direct interest to users. This approach has certain limitations: genetic information provided by morphological characters is often limited and expression of quantitative traits is subjected to strong environmental influence.

With the development of the polymerase chain reaction (PCR) in particular, numerous molecular technologies such as two random amplified polymorphic DNA markers (RAPD) and ISSR techniques have been developed (Zietkiewicz et al., 1994). RAPD markers offered an opportunity to reduce the time and expense taken in RFLP for genetic diversity and molecular mapping. RAPD analysis can be used to characterize DNA variation patterns within species and among closely related taxa. Within grain legume crops alone, RAPD markers have been widely used for the identification of genetic relationships a) among cultivars, b) among wild forms and c) between wild forms and cultivars (Xu et al., 2000). Keeping this in view, the present investigation was undertaken with an objective to assess genetic diversity in common bean genotypes using RAPD markers.

MATERIALS AND METHODS

The research was carried out at the Experimental Farm of Urban Technology Park, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Habak during *Kharif* 2010-2011 and 2011-2012 and molecular analysis was carried out in Molecular Laboratory, Centre for Plant Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir. The experimental material comprised of forty five (45) genotypes of common bean (*Phaseolus vulgaris* L.) collected from different pulse growing areas of Jammu and Kashmir. The field experiment was laid out in a randomized block design with three replications. The experimental materials were provided the cropping geometry of 30 cm distance between the rows and 10 cm within the row. The experimental fields were well prepared and standard recommended package of practices were followed to raise a good crop.

DNA extraction and PCR

Total genomic DNA from 20 gof fresh young leaf tissue, collected from five random plants per accession, was extracted following the cetyl trimethyl ammonium bromide (CTAB) method as described by Saghai-Maroof et al. (1984). Nineteen 10-mer oligonucleotides primers (Operon Technologies Inc., CA. USA) were used for characterization of genotypes (Table 1). *In vitro* amplification using polymerase chain reaction (PCR) was performed in a 96 well BioRad Mode-II T-gradient thermoblock using 50 ng of genomic DNA of each genotype in a final volume of 20 µl per reaction.

To 20 µl of the amplified product, 3.33 µl of 6x loading dye was added so as to make the final concentration of the loading dye in the reaction samples to 1x. The PCR products were resolved on 1.5% super fine resolution agarose gel. The gel was prepared in 0.5x TBE buffer or 1x TAE. Ethidium bromide was added at concentration of 0.5 mg/µl. The gel was run at 10 V, visualized under UV light and photographed (Figure 2a and 2b) using ultra cam digital imaging (A6 rc canon camera).

Table 2. Percentage of polymorphism and polymorphic information content (PIC) obtained by PCR amplification of DNA in common bean (*Phaseolus vulgaris* L.) genotypes using RAPD primers.

Primers	Total number of bands	Number of polymorphic bands	Percentage of polymorphism	Polymorphic information content	Effective multiplex ratio	Marker index
OPA-01	15	13	86.67	0.64	0.07	0.042346
OPA-03	17	16	94.12	0.46	0.06	0.027331
OPA-04	9	9	100.00	0.56	0.11	0.062096
OPA-05	17	14	82.35	0.63	0.06	0.051176
OPA-07	17	15	88.24	0.56	0.06	0.051176
OPA-09	14	14	100.00	0.44	0.07	0.031150
OPA-10	17	16	94.12	0.74	0.06	0.051176
OPA-11	14	14	100.00	0.34	0.07	0.024453
OPA–17	18	14	77.78	0.58	0.06	0.032426
OPB-07	21	20	95.24	0.61	0.05	0.029123
OPB-10	12	11	91.67	0.44	0.08	0.036767
OPC-02	10	10	100.00	0.36	0.10	0.035773
OPC-08	11	11	100.00	0.51	0.09	0.046662
OPD-07	12	12	100.00	0.37	0.08	0.030788
OPG–06	9	9	100.00	0.49	0.11	0.054940
OPG–14	10	10	100.00	0.79	0.10	0.078588
OPE-01	10	10	100.00	0.79	0.10	0.078588
OPE-06	10	10	100.00	0.42	0.10	0.042320
OPE-12	10	8	80.00	0.53	0.10	0.053419
Total	253	236	-	-	-	-
Mean	13.32	12.42	94.22	0.54	0.08	0.045279

Clearly resolved, unambiguous bands were scored visually for their presence or absence. The scores were obtained in the form of a matrix with '1' and '0', which indicate the presence and absence of bands in every accession. The binary data scored was used to calculate the similarity coefficient between the accessions and construct a dendrogram.

Polymorphism information content

Polymorphism information content (PIC) or expected heterozygosity scores for each RAPD marker was calculated based on the formula:

$$PIC = 1 - \sum_{i=1}^{n} (P_{ij})^2$$

Where, Pi is the allele frequency for the j^{th} allele in i^{th} primer and summation extended over 'n' patterns (Nei, 1973).

Cluster analysis

Cluster analysis based on molecular data was conducted using computer software Numerical Taxonomic and Multivariate Analysis System (NTSYS-PC) version 2.02e (Rohlf, 1997). Data from all the 19 primers were used to estimate the similarity based on the number of shared amplified bands. Similarity was estimated using SIMQUAL function of NTSYS, which computes a variety of similarity coefficient for quantitative data (nominal data). Similarity matrix value based on Nei and Li (1979) Sorensen-Dice coefficient of similarity (D_{ij}) was calculated as:

$$D_{ij} = \frac{2a}{(2a + b + c)}$$

Where 'a' represents matched fragments 'b' and 'c' are unmatched fragments. The similarity matrix was then generated and dendrogram was constructed using unweighted pair group method using arithmetic averages (UPGMA) available in NTSYS.

RESULTS AND DISCUSSION

In the present investigation, a total of 253 bands were detected (Table 2). The number of bands detected per primer ranged from 9 to 21 with 13.32 per primer. The highest number of scorable bands (21) was recorded for primer OPB-07 which was closely followed by OPA-17 with 18 bands and OPA-03, OPA-05, OPA-07 and OPA-10 with 17 bands each (Table 2). This value is relatively high in comparison with the other studies performed using RAPD markers. Sadeghi and Cheghamirza (2012) reported that among 33 primers, maximum number of clear bands was 20 bands for primer OPE02. Muthusamy et al. (2008) also reported lower number of clear bands

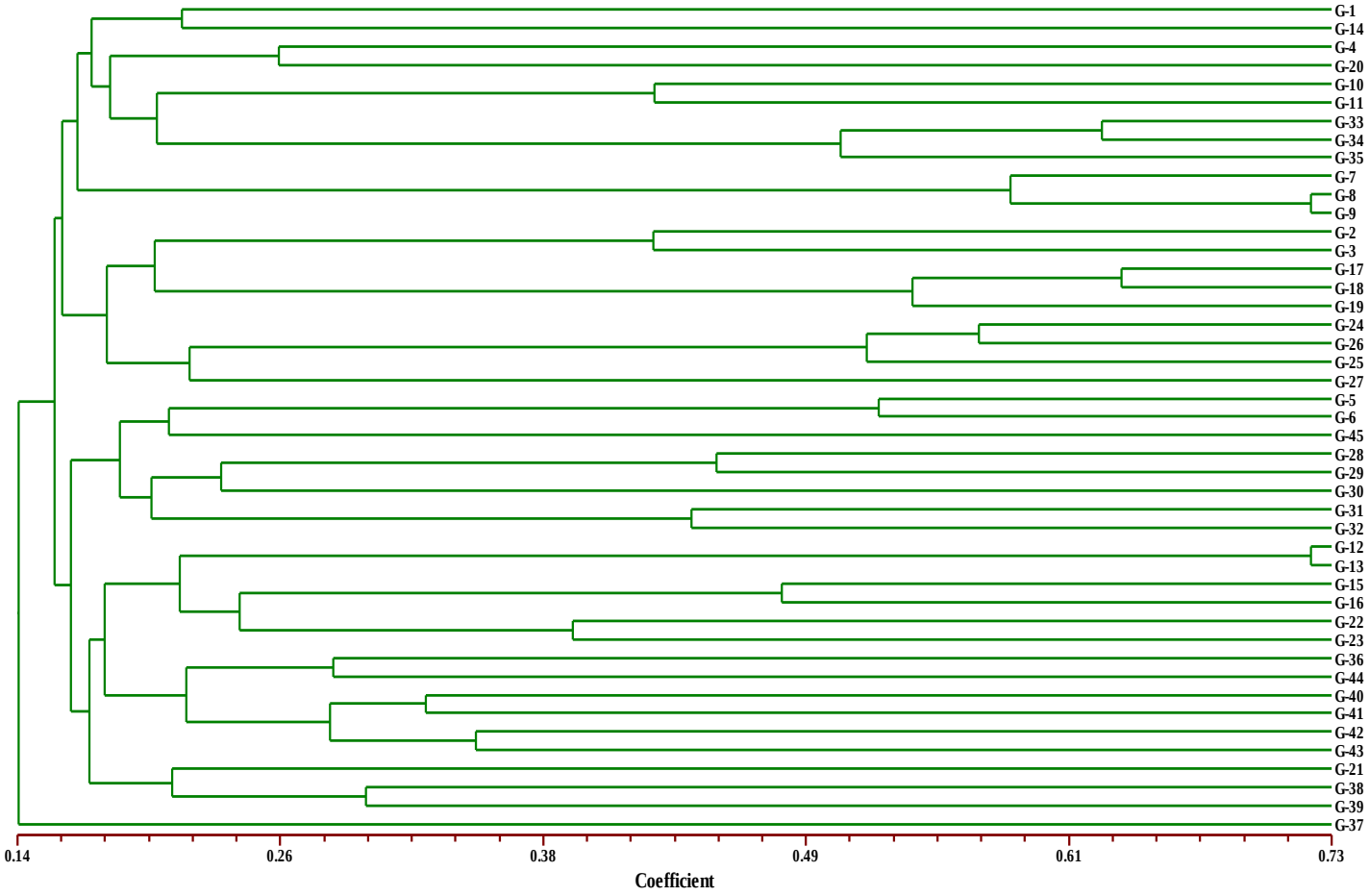

Figure 1. UPGMA dendrogram depicting genetic relationship among 45 genotypes of common bean (*Phaseolus vulgaris L.*) based on RAPD data using Dice similarity coefficient.

as compared to present findings. The possible reason for this could be the utilization of primers with 60 to 70% GC content in our experiments. An increase in the number of amplified bands was observed with increase in primer GC content, and its reason could be the higher stability of G-C complementation with respect to A-T pairing (Fukuoka et al., 1992). The studies of Szilagyi et al. (2011) and Barelli et al. (2011) in common beans also reported less polymorphism by RAPD markers as compared to our study. These high values could be due to nature of RAPD markers. The maximum number of scorable bands 21 was obtained from primers (OPB-07), followed by 18 bands from OPA-17.

The polymorphic information content (PIC) was calculated for all 19 RAPD markers and are presented in Table 2. The PIC values ranged from 0.34 (OPA-11) to 0.79 (OPG-14 and OPE-1) with mean of 0.54. The higher the PIC value, the more informative the RAPD marker is. Hence, the primers OPG-14 and OPE-1 were found to be highly informative, while OPA-11 was least informative. With regard to the effective multiplex ratio (EMR), it was noticed that maximum EMR (0.11) was recorded for the markers OPA-04 and OPG-6; which were closely followed by OPA-4, OPG-14, OPE-1, OPE-6 and OPE-12 with EMR of 0.10 (Plate 2a and b), while the minimum (0.05) EMR was recorded for OP-07 (Table 2). Similarly, the marker index for 19 primers was in the range of 0.024 (OPA-11) to 0.078 (OPG-14 and OPE-1) with mean of 0.045.

Pair-wise genetic similarity coefficients of genotypes varied from 0.56 to 0.92 with average of 0.70 between 45 genotypes. The maximum similarity coefficient (0.92) was noticed between G-9, G-8, G-13 and G-12 which was followed by G-34, G-33 (0.89), G-26 and G-24 (0.88); while minimum similarity coefficient was noticed between G-6 and G-1 (0.56). The difference at molecular level can be explained by the long span of cultivation in the areas by different soil types, climatic conditions and cultivation practices.

Cluster analysis of fingerprints generated by means of RAPD markers resulted in a dendrogram (Figure 1) that grouped bean genotypes into seven clusters using 0.16

Table 3. Composition of the clusters of common bean (*Phaseolus vulgaris* L.) genotypes as observed in the UPGMA dendrogram.

Cluster	Number of genotypes	Name of genotypes
I		
Ia	2	G-1, G-14
Ib	2	G-4, G-1420
Ic	2	G-10, G-141
Id	3	G-33, G-34, G-35
Ie	3	G-7, G-8, G-9
II		
IIa	2	G-2, G-3
IIb	3	G-17, G-18, G-19
IIc	4	G-24, G-25, G-26, G-27
III		
IIIa	3	G-5, G-6, G-45
IIIb	3	G-28, G-29, G-30
IIIc	2	G-31, G-32
IV		
IVa	2	G-12, G-13
IVb	2	G-15, G-16
IVc	2	G-22, G-23
V		
Va	2	G-36, G-44
Vb	4	G-40, G-41, G-42, G-43
VI		
VIa	1	G-21
VIb	2	G-38, G-30
VII	1	G-37

similarity coefficients as a cutoff point. Among these seven clusters, cluster I included the maximum number of genotypes (12) and also showed maximum number of sub-clusters (5); while cluster VI was mono-genotypic. Other clusters included between 3 and 9 genotypes (Table 3). The present findings are in agreement with those of Palomino et al. (2005) who studied genetic diversity of common bean cultivars of the commercialCarioca group using RAPD markers. They reported that the RAPD markers were efficient in differentiating and clustering the genotypes and showed the wide genetic variability within the group of Carioca common bean cultivars. Tiwari et al. (2005) investigated genetic variability in common beans collected in the Central Himalaya region using RAPD markers and obtained 12 groups with different similarity levels.

The close genetic relationship among common bean genotypes from Carioca commercial group and the difficulty in performing a large number of crosses in self-pollinating species suggest the need to cross genotypes that present great genetic variability (Broughton et al., 2003). As recommended by Cruz et al. (2004) the joint use of clustering methods and graph dispersion has been the best suitable alternative in genetic diversity studies. Although the genotypes used in this study were very close genetically, it was possible to differentiate them by using RAPD molecular marker technique. The results have been satisfactory because even with reduced variability, different groups were always formed, showing the efficiency of this technique. Information about genetic diversity is essential for breeding analysis and germplasm conservation. In these cases, molecular markers may be used as an efficient instrument for detecting similarity/divergence and identifying accessions among common bean cultivars (Alzate-Marin et al., 2003).

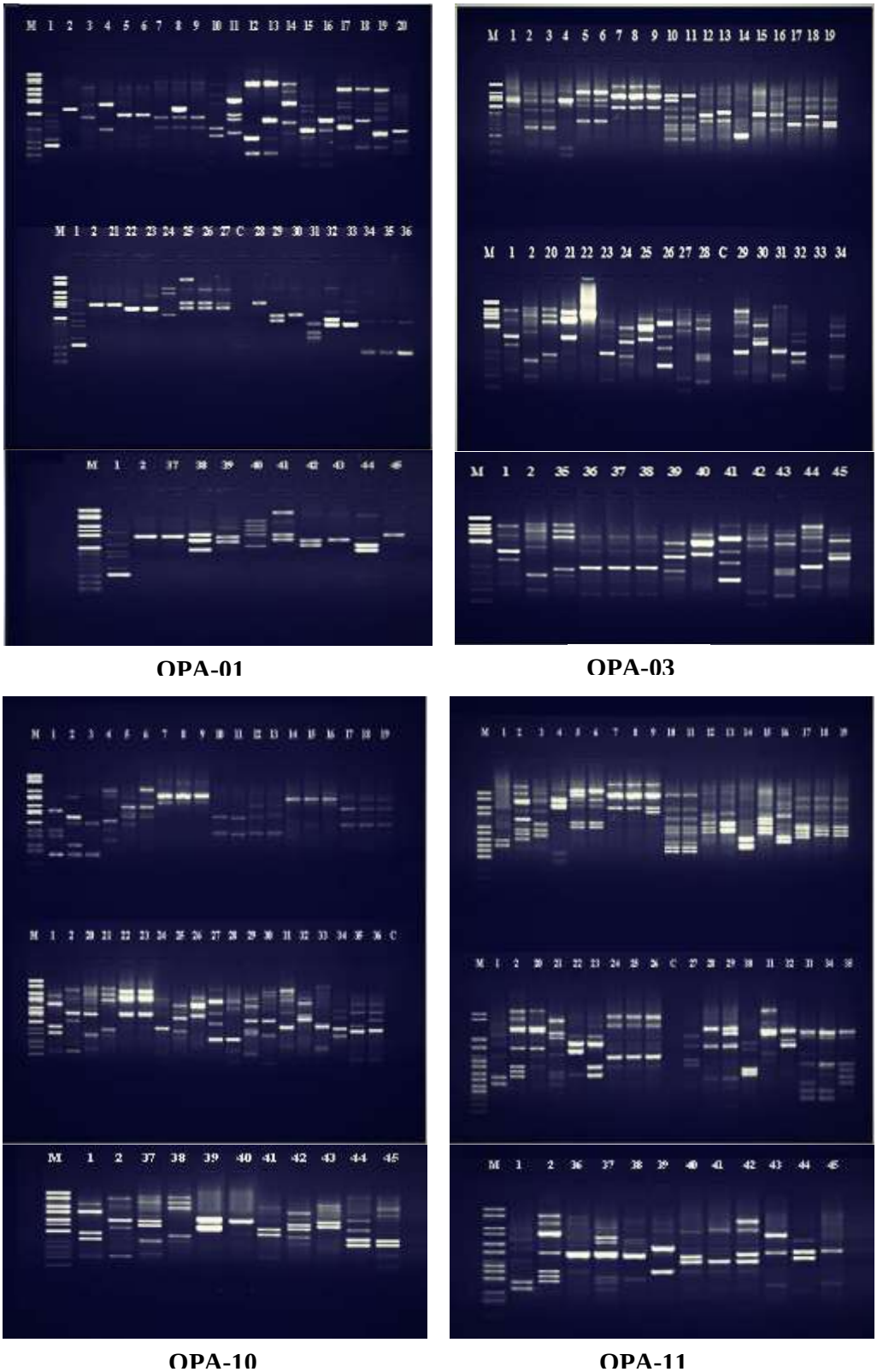

Figure 2a. Images of agarose gels stained by ethidium bromide showing RAPD markers amplified in 45 common bean (*Phaseolus vulgaris* L.) genotypes. M = Ladder; C = Control Sr. No. 1-45 are the 45 genotypes of common bean.

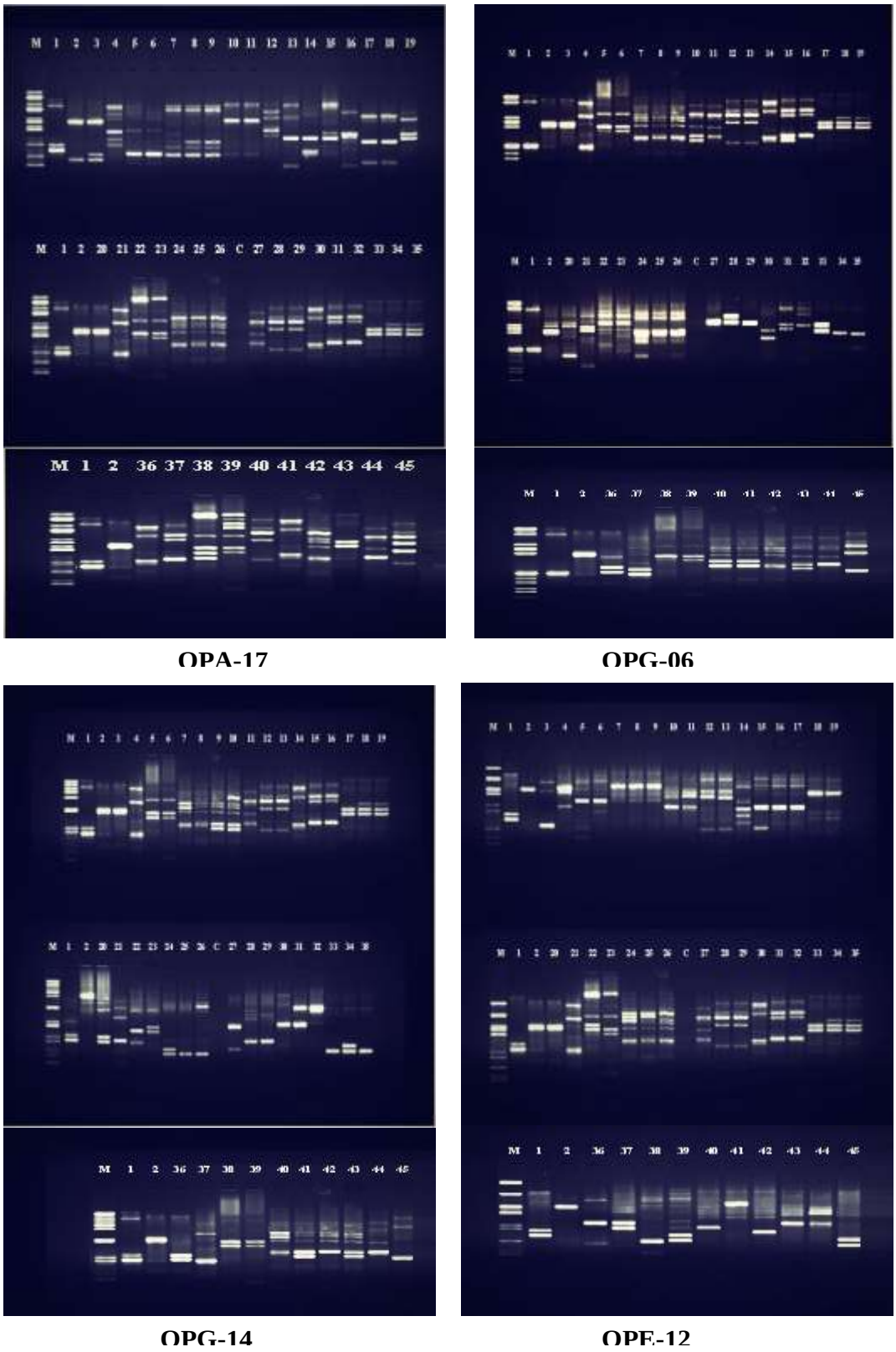

Figure 2b. Images of agarose gels stained by ethidium bromide showing RAPD markers amplified in 45 common bean (*Phaseolus vulgaris* L.) genotypes. M = Ladder; C = Control Sr. No. 1-45 are the 45 genotypes of common bean.

Breeding strategies need to exploit the existing variation within and between wild beans and landraces. Hybridization programme can be initiated between the identified diverse genotypes in order to create variation and for incorporation of the desire trait. The molecular markers, especially SCAR can be utilized for transfer of the desired trait in short duration. These bean germplasm lines when crossed can help to broaden the genetic base of commercial beans to develop high yielding cultivars.

Conflict of interests

The authors did not declare any conflict of interest.

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