Electronic Journal of Plant Breeding, 4(3): 1219-1227 (Sep 2013)
ISSN 0975-928X

Research Article

Studies on genetic diversity in redgram genotypes using rapd markers

K. C. Chandana*, L. Prasanthi, M. Reddi Sekhar and B. V. Bhaskara Reddy
Department of Genetics and Plant Breeding, S.V.Agricultural College, Tirupati 517502, A P
*Email: kalasamudram.chandana@gmail.com

(Received: 101 Jun 2013; Accepted: 24 Sep 2013)

Abstract
Genetic diversity was estimated among fifteen genotypes of redgram using 30 RAPD primers. A total of 172 RAPD amplicons were obtained; of which 114(66%) were polymorphic. The present polymorphism ranged from 25 to 100 per cent. A wide range (18.0 to 95.2%) of Jaccard's similarity coefficient was observed between the pairs of genotypes. A dendrogram constructed based on the UPGMA clustering method revealed two major clusters. Among the 15 genotypes LRG-30 was found in separate cluster (cluster I), while all other genotypes grouped into other major cluster (cluster II) indicating a distinct background of this genotype.

Keywords
Redgram, Dendrogram, Genetic diversity, RAPD

Introduction

Pigeonpea [*Cajanus cajan (L.) Millsp*] belongs to the subtribe *Cajaninae* of the leguminous tribe *Phaseolae* of the family *Fabaceae*. It is rich in protein (22%) and has an important role in vegetarian diet. A better knowledge on genetic diversity of breeding material is prerequisite for an efficient crop improvement programme. Assessment of genetic diversity has traditionally been made through morphological characters that are often limited in number, have complex inheritance and vulnerable to environmental conditions. It is well documented that the DNA markers have many advantages over the traditional morphological and biochemical markers. Among the DNA markers, polymerase chain reaction (PCR) based markers using arbitrary primers, such as, RAPD, have been widely used for investigating genetic relatedness and diversity in plant population and cultivars. It offers a simple, efficient and economic means for diversity analysis (Saini *et al.,* 2010). In the present study, the assessment of genetic diversity and the relationship among redgram genotypes was carried out through RAPD analysis.

Material and Methods

Plant material: The plant material used for this study included fifteen redgram genotypes which belong to different duration groups namely ICPL-85063, LRG-41, TRG-22, ICPL-87119, ICP-15225, PRG-158, ICP-15580, UPAS-120, BDN-2, TRG-7, TRG-59, TRG-38, TRG-33, ICP-7035 and LRG-30. Leaf samples were collected from the plants at 60 DAS for DNA extraction.

DNA isolation**:** DNA was isolated by following modified CTAB (Cetyl Trimethyl Ammonium Bromide) method (Murray and Thompson, 1986) and the quality and concentration of DNA was estimated using nanodrop spectrophotometer (ND 1000) at 260nm and verified DNA by running samples on 1.0% Agarose with 1 Kb ladder.

Polymerase Chain Reaction**:** PCR amplification reactions (Williams et al., 1990) were performed with 30 decamer primers. The reaction mixture consist of 25ng of template DNA, 10X Assay buffer (10 mM Tris, 50 mM KCl, 0.01% of gelatin), 10 mM dNTPs, 2mM $MgCl_2$, 10 picomols primer and 1U Taq DNA polymerase (Fermentas) and final volume was made to 25 µl. Amplification was carried out in a thermo-cycler (Eppendorf, Germany) programmed for 40 cycles with an initial denaturation at 94^0C for 4 minutes, followed by cycling conditions of denaturation at 94^0C for 1 minute, annealing at 1 minute at 37^0C and extension at 72^0C for 2 minutes. After 40 cycles, there was a final extension step of 7 minutes at 72^0C. The amplicons were analyzed on 1% Agarose gel with 1X TBE buffer (89mM Tris-Hcl, 89mM Boric acid and 2mM EDTA p^H - 8) and detected by staining with Ethidium Bromide. UV trans-illuminated gels were photographed with gel documentation system (Alpha Innotech, USA).

Data analysis**:** The amplified products for RAPD analysis were scored visually based on the presence (taken as '1') or absence (taken as '0') of band for each primer. Each RAPD fragment was treated as a unit character and only clear and unambiguous bands were scored. The data were used to generate Jaccard's coefficients for expressed RAPD bands. The Jaccard's coefficients were used to construct a dendrogram using the Unweighted Pair Group Method with Arithmetic averages (UPGMA). The computer package NTSYS-PC was used for cluster analysis.

Results and discussion

Information regarding genetic variation in the available material is essential for selection of suitable genotypes for including them in breeding

programme. Though remarkable improvement in redgram has been achieved through variability and diversity analysis using morphological markers, DNA markers offer precise means to measure genetic diversity and affinity among germplasm lines than the morphological and biochemical markers due to their environmental sensitivity and abundance in genome. Therefore, of late, molecular markers in addition to morphological markers have been proved effective to compliment and accelerate plant breeding programmes. Several molecular markers are available today, including those based on Restriction Fragment Length Polymorphism (RFLP) (Bostein *et al.,* 1980), Random Amplified Polymorphic DNA (RAPD) (Welsh and McClelland, 1990), Amplified Fragment Length Polymorphism (AFLP) (Vos *et al.,* 1995) and Simple Sequence Repeats (SSR) (Tautz, 1989). However, the DNA markers based on RAPD are more advantageous due to its speed, technical simplicity and higher frequency of polymorphism.

By using RAPD, genetic diversity analysis within a species is useful in understanding evolutionary processes at the genomics & population levels. A preliminary effort was made to analyze the genetic diversity of 15 genotypes. 30 RAPD primers (Table 1) belonging to OPA series, OPC series, OPAC-11, and OPAK-19 were used to assess the polymorphism of 15 redgram genotypes. 30 primers generated a total of 172 RAPD fragments showing a total of 66% polymorphism. Out of 30 primers (Table 2), 8 primers (OPA-4, OPA-6, OPA-8, OPA-15, OPA-16, OPA-17, OPA-20 and OPC-2) showed 100% polymorphism and remaining primers showed 60% polymorphism. Malviya and Yadav (2010) and Yadav *et al.* (2012) also reported high amount of polymorphism using RAPD markers in redgram. The number of bands ranged between 2 to 13 corresponding to an average of 7.5. The highest number of RAPD fragments generated for the primer OPA-1 which amplified 13 fragments followed by the primer OPA-2 (11 fragments). The lowest number of fragments was generated by the primer OPA-14 (1 fragment). The RAPD gel profiles of the primers OPA-2 (Plate 1), OPC-3 (Plate 2), OPC-4 (Plate 3) and OPC-6 (Plate 4) were seen with polymorphism.

Cluster analysis was performed using similarity coefficient matrix and calculated from RAPD markers. A dendrogram of 15 genotypes was generated using UPGMA algorithm. The similarity coefficient values ranged between 0.180 and 0.952, indicating the presence of wide range of genetic diversity at molecular level among the fifteen genotypes (Table 3). Lohithaswa *et al.* (2003), Ray choudhury *et al.* (2008) and Ahmad Shah *et al.* (2011) noticed a wide range of genetic diversity by RAPD markers at molecular level in redgram genotypes. The similarity coefficient value was highest (0.952) between the genotypes ICP-15580 and UPAS-120. These genotypes showed maximum degree of similarity in their genetic makeup. However, the minimum value of similarity coefficient was observed between PRG-158 and LRG-30 (0.180), ICP-15225 and LRG-30 (0.184), TRG-59 and LRG-30 (0.197) indicating that, these genotypes were highly diverse pairs.

Based on the dendrogram (Fig. 1) constructed using the similarity coefficient values, all the 15 genotypes were grouped into two major clusters. Among the 15 genotypes LRG-30 was found in separate cluster (cluster I), while all other genotypes were grouped into one major cluster (cluster II) indicating a distinct background of this genotype. This major cluster is divided into two sub clusters A1 and A2. The sub cluster A1 is having two sub sub clusters having 6 genotypes ICP-15580, UPAS-120, BDN-2, TRG-59 and TRG-38 in A1-1 sub cluster and A1-2 sub cluster having ICPL-87119, ICP-15225 and TRG-22. These genotypes were genetically close as they all separated under sub cluster A1. The sub cluster A2 had 2 genotypes i.e., ICP-7035 and TRG-33. The remaining genotypes i.e., LRG-41, TRG-7, PRG-158 and ICPL-85063 showed independent positions in dendrogram. ICP-15580 and LRG-30 were the genotypes placed at the two extremes of the dendrogram.

The genotypes ICP-15580 from Hyderabad and UPAS-120 from Kanpur of sub cluster A1 exhibited maximum similarity based on the present set of markers used in the present investigation. The genotypes TRG-59 and TRG-38 of sub cluster A1 belonging to the same ecological region (Tirupati) exhibited close association. The tendency of genotypes in clusters irrespective of geographic boundaries demonstrates that geographical isolation is not the only factor causing genetic diversity. These results also showed that despite their common origin i.e. Tirupati, the genotypes TRG-22 (sub cluster A1), TRG-33 (sub cluster A2) and TRG-7 (independent position) exhibited diversity at genetic level. Cluster I comprising one genotype, LRG-30 exhibited less similarity with other genotypes and was genetically more distinct and diverse.

The polymorphism studies using Random Amplified Polymorphic DNA (RAPD) marker analysis detected a high level of genetic variation among the 15 redgram genotypes. In this way RAPD markers are used effectively to understand the extent and distribution of the genetic variation available within the redgram genotypes.

References

Ahmad Shah, S., Nabi and Ghand Raideep, K. 2011. Genetic diversity evaluation in Pigeonpea

(*Cajanus cajan* (L.) Millsp.) using protein profiling and RAPD. *Life Sci.*, **11**(3): 326-330.

Bostein, D., White, R. L., Skolnick, M and Davis, R. W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphism. *American J. Human Genet.*,**32**: 314-331.

Lohithaswa, H.C., Shailaja Hittalmani, Shashidhar, H.E and Dharmaraj, P.S. 2003. Assessment of genetic divergence in some pigeonpea [*Cajanus cajan* (L.) Millsp] genotypes using RAPD markers. *Indian J. Agrl. Sci.*, **63**(4): 329-330.

Malviya, N and Yadav, D. 2010. RAPD analysis among Pigeonpea *(Cajanus cajan (L.)* Millsp.*)* cultivars for their genetic diversity. *Genetic Eng. and Biotech. J.*, 1:1-9.

Murray, M.G and Thompson, W.F. 1986. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res.*, **8**: 4321-4325.

Ray choudhury, P., Singh, I.P., George, B., Verma, A.K and Singh, N.P. 2008. Assessment of genetic divergence of pigeonpea cultivars using RAPD analysis. *Biologia Plantarum.* **52**(4): 648-653.

Saini, M., Singh, S., Hussain, Z and Yadav, A. 2010. RAPD analysis in mungbean (*Vigna radiate* (L.) Wilczek): I. Assessment of genetic diversity. *Indian J. Biotech.*, **9**: 137-146.

Tautz, D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Res.*,**17**: 6463-6471.

Vos, P., Hogers, R., Bleccker, M and Hornes, M. 1995. AFLP: A new technique for DNA finger printing. *Nucleic Acids Res.*, **23**: 4407-4414.

Welsh, J and Mc Clelland, M. 1990. Finger Printing Genomes using PCR with arbitrary primers. *Nucleic Acids Res.*, **18**: 7321-7219.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalaski, J.A., Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.*, **18**: 6531–6535.

Yadav, K., Yadav, S.K., Yadav, A., Pandey, V.P and Dwivedi, U. 2012. Genetic diversity of Pigeonpe (*Cajanus cajan* (L.) Millsp.) cultivars and its wild Relatives using Random Amplified Pilymorphic DNA (RAPD) markers. *American J. Plant Sci.*, **3**:322-330.

Table 1. The list of RAPD primers and their sequences were given below

S.No	PRIMER	SEQUENCE (5' -3')
1	OPA 1	CAGGCCCTTC
2	OPA 2	TGCCGAGCTG
3	OPA 3	AGTCAGCCAC
4	OPA 4	AATCGGGCTG
5	OPA 5	AGGGGTCTTG
6	OPA 6	GGTCCCTGAC
7	OPA 7	GAAACGGGTG
8	OPA 8	GTGACGTAGG
9	OPA 9	GGGTAACGCC
10	OPA10	GTGATCGCAG
11	OPA 11	CAATCGCCGT
12	OPA 12	TCGGCGATAG
13	OPA 13	CAGCACCCAC
14	OPA 14	TCTGTGCTGG
15	OPA 15	TTCCGAACCC
16	OPA 16	AGCCAGCGAA
17	OPA 17	GACCGCTTGT
18	OPA 18	AGGTGACCGT
19	OPA 19	CAAACGTCGG
20	OPA 20	GTTGCGATCC
21	OPC 1	TTCGAGCCAG
22	OPC 2	GTGAGGCGTC
23	OPC 3	GGGGGTCTTT
24	OPC 4	CCGCATCTAC
25	OPC 5	GATGACCGCC
26	OPC 6	GAACGGACTC
27	OPC 7	GTCCCGACGA
28	OPC 8	TGGACCGGTG
29	OPAK 19	TCGCAGCGAG
30	OPAC 11	CCTGGGTCAG

Table 2. Characteristics of amplification products obtained from 30 RAPD primers were given below

S.No	Primer	Total No. of bands	Total No. of polymorphic bands	% of polymorphism
1	OPA 1	13	6	46
2	OPA 2	11	9	82
3	OPA 3	6	5	83
4	OPA 4	5	5	100
5	OPA 5	5	3	60
6	OPA 6	8	8	100
7	OPA 7	7	3	43
8	OPA 8	5	5	100
9	OPA 9	8	2	25
10	OPA10	10	5	50
11	OPA 11	4	3	75
12	OPA 12	6	3	50
13	OPA 13	10	7	70
14	OPA 14	1	0	0
15	OPA 15	4	4	100
16	OPA 16	5	5	100
17	OPA 17	2	2	100
18	OPA 18	3	2	67
19	OPA 19	6	3	50
20	OPA 20	2	2	100
21	OPC 1	4	3	75
22	OPC 2	3	3	100
23	OPC 3	6	3	50
24	OPC 4	9	5	56
25	OPC 5	5	2	40
26	OPC 6	8	6	75
27	OPC 7	5	4	80
28	OPC 8	2	1	50
29	OPAK 9	3	1	33
30	OPAC 11	6	4	67

Table 3. Average similarity coefficient values calculated on the basis of similarity matrices of 15 redgram genotypes

Genotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. ICPL-85063	1.000														
2. LRG-41	0.785	1.000													
3. TRG-22	0.820	0.822	1.000												
4. ICPL-87119	0.858	0.787	0.881	1.000											
5. ICP-15225	0.800	0.755	0.870	0.847	1.000										
6. PRG-158	0.742	0.718	0.781	0.768	0.771	1.000									
7. ICP-15580	0. 797	0.752	0.832	0.833	0.859	0.827	1.000								
8. UPAS-120	0.804	0.747	0.851	0.816	0.842	0.809	0.952	1.000							
9. BDN-2	0.801	0.755	0.813	0.838	0.805	0.795	0.899	0.906	1.000						
10. TRG-7	0.725	0.689	0.739	0.787	0.722	0.753	0.775	0.770	0.840	1.000					
11. TRG-59	0.797	0.752	0.844	0.869	0.859	0.779	0.907	0.888	0.899	0.811	1.000				
12. TRG-38	0.792	0.747	0.827	0.852	0.842	0.774	0.901	0.895	0.881	0.781	0.939	1.000			
13. TRG-33	0.705	0.693	0.797	0.799	0.778	0.755	0.822	0.805	0.779	0.738	0.834	0.841	1.000		
14. ICP-7035	0.758	0.723	0.760	0.773	0.787	0.776	0.845	0.815	0.824	0.759	0.820	0.851	0.833	1.000	
15. LRG-30	0.231	0.225	0.208	0.205	0.184	0.180	0.222	0.221	0.218	0.215	0.197	0.212	0.219	0.226	1.000

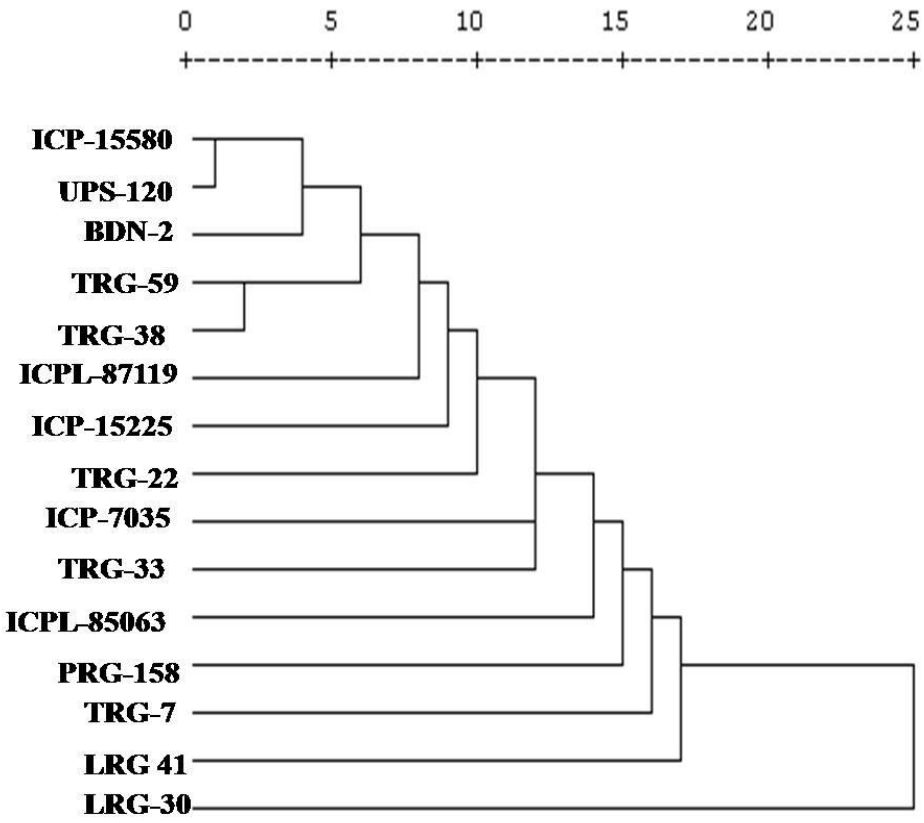

Fig. 1 Dendrogram showing various genetic relationship among 15 redgram genotypes

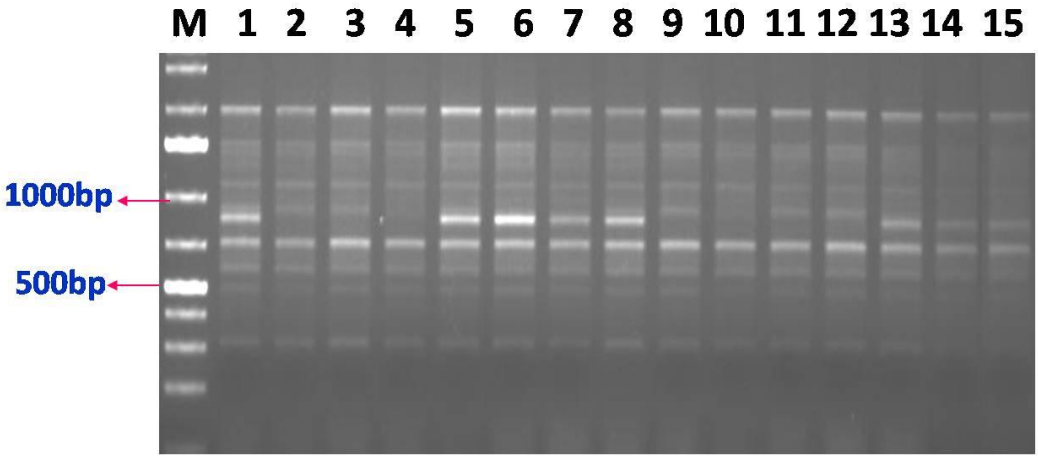

Plate 1. RAPD gel profile of 15 redgram genotypes by using OPA 2 primer

LaneM- 1Kb ladder
Samples: 1) ICPL-85063 2) LRG-41 3) TRG-22 4) ICPL-87119 5) ICP- 15225
6) PRG-158 7) ICP- 15580 8) UPS- 120 9) BDN-2 10) TRG-7
11) TRG-59 12) TRG- 38 13) TRG- 33 14) ICP-7035 15) LRG-30

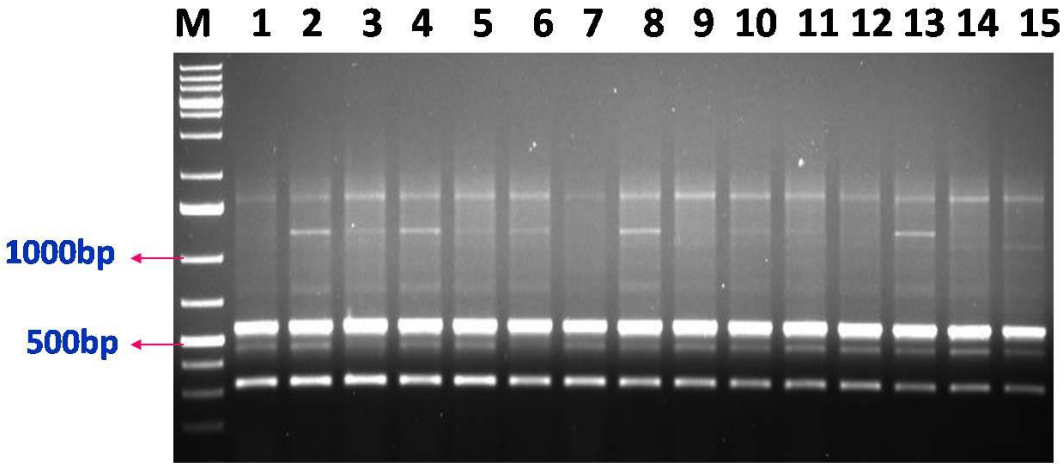

Plate 2. RAPD gel profile of 15 redgram genotypes by using OPC 3 primer

LaneM- 1Kb ladder
Samples: 1) ICPL-85063 2) LRG-41 3) TRG-22 4) ICPL-87119 5) ICP- 15225
6) PRG-158 7) ICP- 15580 8) UPS- 120 9) BDN-2 10) TRG-7
11) TRG-59 12) TRG- 38 13) TRG- 33 14) ICP-7035 15) LRG-30

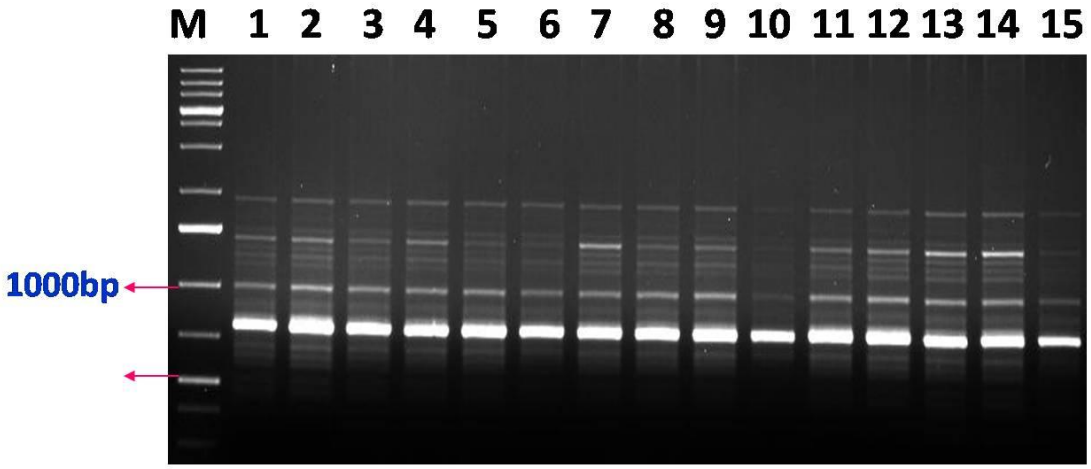

Plate 3. RAPD gel profile of 15 redgram genotypes by using OPC 4 primer

LaneM- 1Kb ladder
Samples: 1) ICPL-85063 2) LRG-41 3) TRG-22 4) ICPL-87119 5) ICP- 15225 6) PRG-158 7) ICP- 15580 8) UPS- 120 9) BDN-2 10) TRG-7 11) TRG-59 12) TRG- 38 13) TRG- 33 14) ICP-7035 15) LRG-30

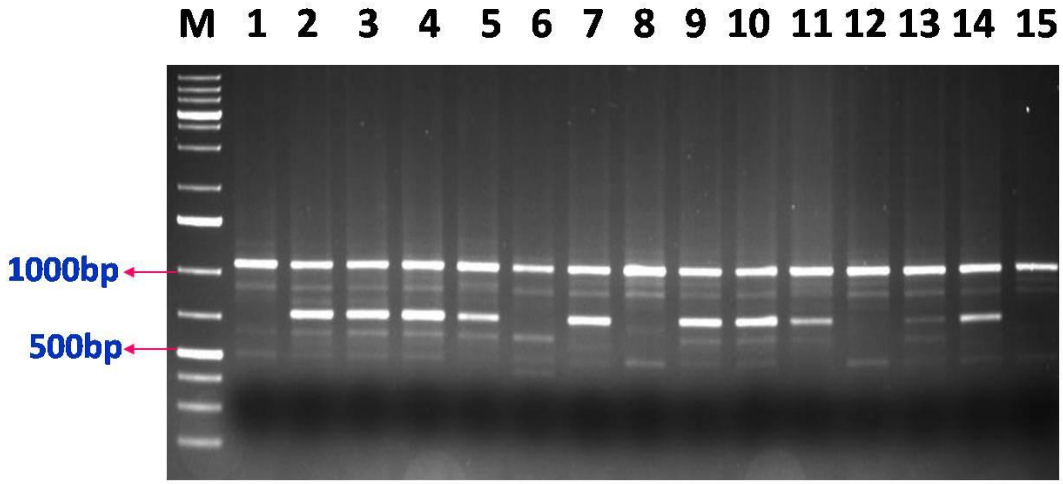

Plate 4. RAPD gel profile of 15 redgram genotypes by using OPC 6 primer

LaneM- 1Kb ladder
Samples: 1) ICPL-85063 2) LRG-41 3) TRG-22 4) ICPL-87119 5) ICP- 15225 6) PRG-158 7) ICP- 15580 8) UPS- 120 9) BDN-2 10) TRG-7 11) TRG-59 12) TRG- 38 13) TRG- 33 14) ICP-7035 15) LRG-30