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Molecular marker analysis of *Oryza sativa* using RAPD markers

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ABSTRACT

Rice is one of the most important crops that provide food for more than half of the world's population and hence their cultivation is expanding drastically due to the increasing demand. There are different varieties of rice distinguished based on their physiological characteristics and geographical area. Identification of these genotypes is important during the genetic breeding programs. Molecular markers play a crucial role in studying genetic variation among different species. The analysis of this diversity among six varieties of rice: Sahabagi Dhan, IR-64, MGD-101, BPT-5204, Azucena, Doddiga was performed by extracting DNA and using 8 RAPD markers on them. The random DNA sequences amplified by PCR are compared and analyzed by the process of electrophoresis. The binary of the gel images is used for analysis of genetic variation between the cultivars. It is observed that the number of polymorphic fragments for each primer varied from 3 to 7 with an average of 4. The average percentage polymorphism was calculated as 77.573%. The values of similarity co-efficient ranged from 0.41 to 0.85. The values of similarity co-efficient ranged from 0.41 between MGD-101 and IR-64 to 0.85 between BPT-5204 and Azucena and an average of 0.71. Cluster analysis showed that IR-64 and MGD-101 are bilious and Azucena is simplifolious indicating that it is a wild variety and is substantially different from all others. Major Allele Frequency ranges from 0.61 to 0.90 with a mean of 0.79, Gene Diversity values range from 0.17 to 0.46 with a mean of 0.29 and PIC values range from 0.14 to 0.36 with a mean of 0.23. The information obtained in this study helps to understand genetic diversity. It can be used to emphasize conservation and propagation of selected natural varieties and to select diverse parents to broaden the germplasm of rice.

Keywords— *Oryza sativa*, RAPD markers, PCR, Polymorphism, Genetic diversity

1. INTRODUCTION

Rice (*Oryza sativa* L.) is the most important cereal crop being grown (over 144.641 million ha) with a production of over 468.275 million tons in the world. It is also probably the world's most versatile crop that grows at more than 3000 m elevations in the Himalayas and at sea level in the deltas of the great rivers of Asia. It feeds more than half the world's population. ⁽¹⁾ White rice is a good source of magnesium, phosphorus, manganese, selenium, iron, folic acid, thiamine, and niacin. ⁽³⁾ There are thousands of varieties of cultivated rice. International Rice Gene Bank has developed more than a thousand improved varieties of rice and it has over 90,000 samples of cultivated rice and wild species are stored making it the world's largest repository of rice. ⁽⁴⁾ Limitations in conventional breeding arise because of the lack of resistance genes in cultivated rice germplasm and inadequate understanding of phenotypic variability. Therefore, transgenic research offers unique opportunities to overcome these problems and to produce improved varieties with reduced yield gaps. ⁽²⁾

Molecular markers provide information that helps in deciding the distinctiveness of species and their ranking according to the number of close relatives and phylogenetic positions. ⁽⁵⁾ Several types of molecular markers available for evaluating the genetic variations in rice are RFLP, AFLP, RAPD, SSR, etc. Of all these, RAPD markers are being employed in genetic research due to its rapid processing and simplicity. ⁽⁶⁾ It also allows the examination of genomic variation without prior knowledge of DNA sequences. It is especially useful for unzipping the variations in species with low genetic variability. RAPD markers are unbiased and neutral for genetic mapping, taxonomy and genetic diagnostics. ⁽⁷⁾ RAPD is a single, short oligonucleotide primer, which binds to different loci and is used to amplify random sequences from a complex DNA template. This means that the amplified fragment generated by

PCR depends on the length and size of both primer and target genome under the assumption that the given DNA sequence (complementary to the primer) will occur in the genome, which is readily amplifiable by PCR. ⁽⁸⁾

Genetic diversity that exists is the foundation of the genetic improvement of crops. Researchers are uncovering new genes and traits in rice that will improve the yield and face challenges like adverse climatic conditions, pests, diseases, etc. Mutation and recombination bring new variations to a population, whereas selection and genetic drift remove some alleles. ⁽⁹⁾

Under the green revolution, we are aiming to develop tools to produce high yielding, drought tolerant, pest resistant, and good nutritional value rice. So, in this study, an attempt is made to assess the molecular diversity using RAPD markers among some common cultivars in Karnataka. Such information will have significance in providing the basis for selection of pre-breeding material, conservation of resource material and useful for rice crop improvement program. ⁽¹¹⁾

2. MATERIALS AND METHODS

2.1 Plant Material and DNA Extraction

The seeds of Sahabagi Dhan, IR-64, MGD-101, BPT-5204, Azucena and Doddiga were selected based on their characteristics (Table 1) and germinated separately in plastic pots labeled with their names and grown in the greenhouse. The leaf samples were collected after 15 days and DNA was extracted using CTAB method (Doyle and Doyle 1990)

2.2 Selection of primers and RAPD optimization

A total of eight RAPD primers were used to assess the genetic diversity between selected rice varieties. The sequences of the primers used in this study are tabulated (Table2). The primers were obtained from Sigma Pvt. Ltd. The PCR reaction mixture and composition used were 1µl of 1X TBE Buffer, 6.2 µl of Sigma water, 0.5 µl of dNTP, 0.7 µl of Primer 0.4 µl of Taq polymerase and 1.2 µl Sample DNA.

The PCR amplification for each RAPD primer was performed at 95°C for 5 minutes (initial denaturation), 95°C for 1 minute(denaturation), 36°C for 1.5 minutes(annealing),72°C for 2 minutes(extension), 72°C for 8 minutes(final extension) in Eppendorf mastercycler nexus gradient.

2.3 Agarose gel electrophoresis

The amplified product was subjected to 1.5% agarose gel electrophoresis with a 100bp ladder and the gel was stained with EtBr. The gels are then analyzed using the gel documentation unit AlphaImager which consists of a UV transilluminator. Alpha imager EP software was used for visualization.

2.4 Data Scoring

The RAPD banding pattern in each gel was subjected to scoring visually by marking the presence (1) or (0) polymorphic bands in individual lanes. The scores with respect to each primer were tabulated and are analyzed using different statistical tools. The NTSYS-PC software ver. 2.02j was used to estimate genetic similarities with the Jaccard coefficient. ⁽¹²⁾ The matrix of generated similarities was analyzed by the UPGMA using SAHN clustering module. The cophenetic module is applied to compute a cophenetic value matrix using the UPGMA matrix. ⁽¹³⁾ The values for major allele frequency, gene diversity and PIC were obtained using the PowerMarker V3.25 (2001-2004 by Jack Liu). Major allele frequency refers to the frequency at which the most common allele occurs in a population. Genetic diversity plays an important role in the populations to adapt to changing environments. ⁽¹⁴⁾ The Polymorphism Information Content (PIC) value is often used to measure the informativeness of a genetic marker for linkage studies. ⁽¹⁵⁾

3. RESULTS AND DISCUSSION

3.1 Analysis of genetic variation

The bands obtained (figure 1 a-h) were analyzed and the following data was obtained with respect to the polymorphism between the different varieties. It was observed that the number of polymorphic bands for each primer varied from 3 to 7. The primer OPA-14 produced maximum polymorphic bands i.e. 7 bands whereas primer OPA-18 produced minimum polymorphic bands i.e. 3 bands. It was also observed that the percentage of polymorphism ranges from 60% to 100%. Out of 8 primers, 5 primers exhibited more than or equal to 80 % polymorphism. The reason for this high level of polymorphism can be due to intraspecies variation among cultivars. It was observed that the level of polymorphism with primers differed between cultivars. ⁽¹⁶⁾

3.2 Similarity Matrix

The genetic relatedness between the different rice varieties was determined using similarity matrix in the present study. ^(17, 18) The values of similarity co-efficient ranged from 0.41 to 0.85 with an average of 0.71. MGD-101 and IR-64 were found to be the most closely related genotypes with similarity index of 0.85. The least value of similarity co-efficient was 0.41 and it was observed between BPT-5204 and Azucena. (Table 3)

3.3 Analysis of genetic analysis

Cluster analysis was performed based on similarity coefficients ⁽¹⁹⁾ in the UPGMA program. Based on the UPGMA dendrogram (Fig. 2), the 6 varieties were grouped into two clusters. Cluster 1 had five varieties: Sahabagi Dhan, IR-64, MGD-101, BPT-5204, and Doddiga. The IR-64 and MGD-101 were grouped into a single cluster indicating that both these varieties were more like each other than they are with Sahabagidhan. While both BPT-5204 and doddiga formed an independent clade and were less similar or related. Cluster 2 was a simplifolious clade with Azucena which was reported as a wild variety and is substantially different from all others. ⁽²⁰⁾

3.4 Allele Frequency and PIC

The major allele frequency is the common frequency at which the most common allele occurs in a given population (table 4). In this study, major allele frequency ranges from 0.61 to 0.90 with a mean of 0.79 and the gene diversity values range from 0.17 to 0.46 with a mean of 0.29.

The Polymorphism Information Content (PIC) of a marker is defined as the probability of marker genotype of the offspring of a heterozygous parent affected with a dominant disease that allows one to deduce the marker allele that was inherited by the offspring from the parent. It represents the effectiveness of marker in linkage analysis. In this study, PIC values range from 0.14 to 0.36 with a mean of 0.23

4. CONCLUSION

The present study revealed the levels of genetic differences between cultivars of rice-based on RAPD markers. We have determined the genetic relationship and the degree of genetic diversity among the six varieties of rice. The data obtained in this study confirmed the efficiency of RAPD markers in assessment of genetic variation in population. The polymorphism detected in this study is necessary to ascertain the germplasm conservation and the development of improved rice genotypes with good quality traits through various breeding programs. Also, the estimation of genetic distance between genotypes can be used for selection of diverse parents to perform appropriate crosses and broaden the germplasm during hybridization programs. The information on intraspecific variation obtained from high level of polymorphism is useful in making decision for improvement of rice cultivars. However, in the present study only six cultivars and ten primers were used in RAPD analysis hence the chance to obtain reliable knowledge about the genetic structure of each variety is reduced. Further studies including large number of primers and cultivars can be conducted to obtain more precise information.

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6. REFERENCES

- [1] Bajaj Y.P.S. Biotechnology in Rice Improvement. In: Bajaj Y.P.S. (EDS) Rice. Biotechnology in Agriculture and Forestry.
- [2] Springer, Berlin, Heidelberg. 1991; vol 14.
- [3] Swapan K. Datta (2004) Rice Biotechnology: A Need for Developing Countries AgBioForum, 2004; 7(1&2) 31-35.
- [4] Dubois, Sirah. "What Nutrition Does Rice Provide?" Healthy Eating | SF Gate, <http://healthyeating.sfgate.com/nutrition-rice-provide-1047.html>. 28 March 2018.
- [5] IRRI facts <http://irri.org/our-work/seeds>
- [6] CHAUHAN, R., Genetic Diversity Analysis of Six Major Cereal Crops Cultivars through RAPD Markers.
- [7] J Bioinfo Proteomics. 2015; Rev 1(1): 1-5.
- [8] Arif IA, Bakir MA, Khan HA. A Brief Review of Molecular Techniques to Assess Plant Diversity.
- [9] International Journal of Molecular Sciences. 2010; 11(5):2079-2096.
- [10] Rupal Chauhan^{1*}, Yogesh Jasrai¹, Archana Mankad¹, Himanshu Pandya¹, Raman Gami² and Kapil Tiwari² (2005) Genetic Diversity Analysis of Six Major Cereal Crops Cultivars through RAPD Markers.
- [11] Adv Crop Sci Tech 2015; 3:4
- [12] Senthil Kumar, N, and Gurusubramanian, Guruswami. (2011). Random amplified polymorphic DNA (RAPD) markers and their applications. Sci Vision. 3.
- [13] Zahid Hassan Pervaiz, M. Ashiq Rabbani, Ishtiaq Khaliq, Stephen R. Pearce, Salman A. Malik. Genetic diversity associated with agronomic traits using microsatellite markers in Pakistani rice landraces.
- [14] Electronic Journal of Biotechnology. 2010;13(3)
- [15] <http://irri.org/about-us/our-people/board-of-trustees/23-our-work/research?start=21>
- [16] NEI M and LI W. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences of the United States of America. 1979; 76(10): 5269-5273
- [17] Mundewadikar DM, Deshmukh P R. Genetic Variability and Diversity Studies in Soybean [Glycine max (L.) Merrill] using RAPD Marker. 2016;4(9)
- [18] Guo XI, Elston R. Linkage information content of polymorphic genetic markers. Hum Hered. 1999; 49:112-118.
- [19] Frankham, Richard. Genetics and Extinction.
- [20] Biological Conservation. 2005; 126(2): 131-140.
- [21] Shuo Zhang, Chanjuan Tang, Qiang Zhao, Jing Li, Lifang Yang, Lufeng Qie, Xingke Fan, Lin Li, Ning Zhang, Meicheng Zhao, Xiaotong Liu, Yang Chai, Xue Zhang, Hailong Wang, Yingtao Li, Development of highly polymorphic simple sequence repeat markers using genome-wide microsatellite variant analysis in Foxtail millet [Setaria italica (L.) P. Beauv.]
- [22] B. K. Chikkaswamy, Rabin Chandra Paramanik. An investigation of RAPD and ISSR molecular marker studies in Mulberry varieties Int.J.Curr.Microbiol.App.Sci. 2014; 3(6): 986-995
- [23] Davierwala, AP Chowdari, KV, Kumar, S Reddy, APK Ranjekar, PK and Gupta. Use of three different marker systems to estimate genetic diversity of Indian elite rice varieties.
- [24] Genetica. 2000; 108 (3): 269-284.
- [25] 16. RAJANI J, DEEPU V, NAIR GM, NAIR AJ. Molecular Characterisation of selected cultivars of rice, *Oryza sativa* L, using RAPD markers. Int. Food Res. J.2013; 20(2): 919-923
- [26] R Skaria, S Sen, PMA Muneer Analysis of Genetic Variability in Rice Varieties (*Oryza sativa* L) of Kerala using RAPD Markers.

[27] Gen Eng Biotech J. 2011; GEBJ-24
 [28] Shamsun Naima Rahman, MD Shahidul Islam MD Samsul Alam1 and Khondoker M Nasiruddin. Genetic polymorphism in rice (*Oryza sativa* L.) through RAPD analysis
 [29] Ind J Biotech. 2007; 6: 224-229.
 [30] Komala NT, Gurumurthy R, Surendra P Omics Technologies towards seed quality improvements.
 [31] Int J Pure App Biosci. 2017; 5(6):1075-1085
 [32] Mackill DJ, Khush GS. IT-64: A high quality and high yielding mega variety. Rice (N-Y) 2018; 11: 18
 [33] Mahesh, Meghana Deepak Shirke, Siddarth Singh, Anantharamanan Rajamani, Shailaja Hittalmani, Guo-Liang Wang and Malali GOWDA. Indica rice genome assembly, annotation, and mining of blast disease resistance genes.
 [34] BMC Genomics 2016; 17:242
 [35] Anantha MS, Patel D, Quintona P, Swain P, Jawarhalal L, Rolando D. Trait combinations that improve rice yield under drought-tolerant varieties in South Asia.
 [36] Crop Sci. 2016; 56 (1): 408-421
 [37] A. Kalakand. Studies on preparation of rice wine. <http://Krishikosh.egranth.ac.in/handle/1/80651>
 [38] Akiyama K, Matsuzaki K, Hayashi H, Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature. (2005) ;435 (7043): 824-827

APPENDIX

Table 1: Selected plant varieties with their special characteristics

S no.	Plant name	Characteristic.
1	Sahabagidhan	Drought-tolerant, high yield rice variety. ⁽²⁴⁾
2	IR-64	Early maturity, disease resistant, excellent cooking quality. ⁽²²⁾
3	MGD-101	Drought and blast resistant properties. ⁽²³⁾
4	BPT-5204	Early maturity, drought-tolerant, fine grains. ⁽²¹⁾
5	Azucena	Symbiosis with arbuscular mycorrhizal (AM) fungi that help the plant to improve nutrient uptake. ⁽²⁶⁾
6	Doddiga	Tolerance to drought and low soil fertility ⁽²⁵⁾

Table 2: RAPD primers, their sequences, size and melting temperature

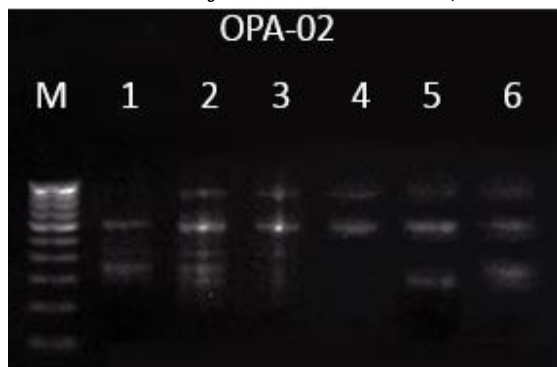
S no.	Marker	Sequence	Total number of bands (loci)	No. of polymorphic bands (loci)	% Polymorphic bands (loci)
1	OPA-02	5'TGCCGAGCTG3'	6	5	83.33
2	OPA-05	5'AGGGGTCTTG3'	6	5	83.33
3	OPA-07	5'GAAACGGGTG3'	5	4	80
4	OPA-12	5'TCGGCGATAG3'	5	4	80
5	OPA-14	5'TCTGTGCTGG3'	7	7	100
6	OPA-18	5'AGGTGACCGT3'	5	3	60
7	OPA-20	5'GTTGCGATCC3'	8	5	62.5
8	OPK-11	5'AATGCCCCAG3'	7	5	71.43

Table 3: Similarity matrix of rice varieties

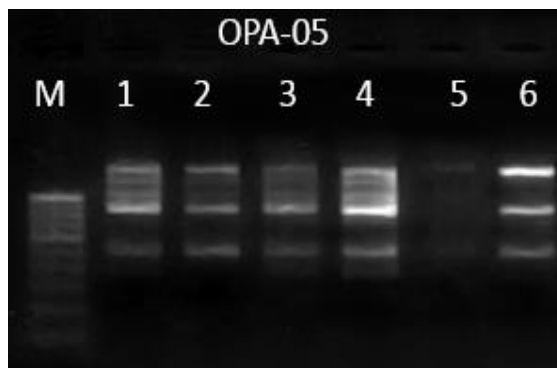
	Sahabagidhan	IR-64	MGD-101	BPT-5204	Azucena	Doddiga
Sahabagidhan	1					
IR-64	0.79	1				
MGD-101	0.77	0.85	1			
BPT-5204	0.73	0.69	0.79	1		
Azucena	0.42	0.42	0.46	0.41	1	
Doddiga	0.58	0.54	0.57	0.54	0.52	1

Table 4: Summary Statistics of different primers

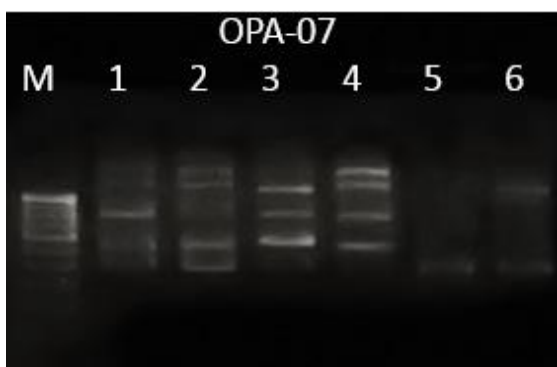
Marker	Major Allele Frequency			Gene Diversity			PIC		
	Average	Highest	Lowest	Average	Highest	Lowest	Average	Highest	Lowest
OPA-02	0.69	1.00	0.50	0.36	0.50	0.00	0.28	0.38	0.00
OPA-05	0.83	1.00	0.67	0.24	0.44	0.00	0.20	0.35	0.00
OPA-07	0.77	1.00	0.67	0.32	0.44	0.00	0.26	0.35	0.00
OPA-12	0.77	1.00	0.67	0.32	0.44	0.00	0.26	0.35	0.00
OPA-14	0.76	0.83	0.67	0.35	0.44	0.28	0.28	0.35	0.24
OPA-18	0.90	1.00	0.83	0.17	0.28	0.00	0.14	0.24	0.00
OPA-20	0.79	1.00	0.50	0.25	0.50	0.00	0.20	0.37	0.00
OPK-11	0.88	1.00	0.83	0.20	0.28	0.00	0.17	0.24	0.00



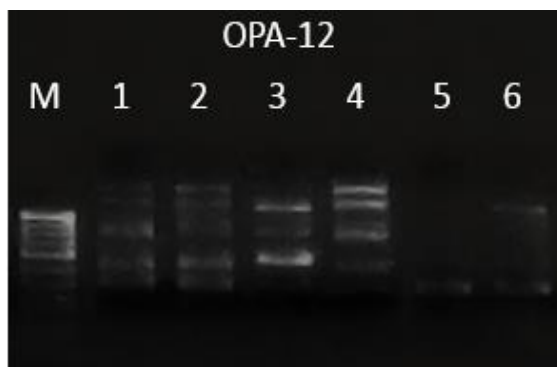
(a)



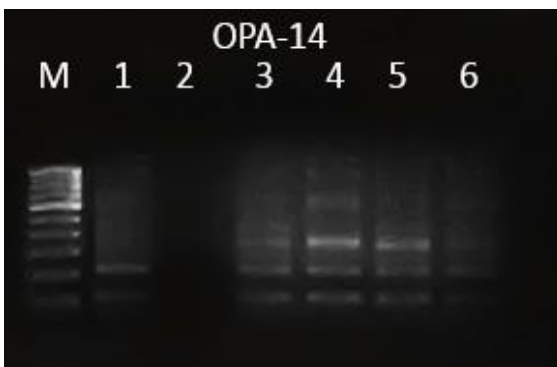
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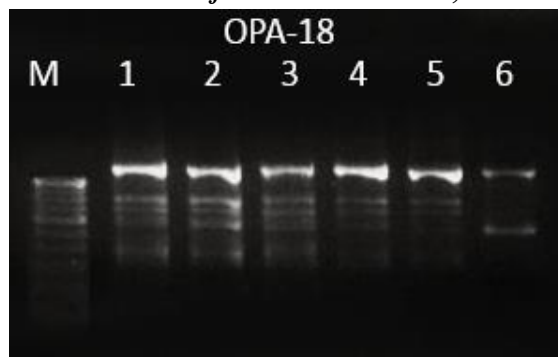
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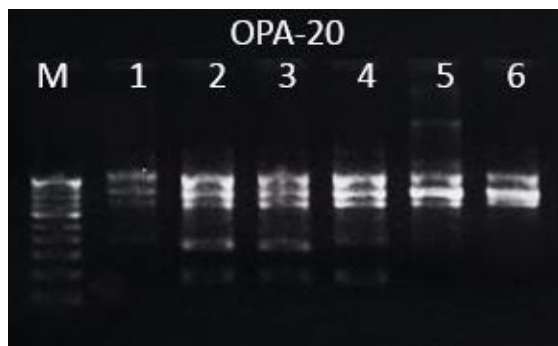
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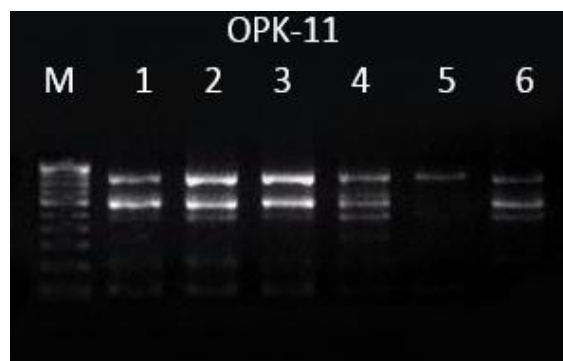
(e)



(f)



(g)



(h)

Fig. 1: RAPD Pattern of the rice varieties with Primer

- (a) OPA-02
- (b) OPA-05
- (c) OPA-07
- (d) OPA-12
- (e) OPA-14
- (f) OPA-18
- (g) OPA-20
- (h) OPK-11

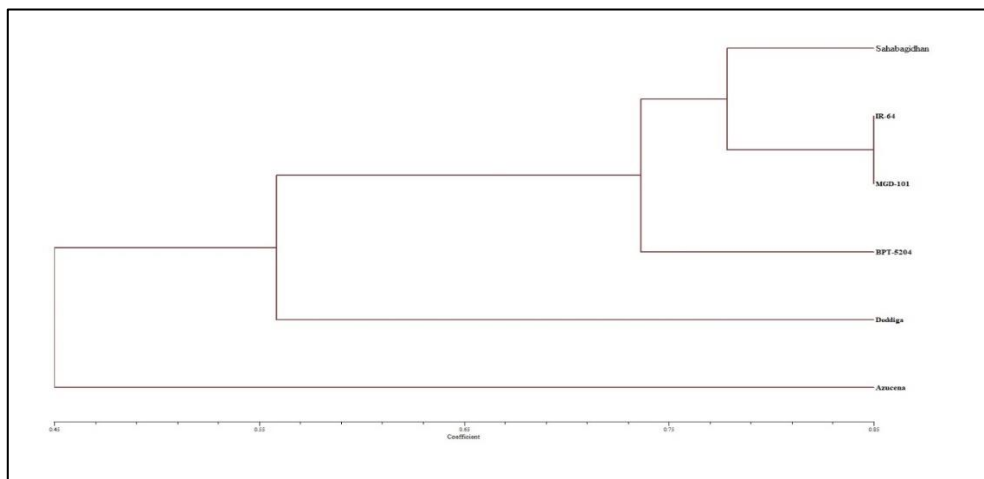


Fig. 2: UPGMA Dendrogram