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Molecular Evaluation of Various Species of Bamboo

Gopalakrishnan M[1], Priya G[2], Karpagam N[3], Rajesh E[4], Sekar T[5]
[1, 2, 3, 4, 5]*PG & Research Department of Botany, Pachaiyappa's College, Chennai - 600030, Tamil Nadu, India*

Abstract: From a 'Poor man's Timber' to Green Gold', From 21st century, Bamboo is considered as a fast-emerging genus and it is one of the fastest growing and highest yielding renewable natural resource which makes it as a good substitute to wood in mitigating pressure on natural forests. Bamboo and its products are largely used for various commercial and industrial products that would prove it as an ideal for raising the economy of the local people without making any compromise to their social - cultural - traditional needs and aspiration. Present study is carried out to investigate the genetic diversity using RAPD molecular markers between fifteen species of Bamboo Bambusa balcooa, Bambusa nutans, Bambusa tulda, Bambusa casarensis, Bambusa pallida, Bambusa multiplex, Bambusa parkisiley, Dendrocalamus hamiltonii, Dendrocalamus asper, Dendrocalamus hookeri, Dendrocalamus gigantius, Dendrocalamus strictus, Oclandra travencorica, Sasa fortune and Guadua angustifolia. In RAPD assay, a short ten nucleotides long decamer primer is used, which generally anneals with multiple sites in different regions of the genome and amplifies several genetic loci simultaneously. This technique is simple, relatively inexpensive and has been employed to analyse the intergeneric diversity of bamboo. The genomic DNA was extracted using Doyle and Doyle method (1990) with some modifications. Out of 40 primers, 10 primers with maximum number of amplifications of OPA9, OPA11, OPA19, OPB18, OPB20, OPC7, OPF5, OPN6, OPN10 and OPN11 were selected and these primers are found to produce total number of 511 distinct amplification products ranging from 100 – 3000 bp, which includes total number of 370 polymorphic bands and total number of 141 monomorphic bands. Number of amplifications per primer was ranging from 3.2 to 8.6 and the average number of amplifications per primer was found to be 5.1. Similarly, average of polymorphism per primer was calculated as 3.7 and percentage of polymorphism as 67.4%. The Dendrogram was generated which shows 70% (30% variability) to 90% (10% variability).

Keywords: Bamboo, RAPD markers, Amplification, Dendrogram, Genetic Similarity

I. INTRODUCTION

Bamboo belongs to family *Poaceae* of sub- family *Bambusoideae.* There are75generaand 1250 species [1]. They are woody, fast growing and grow up to 4000 m above sea level in tropical and in temperate region. In India, there are 23 genera and130species of bamboo, occupying about 10.05 million ha of land, which contribute about12.8%of the forest area of the country [2]. Propagation of bamboo is mainly carried out by seed and its flowering starts after20 - 120years which limit their propagation [3]. It replaces timber in many aspects. It is utilising in the form of pulp in paper industries in mainly India and China [4]. Around 2million tonnes of bamboo are used in handicrafts, construction material in place of timber, matting, ladder, bridges, aqueducts etc [5]. Among Bamboo species, the vegetative growth phase varies from1 year to as longas120yearsand some species have never been known to flower [6]. As it has along sexual cycle and its unavailability of any other diagnostic tool, identification of bamboo is mainly depending on vegetative descriptors such as culm morphology, and the morphology of the culm – sheath including ligule auricle [7]. RAPD assay is one of the cost-effective methods for identifying the genotypes within a limited period and requires only small quantity of DNA. The development of randomly amplified polymorphic DNA (RAPD) markers, generated by amplified polymorphic DNA (RAPD) markers, through polymerase chain reaction (PCR) using arbitrary primers, has provided a new tool for the detection of DNA polymorphism [8]. RAPD analysis has been used to study genetic relationship in several grasses [9]. RAPD markers are used to study the generation among different species and to determine genetic similarities between species. In a RAPD assay, a short usually ten nucleotides long, arbitrary primer is used, which generally anneals with multiple sites in different regions of the genome and amplifies several genetic loci simultaneously. This technique is simple, relatively inexpensive and has been employed to analyse the - and intergeneric genetic diversity of bamboo [10-13]. The present study is aiming to focus on genetic similarity between various species of Bamboo such as Bambusa balcooa, Bambusa nutans, Bambusa tulda, Bambusa casarensis, Bambusa pallida, Bambusa multiplex, Bambusa parkisiley, Dendrocalamus hamiltonii, Dendrocalamus asper, Dendrocalamus hookeri, Dendrocalamus gigantius, Dendrocalamus strictus, Oclandra travencorica, Sasa fortune and Guadua angustifolia.

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II. MATERIALS AND METHODS

A. Collection of Plant Materials

Collection of various species of Bamboo from Genepool Plant Tissue Culture Laboratory, Nagaon Paper Mill, Assam.

B. Isolation of Genomic DNA

Genomic DNA of various species of Bamboo such as Bambusa balcooa, Bambusa nutans, Bambusa tulda, Bambusa casarensis, Bambusa pallida, Bambusa multiplex, Bambusa parkisiley, Dendrocalamus hamiltonii, Dendrocalamus asper, Dendrocalamus hookeri, Dendrocalamus gigantius, Dendrocalamus strictus, Oclandra travencorica, Sasa fortune and Guadua angustifolia were extracted using[14] with some modifications. 1.5g of plant material was ground in a clean mortar and pestle and was frozen with liquid nitrogen. To the extract, 4.5ml of extraction buffer containing 3% CTAB, 100mMTris (pH8), 20mM EDTA (pH8), 1.4M NaCl, 2% PVP and0.2% Mercaptoethanol was added and incubated at 65ºC for 1hr. Samples are then added with equal volume of Chloroform: Isoamyl alcohol (24:1) and was centrifuged at 10,000 rpm for 15mins at 4ºC. The top aqueous layer was carefully transferred to another clean centrifuged tube and 1/10th volume of 3M Sodium acetate and 0.6th volume of ice-cold isopropanol was added and incubated at -20ºC for overnight. Incubated samples were again centrifuge at 10,000rpm for 15mins at 4ºC. The nucleic acid pellet was recovered and was washed with 70% ethanol for twice and remained for drying. Finally, the nucleic acid pellet was dissolved with TE buffer for further use.

C. RNase Treatment

Add 10µg/ml RNase to the DNA samples and was incubated at 37ºC for 1hr. Equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added and centrifuged at 10,000rpm for 2-5mins at 4ºC.Top aqueous layer was transferred and equal volume of chloroform: isoamylalcohol (24:1) was added and centrifuged at 10,000rpm for 2-5mins. 1/10th volume of 3M sodium acetate and 2.5th volume of cold 99% ethanol were added to the aqueous layer and was incubated at -20ºC for 1hr. The incubated samples were centrifuged at 12,00rpm for 10mins and the recovered pellet was washed with 70% ethanol and after drying it was dissolved in TE buffer for further use.

D. Determination of Quality and Quantity of Genomic DNA using UV- Spectrophotometric Method

Purity and Concentration of isolated genomic DNA were determined using UV-Spectrophotometer. The instrument and UV lamp were switched on and the wavelength was set at 260nm and 280nm. The instrument was set at zero absorbance with TE buffer or sterile water as blank. 3µl of the sample is taken in a quartz cuvette and made up to 3ml with TE buffer and the absorbance of the solution with the sample was read. The purity and Concentration of genomic DNA were calculated using following formula:

E. Purity of Genomic DNA

A_{260}:A_{280} ratio = A_{260} / A_{280}

F. Concentration of Genomic DNA

A_{260} × 50µg ×Dilution factor

The ratio of absorbance at 260nm and 280nm hence provides a clear idea about the extent of contamination in the samples. A ratio between 1.7 and 1.9 is pure, less than 1.8 indicates the presence of proteins, greater than 1.8 signify the presence of organic solvent and a ratio of 1.8 determines the pure DNA preparation. Intensity of bands of an isolated DNA was visually assessed using 0.8% agarose gel by comparing with DNA ladder.

G. RAPD Analysis

Decamer primers are used to amplify the DNA fragments and the analysis was carried out in a volume of 20µl mixture containing final concentration of 1X assay buffer, 2.5Mm $Mgcl_2$, 0.2mMdNTP mix, 0.3mMPrimer, 50ng DNA Template and 1U/µl of taq DNA polymerase. Amplification was carried out in a Lark Thermo Cycler under the reaction conditions with program of Initial denaturation at 94ºC for 4mins, 45 cycles of Denaturation at 94ºC for 1mins, annealing of primer at 55ºC, Extension at 72ºC for 2mins and Final extension at 72ºC for 10 mins and finally hold at 4ºC.

The amplified RAPD-PCR products were electrophoresed on a gel made up of 1X TAE buffer and stained with Ethidium Bromide and documented using Lark gel documentation system. The primers which gave reproducible fingerprints were considered to construct dendrogram and similarity matrix to study genetic similarity.

III. RESULTS AND DISCUSSION

Present study was focused to establish the genetic similarity between fifteen species of *Bamboo's*. DNA profiling is an important technique which is used to protect diversity of plants, identification of suitable markers for characterization, identification of genetic diversity and variability [15]. RAPD markers are one of the molecular markers are a part of fingerprinting as they are based on the amplification of unknown DNA sequences using single, short and random oligonucleotide primers RAPD is considered to be a valuable tool in order to access the genetic diversity with the help of the percentage of polymorphism obtained by the decamerprimers. Normally, DNA content is found to be higher in leaves than that of other parts of plants. Xu have reported that in rootstocks, no original RAPD bands were revealed by Ethidium bromide - stained agarose gels, hybridization signals were achieved with a RAPD - derived probe. RAPD is applicable for genus or species for which genetic information is not available [16]. RAPD markers are the dominant markers, so we assumed that each band represents the phenotype at a single biallelic locus. Therefore, amplified fragments were scored for the presence as (1) and absence as (0) of homologous bands.

Forty random primers were used to screen the genomic DNA samples. Various species of Bamboo such as Bambusa balcooa, Bambusa nutans, Bambusa tulda, Bambusa casarensis, Bambusa pallida, Bambusa multiplex, Bambusa parkisiley, Dendrocalamus hamiltonii, Dendrocalamus asper, Dendrocalamus hookeri, Dendrocalamus gigantius, Dendrocalamus strictus, Oclandra travencorica, Sasa fortune and Guadua angustifolia were used for screening.

RAPD is a dominant marker, so we assumed that each band represents the phenotype at a single biallelic locus. Therefore, amplified fragments were scored for the presence as (1) and absence as (0) of homologous bands. Out of 40 primers, 10 primers with maximum number of amplifications were selected. Selected primers are OPA9, OPA11, OPA19, OPB18, OPB20, OPC7, OPF5, OPN6, OPN10 and OPN11. Forty random primers were used for screening RAPD - PCR of various species of Bamboo and out of which 10 primers were selected to develop a final RAPD fingerprint. These 10 primers could elicit reliable banding patterns with high reproducibility and clear band resolution and useful for further analysis. These 10 primers are found to produce total number of 511 distinct amplification products ranging from 100 - 3000 bp, which includes total number of 370 polymorphic bands and total number of 141 monomorphic bands. Number of amplifications per primer was ranging from 3.2 to 8.6 and the average number of amplifications per primer was found to be 5.1. Similarly, average of polymorphism per primer was calculated as 3.7 and percentage of polymorphism as 67.4%.

A. Dendrogram Analysis

The cluster analysis was based on the similarity matrix using UPGMA and the relationship among the genotypes was visualized by dendrogram generated using Biodiversity Professional Software version 2. The genetic similarity ranged from 70% (30% variability) to 90% (10% variability). This was obtained by using random RAPD primers. Dendrogram was developed for fifteen species of Bamboo such as Bambusa balcooa, Bambusa nutans, Bambusa tulda, Bambusa casarensis, Bambusa pallida, Bambusa multiplex, Bambusa parkisiley, Dendrocalamus hamiltonii, Dendrocalamus asper, Dendrocalamus hookeri, Dendrocalamus gigantius, Dendrocalamus strictus, Oclandra travencorica, Sasa fortune and Guadua angustifolia. The analysed dendrogram revealed that fifteen genotypes were divided into two groups of one and six at 70% similarity. First genotype was divided into two clusters (Cluster A and Cluster B). Cluster A consists of six genotypes such as Oclandra travencorica, Bambusa pallida, Guadua angustifolia, Dendrocalamus strictus, Dendrocalamus hamiltoni and Dendrocalamus asper. Cluster B was again divided into sub-clusters (Sub-cluster A_1 and Sub-cluster B_1). Sub-cluster A_1 consists of three species such as Bambusa parkisiley, Bambusa tulda and Sasa fortuni which was again divided into sub-cluster C with Dendrocalamus hookeri and Dendrocalamus gigantius. Sub-cluster B consists of two species such as Bambusa multiplex and Bambusa casarensis. Cluster B consists of two species such as Bambusa balcooa andBambusa nutans.

Table 1. Summary of RAPD amplified products from various species of bamboo

S.NO	DESCRIPTION	RAPD
1.	Total number of bands scored	511
2.	Number of Monomorphic bands	141
3.	Number of Polymorphic bands	370
4.	Percentage of Polymorphism	67.4%
5.	Number of Primers used	10
6.	Average Polymorphism per primer	3.7
7.	Average number of Amplification per primer	5.1
8.	Size range of Amplified fragments	100bp-1500bp

Table2 . Rapd Analysis Of Various Species Of Bamboo

S.no	Primers	Primer sequence	Total No. Of bands per primer	No. Of Polymorphic Bands	Percentage Of Polymorphism	Average Polymorphism Per Primer	Average No. Of Bands Per Primer	Size range (bp)
1	Opa9	5'-gggtaacgcc-3'	35	15	43%	1.5	3.5	150-1000
2	Opa11	5'-caatcgccgt-3'	39	20	51%	2.0	3.9	200-1200
3	Opa19	5'-caaacgtcgg-3'	36	22	61%	2.2	3.6	300-1000
4	Opb18	5'-ccacagcagt-3'	68	61	90%	6.1	6.8	400-1000
5	Opb20	5'-ggacccttac-3'	63	38	60%	3.8	6.3	200-1500
6	Opc7	5'-gtcccgacga-3'	32	10	31%	1.0	3.2	300-1000
7	Opf5	5'-ccgaattccc-3'	38	32	84%	3.2	3.8	300-600
8	Opn6	5'-gagacgcaca-3'	48	37	77%	3.7	4.8	400-1000
9	Opn10	5'-acaactgggg-3'	67	61	91%	6.1	6.7	200-1000
10	Opn11	5'-tcgccgcaaa-3'	86	74	86%	7.4	8.6	400-1000

Dendrogram For Various Species Of B*amboo*

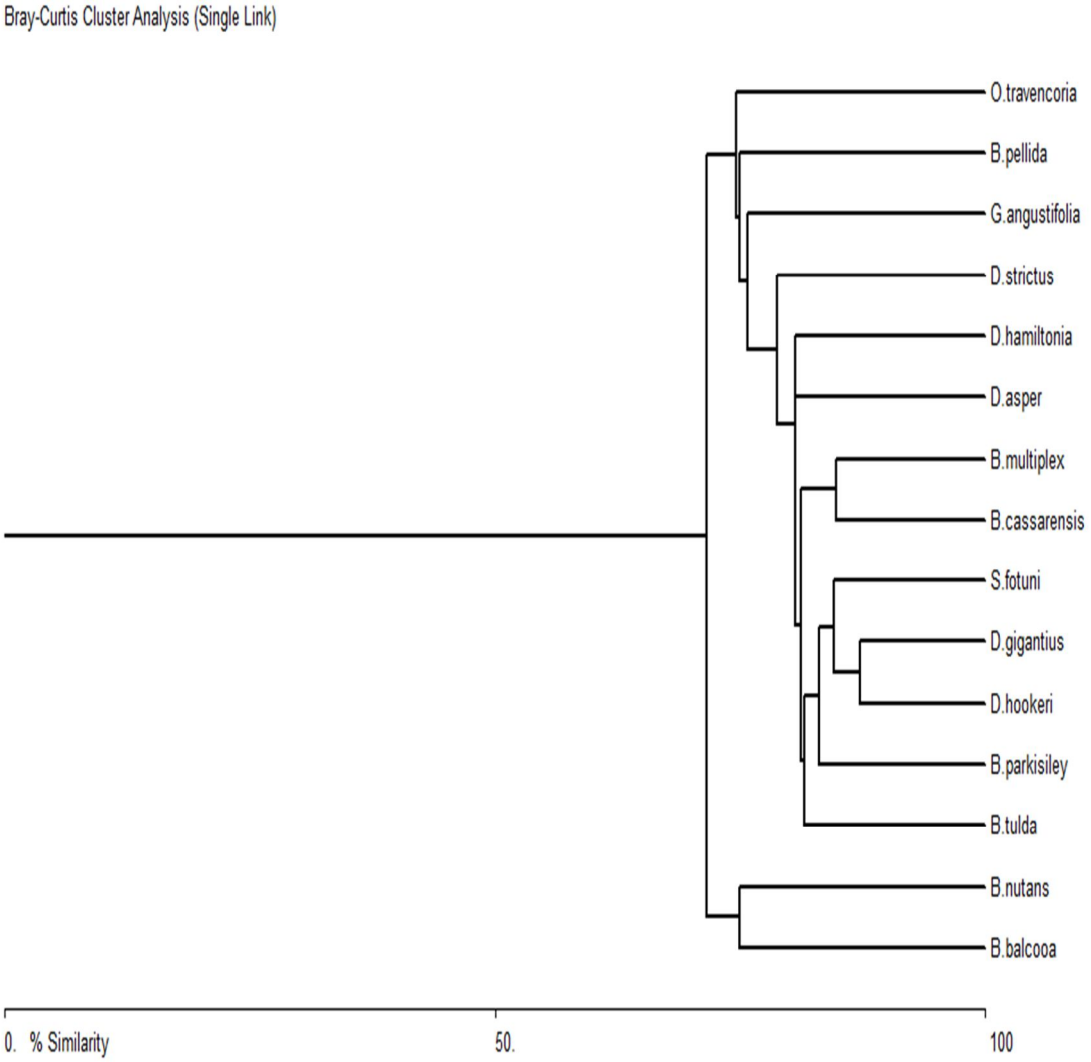

Table 3. SIMILARITY MATRIX FOR VARIOUS SPECIES OF *BAMBOO*

	BB	BN	BT	BC	BP	BM	BPa	DH	DA	DHo	DG	DS	OT	SF	GA
BB		75	65.636	62.5	70.968	65.625	68.656	71.641	64.516	63.888	61.764	66.666	52.459	61.76	59.375
BN			64.706	63.636	62.5	69.697	69.565	63.768	59.375	64.864	62.857	54.838	47.619	54.29	66.666
BT				70.588	57.756	64.706	70.423	73.239	69.697	81.579	77.778	65.625	67.692	75	55.882
BC					75	84.849	75.362	66.666	62.5	75.675	74.285	70.967	69.841	77.14	69.697
BP						75	68.656	71.641	58.065	66.666	67.647	63.333	55.737	70.59	75
BM							81.159	69.565	65.625	70.270	68.571	64.516	63.492	74.29	75.757
BPa								80.555	80.597	83.116	79.452	70.769	69.697	79.45	72.463
DH									80.597	80.519	79.452	67.692	60.606	73.97	66.666
DA										80.555	73.529	66.666	55.737	70.59	62.5
DHo											87.179	71.428	73.239	84.62	72.973
DG												69.697	65.671	81.08	74.285
DS													71.186	78.79	64.516
OT														74.63	60.317
SF															71.428
GA															

IV. CONCLUSIONS

India has the second largest reserve of bamboo population in the world. The fibres of bamboo are mainly used in pulp, paper and charcoal industries, while the culms have several other uses poor man's timber. Hence, this study was carried out to preserve this valuable genus at molecular level using RAPD analysis.

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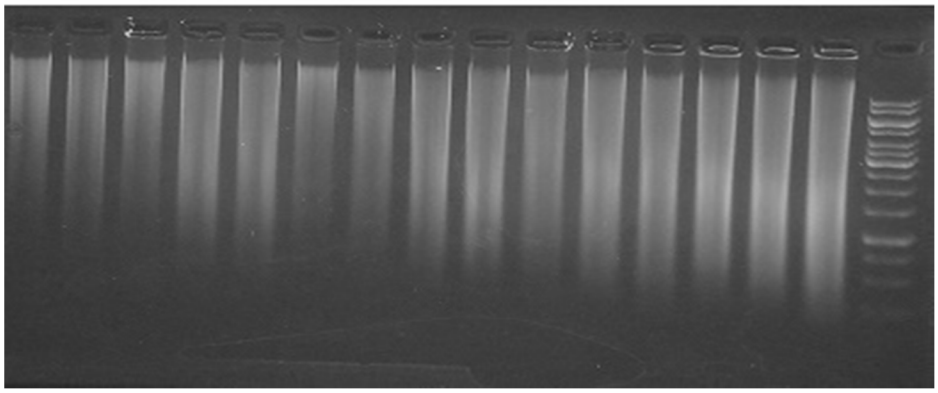
Figure 1. Isolation of genomic dna from various species of bamboo

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 L



1.Bambusabalcooa
2.Bambusanutans
3.Bambusatulda
4.Bambusacasarensis
5.Bambusa pallida
6.Bambusa multiplex
7.Bambusaparkisiley
8.Dendrocalamushamiltonii
9.Dendrocalamus asper
10.Dendrocalamushookeri
11.Dendrocalamusgigantius
12.Dendrocalamusstrictus
13.Oclandratravencorica
14.Sasafortuni
15.Guaduaangustifolia
L. 100kb Ladder

Rapd Profiling Of Different Primers For Various Species Of Bamboo

FIGURE 2. OPA9

L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

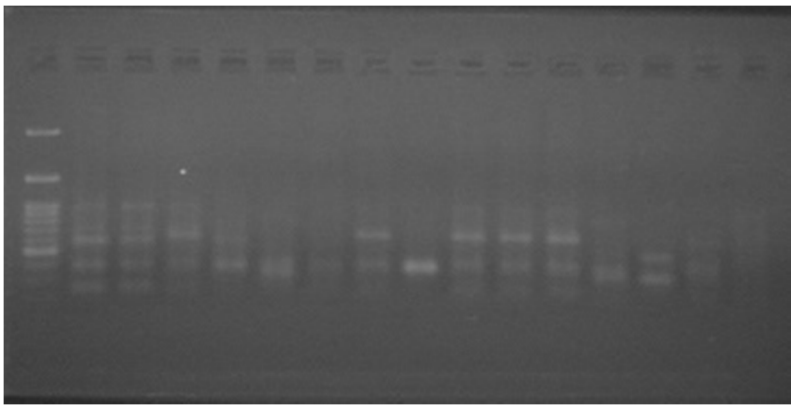


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FIGURE 3. OPA11

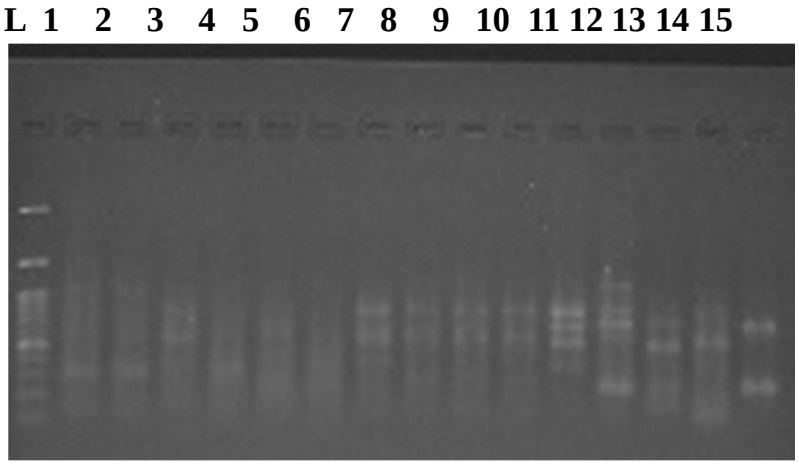

FIGURE 4. OPA19

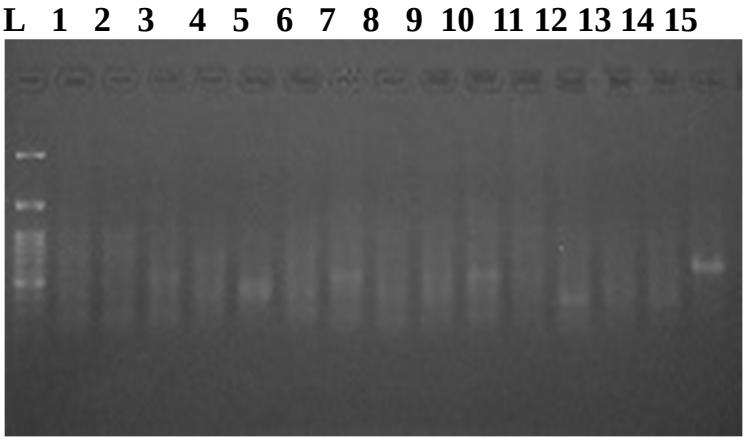

FIGURE 5. OPB18

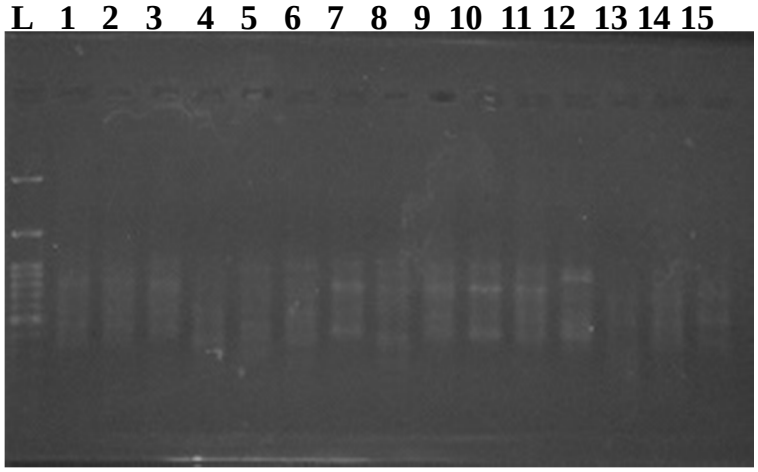

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FIGURE 6. OPB20

L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

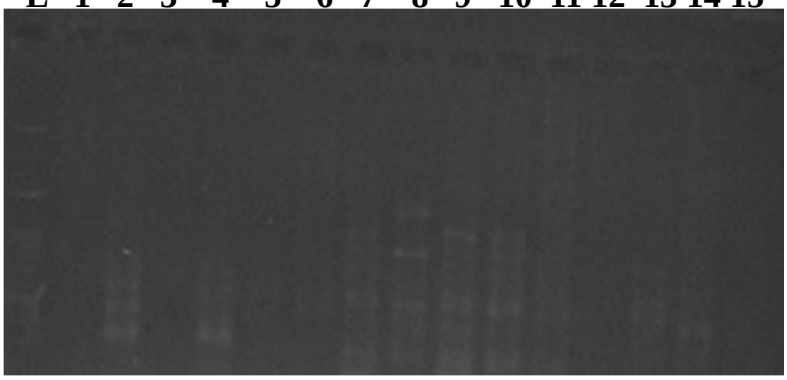


FIGURE 7. OPC7

L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

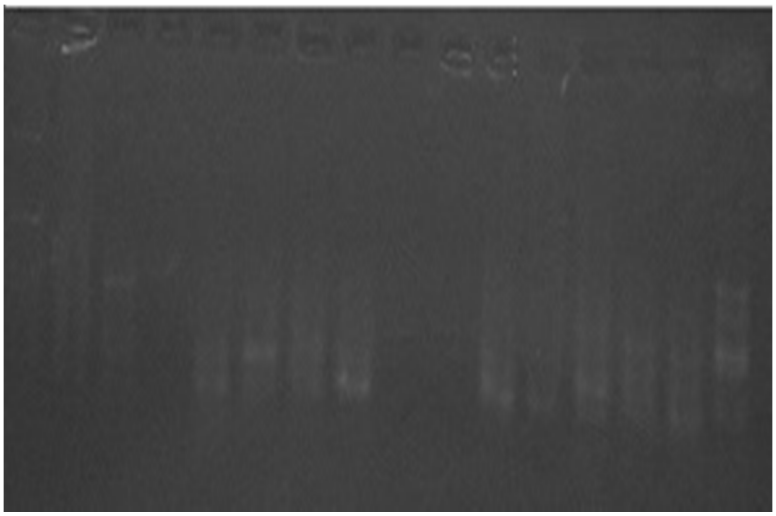


FIGURE 8. OPF5

L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

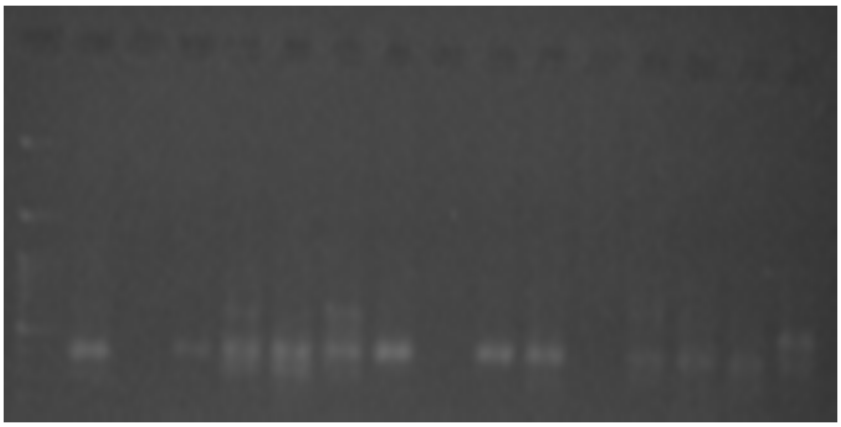


FIGURE 9. OPN6

L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

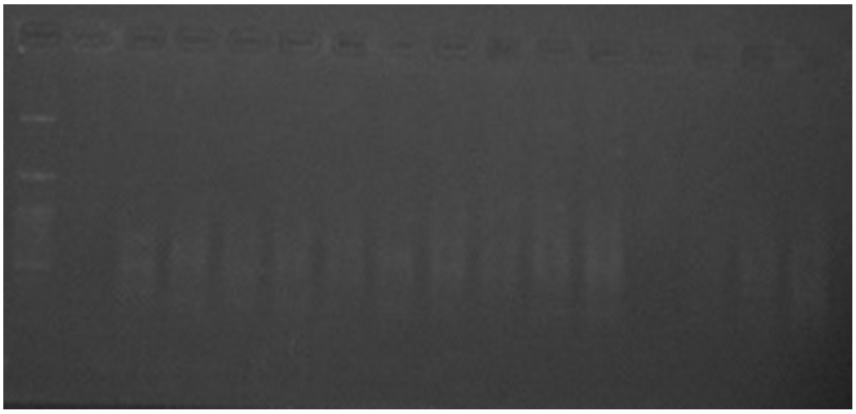


FIGURE 10. OPN10

L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

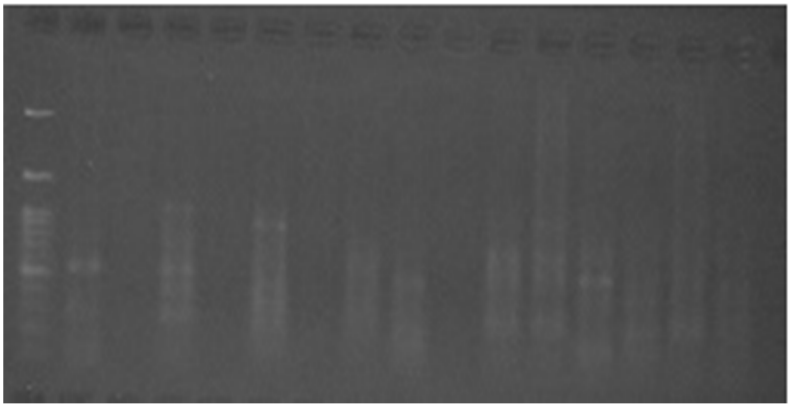
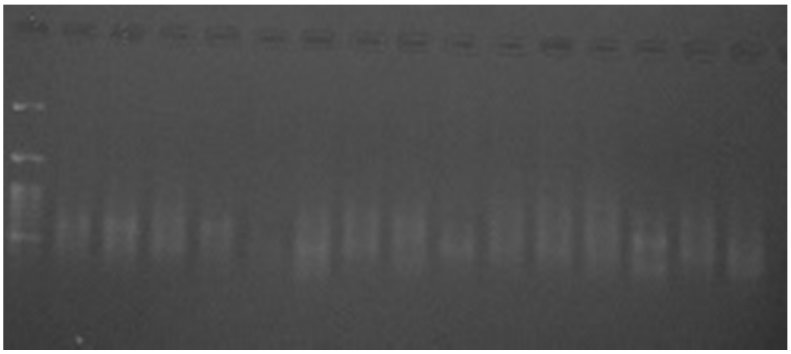
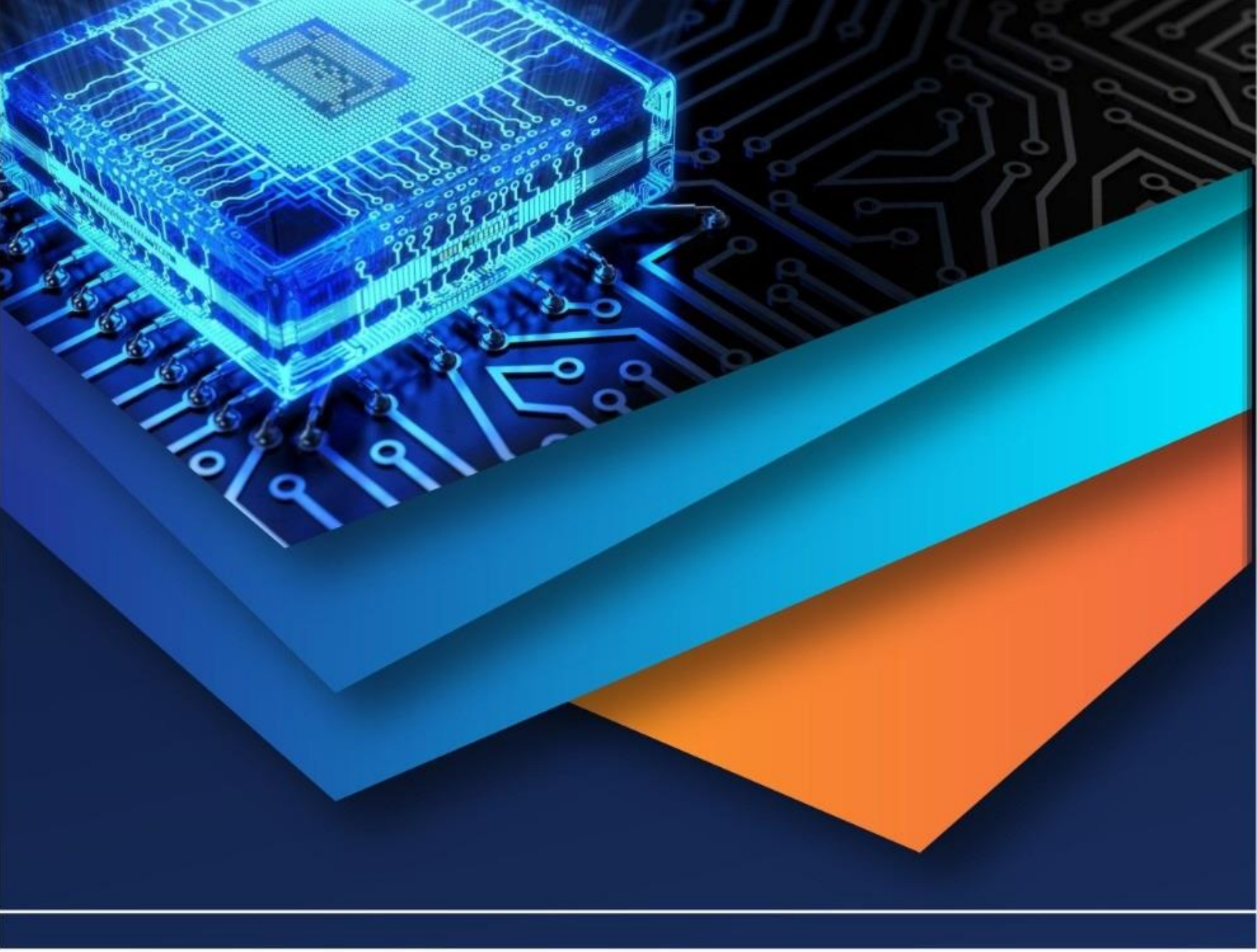


FIGURE 11. OPN11

L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15





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