



ISSN Print: 2664-9926
 ISSN Online: 2664-9934
 NAAS Rating (2026): 4.82
 IJBS 2026; 8(5): 36-39
www.biologyjournal.net
 Received: 12-02-2026
 Accepted: 14-03-2026

MR Shelke

Department of Microbial and
 Environmental Biotechnology,
 College of Agricultural
 Biotechnology, Loni,
 Maharashtra, India

SA Fargade

Department of Microbial and
 Environmental Biotechnology,
 College of Agricultural
 Biotechnology, Loni,
 Maharashtra, India

SR Shelke

Department of Microbial and
 Environmental Biotechnology,
 College of Agricultural
 Biotechnology, Loni,
 Maharashtra, India

Corresponding Author:

MR Shelke

Department of Microbial and
 Environmental Biotechnology,
 College of Agricultural
 Biotechnology, Loni,
 Maharashtra, India

Genetic diversity analysis in cotton i.e. (*Gossypium hirsutum* L.) genotypes using RAPD markers

MR Shelke, SA Fargade and SR Shelke

DOI: <https://www.doi.org/10.33545/26649926.2026.v8.i5a.825>

Abstract

Genetic Diversity and Molecular Characterization of cotton genotypes is essential for breeding and conservation programs. This study evaluates the genetic diversity of eight cotton genotypes (Phule Tarang, Phule Shubhra, Phule Mahi, Phule 688, Phule 388, Phule Anmol, Phule 492, and Phule Dhanwantari) using Random Amplified Polymorphic DNA (RAPD) markers. Genomic DNA was extracted via the CTAB method, with quality assessment through electrophoresis and spectrophotometry (1.7-2.0 ng/μl). PCR amplification using six RAPD primers generated 20 amplicons, 16 of which were polymorphic (80% polymorphism). Jaccard's similarity coefficient ranged from 0.33 to 0.87, with the highest similarity (0.87) between Phule 388 and Phule Anmol and the lowest (0.33) between Phule Tarang and Phule Dhanwantari. Dendrogram analysis classified the genotypes into two major clusters: Cluster A (sub-clusters A1 and A2) and Cluster B. Phule Tarang exhibited genetic similarity ranging from 33% to 50%, indicating moderate to distant relationships. These findings provide valuable insights and highlight the high genetic diversity in cotton varieties, crucial for breeding and genetic improvement.

Keywords: Genetic diversity, molecular characterization, cotton genotypes, RAPD markers, CTAB method

Introduction

Cotton (*Gossypium hirsutum* L.) is one of the most important fibre and textile crop of the world that is cultivated in subtropical and tropical regions of more than 60 nations (Feng *et al.* 2017) [5]. Cotton is also famous for its name as "King of fibre plants". It has many uses in the form of lint fibres and as other cotton by-products. Upland cotton is likewise referred to as "pure white gold" (Kumar and Katageri, 2017) [8].

Cotton belongs to the genus *Gossypium* of the Malvaceae family which contains 50 species. Out of these, four species are under commercial cultivation viz., *Gossypium herbaceum* (2n=26), *Gossypium arboreum* (2n=26), *Gossypium hirsutum* (2n= 52) and *Gossypium barbadense* (2n=52). Cotton is a fibre, oil and protein yielding crop of global significance. Cotton is a multipurpose crop that gives rise to much primary merchandise like seed, lint, oil, hull and linters.

The most economically important product from the cotton plant provides a source of high-quality fibre for the textile industry. The cotton genus *Gossypium* (Malvaceae) comprises four cultivated species: two New World tetraploid species, *Gossypium hirsutum* L. [n=2x=26 (AD)1] and *Gossypium barbadense* L. [n=2x=26(AD)2] and two old World diploid species, *Gossypium arboreum* L. [n=x=13, A1] and *Gossypium herbaceum* L. [n=x=13, A2] (Brubaker *et al.* 1999) [1].

Materials and Methods

- **Plant Materials:** Eight Cotton (*Gossypium hirsutum* L.) genotypes were collected from MPKV, Agricultural Research Station, Rahuri. Cotton genotypes used as experimental material such as 1) Phule Tarang, 2) Phule Shubhra, 3) Phule Mahi, 4) Phule 688, 5) Phule 388, 6) Phule Anmol, 7) Phule 492, 8) Phule Dhanwantari.
- **DNA Isolation:** The isolation Plant genomic DNA from Separately used Eight genotypes of Cotton (*Gossypium hirsutum* L.) leaves by using modified CTAB method. DNA extracted confirmation by Agarose Gel Electrophoresis method.

- **PCR Amplification for RAPD Analysis:** Reaction mixture was prepared thin walled PCR tubes containing the following components as shown in Table No.01 and Table No.02 shown cyclic parameter of thermal cycler for RAPD. The RAPD Primers used for amplification such as 1) OPA-01, 2) OPA-02, 3) OPA-07, 4) OPA-08 and 5) OPA-09 6) OPA-10.

Results and Discussions

The present study entitled 'Genetic Diversity Analysis in Cotton i.e. (*Gossypium hirsutum* L.) genotypes using RAPD

Markers'. Genetic analysis and RAPD polymorphism in soybean genotypes was carried out using 05 RAPD Primer. In these study 8 varieties of cotton crop used to amplification by RAPD primers in PCR in PCR master cycler. The banding pattern thus obtained by RAPD marker clearly distinguished varieties into different clusters showing genetic diversity as shown in Fig 01 to Fig 03. Genetic Similarity estimate based on RAPD banding pattern used for cluster analysis to present genetic relationship in the form of dendrogram Cluster (Fig. 04).

Table 1: Reagents for PCR reaction mix

Sr.no	Components	Volume(μl)
1	PCR Reaction Mix(Taq DNA polymerase, dNTPs, MgCl ₂ and reaction buffer).	5
2	Primer	2
3	Molecular Grade water	1
4	Template DNA	2
	Total	10

Table 2: Temperature Profile for PCR Amplification

Sr. No	Steps	Temperature (°C)	Duration (Minutes)	No of Cycles
1.	Initial Denaturation	94	10	1
2.	Denaturation	94	1	
3.	Annealing	Ta	1.30 secs	35X
4.	Extension	72	1	
5.	Final Extension	72	10	1
6.	Hold	4	∞	

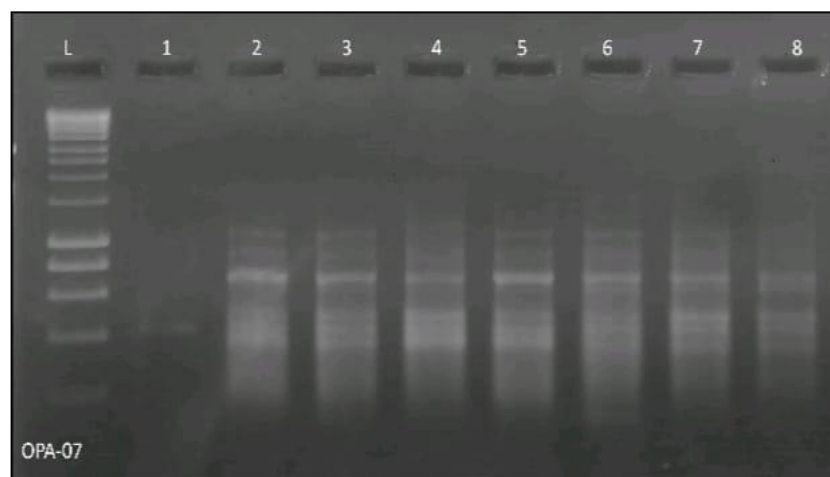


Fig 1: RAPD profile of Cotton genotypes created by OPA 07

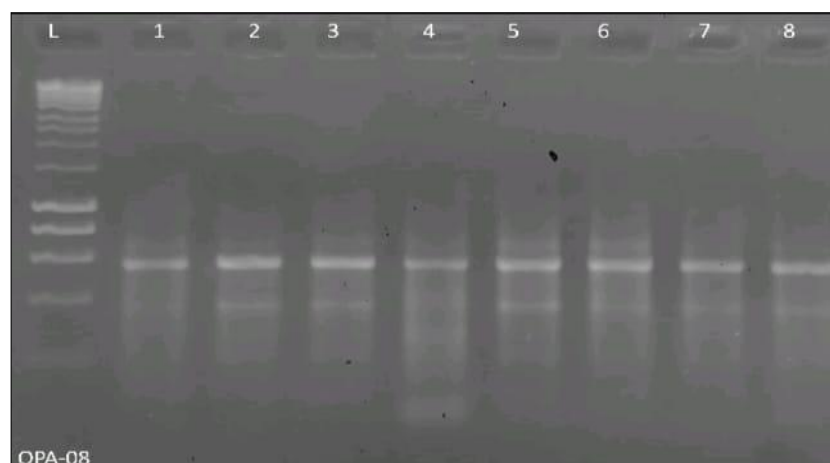


Fig 2: RAPD profile of Cotton genotypes created by OPA 08

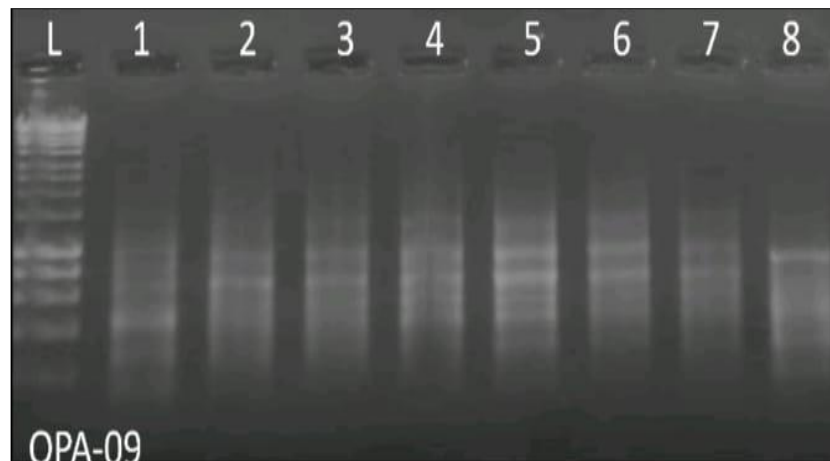


Fig 3: RAPD profile of Cotton genotypes created by OPA 09

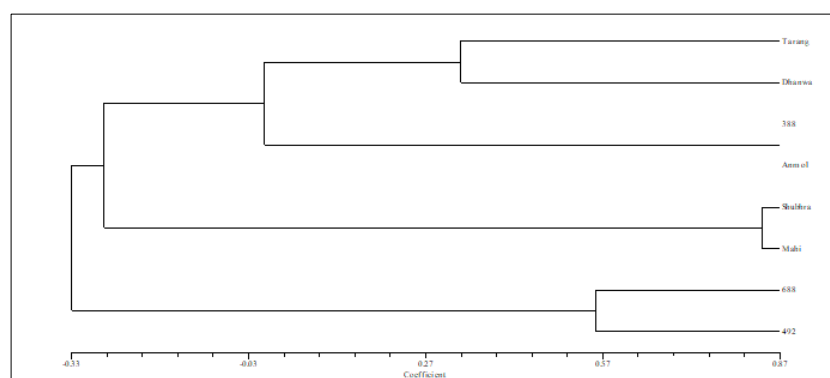


Fig 4: Dendrogram showing results of RAPD analysis of Cotton varieties

Summary and Conclusion

Cotton (*Gossypium hirsutum* L.) is one of the most important fibre and textile crops cultivated in over 60 countries worldwide. Known as the "King of Fibre Plants" and "Pure White Gold," cotton provides high-quality fibre for the textile industry and serves multiple purposes, including oil and protein production. The genus *Gossypium* belongs to the Malvaceae family and consists of 50 species, with four being commercially cultivated: *Gossypium herbaceum* (2n=26), *Gossypium arboreum* (2n=26), *Gossypium hirsutum* (2n=52), and *Gossypium barbadense* (2n=52). Apart from fibre, cotton by-products have applications in food, pharmaceuticals, cosmetics, and industrial manufacturing. In this study, genomic DNA was extracted from eight cotton genotypes (Phule Tarang, Phule Shubhara, Phule Mahi, Phule 688, Phule 388, Phule Anmol, Phule 492, and Phule Dhanwantari) using the CTAB method. DNA quality was confirmed through electrophoresis, with spectrometer measurements ranging from 1.7 to 2.0 ng/μl. The DNA samples were diluted to 20 ng/μl for further genetic diversity analysis using six RAPD primers. The amplification process generated 20 amplicons, with an average of 3.3 per primer, out of which 16 were polymorphic, leading to an 80% polymorphism rate. The Jaccard's similarity coefficient ranged from 0.33 to 0.87, with the highest genetic similarity (0.87) observed between Phule 388 and Phule Anmol and the lowest similarity (0.33) between Phule Tarang and Phule Dhanwantari.

References

1. Brubaker CL, Paterson AH, Wendel JF. Comparative genetic mapping of allotetraploid cotton and its diploid progenitors. *Genome*. 1999;42(2):184-203.
2. Bukhari SA, Iqbal MA, Naz S. Studies on genetic diversity of cotton using RAPD markers. *Pure Appl Biol*. 2021;3(3):95-100.
3. Chaudhary L, Sindhu A, Kumar M, Kumar R, Saini M. Estimation of genetic divergence among some cotton varieties by RAPD analysis. *J Plant Breed Crop Sci*. 2010;2(3):39-43.
4. Esmail RM, Zhang JF, Abdel-Hamid AM. Genetic diversity in elite cotton germplasm lines using field performance and RAPD markers. *World J Agric Sci*. 2008;4(3):369-375.
5. Feng L, Dai J, Tian L, Zhang H, Li W, Dong H. Review of the technology for high-yielding and efficient cotton cultivation in the northwest inland cotton-growing region of China. *Field Crops Res*. 2017;208:18-26.
6. Iqbal MJ, Aziz N, Saeed NA, Zafar Y, Malik KA. Genetic diversity evaluation of some elite cotton varieties by RAPD analysis. *Theor Appl Genet*. 1997;94:139-144.
7. Khan AI, Awan FS, Sadia B, Rana RM, Khan IA. Genetic diversity studies among coloured cotton genotypes by using RAPD markers. *Pak J Bot*. 2010;42(1):71-77.
8. Kumar NM, Katageri IS. Genetic variability and heritability study in F2 population of *Gossypium barbadense* L. cotton for yield and its components. *Int J Curr Microbiol Appl Sci*. 2017;6(6):975-983.
9. Kumar P, Singh K, Vikal Y, Randhawa LS, Chahal GS. Genetic diversity studies of elite cotton germplasm lines using RAPD markers and morphological characteristics. *Indian J Genet*. 2003;63(1):5-10.

10. Parre S, Patil RS. Assessment of molecular diversity using RAPD markers in elite genotypes of cotton (*Gossypium hirsutum* L.). 2021.
11. Patil MD, Biradar DP, Patil VC, Janagoudar BS, Nadaf HL. Analysis of genetic diversity of cotton genotypes using RAPD PCR technique. Karnataka J Agric Sci. 2007;20(2):215.
12. Tyagi S, Verma SK, Nehra K, Bajya DR. Molecular marker based genetic diversity analysis in cotton using RAPD and SSR markers. J Cotton Res Dev. 2015;29(2):232-236.
13. Verma SK, Tuteja OP. Genetic diversity analysis of CGMS lines A, B and restorer lines in tetraploid cotton (*Gossypium hirsutum* L.) using RAPD markers. J Pharmacogn Phytochem. 2021;10(1):2549-2554.
14. Welsh J, McClelland M. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 1990;18(24):7213-7218.
15. Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 1990;18(22):6531-6535.