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Molecular Characterization of *Rabi* Sorghum [*Sorghum bicolor* (L) Moench] Cultivars Using RAPD Markers

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Genetic diversity among six cultivars of rabi sorghum was studied by using 10 random primers. The total number of amplified PCR bands was 44 of which 30(68.18%) were polymorphic displaying PIC values ranging from 0.07 to 0.40 with an average of 0.30. Similarity coefficients among the genotypes ranged from 0.43 to 0.94. The similarity coefficient was used to construct a UPGMA based dendrogram using average linkage between groups. The analysis illustrates considerable potential of RAPD markers for estimating genetic diversity among six genotypes of rabi sorghum. The maximum genetic distance was found between the M-35-1 and Phule Rewati (0.43) while the lowest genetic distance was between Phule Vasundhara and Phule Vasudha (0.94). Selecting parents of diverse genetic base with contrasting phenotypes of various biotic and abiotic stresses for developing mapping populations for quantitative trait loci (QTL) detection and marker-assisted selection. RAPD markers used to reveal genetic diversity and variations within and among them the improved cultivars through the diverse gene pool for future breeding program.

Introduction

Sorghum (*Sorghum bicolor*) is a cereal of Poaceae grass family native to Northeastern Africa and was first cultivated from 3700 to 4000 years ago (Yun Xiong 2019). Sorghum is one of the leading cereal crops worldwide and ranked the fifth highest production of cereal crops, following maize, wheat, rice, and barley, with 57.6 million tons of annual production globally in 2017 (FAO, 2017). Sorghum has been widely grown in tropical and subtropical regions. In Asian and African countries such as India and Nigeria, sorghum is one of the important crops and is used to make foods such as bread and porridges; especially in some under-developed and semiarid regions, it is the principal source of energy and nutrition for humans (Rooney & Waniska, 2000). Important sorghum growing countries are U.S.A., India, China, Nigeria, Sudan and Argentina. India ranks fifth in total Sorghum production in world. In India, important sorghum growing states are Maharashtra, Andhra Pradesh, Karnataka, Madhya Pradesh, Gujarat, Tamil Nadu, Rajasthan, and Uttar Pradesh. It is one of the most widely grown dry land food grains in India. It has capacity to withstand drought. It makes comparatively

quick growth and gives not only good yields of grain but also very large quantities of fodder (Choudhary Manju Kumari 2022).

However, there is increasing interest in cultivating sorghum for biofuel production, as well as food for human consumption, due to the natural ingredients contained in sorghum, which are beneficial for the development of healthy and functional foods (Yun Xiong 2019). Sorghum is well known for its outstanding agronomic performance, that is, adaptability to grow in a variety of environments. It is drought tolerant, heat tolerant, and can grow in high altitudes and saline–alkaline and barren soil. This is because sorghum has a well-developed root system with a high root to leaf ratio, and the leaves are protected by wax and can also roll themselves in response to external threat/stimulus (Yun Xiong 2019). Sorghum is a famous C4 grain crop with high productivity. Sorghum is the main source of food security in the semi-arid zone. Sorghum was domesticated in Africa, from where it was introduced to other regions of the world with diverse agro-climatic conditions. Therefore, a wide diversity is found within and among the sorghum cultivars at both phenotypic and genotypic level (Ruiz- Chután *et al.,* 2019).

Genetic improvement of sorghum can help farmers in semi-arid regions where sorghum is key food crop. Cultivated sorghum is an important food security in semi-arid regions of the world including Asia and Africa. Climate change will modify the length of growing period and increase the temperature across the sorghum growing regions need on development of drought resistance and heat tolerant cultivars by using modern with the use of marker it would now be possible to hasten the transfer of desirable genes among varieties and to introduce novel genes from wide species (Sinha Sweta *et al.,* 2014).

The development of DNA marker technology has provided an efficient tool to facilitate plant genetic resource conservation and management. Compared to morphological analysis, molecular markers can reveal differences among accessions at DNA level. They represent an opportunity to provide information on the variation that exists in a particular species within a local region as well as among different countries. A RAPD marker is increasingly being employed in genetic research owing to its speedy process and simplicity. This technique always allows the examination of genomic variation without prior knowledge of DNA sequences and is especially useful for unzipping the variations in species with low genetic variability when other techniques such as isozyme analysis fail to reveal differences among the individuals. Moreover, varietal distinctiveness and relativeness can unambiguously be estimated by RAPD fingerprinting in commercially important crops. RAPD markers are unbiased and neutral markers for genetic mapping applications, in population genetics, taxonomy as well as for genetic diagnostics. RAPD has been used for classification and assessing diversity and relatedness of six major cereal crops genotypes by several groups (Rupal Chauhan 2015). This study aimed to use RAPD markers to evaluate the genetic variation within a collection of improved six improved *Rabi* sorghum cultivars and to reveal genetic relationships among them for future use in selection, hybridization, biodiversity assessment and conservation of diverse gene pools.

Materials and Methods

Plant Materials

The plant material used for the seeds of six improved *Rabi* sorghum cultivars were collected from All India Co-ordinated Sorghum Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri (Table 1), which have different genotypic and phenotypic characters. All *Rabi* sorghum cultivars were sown at Research Farn Unit at College of Agricultural Biotechnology, Loni, Ahilyanagar in November 2025.

Genomic DNA Extraction

Total genomic DNA was extracted using cetyltrimethylammonium ammonium bromide (CTAB) protocol given by (Saghai-Maroof *et al.,* 1984) with some modifications. DNA was isolated from 0.5 g of fresh leaves of the 10-15 days seedlings. Tissue was crushed into a fine powder using liquid nitrogen and dispersed in 1 ml pre-warmed (60ºC) extraction buffer (2% CTAB, 1.4 M NaCl, 0.5 M EDTA, pH 8.0, 100 mM Tris-HCl, PVP 2% and 0.2% beta-mercaptoethanol). After incubation for 1 hr at 60°C with intermittent swirling, the mixture was emulsified with an equal volume of chloroform: isoamyl alcohol (24:1). Following centrifugation, the supernatant was collected and mixed with 0.6 volume of isopropanol. The precipitated nucleic acid was spooled out, washed twice in 70% ethanol, dried under vacuum, dissolved in TE buffer (1 M Tris-HCl and 0.5 M EDTA pH 8.0) and treated with RNase

and proteinase-K to remove RNA and protein respectively. DNA was tested for its quality and integrity on 1.5% agarose gel, quantified by spectrophotometer, diluted in TE buffer to a concentration of 25ng/µl and utilized for PCR analysis.

Quantification of Genomic DNA

The quantity of DNA was checked using 1µl of DNA of each 10 soybean genotypes by using Nano Drop (ND-1000 Spectrophotometer) machine (Table 2). Stock DNA was diluted in TE buffer to make a working solution of 100ng/µl for PCR reaction. A part of DNA sample was diluted with appropriate quantity of TE buffer to yield a working concentration of 100ng/µl and stored at $4^{O}C$ for further work until PCR amplification.

PCR amplification for RAPD analysis

Requirements

Template DNA, RAPD primers, 2X PCR master mix, sterile distilled water, el loading dye (6X), 50X TAE buffer, Ethidium bromide, DNA Ladder. The analysis of PCR amplification using RAPD markers with the certain modifications (Williams *et al.,* 1990). The optimum specifications followed for DNA amplifications were as follows. RAPD primers were used for the PCR amplification. The amplification was carried out in thermal cycler for 35 cycles under following PCR conditions. Reaction mixture was prepared in 0.2ml thin-walled PCR tubes containing the following components. The total volume of each reaction mixture was 15µl.

Data analysis

Submerged gel electrophoresis unit was used for fractionating RAPD markers on 1.5% agarose gel. 4µl loading dye was added to the amplified products in each tube and mixed well. 15µl of amplified products of each sample were loaded on 1.5% agarose gel containing 1x TAE buffer to separate the amplified fragments. The amplified products were resolved at 1.5% agarose gel at 50V for 2 hours. The gel was stained with ethidium bromide (0.5µl) after electrophoresis, the gel was carefully taken out of the casting tray and photograph was taken from the gel documentation. The Gene ruler 100bp DNA ladder plus was used as the standard to determine the size of the polymorphic fragments. The gel was visualized under UV- transilluminator (JASCO) and photographed using Gel-Doc system (UV-Tech Ltd). The amplified fragment profiles were visually scored for the presence (1) or absence (0) of bands and entered in a scoring matrix. Pair-wise genetic similarities between soybean genotypes and polymorphic band were estimated by Jaccard's similarity coefficient. Clustering was done using the symmetric matrix of similarity coefficient and cluster obtained was based on unweighted pair group method for arithmetic mean (UPGMA) using sequential agglomerative hierarchical nested (SAHN) cluster analysis of NTSYS-PC version 2.02 (Rohlf 2000). The computer package NT SYS-pc version 2.1 was used to carry out cluster analysis. Molecular weights of the bands were estimated by using 100bp DNA ladder as standard. Percent polymorphism was calculated by using the formula,

$$\text{Polymorphic Percent} = \frac{\text{Polymorphic Amplicons}}{\text{Total Amplicons}} \times 100$$

Results and Discussion

'Molecular characterization of *Rabi* sorghum (*Sorghum bicolor*) cultivars through RAPD marker' was carried out by using 10 RAPD primers. Universal primers of OPA series were used to evaluate polymorphism of 6 *Rabi* sorghum cultivars. PCR amplified products of each RAPD primer were resolved on 1.5% agarose gel, and the size of the amplified products was compared with marker DNA 10 primers were screened (Table 3).

DNA fingerprinting

Universal random primers like OPA-01, OPA-02, OPA-03, OPA-06, OPX-03, OPA-07, OPA-05, OPB-19, OPA-12 and OPA-15 were used. Total 44 RAPD amplified amplicons were generated (Table 6). OPB-19 produced maximum number of amplicons (09 from all cultivars) followed by OPA-05 & OPA-01 (06), OPA-12, OPA-06 and OPA-15 (04) while OPA-02, OPA-03 & OPA-07 (02) generated minimum number of amplicons in the genomic pool.

Higher numbers of polymorphic amplicons were observed in OPB-19 (9), OPA-01 (6), OPA-06 & OPA-12 (4) and lower numbers of polymorphic amplicons were observed in OPA-05 (1) & OPA-05 (1) & OPA-15 (1). The polymorphic percentage was calculated which was highest in OPA-01 (100%), OPA-03 (100%), OPA-

06 (100%), OPB-19 (100%) and OPA-112 (100%) and lowest in OPA-15 (25%). Highest polymorphism information content (PIC) was observed in OPB-19 (0.60), OPA-12 (0.27) & OPA-06 (0.26) and lowest was observed in OPA-02 (0.06) & OPA-15 (0.07) (Table 6).

Higher numbers of monomorphic amplicons (05) were observed in OPA-05, and lower numbers of monomorphic amplicons were observed in OPA-02 (01) and OPA-07 (02). The percentage of monomorphic amplicons in banding pattern was calculated, which was highest in OPA-07 (100%), OPA-05 (83.33%), OPA-15 (75%) and OPX-03 (60%) while lowest was recorded OPA-2 (50%) (Table 6).

Similarity matrix for Jaccard's coefficient

A Jaccard's binary similarity matrix of combined data for 10 primers for the six *Rabi* sorghum cultivars was prepared by scoring bands for presence or absence. DNA bands of same mobility (molecular weight) were assumed to be identical. Genetic Similarity estimate (Jaccard's coefficient) based on RAPD banding pattern used for cluster analysis to present genetic relationships in the form of dendrogram. In the present study, the similarity coefficient values ranged from 0.43 to 0.94 across six *Rabi* sorghum cultivars indicating high degree of genetic variation and diversity.

The highest genetic similarity to an extent of 0.94 was recorded between Phule Vasundhara & Phule Vasudha and least genetic similarity 0.43 was observed in between M-35-1 (Maldandi) & Phule Rewati. Genetic similarity estimate (Jaccard's coefficient) based on RAPD banding pattern used for cluster analysis to present genetic relationship in the form of dendrogram (Fig.1) and Jaccard's coefficient (Table 7).

Distribution-based Clustering:

We first computed the cophenetic correlation coefficients between the similar matrices and the respective dendrograms (Table 7). Randomly amplified polymorphic DNA, or RAPD, marker analysis utilizes short PCR primers consisting of random sequences usually in the size range of 8 to 15 nucleotides in length. The RAPD markers *were scored* visually based on their *presence* (*1) or absence* (*0*), separately for *everyone* and *each* primer in Microsoft excel sheet and this scored data were used in NTSYS-pc software v.2.2 for cluster analysis. Dendrogram was generated by UPGMA cluster analysis based on Jaccard's similarity coefficients (Table 7). The dendrogram of *Rabi* sorghum cultivars showed two major clusters (Cluster A & Cluster B). Cluster A divided into two sub clusters (Cluster A1 & Cluster A2) and Cluster A1 also subdivided into the two sub clusters (Cluster A1a & Cluster A1b); Cluster A1a again divided into two sub clusters *via*., Cluster A1a1 & Cluster A1a2 which consist two *Rabi* sorghum cultivars Phule Vasudha & Phule Yashodha respectively and A1b also again divided into two sub clusters *via*., Cluster A1b1 & Cluster A1b2 which consist two *Rabi* sorghum cultivars Phule Rewati & Phule Vasundhara respectively. Cluster A1 consists of four *Rabi* sorghum cultivars *via*., Phule Vasudha, Phule Yashodha, Phule Rewati and Phule Vasundhara & Cluster A2 shows one *Rabi* sorghum cultivar *via*., Phule Suchitra) Cluster B consists of single *Rabi* sorghum cultivar *via*., M-35-1 (Maldandi) (Fig 1). The maximum genetic distance of *Rabi* sorghum cultivars was found between the M-35-1 (Maldandi) and Phule Rewati (0.43) while the lowest genetic distance was between Phule Vasundhara and Phule Vasudha (0.94).

In this study 44 DNA fragments, reproducible and scorable amplification products, were generated across 6 *Rabi* sorghum cultivars. Out of 44 bands, 30 (68.18%) (Table 6) were found to be polymorphic for one or more cultivars ranging from 3.00 polymorphic bands per primer. The average number of polymorphic fragments per primer among the 6 *Rabi* sorghum cultivars was 3.00. This percentage of polymorphic bands was observed in the study (Prakash SPJ 2006 & Suresh H. 2013). An assessment of molecular characterization between the six *Rabi* sorghum cultivars was analyzed by using 10 RAPD primers where the polymorphic RAPD markers have clearly observed. Dendrogram were generated based on data recorded by the one marker systems. The result of cluster analysis indicated the separation of two different Cluster A and Cluster B. Cluster A has 5 cultivars within same species. Cluster A1 consists of four *Rabi* sorghum cultivars *via*., Phule Vasudha, Phule Yashodha, Phule Rewati and Phule Vasundhara & Cluster A2 shows one *Rabi* sorghum cultivar *via*., Phule Suchitra; Cluster B consists of single *Rabi* sorghum cultivar *via*., M-35-1 (Maldandi). The maximum genetic distance of *Rabi* sorghum cultivars was found between the M-35-1 (Maldandi) and Phule Rewati (0.43) while the lowest genetic distance was between Phule Vasundhara and Phule Vasudha (0.94) in the RAPD marker analysis.

Fig.1 Dendrogram is generated by UPGMA cluster analysis using similarities percents that obtained from 10 RAPD markers

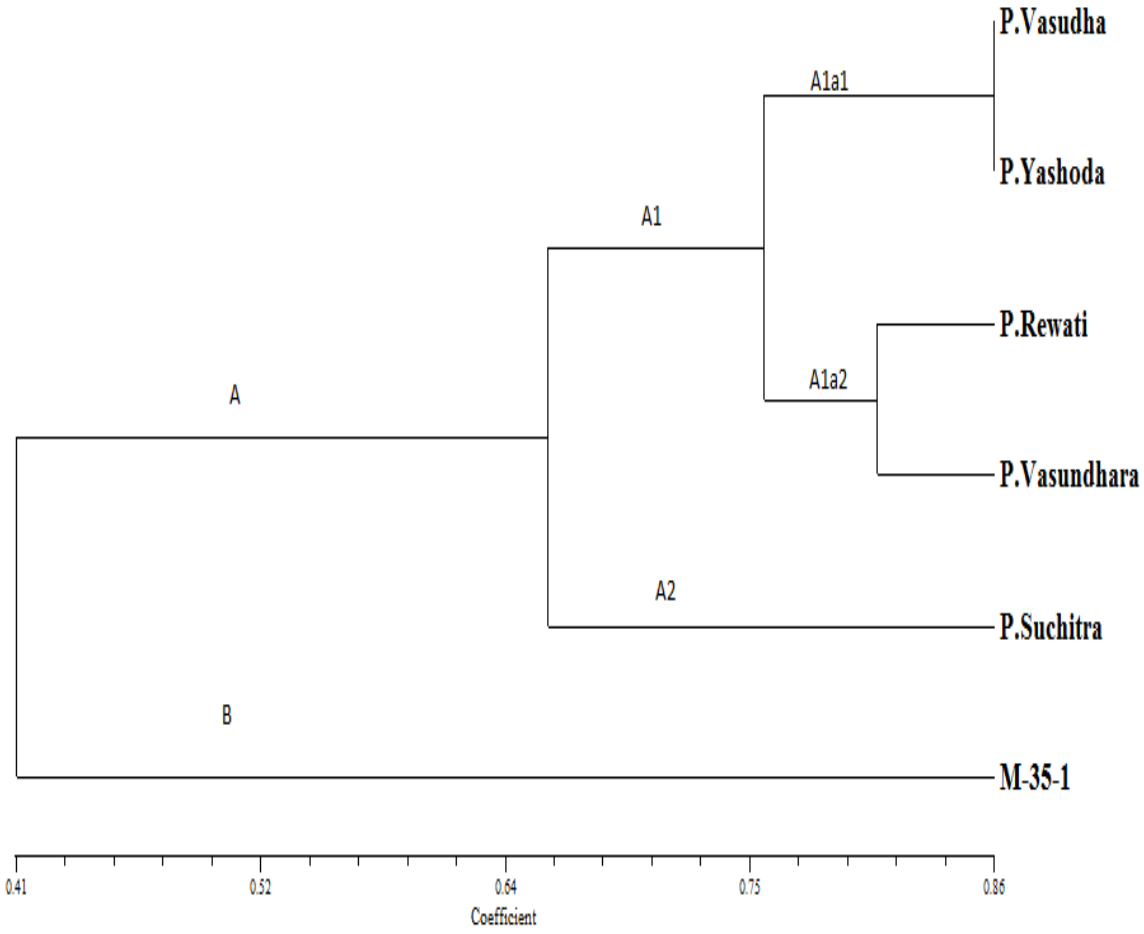
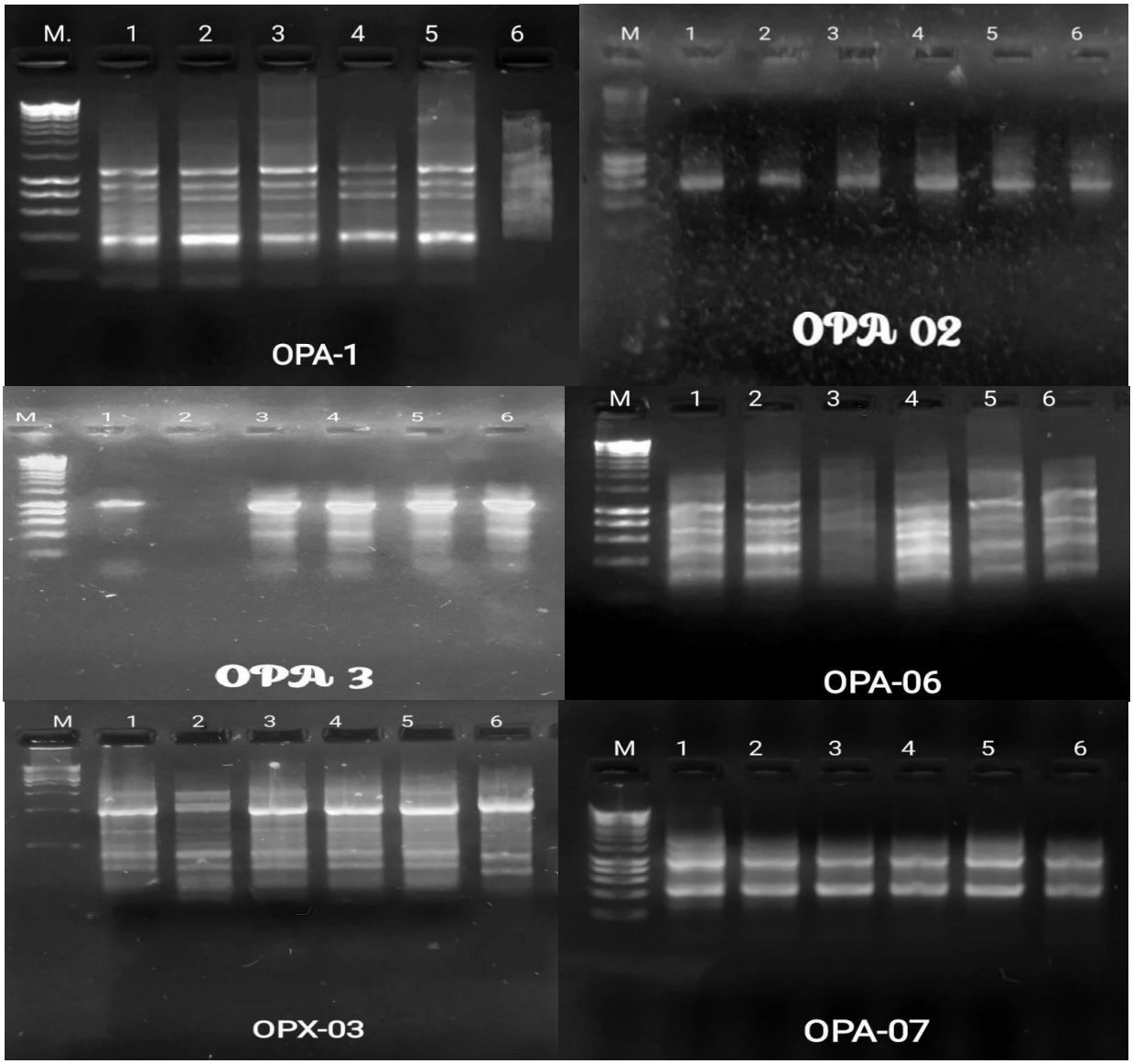


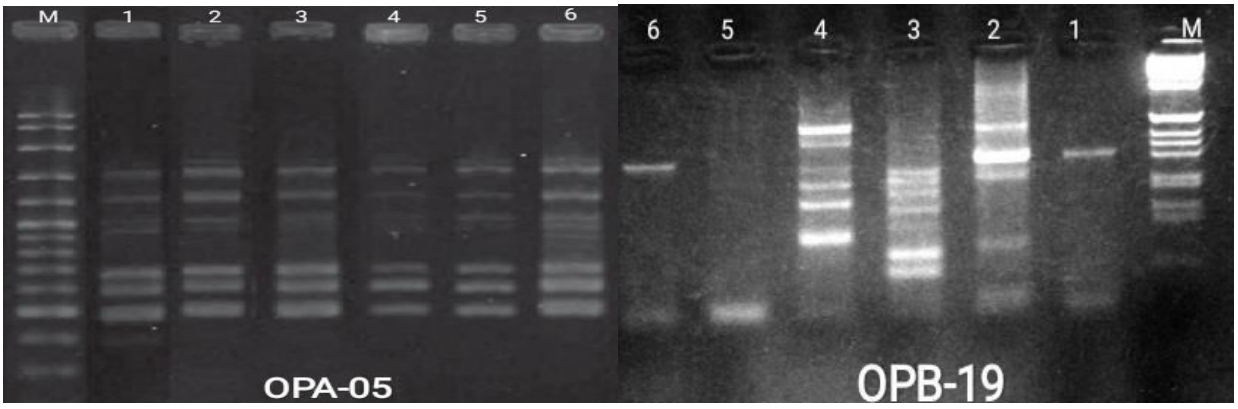
Table.1 Rabi Sorghum cultivars and their source:

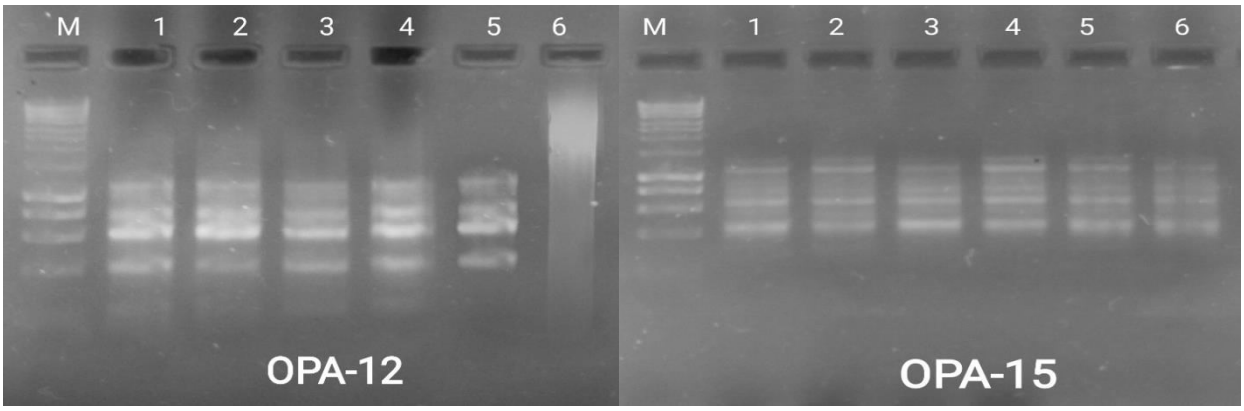
Sr. No.	Name of Cultivars	Source
1.	Phule Vasudha	All India Co-ordinated Sorghum Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahilyanagar (M.S.) India
2.	Phule Yashodha	
3.	Phule Suchitra	
4.	Phule Rewati	
5.	Phule Vasundhara	
6.	M-35-1 (Maldandi)	

Fig.2 RAPD marker profile *Rabi* sorghum cultivars



1. Phule Vasudha, 2. Phule Yashoda, 3. Phule Suchitra, 4. Phule Rewati 5. Phule Vasundhara, 6. M-35-1 (Maldandi); M=100bp ladder





1. Phule Vasudha, 2. Phule Yashoda, 3. Phule Suchitra, 4. Phule Rewati 5. Phule Vasundhara, 6. M-35-1 (Maldandi); M=100bp ladder

Table.2 Genomic DNA Quantification of *Rabi* Sorghum cultivars:

Sr. No.	Rabi Sorghum Cultivars	Concentration of DNA	Optical Density (260/280)
1.	Phule Vasudha	811.2	1.87
2.	Phule Yashodha	868.2	1.81
3.	Phule Suchitra	501.9	1.82
4.	Phule Rewati	322.3	1.73
5.	Phule Vasundhara	265.7	1.73
6.	M-35-1 (Maldandi)	581.3	1.81

Table.3 RAPD primers along with their sequences:

Sr. No.	(RAPD) Primers	Sequence (5' to 3'	Tm (^{O}C)
1.	OPA-01	CAGGCCCTTC	34
2.	OPA-02	TGCCGAGCTG	34
3.	OPA-03	AGTCAGCCAC	32
4.	OPA-05	AGGGGTCTTG	32
5.	OPA-06	GGTCCCTGAC	32
6.	0PA-07	GAAACGGGTG	32
7.	OPA-12	TCGGCGATAG	34
8.	OPA-15	TTCCGAACCC	34
9.	OPX-03	TGGCGCAGTG	32
10.	OPB-19	ACCCCCGAAG	34

Table.4 Temperature Profile for PCR Amplification.

Sr. No.	Steps	Temperature (^{O}C)	Duration (Min)	Number of Cycle
1.	Initial denaturation	94	10	1
2.	Denaturation	94	1	35
3.	Annealing	28-34	1	
4.	Extension	72	1	
5.	Final Extension	72	10	1
6.	Hold	4	-	-

Table.5 Components, Stock and Volume of PCR reaction mixture.

Components	Final Concentration	Volume for one tube (µl)
2 X PCR Master Mix	2X Master mix	7.5
Primer	16 Pmol	1.0
Grade water	----------	5.5
Template DNA	30ng/µl	1
Total	**-**	**15 µl**

Table.6 RAPD primers and polymorphic amplicons generated:

Sr. No.	Primer (RAPD)	Monomorphic Amplicons	Monomorphic (%)	Polymorphic Amplicoins	Polymorphic (%)	Total Amplicons	PIC Value
1.	OPA-01	-	-	6	100	6	0.40
2.	OPA-02	1	50	1	50	2	0.06
3.	OPA-03	-	-	2	100	2	0.13
4.	OPA-06	-	-	4	100	4	0.26
5.	OPX-03	3	60	2	40	5	0.13
6.	OPA-07	2	100	-	-	02	-
7.	OPA-05	5	83.33	1	16.67	06	0.07
8.	OPB-19	-	-	9	100	09	0.60
9.	OPA-12	-	-	4	100	04	0.27
10.	OPA-15	3	75	1	25	04	0.07
Total		**14**	**31.81**	**30**	**68.18**	**44**	-

Table.7 Jaccard's Binary similarity matrix for RAPD analysis:

	Phule Vasudha	Phule Yashodha	Phule Suchitra	Phule Rewati	Phule Vasundhara	M-35-1 (Maldandi)
Phule Vasudha	1.00					
Phule Yashodha	0.85	1.00				
Phule Suchitra	0.68	0.66	1.00			
Phule Rewati	0.65	0.78	0.69	1.00		
Phule Vasundhara	**0.94**	0.80	0.71	0.68	1.00	
M-35-1 (Maldandi)	0.72	0.61	0.52	**0.43**	0.66	1.00

Genetic diversity and variability tool for evaluating and determining cultivar's identity. Generations of new and improved *Rabi* sorghum cultivars can be enhanced by new sources of genetic variation; therefore, criteria for the need parental stock selection need to be considered not only by agronomic value, but also for genetic dissimilarity.

RAPD analysis showed good potentiality to determine phylogenetic relationships among six *Rabi* sorghum cultivars and the information about genetic similarity will be helpful to avoid any chance of elite germplasm becoming genetically uniform and endangering long term productivity gains during breeding programs. Germplasm and mutant lines demonstrated wide variability for most of the quantitative traits and can be utilised for developing new cultivars or used as parents in the recombination breeding (G Somu, N Meena, and Ashok Badigannavar 2025). Cultivars with the most distinct DNA profiles were likely to contain the greatest number of novel genes. To evaluate genetic distance between cultivars and identification of parents for

performing appropriate crosses to the reaching maximum heterosis in hybridization programs.

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Author Contributions

Amit T. Adsul: Conceptualization, Methodology, Investigation, Formal analysis, Writing -Original Draft & Editing

Consent to Publish Declaration

All authors have full consent to publish this article.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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