

Research Article

Molecular Markers in determining Phylogenetic relationship in Foxtail Millet (*Setaria italica* (L.) P. Beauv.) Var. Co(Te)7

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Abstract: The degree of polymorphism in seven different mutants were assessed along with untreated population, control in *Setaria italica* var. Co(Te)7. The mutants identified in M₂ generation were selected for RAPD analysis to examine the genetic variation between them. The mutant like multiple branched panicle, long Panicle, tall plant, anthocyanin pigmentation in panicle, thumb branched panicle, bold seed, tiller were chosen to compare the banding pattern and distinguishing to evaluate their phylogenetic relationship. The primers chosen for their amplification were OPC11, OPA05, OPA08 and OPA13. All the mutants showed the polymorphism depicting genetic diversity and phylogenetic relationship in the foxtail millet var. Co(Te)7. Due to the exclusive design of genetic background, therapeutic potential, nutritional benefits and the traits of agronomic values like the need for cultivation methodology in Co(Te)7 variety in foxtail millet. The dendrogram constructed based on UPGMA clustering method revealed the diverse genotype between the essential mutants utilized for increased crop improvement program.

Keywords: *Setaria italica*, M₂ generation, RAPD, Primers, Mutants.

1. Introduction

Foxtail millet, (*Setaria italica* (L.) P. Beauv.) var. Co(Te)7 is a member of the subfamily Panicoideae with the chromosome number of $2n=2x=18(AA)$. It is an important ancient crop of dryland agriculture, grown since >10,500 years ago in China [1]. Mutation breeding a much-heralded shortcut breeding technique experimented with mutagens in stabilizing the phenotype to produce variability in crop improvement [2]. Breeders use mutation induction to widen the genetic base of germplasm and avail their lineage as a source of raw material in plant breeding programs. Induced mutagenesis in *Setaria italica* quenches the thrust area in multifaceted approaches in sustainable crop breeding. Foxtail millet is used to make different food recipes such as roti (Bread), jaula, singal. Jaula made from foxtail millet has a special medicinal value for chicken pox patients. Various detrimental features and intricate phenomenon along with the complicated regulatory gene networking thrived in *Setaria italica* proved to be an interesting model plant for genomic studies [3]. Foxtail millet has very low genetic diversity which hinders in crop research program [4,5]. Henceforth it is essential for a look to detect the beneficial traits for the betterment of the millet

research. The incredible character stress bearing property to withstand heavy drought and salinity [6,7] were well suited to thrive *Setaria italica* in dryland areas in south in regional locations. Analyzing the therapeutic potentiality of *Setaria italica* in low glycemic index [8], high dietary fiber and protein [9] contains antioxidant [10], prolamine content [11] all types of dishes produced out of foxtail millet were effective in reducing blood glucose level in both normal and diabetic patients [12], therefore, the need and curiosity of improving this plant for both aliment of disease and for agriculture importance is indispensably necessary. Characterizing this valuable crop in the germplasm for the genetic improvement will establish intensive sustainable farming and food security for the huge population with requirement in a balanced diet. Genetic variation has led to an increase in the quantitative trait of crop, the variability on genome is induced by mutation enhancing productivity [13]. The present investigation with RAPD technique found to be more successful in identifying polymorphism in foxtail millet [14]. The results obtained from RAPD analysis is based on the PCR product showing gene variation at DNA level [15]. Thus the research promoted in screening and locating

the genetic diversity of seven different mutants in foxtail millet is achieved by using RAPD as marker.

2. Materials and Methods

2.1 Collection of plant materials

The seeds of *Setaria italica* var. Co(Te)7 were collected from TNAU Coimbatore. The morphological mutants isolated from M₂ generations were allowed to raise M₃ generations. The mutants selected were tall plant, long panicle, thumb branch panicle, multiple branched panicle, anthocyanin pigmentation in panicle, bold seed, tiller along with the control. The seedlings grow up to three leafy stages were used for the isolation of DNA.

2.2 DNA isolation

Total genomic DNA was isolated from selected mutants and DNA isolation carried out by using CTAB method [16]. 1g of mutant and leaf was ground in CTAB buffer (120mM Tris, pH-8.0, 1.3M NaCl, 25mM EDTA pH-8.0, 0.2% β-mercaptoethanol, 2% CTAB), incubated at 65°C for 30 minutes. The extract was purified with 24:1 mixture of chloroform-isoamyl alcohol before precipitation of DNA with double volume of isopropanol. The precipitate was spooled out; the precipitate was dissolved in 1ml of TE buffer with RNase then followed by addition of 70% ethanol, sodium acetate and dissolved with TE buffer.

2.3 Optimization of RAPD reaction

A total of ten RAPD primers (Operon Technologies (OT), Eurofins, Bangalore) were used. DNA amplification was performed based on Williams's methodology [17]. Polymerase chain reaction was performed in a PCR mixture of 20μl having composition 10mM Tris HCl (pH-8.3), 2.5mM MgCl₂, 25mM dNTPs mix, 0.2μM of each primer, 10x Taq buffer, 1 U of Taq DNA Polymerase and 50ng of template DNA. RAPD-PCR was performed in Mastercycler nexus (Eppendorf) for 35 cycles consisting of denaturation at 94°C for 45 sec, annealing at 36°C for 50 sec, and extension at 72°C for 60 sec.

The final extension was carried out at the same temperature for 10 min and holds the temperature at 4°C at the end. The PCR amplified products were electrophoresed on 1.2% (w/v) agarose gels, in 1x TAE buffer at 65 V for 3 hrs and then stained with ethidium bromide (0.5μg/ml). Gels with amplification fragments were visualized and photographed under UV gel documentation system (Alpha Innotech). DNA is classified with the reference to their bandwidth and length compared to their markers and control lane along with the seven mutants to identify the phylogenetic relationship using the software UPGMA.

3. Results

The mutants selected are tall plant, long panicle, multiple branched panicle, thumb branched panicle, bold grain, anthocyanin pigmentation in panicle, tiller along with control plant. RAPD technique was used to detect polymorphism in the base pair of DNA among the mutants. Ten primers were used to screen the polymorphism. Total number of 111 bands scored for which 66 polymorphic and 45 monomorphic bands were obtained. Out of four primers (OPC11, OPA05, OPA08 and OPA13) were amplified in a good manner in all the samples used for the studies (Table 1) and (Fig. 1, 2, 3 and 4).

The eight genotypes were used to construct a Jaccard coefficient based on the similarity matrix, revealing a genetic distance varying from 0.368 – 0.650. Based on the dendrogram constructed by Jaccard coefficient similarity in *Setaria italica* mutants indicated two main clusters one comparing tall plant and other main cluster subdivided into three major clusters of traits bold grain, long panicle, anthocyanin pigmentation in the panicle, one of the major cluster further divided into three minor clusters representing the traits thumb branched panicle, control, tiller and multiple branched panicle. Thus the result explains a wide variation in the tall plant to that of the control other mutants show a less variation to that of the control (Fig. 5).

Table 1: Analysis of RAPD banding pattern for *Setaria italica* Var. Co(Te)7.

S. No.	Primer name	Primer Sequence (5'-3')	Total number of bands	Number of Polymorphic bands	Number of monomorphic bands	% of polymorphic bands	GC content (%)
1	OPC11	AAAGCTGCCG	14	12	02	85.71	70
2	OPA05	AGGGGTCTTG	15	11	04	73.33	60
3	OPA08	GTGACGTAGG	12	08	04	66.66	60
4	OPA13	CAGCACCCAC	16	10	06	62.50	70
5	OPA04	AATCGGGCTG	10	05	05	50.00	60
6	OPM10	TCTGGCGCAC	11	06	05	54.54	70
7	OPD02	GGACCCAACC	09	05	04	55.55	60
8	OPO12	CAGTGTGTG	07	03	04	42.85	70
9	OPC05	GATGACCGCC	08	02	06	25.00	60
10	OPG11	TGCCGTCGT	09	04	05	44.44	70
Total			111	66	45	560.58	650

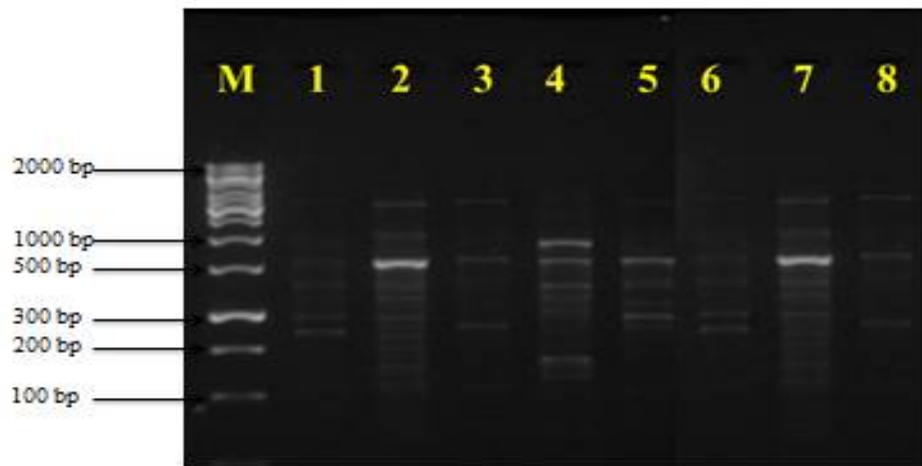


Fig. 1: Electrophoretic banding pattern of 8 *Setaria italica* genotypes amplified with RAPD primer OPC11.

M - 2 kb DNA Ladder; 1 – Control; 2 - Tall Plant; 3 - Long Panicle; 4 - Multiple Branched Panicle; 5 - Thumb Branched Panicle; 6 - Bold grain; 7 - Anthocyanin Pigment in Panicle; 8 – Tiller.

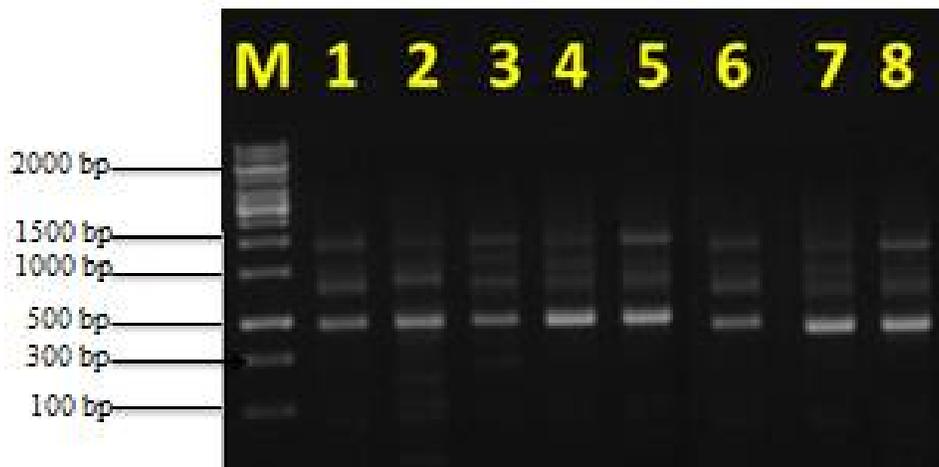


Fig. 2: Electrophoretic banding pattern of 8 *Setaria italica* genotypes amplified with RAPD primer OPA05.

M - 2 kb DNA Ladder; 1 – Control; 2 - Tall Plant; 3 - Long Panicle; 4 - Multiple Branched Panicle; 5 - Thumb Branched Panicle; 6 - Bold grain; 7 - Anthocyanin Pigment in Panicle; 8 – Tiller.

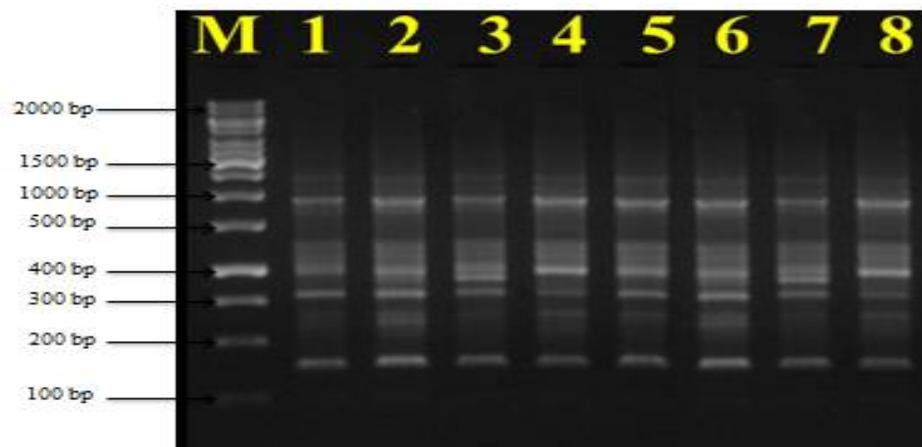


Fig. 3: Electrophoretic banding pattern of 8 *Setaria italica* genotypes amplified with RAPD primer OPA08.

M - 2 kb DNA Ladder; 1 – Control; 2 - Tall Plant; 3 - Long Panicle; 4 - Multiple Branched Panicle; 5 - Thumb Branched Panicle; 6 - Bold grain; 7 - Anthocyanin Pigment in Panicle; 8 – Tiller.

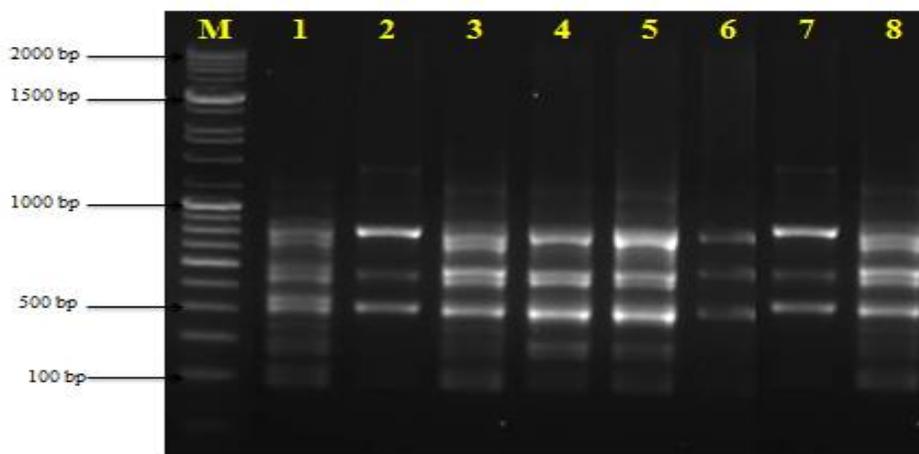


Fig. 4: Electrophoretic banding pattern of 8 *Setaria italica* genotypes amplified with RAPD primer OPA13.

M - 2 kb DNA Ladder; 1 – Control; 2 - Tall Plant; 3 - Long Panicle; 4 - Multiple Branched Panicle; 5 - Thumb Branched Panicle; 6 - Bold grain; 7 - Anthocyanin Pigment in Panicle; 8 – Tiller.

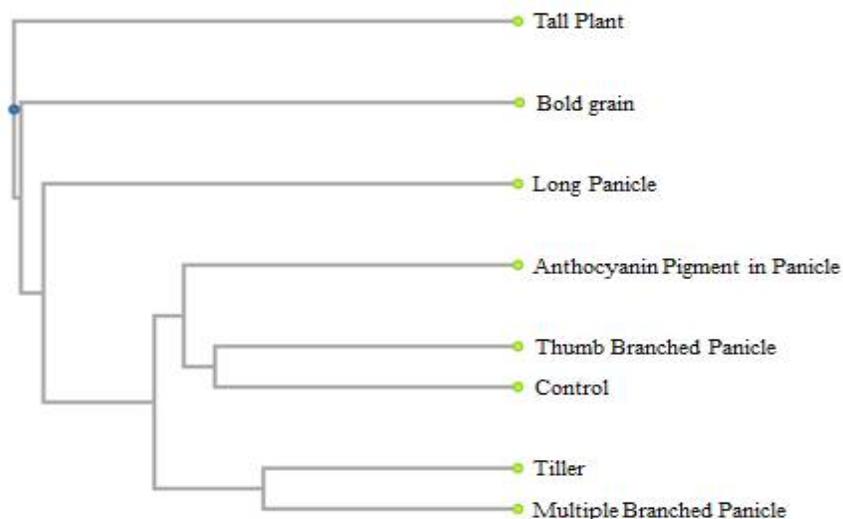


Fig. 5: Dendrogram generated among *Setaria italica* genotypes using UPGMA cluster analysis based on RAPD data matrix.

4. Discussion

The core research work is scrutinized to establish an efficient amplification of the primers with the better reproducible amplicon with the primers by RAPD [18]. The size and the number of bands produced were totally controlled by the nucleotide sequence. The simple and quick sample processing of RAPD made it to be an efficient technique to assess genetic diversity between mutants [19]. The base substitution in the primer binding site due to indels in the base sequence at the site of the polymorphic DNA [20]. However, four primers responded to produce amplicon in *Setaria italica* var. Co(Te)7 (OPC11, OPA05, OPA08, OPA13). Among the primer, monomorphic and polymorphic bands were obtained. The results obtained with this technique confirm the existence of six distinct genetic groups closely related to seven mutant appear to

be linked in their structure and function. The study is more predominant using the RAPD for determining various accessions [21] were correlated with that of the results obtained in *Setaria italica*. The disappearance of some bands and variation shown in band intensity may correlate with the effect of mutagen [22]. Appearance of new bands can be explained as a result of different DNA structural changes (breaks, transpositions, deletions etc.). Therefore the existence of mutation and structural alterations in plant DNA after the impact of EMS and DES on the bases of DNA patterns could be obtained after RAPD markers with the set of primers. A similar opinion was reported by Wachira *et al.*, (1995). Arulbalachandran *et al.*, (2009) also reported the polymorphism revealed by RAPD due to deletion and/or addition may be caused by variation in DNA binding pattern by gamma rays and EMS. The findings of this study are in agreement with the earlier

conclusions that despite the presence of high morphological variations, discrete grouping can be tied up together [23,24]. Jaccard coefficient reveals the wide variation between tall plant than control and close relationship between other mutants of *Setaria italica*.

5. Conclusion

The RAPD analysis in foxtail millet Var. Co(Te)7 clearly revealed the genetic variation in the polymorphic DNA of all the mutants through flexible DNA banding pattern predominantly in the four primers OPC11, OPA08, OPA05, OPA13. The results of RAPD imply the mutants have slight variation with each other than control. The phylogenetic relationship among the mutants is established by dendrogram augment the close association of similar and dissimilar gene among the mutant, the result concluded the raising of mutants of foxtail millet towards the attribution of yield traits in crop improvement.

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