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Genetic Variation in Mutants of Finger Millet (Eleusine Coracana (L.) Gaertn.) Var- Co 13 Evaluated by Rapd Markers

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ABSTRACT

The present study was under taken in order to analyze the chemical mutagenesis on finger millet germplasm. In this regard, Co-13 Variety of Finger millet was subjected to different mutagenic concentration for inducing mutagenesis. The M4 plants exposed to EMS and DES to produce clear difference from the control, thus indicating that mutagenic treatment produce polymorphic regions in the Finger millet. For extraction of genomic DNA was adopted an improved protocol of CTAB method with slight modification. A total of ten primers were used to screen the polymorphism among the treated populations line tall mutant, dwarf mutant, early maturity mutant, seed colour mutant and high yielding mutant were analyzed with control. Out of ten primers, five primers (OPC04, OPC08, OPA13, OPC12 and OPD08) were successfully amplified in all the samples used for this study. The successful primers were amplified in to 145 products showing an average of 14.5 bands.

Keywords : Induced Mutation, Finger Millet, EMS, DES, RAPD Analysis.

I. INTRODUCTION

Eleusine coracana, commonly called finger millet, is an annual allotetraploid (2n=4x=36; genome constitution AABB) cultivated plant that belongs to the grass family Poaceae, subfamily Chloridoideae. There are about nine species under the genus, Eleusine Gaertn. Two species, Eleusine indica and Eleusine floccifolia, are believed to be the genome donors to the cultivated species, E. coracana (Bisht and Mukai, 2001). It is extensively cultivated in the tropical and sub-tropical regions of Africa and India and is known to save the lives of poor farmers from starvation at times of extreme drought (Kotschi, 2006). It is adapted to a wide range of environments and grown mainly by subsistence farmers. Finger millet serves as a food security crop because of its high nutritional value, excellent storage qualities and its importance as a low input crop (Dida et al., 2007). Finger millet plays an important role in both the dietary needs and incomes of many rural households like other African countries due to its richness in fiber, iron and calcium (Babu et al., 2007).

The induced mutations are caused artificially by mutagenic factors. The agents that induce mutations are called mutagens and it mainly consists of two different kinds; physical (radiation) and certain chemical mutagens. Mutagens are not only beneficial to create genetic variability in a crop species, but also useful for the effective control of pests during post-harvest storage (Chaudhuri, 2002). Use of ionizing radiations, such as X-rays, gamma rays, neutrons and chemical mutagens for inducing genetic variation is well established (Micochova et al., 2004). In agriculture, mutation breeding is accomplished by chemical or physical treatment followed by selection of heritable changes in specific phenotypes and this method has been used successfully in the genetic improvement of crop plants (Micke et al., 1985).

RAPD is a fast and sensitive method and is able to provide reproducible and characteristic fingerprints of complex genomes without prior sequence information. The use of short primers of arbitrary sequence during PCR results in amplification of different segments of genomic DNA, which after gel electrophoresis gives characteristic band patterns. Most informative DNA bands on RAPD are usually of the 300- 3000bp range. RAPD provides a cost-effective method for the precise and routine evaluation of variability. It may also be used to identify areas of maximum diversity. Thus molecular technique provides a powerful tool for the study of plant population genetics. RAPD has been used for genetic fingerprinting creating linkage maps (Rafalski et al., 1991) locating disease resistance genes (Martin et al.,1991); (Michelmore et al.,1991) identifying chromosome-specific markers (Quiros et al.,1991) and characterization of somatic hybrids (Baird et al.,1992).

In the past few years, the RAPD technique has been widely accepted in labs throughout the world. Many agronomically important species have been analyzed by this technique. This technique helps to identify a large number of markers which can be used for estimating genetic variation (Powell et al., 1991).

II. MATERIAL AND METHODS

The seeds of Finger millet (Eleusine coracana (L.) Gaertn.) Var- Co 13. Varieties collected from Tamilnadu Agricultural Research Institute Villupuram. Was used for the present study. The healthy seeds treated with various concentrations of chemical mutagens.

EMS ($CH_3SO_2OC_2H_5$), an alkylating agent having molecular weight 124.16 was used in the present study. For the treatment of EMS, the seeds were pre-soaked in distilled water for 6 hours in order to make them relatively more sensitive to mutagenic action. Pre soaked seeds were treated with different concentrations of EMS (10, 20, 30, 40 and 50mM) for 4hours with repeated stirring. After the chemical treatment, the treated seeds were washed throughly in running tap water to remove the residues of the chemicals. Healthy, well- matured and untreated seeds were used as control.

Seeds of Finger millet were subjected to different treatment levels (20, 30, 40, 50 and 60mM) of Diethyl sulphate for induced mutagenesis. Before treatment, seeds were pre-soaked in distilled water for 12hrs at room temperature. Later on these seeds were dried on filter paper. All seeds were uniformly exposed to Diethyl sulphate solution by stirring with a glass rod. After treatment seeds were rinsed thoroughly with distilled water, air-dried and stored for further studies. The treated seeds were sown in seed beds along with control. After 30 days, old seedlings were transplanted to experimental field in Completely Randomized Block Designs with three replicates to raise M_1 population. The M_1 generation (produced directly from mutagen treated seeds) was grown in the seed bed culture experiment at the Botanical Garden, Department of Botany, Annamalai University. All the recommended cultural practices were carried out during the plant growth period. The M_2 generation (produced directly from M_1 seeds, M_3 generation produced directly from M_2 seeds and M_4 generation produced directly from M_3 seeds) was grown in the field experiment.

III. RESULT

Five Finger millet Var-Co 13 mutants were subjected to RAPD assay to analyze for identifying DNA polymorphism. Ten random decamer primers revealed a high DNA polymorphism among the mutant populations like tall, dwarf, yearly maturity, seed colour and high yielding mutant were analyzed. A total of 145 bands scored of which 100 bands were polymorphic with an average of 67.97 per centage polymorphism. Only four primers (OPC04, OPC08, OPA13 and OPC12) showed

highest polymorphism and the primer OPC05 gave the lowest polymorphism (55.55%) (Table-1). The Jaccard's coefficient similarity varied from 0.556 to 0.718 (Table-2).

Based on the dendrogram constructed by Jaccard's coefficient similarity in Finger millet Var-Co 13 These results indicated that a moderate to a high extent of genetic polymorphism was observed between the control plant and the mutants used for the analysis. Dendrogram analysis using UPGMA method exhibited clustering of all the mutants and the control plant mutants indicated two clusters; one comparing control and dwarf mutant. The second cluster consists of early maturity mutant, tall mutant, seed colour mutant and high yielding mutant. The high yielding mutant has lowest similarity with more variation from other mutant characters (Table-2; Fig. 1).

IV. DISCUSSION

Random Amplified Polymorphic DNA analysis is suitable for genotyping, phylogenetic analysis and molecular selection (Williams et al., 1990; Gokturk et al., 2003; Atak et al., 2004; Akcicek et al., 2005; Yuzbasioglu et al., 2006). RAPD among other molecular marker methods has considerable advantages because it is fast, less expensive, applicable to any plants without prior information on the nucleotide sequence and in the potential detection of DNA damage and mutation (Ahloowalia and Maluszynski, 2001; Atienzar et al., 2002). It has been widely used in the phylogenetic analysis of many plants and a general concordance was demonstrated among the results derived from RAPD and other techniques (Naugzemys et al., 2007). The presence or absence of RAPD bands are used to estimate diversity and in measurement of similarity (Upadhya et al., 2004; Atienzar and Jha, 2006; Wang et al., 2009).

In the present investigation, the RAPD analysis of the M_4 generation plants exposed to EMS and DES produce clear difference from the untreated control, thus indicating that mutagenic treatments produce polymorphic regions in the Finger millet mutant. This may be due to the reason that the variation might be stabilized upon consecutive generations.

According to Danylchenko and Sorochinsky (2005), RAPD molecular marker method is applicable for the detection of changes in the DNA structure after different genotoxical treatments. The variation in band intensity and disappearance of some bands may correlate with the level of photoproducts in DNA template after treatment, which can reduce the number of binding sites for Taq polymerase. Appearance of new bands in some cases can be explained as a result of different DNA structural changes (breaks, transpositions, deletions etc.). Thus, the estimate on the existence of mutation and structural alterations in plant DNA after impact of treatment 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 EMS and DES.

Hegazi and Hamideldin (2010) observed that the changes in the DNA bands, where the main changes in the RAPD profiles of the appearance or disappearance of different bands with variation in their intensity. These effects might be due to the structural rearrangements in DNA caused by different types of DNA damages in Abelmoschus.

A total of ten primers were used to screen the polymorphism between the mutants and control. It revealed a high DNA polymorphism among the treated populations like tall, dwarf, early maturity, seed colour and high yielding mutants were analysed with control. The DNA fragments with different molecular weights were amplified and the product

size ranged between 250 – 6000 bp. Out of ten primers, four primers have successfully amplified in all the samples used for the study. These four successful primers have amplified products showing an average of bands.

In the present investigation, the RAPD analysis of the M_4 generation plants exposed to EMS and DES treatments produce clear difference from the mutant and untreated control, thus indicating that mutagenic treatments produce polymorphic regions in the finger millet mutant. Similar varied levels of polymorphism have been reported in chemical mutagens and combination of both treated seeds by Ashok Kumar and Ponnuswami (2010) in paprika, Subramanian et al., (2000) in groundnut, Mullainathan et al., (2014) in Chilli and Thilagavathi (2011) in Black gram.

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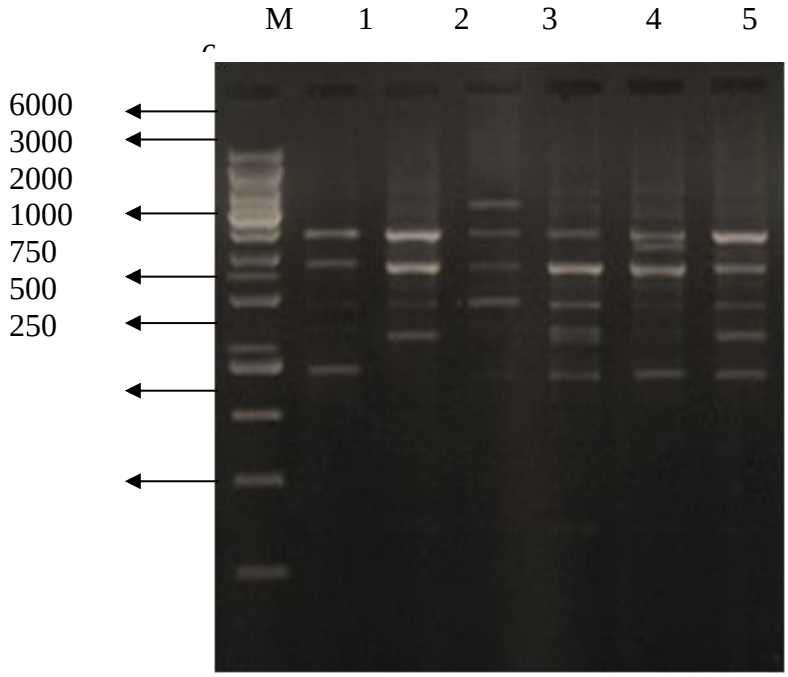
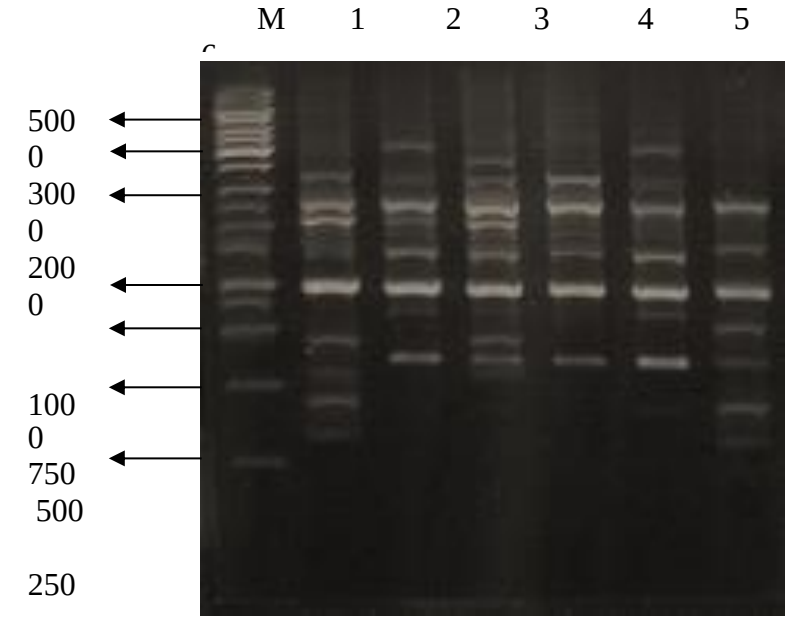
1) **PLATE 1**

Gel electrophoresis showing PCR profiles of amplified DNA from control and mutants using primer-OPC04



Lane1- Marker, Lane 2-Control; Lane 3- Tall mutant, Lane 4- Dwarf mutant, Lane 5- Early maturity mutant, Lane 6-Seed colour mutant and Lane 7- High yielding mutant

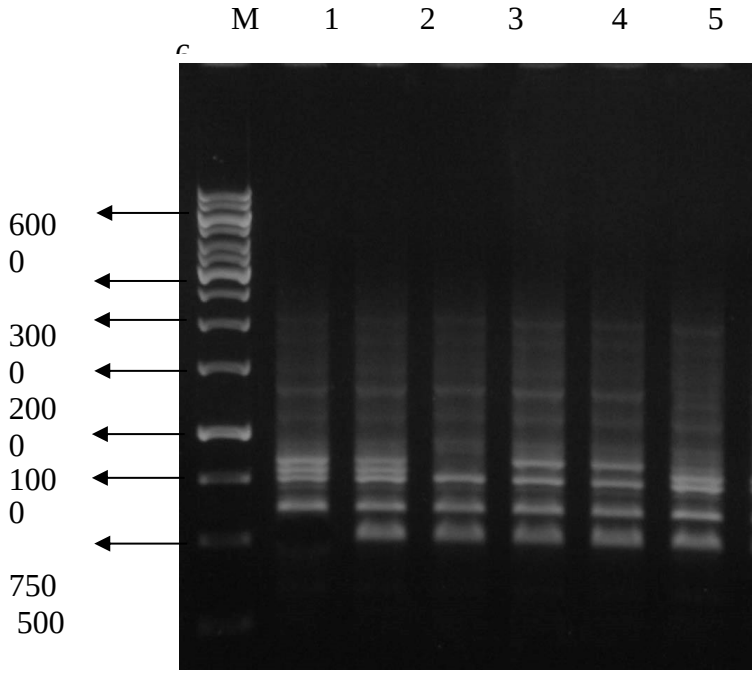
Gel electrophoresis showing PCR profiles of amplified DNA from control and mutants using primer-OPC08



Lane1- Marker, Lane 2-Control; Lane 3- Tall mutant, Lane 4- Dwarf mutant, Lane 5- Early maturity mutant, Lane 6-Seed colour mutant and Lane 7- High yielding mutant

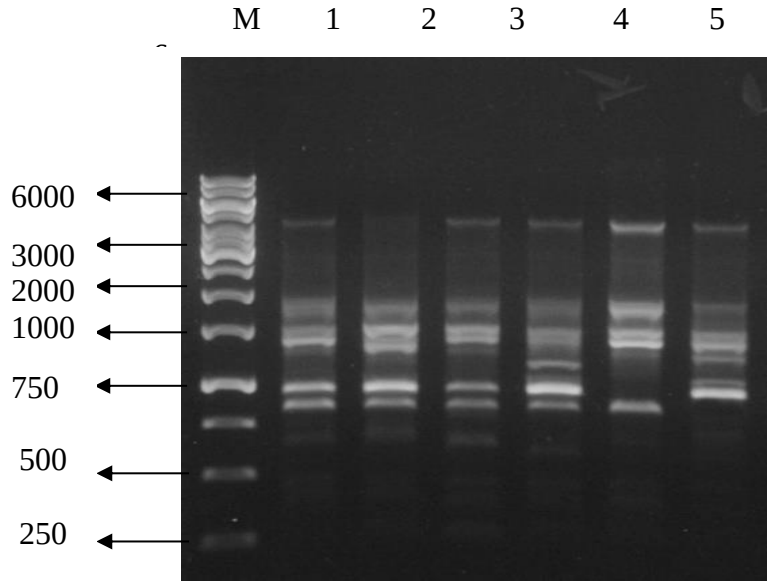
2) **PLATE 2**

Gel electrophoresis showing PCR profiles of amplified DNA from control and mutants using primer-OPA13



Lane1- Marker, Lane 2-Control; Lane 3- Tall mutant, Lane 4- Dwarf mutant, Lane 5- Early maturity mutant, Lane 6-Seed colour mutant and Lane 7- High yielding mutant

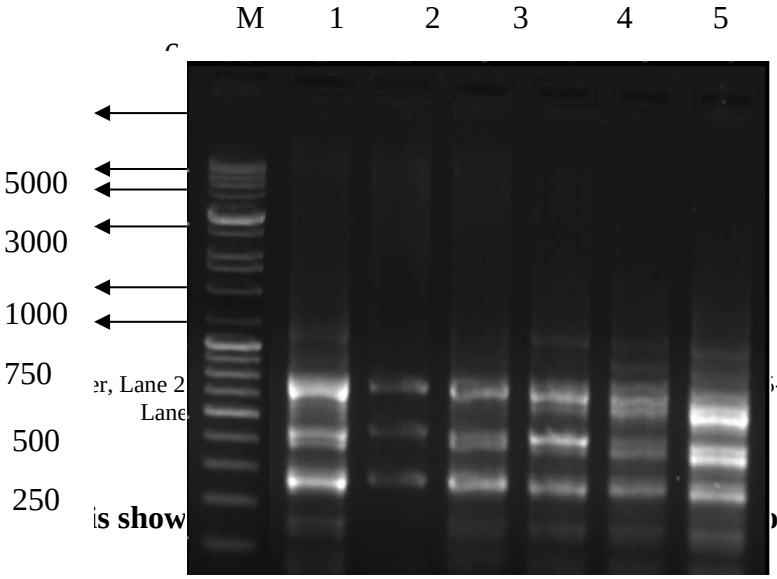
Gel electrophoresis showing PCR profiles of amplified DNA from control and mutants using primer-OPC12



Lane1- Marker, Lane 2-Control; Lane 3- Tall mutant, Lane 4- Dwarf mutant, Lane 5- Early maturity mutant, Lane 6-Seed colour mutant and Lane 7- High yielding mutant

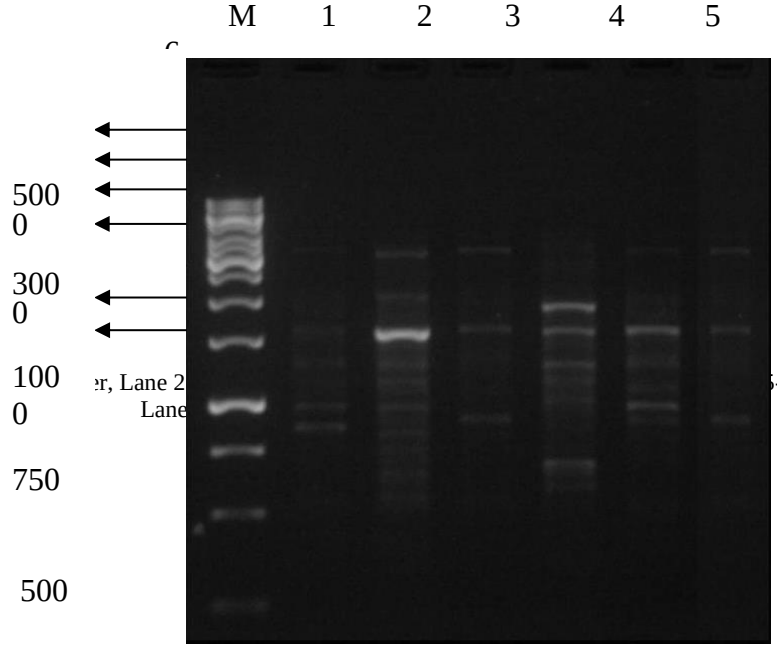
3) **PLATE 3**

Gel electrophoresis showing PCR profiles of amplified DNA from control and mutants using primer-OPD08



La er, Lane 2 - Early maturity mutant, Lane

Gel electr is show ontrol and mutants



Lar er, Lane 2 - Early maturity mutant, Lane

Table-1. Analysis of RAPD banding pattern for Finger millet mutants isolated from M_4 generation

S. No	Primer Code	Sequence 5 'to 3'	Total number of bands	Polymorphic bands	Monomorphic bands	Percentage of polymorphism
1	OPA-04	5'-AATCGGGCTG-3'	11	7	4	63.63

2	OPA-13	5'-CAGCACCCAC-3'	18	13	5	72.22
3	OPC-04	5'-CCGCATCTAC-3'	10	8	2	80.00
4	OPC-05	5'-GATGACCGCC-3'	9	5	4	55.55
5	OPC-08	5'-TGGACCGGTG-3'	23	17	6	73.91
6	OPC-12	5'-TGTCATCCCC-3'	21	15	6	71.42
8	OPC-18	5'-TGAGTGGGTG-3'	9	6	3	66.67
7	OPD-08	5'-GTGTGCCCCA-3'	17	12	5	70.58
9	OPN-03	5'-GGTACTCCCC-3'	14	9	4	64.28
10	OPN-08	5'-ACCTCAGCTC-3'	13	8	5	61.53
Total			**145**	**100**	**44**	**679.79**
Average			**14.5**	**10**	**4.4**	**67.97**

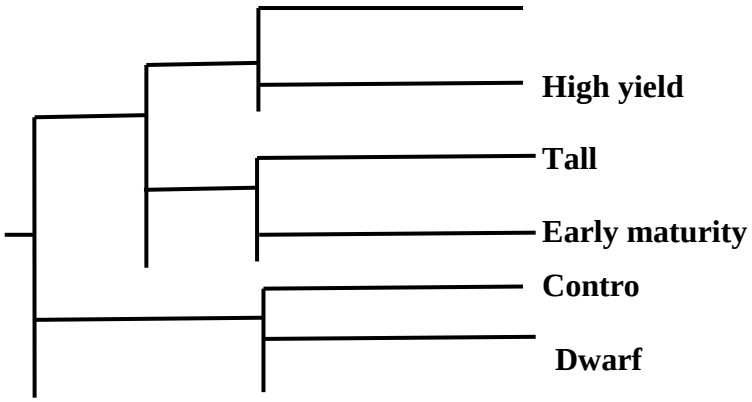
Table-2. Similarity matrix based on Jaccard's coefficient of six mutants and control (Finger millet Var-Co 13)

	Ctrl	**TM**	**DM**	**EMM**	**SCM**	**HYM**
Ctrl	1.000	0.556	0.625	0.523	0.610	0.634
TM		1.000	0.643	0.690	0.667	0.614
DM			1.000	0.571	0.585	0.571
EMM				1.000	0.634	0.659
SCM					1.000	0.718

HYM						1.000

Ctrl – Control; TM-Tall mutant; DM- Dwarf mutant; EMM- Early maturity mutant; SCM- Seed colour mutant and HYM- High yield mutant

Fig. 1: Dendrogram analysis of five mutants and control in finger millet



Cophenetic Correlation Coefficient (CP) = 0.81407