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Assessment of Genetic Diversity among the Promising Mutants of Finger Millet (*Eleusine coracana* L. Gaertn.) by using ISSR Markers

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ABSTRACT

Ten promising cultures from M_5 generation were evaluated for the genetic variation in finger millet (Eleusine coracana L. Gaertn.) mutants using Inter Simple Sequence Repeat (ISSR) markers. Ten primers showing reliable polymorphism were used to produce a total of 283 bands of which 206 (72.79 %) were polymorphic. The size range of bands was 360-1450vbp. The overall range of the similarity among 10 genotypes was found to be very wide ranging from 0.281 to 0.889 which indicates there was high variability among the finger millet mutants under study. The dendrogram constructed using the UPGMA method separated the mutant cultures in two main groups each having two sub groups. The study also demonstrates high reliability, ease of applicability and importance of ISSR markers in evaluating genetic variation among finger millet mutants.

Key words: *Finger millet, Variability, ISSR markers, Genetic variability.*

INTRODUCTION

Millet is a general category for several species of small grained cereal crops and is a staple food in various parts of India, Africa, China and elsewhere. Millet has been cultivated since prehistoric times in regions of North Africa and Central Asia, though its origin is ambiguous. Most of the millets are produced in Asia and Africa. According to De Candolle (1986), finger millet (*Eleusine coracana* L. gaertn.) probably originated in India as many of the forms exists in this country. Genetic improvement in this crop has been restricted through pureline selection in land races. Recombination breeding has not been practiced extensively in the crop due to inherent difficulties of emasculation and hybridization, owing to tiny florets closely packed in spike. Since in many species mutagenesis has been found to be as effective as hybridization in creating genetic variability in different traits, proper selection procedure would be effective in identification of superior mutant lines which can be released as varieties for cultivation. Similarly, detection of point mutation at phenotypic level is very difficult and there is chances of loosing desirable mutant lines.

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Molecular markers have proven to be powerful tools in the assessment of genetic variation and in the elucidation of genetic relationships within and among the species. Several reports are available assessing the genetic diversity in finger millet using DNA based molecular markers namely RAPD (Fakrudin *et al.,* 2004) and ISSR (Prabhu and Ganesan, 2013). The ISSR markers are multilocus, more reproducible, provide highly polymorphic fingerprints, are easy to develop in large numbers, have a simple assay, and are random, so that no prior sequence information is required, due to these advantages; they are effective for genetic variability analysis, fingerprinting and genome mapping. Hence in the present study ISSR markers were used to assess the genetic variability among the promising mutants of finger millet.

MATERIAL AND METHODS

The DNA was isolated from the 10 promising mutants and parent variety, Dapoli-1 of finger millet by rapid DNA extraction method following the protocol of Doyle and Doyle (1990) with slight modifications of buffer composition and concentration. The quality and quantity of DNA was ascertained through agarose gel electrophoresis with standard DNA i.e. uncut lamda Hind III DNA on 1% agarose gel and by comparison of the intensity of staining with ethidium bromide. The master mix was prepared by using 35 ng DNA template, 10X PCR buffer, 15 mM $MgCl_2$, 10 mM dNTPs, 5 PMol ISSR primers and 3 U Taq polymerase enzyme. A total of 10 ISSR primers were used for genetic diversity analysis in promising mutant lines of finger millet (Table 1). PCR was performed in master thermal cycler under desired temperature condition and amplified products were separated by 2% agarose gel at 80V in 1X TAE buffer. Gels were stained in ethidium bromide solution and visualized under UV light using the gel documentation system (Uvi Tech, Fire reader, Cambridge, England) and saved in computer for further analysis. Markers were scored for the presence (1) or absence (0) of the corresponding band among the mutants. A pairwise similarity index (SI) was calculated and the UPGMA based dendrogram (Fig. 1) of 10 mutants and control parent was generated with Multivariate Statistical Package (MVSP).

RESULTS AND DISCUSSION

Marker analysis helps to understand the genetic makeup of the accessions and also make it possible to analyze the global organization of genetic diversity within a species. The ISSR pattern of genomic DNA of 10 mutants and one control parent (Dapoli-1) were analyzed with respect to the fragments, informativeness of the markers and polymorphism for the assessment of genetic diversity present among the mutants. For the present study 10 ISSR primers were used for molecular characterization and to assess genetic diversity. A total of 211 scorable DNA fragments were produced and among them 206 DNA fragments were found to be polymorphic in the finger millet mutants and its control parent. The primers produced high degree of polymorphism with an average of 72.79 per cent. Average 28.3 bands per primer were amplified. Among the 10 generic primers UBC-811, UBC-824, UBC-878, UBC-879 revealed 100 per cent polymorphism with the maximum number of DNA fragments. The percentage of polymorphism across the finger millet genotypes ranged from 38.89-100 per cent. Similar results were obtained by Kumari and Pande (2010) which indicated that the per cent polymorphism ranged from 6.6–100 per cent in eleven finger millet genotypes. Comparison of more primers generally provides additional confirmatory evidence for genetic variation (Bezaweletaw, 2011). The Polymorphism Information Content (PIC) value calculated for the 10 ISSR primers. In the present study the maximum PIC information produced by the primer UBC-807 (0.75) while the minimum PIC value was given by the primer UBC-879 (0.33) while the average PIC value obtained for each primer was 0.58 (Table 1). The overall range of the similarity among 10 mutants and one parent line of finger millet was found to be very wide

ranging from 0.281 to 0.889 which indicates there was high variability among the finger millet mutants under study (Table 3). The cluster analysis was carried out based on the ISSR profile. The results based on the ISSR profile broadly grouped the 10 finger millet mutants into two main clusters (I and II). The first cluster (I) was formed by only two mutants. The second cluster (II) was again subdivided in to two classes in which the first sub class of the second cluster containing 1 mutant line DML-33 while the second sub class was divided in two categories including four mutant lines each. It was observed that, the mutant line DML-58 and DML-10 occupied a unique position and was most diverse from rest of 8 mutant lines of finger millet and one parent line Dapoli-1. Similar results have been found by Gupta *et al.*, (2012) for finger millet accessions.The study indicated that ISSR markers are suitable for the assessment of genetic variability among different mutant lines of finger millet. The ISSR analysis revealed substantial polymorphism in finger millet. The results of the present study indicated the efficiency of ISSR markers in investigating genetic variability at molecular level, which is important for detecting distinctness of mutants and also for the identification of desirable mutants and its utilization for further breeding programme.

Table 1: List of ISSR primers with their sequences used in the study

Sr. No.	Primer	Primer sequence (5' – 3')	GC %
1.	UBC-807	AGAGAGAGAGAGAGAGT	47.05
2.	UBC-811	GAGAGAGAGAGAGAGAC	52.94
3.	UBC-816	CACACACACACACACAT	47.05
4.	UBC-820	GTGTGTGTGTGTGTGTC	52.94
5.	UBC-824	TCTCTCTCTCTCTCTCG	52.94
6.	UBC-825	ACACACACACACACACT	47.05
7.	UBC-857	ACACACACACACACACCG	58.82
8.	UBC-878	GGATGGATGGATGGAT	50.00
9.	UBC-879	CTTCACTTCACTTCA	40.00
10.	UBC-891	AGATGTGTGTGTGTGTG	47.05

Table 2: Molecular information obtained by 10 ISSR primers

Sr. No.	Primer	Total No. of Bands	No. of Monomorphic Bands	No. of Polymorphic Bands	% Polymorphism	No. of Alleles	PIC
1	UBC-807	45	11	34	75.56	6	0.75
2	UBC-811	27	0	27	100.00	4	0.62
3	UBC-816	43	22	21	48.84	6	0.74
4	UBC-820	18	11	7	38.89	2	0.38
5	UBC-824	16	0	16	100.00	2	0.43
6	UBC-825	30	11	19	63.33	4	0.63
7	UBC-857	30	11	19	63.33	4	0.63
8	UBC-878	22	0	22	100.00	3	0.59
9	UBC-879	12	0	12	100.00	2	0.33
10	UBC-891	40	11	29	72.50	6	0.72
	Total	**283**	**77**	**206**	**-**	**37**	**-**
	Average	**28.3**	**7.7**	**20.6**	**72.79**	**3.7**	**0.58**

Table 3: Genetic distances based on ISSR pooled over the 10 primers in mutant lines of finger millet

	DML-4	DML-7	DML-8	DML-10	DML-33	DML-55	DML-58	DML-62	DML-70	DML-75	DPL-1
DML-4	1.000										
DML-7	0.821	1.000									
DML-8	0.742	0.839	1.000								
DML-10	0.423	0.379	0.344	1.000							
DML-33	0.586	0.531	0.529	0.458	1.000						
DML-55	0.821	0.800	0.676	0.333	0.581	1.000					
DML-58	0.560	0.556	0.500	0.474	0.370	0.500	1.000				
DML-62	0.656	0.647	0.686	0.448	0.645	0.647	0.375	1.000			
DML-70	0.568	0.605	0.684	0.382	0.600	0.649	0.361	0.853	1.000		
DML-75	0.568	0.605	0.684	0.382	0.556	0.605	0.361	0.800	0.889	1.000	
DPL-1	0.529	0.528	0.611	0.281	0.563	0.571	0.344	0.629	0.722	0.824	1.000

DML; Dapoli mutant line and DPL-1; Dapoli-1(Parent variety)

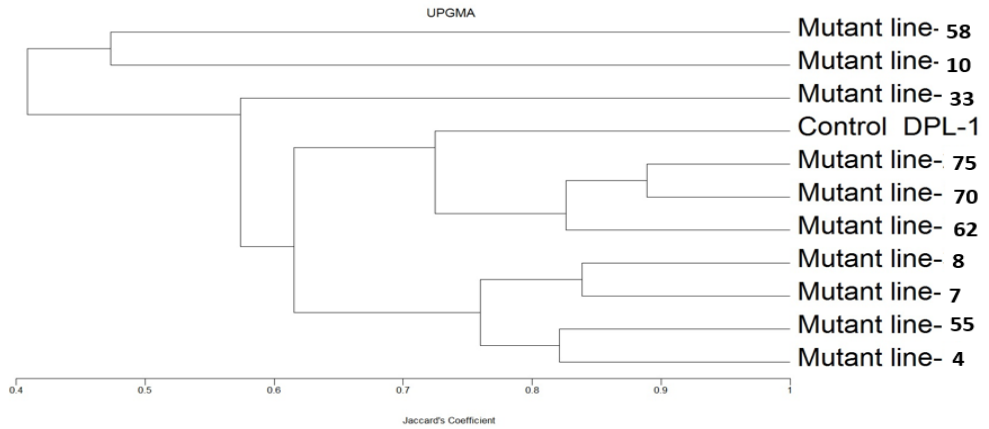

Fig. 1: Dendrogram constructed using Jaccards Similarity Coefficient

REFERENCES

1. De Candolle A, Origine of cultivated plants. (Hafner Publication Co, New York) 1886.
2. Fakrudin B, Shashidhar H E, Kulkarni R S and Hittalmani S, Genetic diversity assessment of finger millet, *Eleusine coracana* (Gaertn.), germplasm through RAPD analysis, *PGR Newsletter*, **138:** 50–54 (2004).
3. Prabhu R and Ganesan N M, Genetic diversity studies in ragi (*Eleusine coracana* (L.) Gaertn.) with SSR and ISSR Markers, *Molecular plant Breeding*,**17:** 141-145 (2013).
4. Doyle J J and Doyle J L, Isolation of plant DNA from fresh tissue, *Focus*, **12:** 13-15 (1990).
5. Kumari K and Pande A, Study of genetic diversity in finger millet (*Eleusine coracana* L. Gaertn) using RAPD markers, *African Journal of Biotechnology*, **29:** 4542-4549 (2010).
6. Bezaweletaw K, Genetic Diversity of Finger Millet (*Eleusine coracana* L. Gaertn) landraces characterized by random amplified polymorphic DNA analysis, *Inno Syss Des and Engin*, **4:** 207-218 (2011).
7. Gupta R, Verma K K and Singh M, Molecular characterization of different seed coat colour varieties of finger millet (*Eleusine coracana* L.) using ISSR marker, *Current Botany*, 1- 4 (2012).