Environment & Ecology 35 (2C) : 1233—1237, April—June 2017
Website: environmentandecology.com ISSN 0970-0420

Analysis of Genrtic Variability among the Finger Millet Germplasm by using ISSR Markers

V. G. Kelkar, S. V. Sawardekar, S. G. Bhave, N. B. Gokhale D. V. Rasam, S. S. Sawant

Received 18 July 2016; Accepted 22 August 2016; Published online 6 September 2016

Abstract In the present study forty germplasms of finger millet were screened by using 15 ISSR (Inter simple sequence repeats) markers to study the genetic variability among the germplasms. The DNA was extracted from the green leaf of 15 days oil seedlings by rapid DNA extraction method with slight modification. The standard PCR components with varying concentrations were used for further analysis. A total of 1876 bands were scored out of which 1552 were polymorphic, which showed 83.32% polymorphism. The average size of amplified fragment ranged from 200 bp to 1650 bp. The primer UBC-872 recorded minimum PIC (Polymorphic information content) value 0.20, whereas primer UBC-841 gave maximum PIC value 0.88. The average PIC value was 0.70 among the all 40 germplasms. The similar data of finger millet were used to generate pair-wise matrix based on the Jaccards's Similarity Co-efficient. The similarity co-efficient ranged from 0.197 (between germplasm Nagali-55 and KMR-204) to 0.679 (between germplasm VR-762 and PR-202) indicating the distinctness and similarities of these germplasms.

Keywords Finger millet, ISSR, Polymorphism, Polymorphic information content, Jaccard's Similarity Co-efficient.

V. G. Kelkar*, S. V. Sawardekar, S. G. Bhave,
N. B. Gokhale, D. V. Rasam, S. S. Sawant
Plant Biotechnology Center,
College of Agriculture,
Dr Balasaheb Sawant Konkan Krishi Vidyapeeth,
Dapoli 415712, India
e-mail : vipulkelkar221292@gmail.com
*Correspondence

Introduction

Finger millet (*Eleusine coracana* (L.) Gaertn.) 2n=36, is a poor man's crop, originated in Ethiopia [1]. It belongs to the tribe chloridae of the family Poaceae. The global annual planting area of finger millet is estimated at around 4-4.5 million hectare with a total production of 5 million tonnes. The area and production of ragi in India in *kharif* of 2012-13 was 1.12 million hectare 1.57 million tonnes respectively. In Maharashtra, area under finger millet was 166.8 thousand hectare with production of 170.2 thousand tones. In the Konkan region of Maharashtra it is cultivated in the area of 38.488 thousand hectare comprising Raigad, Thane, Palghar, Sindhudurga and Ratnagiri district with production of 41.136 thousand tones

Table 1. Details of germplasm used in the study.

Sl. No.	Name of germplasm	Sl. No.	Name of germplasm	Sl. No.	Name of germplasm	Sl. No.	Name of germplasm
1	Nagali-35	11	Dapoli-1	21	GPU-69	31	VR-762
2	Nagali-52	12	Dapl Safed	22	IGPSM-10	32	PR-202
3	Nagal-55	13	Vakavali-02	23	IGPSM-18	33	PR-1044
4	Nagali-56	14	Kolhapur	24	OEB-54	34	MR-06
5	Nagali-61	15	GPU-28	25	OEB-265	35	GSIS-01
6	Nagali-62	16	GPU-45	26	L-5	36	GOA-712
7	Nagali-66	17	GPU-48	27	L-481	37	PNV-5
8	Nagali-67	18	GPU-65	28	VL-149	38	ACCR-33
9	Nagali-69	19	GPU-66	29	VL-324	39	KOPN-235
10	Nagali-2RJ	20	GPU-67	30	VR-708	40	KMR-204

(agrimaha.com 2015). Finger millet is an energy feed valuable for its high carbohydrate content (80%). It is rich source of iron (380 ppm) and calcium (275 ppm).

Breeding is powerful tool for genetic improvement in cereals crops like rice, wheat. In case of finger millet due to very small size flowers the breeding is very difficult, hence instead of breeding, mutation is better option for crop improvement. The molecular approach for identification of plant genotypes seems to be more effective than traditional morphological markers because it allows direct access to the hereditary material and makes it possible to understand the relationships between plants [2]. The ISSR analysis revealed substantial polymorphism in finger millet [3].

The information on genetic variability and component analysis can be of great help in formulating appropriate breeding strategy for genetic upgradation of ragi [4]. Thus, the present study was undertaken with the objective to analyze the genetic variability among the finger millet germplasms through ISSR marker.

Materials and Methods

Plant material

In the present investigation 40 germplasms of finger millet available at Department of Agriculture Botany, Dr Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, were used for variability analysis.

DNA isolation

The tender leaf samples were collected from 15 days old seedlings for the extraction of genomic DNA by rapid DNA extraction method. The various solutions, buffers and its concentration used for extraction of the DNA are 1) Extraction buffer stock solutions : 200 mM Tris-HCL, 25 mM EDTA, 250 mM NaCl, 2) Composition of extraction buffer : 0.5 M glucose, 0.5% SDS, 3% PVP, 0.4% sodium bisulfate, 5% lauroyl sarcosine, chloroform isoamyl alcohol mixture (24:1), 100% chilled isopropanol, 70% ethyl alcohol, IX TE buffer, Purification of DNA was done to remove RNA and proteins which were the major contaminants. RNA was removed by RNase treatment and proteins were removed by proteinase-K treatment.

DNA amplification and gel electrophoresis

A set of 15 primers composed wholly of defined, short tandem repeat sequence with anchor and representing different microsatellites (di and tri repeats) have been used as genetic primers in PCR amplification of inter simple sequence repeats (ISSR) regions as according to method of Adawy et al. [5]. Amplification was achieved in eppendorf thermal cycler using 20 µl reaction containing 3U of taq polymerase (Banglore genei ltd), 2.5 µl of 10X taq assay buffer, 0.5 µl of 25 mM $MgCl_2$, 1 µl of 10 mM dNTPs, 1 µl of 5 pmoles concentration ISSR primer and approximately 1 µl (50 ng) of template DNA. The PCR thermal cycler was

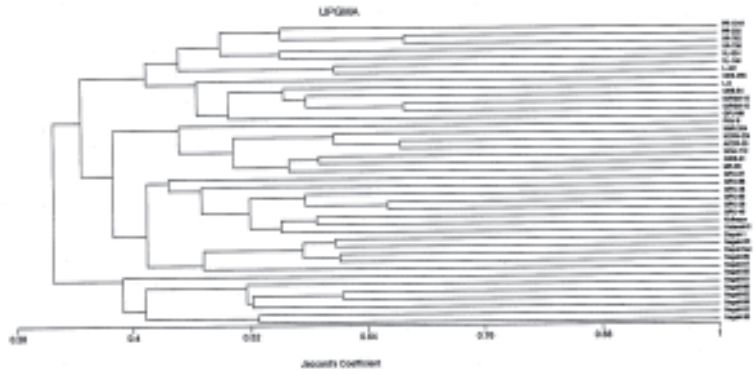

Fig. 1. Dendrogram constructed using Jaccard's Similarity Co-efficient.

programed for initially 5 minute denaturation step at 94°C, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing 40°C to 56.7°C for 1 minute and extension at 72°C for 1 minute and finally 72°C for 7 minute.

The amplified products in ISSR reaction were separated by electrophoresis in 2% agarose gel (SRL, India), containing ethidium bromide in IX TAE buffer (pH 8.0) and separation were carried out by applying constant voltage of 100 volts for 1 h. The standard DNA ladder used was Φ × 174/Hae III digest (1.3 kb). The gel images were taken by the documentation systems (Uvi-Tech. Fire reader, Cambridge, England) and saved in computer for further analysis.

Data analysis

ISSR markers across the 40 germplasms were scored for their presence (1) or absence (0) of bands for each primer. The binary data so generated was used to estimate the levels of polymorphism by dividing the number of polymorphic bands by the total number of scored bands. Jaccard's similarity co-efficients for each pair wise comparison between germplasms were calculated and similarity co-efficient matrix was generated. This matrix was subjected to unweighted pair group method for arithmetic average analysis (UPGMA) to construct a dendrogram. The similarity co-efficient analysis and dendrogram construction were carried out by using MVSP-A multi variate statistical package-5785 (version 3.1).

Results and Discussion

ISSR analysis

The marker analysis helps to understand the genetic makeup of the germplasm and also make it possible to analyze genetic diversity within species as well as between species. The present study utilized 40 germplasms (Table 1) for ISSR analysis with 15 random primers which gave scorable DNA bands and each of the 15 random primers revealed polymorphism (Table 2). The primers produced high degree of polymorphism with an average of 83.32%. Average 125 bands per primer were amplified. Among the 15 generic primers 7 primers viz. UBC-807, UBC-815, UBC-816, UBC-853, UBC-854, UBC-857 and IBC-872 revealed 100% polymorphism. The percentage of polymorphism across the finger millet genotypes ranged from 29.82–100%. Primer wise amplification and % polymorphism given in Table 3.

The PIC value was calculated for the 15 ISSR primers given in Table 2. In the present study the maximum PIC information produced by the primer UBC-841 (0.88) while the minimum PIC value was given by the primer UBC-872 (0.20) the average PIC value obtained for each primer was 0.70. The similar result was obtained by Prabha and Ganesan [6], the higher PIC value indicated the in formativeness of the prim-

Table 2. Optimization of annealing temperature of ISSR markers. Single letter abbreviations for mixed base positions.

Sl. No.	Primer	Primer sequence (5′ – 3′)	Range of temp (°C)	Standardized annealing temperature (°C)
1	UBC-807	AGA GAG AGA GAG AGA GT	45-55	50.4
2	UBC-815	CTC TCT CTC TCT CTC TG	40-50	49.5
3	UBC-816	CAC ACA CAC ACA CAC AT	45-55	51.4
4	UBC-818	CAC ACA CAC ACA CAC AG	45-55	45.9
5	UBC-824	TCT CTC TCT CTC TCT CG	45-55	56.7
6	UBC-834	AGA GAG AGA GAG AGA GT	45-55	50.4
7	UBC-841	GAG AGA GAG AGA GAG AC	42-52	47.4
8	UBC-853	TCT CTC TCT CTC TCT CRT	45-55	54.4
9	UBC-854	TCT CTC TCT CTC TCT CRG	45-55	54.8
10	UBC-857	ACA CAC ACA CAC ACA CYG	45-55	51.7
11	UBC-872	GAT AGA TAG ATA GAT A	40-50	43.0
12	UBC-884	HBH AGA GAG AGA GAG AG	40-50	40.4
13	UBC-885	BHB AGA GAG AGA GAG AG	40-50	40.7
14	UBC-886	VDV CTC TCT CTC TCT CT	45-55	51.4
15	UBC-891	HVH TGT GTG TGT GTG TG	45-55	52.8

B=(C, G, T) (i.e. not A) H=(A, C, T) (i.e. not G)
V=(A, C, G) (i.e. not T) D=(A, G, T) (i.e. not C)
Y=C OR T (i.e. not A, G) R=A OR G (i.e. not C, T)

Table 3. Primer wise amplification and % polymorphism of finger millet germplasm.

Sl. No.	Primer name	No of polymorphic bands	No of monomorphic bands	Total no of bands	Polymorphism %	Range of amplification (bp)	PIC
1	UBC-807	161	0	161	100	600-1600	0.78
2	UBC-815	104	0	104	100	500-1100	0.73
3	UBC-816	113	0	113	100	550-1150	0.74
4	UBC-818	68	40	108	62.96	300-1150	0.65
5	UBC-824	73	40	113	64.60	200-1250	0.71
6	UBC-834	17	40	57	29.82	350-900	0.71
7	UBC-841	173	40	213	81.22	200-1000	0.88
8	UBC-853	102	0	102	100	700-1250	0.72
9	UBC-854	40	0	40	100	500-600	0.40
10	UBC-857	87	0	87	100	900-1500	0.76
11	UBC-872	40	0	40	100	100-300	0.20
12	UBC-884	162	40	202	80.20	400-1650	0.84
13	UBC-885	140	40	180	77.77	300-1000	0.78
14	UBC-886	107	40	147	72.79	700-1650	0.71
15	UBC-891	165	40	205	80.49	300-1400	0.81
	Total	1552	320	1872	–	–	–
	Average	103.46	21.33	124.8	83.32	200-1650	0.70

ers. Hence, the primers UBC-891, UBC-810 and UBC-825 can be of use in future studies in the field of taxonomical and genetic resource management. The result of Kumari and Pande [7] indicated that the % polymorphism ranged from 6.6–100% in eleven finger millet genotypes.

Genetic relationship among germplasms

The genetic distance was computed considering the 40 germplasms from the pooled data. The overall range of the similarity among 40 germplasms of finger millet was found to be very wide ranging from 0.197 to 0.679 which indicates there was high variability among the finger millet cultivars under study. Based on the similarity matrix and clustering pattern, the germplasms VR-762 and PR-202 were found to have maximum similarity coefficient 0.679. While, the lowest similarity coefficient (0.197) were observed in between the germplasms Nagali-55 and KMR-204 which was suggesting a large differentiation in the germplasm of finger millet. Similar observations were also recorded by Gupta et al. [8] while carried out the study on assessment of genetic relatedness among three varieties of finger millet with variable seed coat color using RAPD and ISSR markers. The ISSR primers have a high potential to reveal polymorphism and to determine intra and inter genomic diversity [9].

Cluster analysis

The cluster analysis was carried out based on the ISSR profile. The results based on the ISSR profile broadly grouped the 40 finger millet germplasms into two main clusters (I and II). The first cluster (I) was

Table 4. Clustering pattern of 40 germplasms of finger millet.

Cluser		No of genotypes	Name of the genotype
I	IA	13	PR-1044, PR-202, VR-762, VR-708, VL-324, VL-149, L-481, OEB-265, L-5, OEB-54, IGPSM-18, IGPSM-10, GPU-69.
	IB	20	PNV-5, KMR-204, KOPN-235, ACCR-33, GOA-712, GSIS-01, MR-06, GPU-67, GPU-66, GPU-48, GPU-65, GPU-28, GPU-45. Kolhapur, Vakavali-02, Dapoli-1, Nagali-2RJ, Dapoli Safed Nagali-69, Nagali-67.
II	IIA	1	Nagali-55.
	IIB	6	Nagali-61, Nagali-66, Nagali-62, Nagali-56, Nagali-52, Nagali-35.

formed by the two subclasses. The first sub class of the first cluster containing 13 germplasms while the second sub class consists of 20 germplasms. The second cluster (II) further subdivided into two subclasses. The first sub class of the second cluster containing 1 germplasm while the second sub class consists of 6 germplasms. The clustering pattern of 40 germplasms of finger millet given in Table 4. Similar results have been found by Gupta et al. [10] for finger millet accessions in which the cluster one contain single variety PRM-1 and cluster two contain two varieties PRM-701 and PRM-801 respectively based on ISSR analysis. This study could be used to identify the diverse genotypes like Nagali-55 and their use in hybridization program of ragi. The genetic diversity in this study might be useful in future strategies for development of desired genotypes.

The dendrogram based on Jaccard's similarity Coefficient was constructed using UPGMA after analysis of banding patterns generated by all the accessions with 15 primers across the 40 germplasms of finger millet genotypes. The dendrogram and similarity coefficient values give an idea about the nature of the individual sample in the whole sample set and all genotypes into two main cluster were presented in Fig. 1.

Conclusion

The study indicated that ISSR markers are suitable for the assessment of genetic variability among different germplasms of finger millet. The results of the present study indicated the efficiency of ISSR markers in investigation genetic variability at molecular level, which is important for detecting distinctness of germplasms also for the identification of desirable germplasms and its utilization for further breeding program. Such information may be useful for selecting the diverse parents and monitoring the genetic diversity periodically for improvement of finger millet.

References

1. Vavilov NI (1951) The origin, variation immunity and breeding of cultivated plants. Chronica Botanica 13 : 1—366.
2. Das S, Misra RC, Rout GR, Pattanaik MC, Aparajita S (2009) Relationship of status of polymorphic RAPD bands with genotypic adaptation in early finger millet genotypes. Afr Crop Sci J 17 : 61—69.
3. Gilande HG, Ghokhale NB, Sawardekar SV, Patil DM, (2015) Analysis of genetic variability in finger mutant lines using ISSR markers. Agric Res Tech 40 : 233—238.
4. Lal C, Dawa T, Plaha P, Sharma SK (1996) Studies on genetic variability arid componant analysis in ragi (*Eleusine coracana* Gaertn.). Ind J Genet 56 : 162—168.
5. Adawy SS, Hussein, Ebtissam HA, Saker MM, El-Itriby Hanaiya A (2004) Intra- and Inter-varietal variation of Upper Egypt date palm cultivars (*Phoenixdactylifera* L.): I as revealed by RAPD and ISSR markers. Int Conf Genet Engg 11 : 165—179.
6. Prabhu R, Ganesan NM (2013) Genetic diversity studies in ragi (*Eleusine coracana* (L.) Gaertn.) with SSR and ISSR markers. Mol Pl Breed 4 : 141—145.
7. Kumari K, Pande A (2010) Study of genetic diversity in finger millet (*Eleusine coracana* L. Gaertn) using RAPD markers. Afr J Biotech 9 : 4542—4549.
8. Gupta R, Verma K, Joshi DC, Yadav D, Singh M (2010) Assessment of genetic relatedness among three varieties of finger millet with variable seed coat color using RAPD and ISSR markers. Genet Engg and Biotech J 2 : 1—9.
9. Paul AJ, Panneerselvam R (2013) Analysis of intra specific variation in *Setaria italica* (L.) P. Beauv landraces using RAPD and ISSR markers. Int J Res in Biochem and Biophy 3 : 15—20.
10. Gupta R, Verma KK, Singh M (2012) Molecular characterization of different seed coat color varieties of finger millet (*Eleusine coracana* L.) using ISSR marker. Curr Botany, pp 1—4.