Trends in Biosciences 11(25), Print : ISSN 0974-8431, 3369-3373, 2018

Genotypic Diversity and Population Structure of ICRISAT Composite Collection of Finger Millet [*Eleusine coracana* (L.) Gaertn]

A. BHARATHI[1*], HD. UPADHAYA[2], RK VARSHNEY[2] AND CLL GOWDA[2]

[1]*Agricultural Research Station, Pattukkottai, Tamil Nadu*
[2]*International Crop Research Institute for Semi-Arid Tropics (ICRISAT), Hyderabad*
email : bharat22880@yahoo.co.uk

ABSTRACT

Finger millet [*Eleusine coracana* (L.) Gaertn] is an important crop used for food, fodder and industrial purposes. With the objective of increasing the utilization of finger millet germplasm in crop improvement, A composite collection consisting of 1000 accessions was developed and genotypically and profiled using 20 SSR markers. This study reported the genetic diversity and development of genotype based reference set with most diverse 300 accessions. Allelic data on 959 accessions and 20 markers based on quality index was used for further statistical analysis. A total of 231 (121 common and 110 rare) alleles were detected in the composite collection. Gene diversity varied from 0.200 to 0.850. The average frequency of multiple alleles were maximum (13.6%) in race *spontanea* wild types and ranged from 1.7 to 9.5% in other races. A reference set consisting 300 genetically most diverse accessions was established. This reference set had 206 (89.2%) of the 231 alleles detected in the composite collection, and showed high gene diversity (0.307 to 0.852).

Key words *Genotypic Diversity, Population Structure, ICRISAT, Composite Collection, Finger Millet, Eleusine coracana (L.) Gaertn*

Finger millet (*Eleusine coracana L. Gaertn*) is an important crop in several countries of Asia and Africa used for food, fodder, and industrial purpose. In finger millet the diversity has been studied using morphological characters like growth habit, leaf architecture or floral morphology (Rachie and Peters, 1997). At molecular level, DNA markers such as RFLP (Muza et al 1995, RAPD (Das et al., 2007), SSRs (Dida et al., 2007) have been used to determine genetic diversity. The genetic map of finger millet has been developed recently by using RFLP, AFLP, EST and SSR markers (IRGSP.2005). Comparative analysis of finger millet genetic map with rice genetic map was a novel attempt that reported high level of conserved co-linearity between the two genomes (Srinivasachary et al., 2007). Low molecular variation was reported in the cultivated finger millet in the past as the results were based on limited number of germplasm and markers. With the discovery of large numbers of genomic SSR markers (Dida et al., 2007), it is now possible to conduct extensive molecular diversity and QTL analysis in finger millet. Population structure using 79 finger millet accessions and 45 SSR markers has been reported (Dida et al., 2008). The present study aimed to assess the genotypic diversity nature of the composite collection and development of a reference set (300 accessions) based on genotypic data.

MATERIALS AND METHODS

All the 1000 accessions of the finger millet composite collection including four internal checks (VR708, VL149, PR202 and RAU8) were grown in the field. The DNA was extracted from single seedling of each accession by high throughput 96- well plate mini preparation method. From the preliminary screening of 31 SSR markers on an eight diverse finger millet genotypes (IE4709, IE6082, IE2921, IE5177, IE4057, IE4443, IE2564 and IE3025), 20 polymorphic SSR markers were selected to genotype the composite collection. Of these, 19 SSRs belong to dinucleotide repeats and one to trinucleotide repeats. The 20 SSR markers used for genotyping were mapped on nine chromosomes.

Polymerase chain reaction (PCR)

The PCR reactions were conducted in 96-well and 384-well micro-titer plates in a GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA, USA) Thermal Cycler. The PCR reactions were performed in 5 ml volume in 384-well PCR plates. The reaction mixtures contained 10 pmol of primer, 25 mM $MgCl_2$, 2mM dNTP, 0.3 unit of *Taq* polymerase and 1x PCR buffer (Applied Biosystems, Foster City, CA, USA). The touch down PCR protocol was used for the following reaction of following: three-minute denaturation cycle, followed by first five cycles of 94°C for 20 seconds, 60 °C for 20 seconds and 72 °C for 30 seconds, then by 30 cycles of 94 °C for 20 seconds. After completion of 30 cycles, a final extension of 20 min at 72 °C to ensure amplification to equal lengths of both DNA strands. The amplified PCR products were tested on 1.2 per cent agarose gel to check for the amplification of the products.

Genotyping

The PCR products were size-separated by capillary electrophoresis using an ABI 3700 DNA analyzer (Applied Biosystems, Foster City, CA, USA). The PCR products of 4 primer pairs labeled with different dyes (FAM, VIC, NED and PET) could be pooled (post-PCR), because of the different signal spectra of the fluorophores used. The products of the same fluorophore-labeled primers were also pooled, when they had non-overlapping amplicons in terms

Table 1. Allelic composition, PIC, gene diversity and multiple allele frequency (%) of 20 SSRs in global finger millet composite collection and reference set.

Marker	Total alleles		Rare allele (<1%)		Common allele (>1%)		PIC value		Gene diversity		Multiple allele frequency (%)	
	Com	Ref	Com	Ref	Com	Ref	Com	Ref	Com	Ref	Com	Ref
UGEP56	8	7	5	4	3	3	0.313	0.402	0.360	0.458	1.00	2.08
UGEP8	10	8	5	3	5	5	0.452	0.336	0.350	0.372	13.00	12.50
UGEP11	7	7	4	3	3	4	0.316	0.464	0.550	0.560	14.00	14.44
UGEP15	11	11	2	3	9	8	0.753	0.774	0.780	0.800	10.00	8.07
UGEP3	10	9	5	3	5	6	0.568	0.612	0.620	0.667	12.00	16.67
UGEP81	10	10	5	4	5	5	0.388	0.66	0.670	0.699	19.00	17.69
UGEP5	21	18	13	9	8	9	0.636	0.482	0.420	0.522	4.00	4.44
UGEP107	9	9	5	5	4	4	0.196	0.404	0.390	0.430	2.00	1.06
UGEP31	10	10	3	3	7	7	0.522	0.297	0.200	0.307	4.00	7.39
UGEP104	8	7	4	1	4	6	0.564	0.586	0.590	0.643	10.00	15.86
UGEP90	8	8	3	3	5	5	0.593	0.683	0.720	0.728	17.00	1.56
UGEP18	12	12	4	4	8	8	0.669	0.462	0.420	0.528	0.00	0.34
UGEP68	8	8	3	2	5	6	0.543	0.665	0.590	0.709	2.00	2.75
UGEP65	10	10	5	4	5	6	0.369	0.653	0.590	0.700	2.00	2.74
UGEP1	8	8	3	3	5	5	0.559	0.558	0.630	0.631	2.00	17.9
UGEP10	16	14	9	5	7	9	0.571	0.674	0.600	0.704	12.00	13.09
UGEP102	16	12	9	6	7	6	0.630	0.669	0.670	0.708	17.00	16.55
UGEP26	12	11	6	4	6	7	0.497	0.837	0.850	0.852	9.00	8.58
UGEP77	17	13	8	3	9	10	0.644	0.567	0.520	0.589	16.00	16.43
UGEP12	20	15	9	3	11	12	0.834	0.688	0.680	0.717	9.00	5.90
Mean	11.55	10.3	5.50	3.75	6.05	6.55	0.530	0.574	0.560	0.616	9.00	9.18
Range	7 - 21	7 - 18	2-13	1-9	3 -11	3 -12	0.196 - 0.834	0.297 - 0.837	0.200 - 0.850	0.307 - 0.852	- 0 - 19.00	0.34 - 17.9
Total	231	206	110	75	121	131	-	-	-	-	-	-

Com – composite collection

Ref – Reference set

of size. The pooled PCR products were mixed with 0.25 µl of the GeneScan 500™ LIZ® internal size standard and 7 µl of Hi-Di™ Formamide (Applied Biosystems, Foster City, CA, USA). The final volume was made up to 12 µl with sterile double-distilled water. This mixture was denatured for 5 minutes at 95°C and cooled immediately on ice.

Fragment size fractionation

After denaturation, the plate with samples was placed into the sequencer machine (ABI Prism 3700 DNA analyzer). The capillary run was performed using the "GeneScan2_POP6 Default" run module and "G5" filter-set. The analysis module used was "GS500 analysis". The fragments were separated in a 50 cm capillary array using POP6 (Performance Optimized Polymer) as the separation matrix.

Table 2 Molecular diversity based on biological and geographical grouping of finger millet composite collection using 20 SSRs.

Geographical origin	Number of accessions	Allele Frequency (%)	Allele No	PIC	Gene Diversity	Multiple allele frequency (%)
Biological races						
Compacta	127	58.74	7.4	0.509 (0.153 - 0.810)	0.553 (0.156 - 0.829)	9.40
Elongata	69	60.22	6.5	0.501 (0.191 - 0.816)	0.542 (0.197 - 0.834)	7.70
Plana	201	58.08	8.0	0.521 (0.259 - 0.805)	0.563 (0.267 - 0.825)	7.30
Vulgaris	551	59.00	10.3	0.509 (0.177 - 0.840)	0.552 (0.182 - 0.854)	8.90
Spontanea	7	51.61	4.0	0.566 (0.214 - 0.811)	0.611 (0.244 - 0.833)	13.50
Africana	3	63.33	2.1	0.349 (0.000 - 0.592)	0.422 (0.000 - 0.666)	1.60
Mean	-	58.40	6.3	0.429	0.540	8.30
Range	-	51.61 – 63.33	2.1 -10.3	0.349 – 0.566	0.422 – 0.611	1.60 -13.50
Total	959	959	38.3	2.957	3.245	4.87
Geographical origin						
Central Africa	55.13	8	3.7	0.517 (0.214 - 0.778)	0.568 (0.244 - 0.806)	12.80
East Africa	57.77	429	9.9	0.523 (0.222 - 0.831)	0.564 (0.229 - 0.846)	8.60
Southern Africa	61.89	164	8.1	0.484 (0.163 - 0.823)	0.524 (0.167 – 0.841)	9.00
West Africa	68.15	7	2.6	0.370 (0.00 - 0.671)	0.419 (0.000 – 0.722)	5.80
South Asia	61.50	316	8.8	0.496 (0.158 - 0.823)	0.537 (0.160 – 0.841)	8.30
Europe	60.21	4	2.2	0.359 (0.00 - 0.703)	0.435 (0.000 – 0.750)	4.50
America	59.83	4	2.6	0.427 (0.00 - 0.745)	0.496 (0.000 – 0.781)	11.60
Unknown	60.12	27	5.3	0.525 (0.211- 0.816)	0.572 (0.240 – 0.834)	8.40
Minimum	55.13	4	2.2	0.359	0.419	4.50
Maximum	68.15	429	9.9	0.525	0.572	12.80
Mean	60.00	0	5.4	0.462	0.514	8.60
Range	55.13 – 68.15	4 -429	2.2 – 9.9	0.359 – 0.525	0.419 -0.572	4.50 – 12.80
Total	--	959	-	3.703	4.119	69.40

Values in parenthesis indicate the range for particular group

Data processing

After the capillary runs were over, the raw data were processed with Genescan 3.1 software (Applied Biosystems) to size the peak patterns in relation to the internal size standard GeneScan 500™ LIZ®. The principle behind this is that standards are run in the same lane or capillary injection as the samples, which contain fragments of unknown sizes labeled with different fluorophores. Genescan® analysis software automatically calculates the size of the unknown DNA sample fragments by generating a calibration sizing curve based upon the migration times of the known fragments in the standard. The unknown fragments are mapped onto the curve and the sample data is converted from migration times to fragment size. Genotyper 3.7 (Applied Biosystems) was used for allele calling. The peaks were displayed with base pair values and height (amplitude) in a chromatogram and the allelic data were exported in to Excel spread sheet for further analysis.

Data analysis

The binned data were subjected to diversity analysis in DARwin5. The 19180 data points (959 accessions x 20 SSR loci data) validated for final diversity analysis. The accessions with >5% missing data were excluded for the analysis. Statistical analysis was done using Power Marker V3.0 (Lie and Muse., 2008) for analysis estimating basic statistics (PIC value, allelic richness as determined by a total number of the detected alleles and a number of alleles per locus, gene diversity (H_e), occurrence of unique allele, rare and common alleles, and multiple allele percentage (%).

RESULTS AND DISCUSSION

Allelic richness

Twenty SSR loci data on 959 finger millet accessions detected a total of 231 alleles with allelic frequency of 11.55 alleles per SSR loci. The allele number per SSR locus was higher than the earlier studies in finger millet (Dida et al., 2008) in which higher number of polymorphic SSR markers and large population were used. The presence of many rare alleles could be due to the higher rate of mutation at SSR loci (Cho, 2000).

The markers, UGEP8, UGEP3, and UGEP31 had high PIC value, whereas the markers UGEP81 and UGEP65 had low PIC for the similar number of alleles. This result indicated that PIC values depend not only on the number of alleles but also the gene diversity [Varshney et al., 2001; Smith et al., 2002). Normally in inbreeding species, the level of polymorphism is expected to be generally lower than in out crossing species (Casa et al., 2005). Though the number of SSR markers in this study was limited, high polymorphism was revealed as SSRs are more variable, and provide higher resolution and higher expected heterozygosity than the RFLPs, RAPDs or AFLPs [Powel et al 1996)

Similarly mean gene diversity was higher than RAPD (Babu et al., 2006). As random genomic DNA markers (RFLP and RAPD) may assay polymorphism located in the non-coding regions of the genome that are poorly conserved among species (Brown et al., 1996). Therefore, the genetic diversity detected by these markers may not represent true or functional diversity. In contrast, functional markers such as EST/SSR assay polymorphism are associated with the coding regions of the genome and should detect "true gene diversity" available inside or adjacent to the genes (Thiel et al., 2003). A wide range of multiple allele frequency was detected in the composite collection, even though DNA was extracted from a single plant per accession and crop is highly self-pollinated. The reason could be that the allotetraploid nature of the finger millet having A and B genomes. The loci with large number of repeat units (SSR units) tend to show high mutational rate. As a result, any mutations in any one of the alleles may create a heterozygous condition. Many of the loci which displayed multiple allele status have a large number of SSR units. High allelic variability and low heterozygosity observed in this study implies the inbreeding nature of finger millet as reported in sorghum (Dje et al., 2000).

Most of the accessions carrying rare alleles were from East Africa, which is believed to be centre of origin. It might be the founder alleles which are retained in the gene pool even after so many years of crop evolution (de Wet et al., 1984). Such rare alleles are important, because they may be diagnostic for particular regions of the genome specific to a particular trait/type of finger millet.

Multiple allele frequency in germplasm accessions

Finger millet is a self pollinated crop. Moreover, in this study, seeds from single plant from each accession were harvested and the seeds obtained from such plants were sown to raise seedlings for DNA extraction. Extreme care was taken to avoid inadvertent seed mixtures. In spite of this, a wide range of multiple allele frequency (%) was detected in the composite collection materials, from 0 to 19%, with an average of 9 %. A large collection of landraces was involved in this study and it is possible that these accessions still possess some residual heterozygosity at some SSR loci reported. A landrace is defined as a primitive variety with capacity to tolerate biotic and abiotic stresses, resulting in intermediate productivity and stability of yield under a low input agricultural system. As finger millet is a tetraploid, having A and B genomes, loci UGEP3 was mapped in both the chromosome and this could be the reason for the occurrence two alleles and high multiple allele frequency for this loci. The multiple allele frequency observed at some of the loci could also be due to high mutational rate and mutational bias at SSR loci. The loci with large number of repeat units (SSR units) tend to show high mutational rate (cha et al., 2000). As a result, any mutations in any one of the alleles may create a heterozygous condition. Many of the loci which displayed heterozygous status have a large number of SSR units.

CONCLUSION

The global finger millet composite collection showed rich allelic diversity (231 alleles, 11.6 alleles per locus, 121 common alleles and 110 rare alleles at 1%) and genotype based reference set (300 accessions) captured 206 (89.2%) of the 231 alleles detected in the composite collection. This reference set can be used for diverse applications in genomics and breeding in finger millet in future.

ABBREVIATIONS

RFLP, Restriction Fragment Length Polymorphisms; RAPD, Random Amplified Polymorphic DNA; SSR, Simple Sequence Repeats; QTL, Quantitative Trait Loci; ICRISAT, International Crop Research Institute for the Semi-Arid Tropics; TNAU, Tamil Nadu Agricultural University, PIC, Polymorphic Information Content; PCR, Polymerase Chain Reaction: LG, Linkage group, MLM, Mixed Linear Model

LITERATURE CITED

Babu BK, Senthil N, Gomez SM, Biji KR, Rajendra Prasad NS, Satheesh Kumar S, Chandra Babu R: Assessment of genetic diversity among finger millet (*Eleusine coracana* (L.) Gaertn.) Accessions using molecular markers. *Genetic Resources and Crop Evolution,* 2006, **54:399**–404.

Brown SM, Hopkins MS, Mitchell SE, Senior ML, Wang TY, Duncan RR, Gonzalez- Candelas F, Kresovich S : Multiple methods for the identification of polymorphic simple sequence repeats (SSRs) in sorghum [*Sorghum bicolor* (L.) Moench]. *Theor. Appl. Genet*. 1996, **93:** 190-198.

Cho YG; Diversity of microsatellites derived from genomic libraries and GenBank sequences in rice (Oryza sativa L.). *Theor. Appl. Genet.*, 2000, **100**: 713–722.

Casa AM, Mitchell SE, Hamblin MT, Sun H, Bowers JE, Paterson AH, Aquadra CF, Kresovich S: Diversity and selection in sorghum: simultaneous analyses using simple sequence repeats. *Theoretical and Applied Genetics,* 2005, **111 (1):** 23-30.

Das, Mishra RC, Routb GR, Aparajitab S: Genetic variability and relationships among thirty genotypes of finger millet (*Eleusine coracana* L. Gaertn.) using RAPD markers. *Z. Naturforsch,* 2007, **62c**, 116 – 122.

de Wet JMJ, Prasada Rao KE , Brink DE, Mengesha MH: **Systematic and evolution of *Eleusine coracana* (Gramineae)**. *Amer. J Bot., 1984*, **71:**550–557.

Dida MM, Srinivasachary, Sujatha Ramakrishna, Bennetzen JL, Gale MD, Devos KM: The genetic map of finger millet. *Eleusine coracana*, *Theo. Appl. genet,* 2007, **114:**321-332.

Dida, MM, Wanyera N, Harrison Dunn MLN, Bennetzen JL, Devos KM: Population structure and diversity in finger millet (*Eleusine coracana*) germplasm. *Tropical Plant Biology*, 2008, DOI 10.1007/s1 2042-008-9012-3.

Dje Y, Heuertz M, Levfebvre C, Vekemans, X: Assessment of genetic diversity within and among germplasm accessions in cultivated sorghum using microsatellite markers. *Theor. Appl. Genet,* 2000, **100**: 918-925.

Hilu KW, DeWet JMJ: Racial evolution of finger millet, *Eleusine Coracana*, *Am. J. Bot* 1979b**, 63:**1311–1318.

International Rice Genome Sequencing Project; Mapped based sequence of rice genome**,** *Nature,* 2005, 465: 793-800

Liu K, Muse SV: Power marker: Integrated analysis environment for genetic marker data. *Bioinformatics,* 2005, **21:**2128-2129.

Muza FR, Lee DJ, Andrews DJ, Gupta SC: Mitochondrial DNA variation in finger millet (*Eleusine coracana*). *Euphytica,*1995, **81:**199–205.

Powell W, Morgante M, Andre C, Hanafey M, Vogel T, Tingey S, Rafalski JA: The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Mol. Breed,* 1996, **3**: 225-238.

Rachie KO, Peters LV: The *Eleusines*. A review of world literature. ICRISAT. Nairobi,

1997 Smith JSC, Kresovich S, Hopkins MS, Mitchell SE, Dean RE, Woodman WL, Lee W, Porter K: Genetic diversity among elite sorghum inbred lines assessed with simple sequence repeats. *Crop Sci.,* 2002, **40:** 226–232.

Srinivasachary, Dida MM, Gale MD, Devos KM: Comparative analyses reveal high level of conserved co-linearity between the finger millet and rice genomes. *Theor. Appl. Genet,* 2007, **115:** 489-499.

Varshney RK, Kumar A, Balyan HS, Roy JK, Prasad M, Gupta PK: Characterization of microsatellites and development of chromosome specific STMS markers in bread wheat. *Plant Mol Biol Rep,* 2001, **18:** 5- 16.

Thiel T, Michalek W, Varshney RK, Graner A: Exploiting EST database for the development and characterization of gene derived SSR markers in barley (*Hordeum vulgare L.*). *Theor. Appl. Genet,* 2003 **106:** 411-422.

Received on 15-06-2018 Accepted on 05-07-2018