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Development and genotyping potentials of EST-SSRs in Finger Millet (*E. coracana* (L.) Gaertn.)

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Abstract: Finger millet (*E. coracana* (L.) Gaertn.) provides food for millions of people in Africa and Asia. In this study, sequence data were mined at the database of National Center for Biotechnology Information (NCBI) with the aim of developing polymorphic expressed sequence tags simple sequence repeat (EST-SSRs) markers. Three selected markers which showed clear polymorphism in pre-testing with 5 accessions were used to characterize some randomly selected 48 accessions from the pool of finger millet core set. The polymorphic information content (PIC) of the developed markers gave a value range of 0.6741 for marker UH-Ec-931 to 0.7658 for marker UH-Ec-958. The mean PIC value of 0.7171 was recorded. Marker UH-Ec-958 showed 13 alleles per locus while marker UH-Ec-956 showed 20 alleles per locus. The mean average allele per locus was 17. Following Nei's approach, the mean gene diversity value of 0.7638 was captured by the three markers. Cluster analysis for the 48 selected accessions of finger millet showed four major clusters. Accessions from Zimbabwe and Zambia are distributed on the cluster I. Accessions from India are mostly found in cluster IV. Accessions from Nepal were found mostly on cluster III while Ugandan accessions are found in cluster II and III respectively. Our investigation showed that the developed EST-SSRs are quite effective in unraveling the nature of diversity in our studied population.

Keywords: EST-SSRs, Finger Millet (*E. Coracana* (L.) Gaertn.), Gene Diversity, Polymorphic Information Content (PIC)

1. Introduction

Finger millet (*E. coracana* (L.) Gaertn.) is a tetraploid (2n= 4x= 36; genome constitution AABB) belonging to the grass family of Poaceae [1] . The crop is grown as an annual robust grass on marginal lands of Africa and Asia providing food for millions of households. The grains are rich source of protein, fiber, minerals, and amino acid [2]. The grains are also used in therapeutic management of patients with immune related problems in east Africa [2]. The nutritional quality inherent in finger millet makes it an ideal supplement for expectant mothers, breastfeeding mothers, children, the sick and diabetics [3]. The grains are used in beer and liquor production [4]. Developing superior, high yielding and disease tolerance cultivars that are highly adaptable to varying environmental conditions can be achieved through understanding genetic variations in available germplasm. Several studies have assessed the genetic diversity in finger millet using DNA based molecular markers like RAPD, RFLP and SSR [5-8]. Despite these achievement there is need to intensify more research efforts towards developing markers for crop improvement. SSR based EST libraries are powerful tools for genetic research in genetic variation, gene tagging and evolution, mapping and analysis of quantitative traits [9]. ESTs derived SSRs are quickly obtained, unbiased in their repeat type, and they have a higher probability of being functionally associated with differences in gene expression than the genomic DNA or cDNA derived SSRs [10]. Furthermore, SSRs acquired through genomic DNA or cDNA sequences are limited to those probed SSR motifs thus restricting the marker utility [10]. EST-SSRs are

obtained from transcribed regions of DNA, and are more conserved and have a higher rate of transferability to close related specie and genera [9]. The mining of these abundant resources provides an opportunity to rapidly expand the database of molecular markers in finger millet at a minimal cost. The objective of this study is to (i) develop EST-SSR markers for finger millet and (ii) validate their polymorphic potential by using them to describe the diversity of 48 randomly selected representatives from a core set.

2. Material and Methods

2.1. Plant Material and DNA Extraction

The plant material consists of 48 randomly selected accessions from the pool of 622 core accessions of finger millet [11]. This comprised entries from 7 countries namely Kenya, Malawi, Zambia, Uganda, India, Zimbabwe and Nepal (Table 1). The seeds were planted in greenhouse at the University of Hohenheim, Germany in spring 2009. The leaves were harvested at two-leaf stage at 15 days after the planting (DAP) and a modified CTAB protocol was used for genomic DNA extraction from the tissues [12]. Standard lambda DNA was used to determine the DNA concentration on 3% agarose gels. The relative purity of the extraction was validated using NanoDrop ND-1000 (NanoDrop Technologies Inc., USA). A final working concentration of 20ng/µl was adjusted from the stock for each sample. DNA samples were stored at a temperature of -20°C.

Table 1. *List of core collections of E. coracana used in the study*

Serial number	Genotype identification number	Country of origin
1	2384	Kenya
2	2399	Kenya
3	2416	Kenya
4	2425	Kenya
5	2437	Kenya
6	2440	Kenya
7	2476	Kenya
8	2487	Kenya
9	2503	Kenya
10	2551	Kenya
11	2606	Malawi
12	2608	Malawi
13	2622	Malawi
14	2633	Malawi
15	2652	Malawi
16	2732	Malawi
17	2857	Zambia
18	2861	Zambia
19	2869	Zambia
20	2871	Zambia
21	2896	Zambia
22	3779	Uganda
23	3780	Uganda
24	3808	Uganda
25	3817	Uganda
26	3826	Uganda
27	3947	Uganda
28	3973	Uganda
29	4057	Uganda
30	2299	India
31	2322	India
32	2264	India
33	2212	India
34	2223	India
35	3062	India
36	3127	India
37	3135	India
38	4245	Zimbabwe
39	4274	Zimbabwe
40	4296	Zimbabwe
41	4312	Zimbabwe
42	4339	Zimbabwe
43	4383	Zimbabwe
44	4403	Zimbabwe
45	5542	Nepal
46	5635	Nepal
47	5537	Nepal
48	5896	Nepal

2.2. Data Mining for Microsatellites

The databases of the National Center for Biotechnology Information (NCBI) were screened for SSRs. Similarly, a total of 194 nucleotide and 1,927 EST sequences were registered for *E. coracana* at NCBI databases. Using the RepeatMasker Open-3.0 program of the Institute for Systems Biology [13] the sequences were screened for interspersed repeats. The parameters were set for the detection of perfect di, tri, tetra, penta, and hexa-nucleotide motifs with a minimum repeat length of 10, 7, 6, 5 and 4 respectively. The output of the program is a detailed annotation of the repeats that are present in the query sequence as well as a modified version of the query sequence in which all the annotated repeats have been masked [13]. The output was further controlled visually to ensure its conformity with the set parameters.

2.3. Oligonucleotide Primer Design and Blasting for Specificity

Primers were designed around the flanking regions of SSRs of interest using the primer premier 5.0 software. Because of the need for amplification specificity and ease of primer template annealing, parameters were set for primer length of 17 to 24 bp. The program further estimates the required annealing temperature based on the melting temperature of the constituent bases. Primer-template overlap was avoided during primer design by ensuring that the region to be amplified was between the forward and reverse primer pair. Efforts were made to reduce the occurrence of secondary structures such as dimers and hairpins. The designed primers were subsequently compared for sequence similarity against published primers

sequences. This was done using the Basic Local Alignment Search Tool (BLAST). Default parameters for minimum homology ratio, length and power threshold were used for the blast. Sequence alignments are significant, if its "power" exceeds 7 Standard Deviation units (SD).

2.4. Optimization of PCR for Designed Primers

The PCR was set up in a 25 µl reaction mixture constituting of; 1 x PCR buffer (1.5 mM $MgCl_2$), 20 ng DNA template, 250 nM of each of the forward and reverse primers, 0.2 mM dNTPs, and 0.5 U of Taq DNA polymerase (GENAXXON biosciences). The mixture was assembled on ice. Optimum annealing (T_A) temperature was determined after first running the PCR on a gradient thermo-cycler (MJ Research, Inc), which allows a temperature range of 5°C above and below the estimated T_A of the primers. The PCR product was evaluated on a gel and the clearest band within the expected fragment range was chosen as the optimum T_A for the amplification. Optimum conditions for the $MgCl_2$, dNTPs, and Taq DNA polymerase and the template DNA concentrations were determined empirically, after repeated trials. The primers whose PCR products were specific using pre-selected 5 accessions were used to genotype 48 accessions of the finger millet.

2.5. Setting up PCR using Labelled Primers and Capillary Electrophoresis

Following a successful optimization for the PCR conditions, the forward primers were labeled with fluorescent dyes namely, tetrachloro-6-carboxyfluorescien (TET; green), hexachloro-6-carboxyfluorescein (HEX; black) or 6-carboxyfluorescein (6-FAM; blue). The labeled primers were used in a new PCR amplification using DNA templates from the 48 accessions of finger millet. A MegaBACE sequencer (Amersham Biosciences) was used in the separation of the labeled PCR product. Three differently labeled PCR products (TET, HEX, and FAM) were pooled per run on a 96 well ABI plate. An energy transfer dye standard (ET400-R, Amersham Biosciences) was used for fragment size estimation. The final cocktail for capillary electrophoresis constituted of 0.6 µl of multiplexed PCR products and 5 µl of the diluted ET-ROX standard (1:20 dilution with loading solution). The multiplexed product was denatured at 94°C for 1 minute, cooled and centrifuged for another 1 minute at 2,500 rpm and was loaded in the MegaBACE. The run time lasted for 75 minutes. At the end of the capillary electrophoresis, the information on the peak value of the amplified PCR fragments was assessed using the MegaBACE fragment profiler (Amersham Biosciences) software. Varying allele peak value for a given locus across a given population corresponds to the possibility of varying lengths of the repetitive base sequences of the SSR. Allele calling was performed by selecting the allele peaks from the sized peak list for each trace. The peak selection was based on the fragment size range of the respective marker and the expected peak pattern of the SSRs. Peak selections that fall within the same allele bin of the fragment profiler were called as one allele. In the event of multiple stuttering peaks, alleles with the highest fluorescent intensity were called.

2.6. Statistical Analysis

The generated data was analyzed using the software Tool for Population Genetics Analysis (TFPGA) version 1.3 [14]. to calculate the genetic distance between accessions using Nei's average gene diversity measure [15]. Polymorphic information content (PIC) of each of the developed markers was calculated [16]. A dendrogram was constructed using DARwin 5.0.

3. Results and Discussion

3.1. Informativeness of the Developed EST-SSR Markers for Finger Millet

Out of the 70 SSR enriched sequences found, primers were developed for 45 of the sequences. Majority of the sequences had a lot of secondary structures and primer template-overlap. PCR conditions were further optimized for 28 of the primers but only 3 primers showed clear polymorphism across the pre-selected 5 accessions and 48 accessions of finger millet on 1.5 % agarose gel. The 3 primers were derived from di-nucleotide sequences (Table 2). The polymorphic information content (PIC) of the developed markers had a value range of 0.6741 for marker UH-Ec-931 to 0.7658 for marker UH-Ec-958. The mean PIC value is 0.7171 (Table 3). Marker UH-Ec-958 showed 13 alleles per locus while marker UH-Ec-956 showed 20 alleles per locus. The mean average of alleles per locus was 17 (Table 3). Following Nei's approach, the mean gene diversity value of 0.7638 was captured by the three markers from the 50 populations of finger millet studied. The highest diversity value of 0.7970 was captured with Marker UH-Ec-958 while the minimum score of 0.7337 was recorded from marker UH-Ec-931(Table 3). The three developed EST-SSRs used for the characterization revealed a total of 51 alleles with an

average of 17 alleles per locus. This average number per locus represents a high value considering the findings of Babu *et al*., (2007) who generated an average allele of 10.58 per locus in their studied population. Gupta *et al*., (2010) used 10 RAPD primers to get an average of 8.6 alleles per locus while with 10 inter simple sequence repeats (ISSR), they generated 5.7 allele per locus. These previous results when compared to our findings shows that our newly developed markers are highly informative in terms of capturing allelic richness. The polymorphic information content (PIC) of the developed markers gave a value range of 0.6741 for marker UH-Ec-931 to 0.7658 for marker UH-Ec-958. The mean PIC value is 0.7171. This result is high when compared to finding of [17,8,18] who reported a maximum PIC of 0.50, 0.50 and 0.26 respectively.. The mean gene diversity value of 0.7638 was captured by the three markers from the48 populations of finger millet studied. The highest

diversity value of 0.7970 was captured with Marker UH-Ec-958 while the minimum score of 0.7337 was recorded from marker UH-Ec-931. This high genetic variation within the 48 randomly selected accessions from the core set of finger millet indicates substantial variance for genetic improvement. This result further shows that the morphological and phenotypical descriptors used for the development of the 622 core collections of finger millet as reported by [11]. was optimal enough and captured as much diversity.

Table 2. *Developed ESTs derived SSR markers for finger millet and their characteristics*

Marker	Foward primer (5´-3´)	Reverse primer (5´-3´)	Repeat motif	Annealing Temp[°C]	Expected size (bp)
UH-Ec-956	TCCGTGTTCGGTTGCT	ATGGGTTCACTGACTCTGC	$(AG)^{23}$ + $(AC)^{11}$	54	190
UH-Ec-958	AACGATCTGGCCTTCCG	TGCCGTGCTGCTCCTCT	$(GA)^{21}$	54	191
UH-Ec-931	GGAAGTTATCACCAGAA	AGACGGACAAATACACA	$(TC)^{14}$	54	236

Table 3. *Result of statistical analysis for E. coracana*

Marker	N_A	PIC	J
UH-Ec-956	20	0.7115	0.7606
UH-Ec-958	13	0.7658	0.7970
UH-Ec-931	18	0.6741	0.7337
Mean	17	0.7171	0.7638

N_A=Number of allele
PIC=Polymorphic information content
J =Nei´s gene diversity

3.2. Cluster Analysis

Cluster analysis for the 48 selected genotypes of finger millet is given in Figure 1. The cluster analysis from the weighted neighbor joining dendrogram generated 4 clusters (Figure 1). Cluster I and II are quite close in similarity. Genotypes from Zimbabwe and Zambia are distributed on the cluster I. Accessions from India are mostly found in cluster IV. Accessions from Nepal were found mostly on cluster III while Ugandan accessions are found in cluster II and III respectively.. Cluster I and II are quite close suggesting a close ancestral lineage. Cluster I major entries are from Zimbabwe and Zambia respectively. The unique placement of most accessions from Zimbabwe in cluster I suggests limited introduction and cross breeding and perhaps limited adaptation of foreign accessions into the region. Ugandan accessions are found only in clusters II and III. This draws to hypothesis that accessions in the regions must have had limited or minimal success in hybridizing with accessions from other regions. A second hypothesis could be that the domestication process has been restricted to a particular region. East Africa is considered a primary center of diversity [4]. and spread of accessions from Zimbabwe, Uganda and Zambia must not have been favored by varying environmental variables. Accessions from Nepal are mostly observed in cluster III suggesting distinctiveness in this region and minimal dispersion. India accessions are found mainly on cluster IV suggesting a closed breeding system. Cultivars introduced from Africa into India through the sea trade of around 1000 BC must have been shaped by adaptive forces of evolution. India had earlier been known as a secondary center of diversity [19]. The accessions from Kenya are fairly represented within all clusters suggesting that dispersion from Kenya to other regions of cultivation has been quite successful. Perhaps human migration through the developed tourist industry and sea access in Mombassa, Kenya must have facilitated this. Our developed EST-SSRs are quite effective in the evaluation of diversity in our studied population. These SSRs represent a significant contribution to the enrichment of markers. The exploitation of these markers will contribute to the genetic advancement of finger millet and closely related genera.

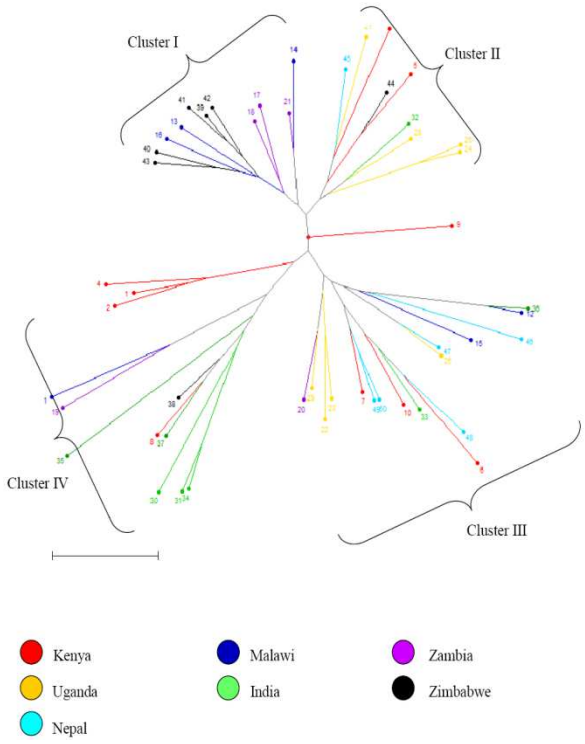

Figure 1. *Weighted neighbour-joining dendrogram illustrating genetic distance of 48 populations of E. coracana*

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References

[1] MM. Dida, N. Wanyera, MLH. Dunn, JL. Bennetzen, KM. Devos, "Population structure and diversity in finger millet (*Eleusine coracona*) germplasm". Trop. Plant. Biol. Vol. 1, pp.131–141, 2008

[2] HD**.** Upadhyaya, NDRK. Sarma, CR. Ravishankar, T. Albrecht, Y. Narasimhudu, SK. Singh, SK. Varshney, VG. Reddy, S. Singh, SL. Dwivedi, N. Wanyera, COA. Oduri, MA. Mgonja, DB. Kisandu, HK. Parzies, CLL. Gowda, " Developing minicore collection in finger millet using multi-location data". Crop. Sci. Vol. 50, pp. 1924-1931, 2010

[3] National Research Council, "Finger millet, In FR Ruskin, eds, Lost crops of Africa, Grains" Nat. Acad. Press. USA. Vol. 1, pp. 39–58,1996

[4] KW. Hilu, JMJ. deWet, "Domestication of *Eleusine coracana*" Econ. Bot. Vol. 30, pp. 199–208, 1979

[5] B. Babu, N. Senthil, S. Gomez, K. Biji, N. Rajendraprasad, S. Kumar, R. Babu, "Assessment of genetic diversity among finger millet (*E. coracana* (L.) Gaertn.) accessions using molecular markers". Genet. Resour. Crop. Evol. Vol. 54, pp. 399–404, 2007

[6] M. Dida, M. Srinivasachary, S. Ramakrishnan, JL. Bennetzen, MD. Gale, KM. Devos, "The genetic map of finger millet, *Eleusine coracana*" Theor. Appl. Genet. Vol. 114, pp. 321–332, 2007

[7] R. Gupta, K. Verma, DC. Joshi, D. Yadav, M. Singh, "Assessment of genetic relatedness among three varieties of finger millet with variable seed coat color using RAPD and ISSR markers". Genet. Eng. Biotech. J. Vol. 2, pp. 1-5, 2010

[8] K. Bezaweletaw, "Genetic diversity of finger millet (*E. coracana* (L.) Gaertn.) landraces characterized by random amplified polymorphic DNA analysis" Inn. Sys. Des. Eng. Vol. 2(4), pp. 207-217, 2011

[9] KD. Scott, P. Eggler, G. Seaton, M. Rossetto, EM. Ablett, LS. Lee, RJ. Henry, "Analysis of SSRs derived from grape ESTs" Theor. Appl. Genet. Vol. 100, pp. 723-726, 2000

[10] C. Chen, P. Zhou, YA. Choi, S. Huang, FG. Jr. Gmitter, "Mining and characterizing microsatelites from *Citrus* ESTs" Theor. Appl. Genet. Vol.112, pp. 1248-1257, 2006

[11] HD. Upadhyaya, CLL. Gowda, RPS. Pundir, VG. Reddy, S. Singh, "Development of core subset of finger millet germplasm using geographical origin and data on 14 quantitative traits" Genet. Resour. and Crop Evol. Vol. 53, pp. 679–685, 2006

[12] JJ. Doyle "DNA protocols for plants-CTAB total DNA isolation", In molecular techniques in Taxonomy, GM Hewitt , eds , Springer, Germany, 1991

[13] AFA. Smit, R. Hubley, P. Green, "RepeatMasker Open-3.0. http://www.repeatmasker.org", 2004

[14] P. Miller, "Tools for population genetic analysis (TFPGA) 1.3, a windows program for the analysis of allozyme and molecular population genetic data", 1997

[15] M. Nei, "Analysis of gene diversity in subdivided populations" Proc. Nat. Acad. Sci. Vol.70 (12), pp. 3321-3323, 1973

[16] D. Botstein, RL. White, M. Skolnick, RW. Davis, "Construction of a genetic linkage map in man using restriction fragment length polymorphisms" Am. J. Hum. Genet. Vol. 32, pp. 314-331, 1980

[17] P. Panwar, M. Nath, VK.Yadav, A. Kumar, "Comparative evaluation of genetic diversity using RAPD, SSR and cytochome P450 gene based markers with respect to calcium content in finger millet (*Eleusine coracana* L. Gaertn.)". J. Genet. Vol. 89, pp. 121–133, 2010

[18] M. Nirgude, BB. Kalyana, Y. Shambhavi, UM. Singh, HD. Upadhyaya, A. Kumar, "Development and molecular characterization of genic molecular markers for grain protein and calcium content in finger millet (*Eleusine coracana* (L.) Gaertn.)" Mol. Biol. Rep. DOI-10.1007/s11033-013-2825-7.

[19] KW. Hilu, JMJ. de Wet, JR. Harlan, " Archaeobotanical studies of *Eleusine coracana"*. Am. J. Bot. Vol. 66, pp. 330-333, 1976