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Genetic diversity analysis and population structure of some African and Asian Finger Millet (*Eleusine coracana* L.) accessions using Expressed Sequence Tags – Simple Sequence Repeat (EST-SSR) markers

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Abstract*— *Finger millet is a high nutritious cereal compared to maize, wheat, rice and sorghum and adaptable to different abiotic and biotic stresses. Understanding the molecular basis of unique traits of finger millets, is key in harnessing its potential as a nutritional security crop among other important aspects. In this study some accession from Africa and Asia were used to research the genetic diversity and population structure of finger millet using EST-SSR Markers. Twenty four accessions of finger millet were tested for polymorphism and highly polymorphic bands were generated in 27 EST markers. A total of 46 alleles were amplified and ranged from 2 to 3 with average of 1.703 per primer pair. The observed heterozygosity value of EST-SSR markers (mean = 0.004) was from 0 to 0.125 and the range of expected heterozygosity value was from 0.16 to 0.582 (mean=0.233). The range of PIC values were from 0.077 to 0.477 and the average PIC value was 0.273.The genetic relationship was divided into three major groups, with accessions from Africa showing a high level of polymorphism and unique population structure compared to Asian ones. These results echos the need for strategic continued colaborative breeding and other crop research programmes between Africa and Asia. The results from futher molecular evaluation will serve as important information for better and efficient management of genetic resources of finger millet for; conservation, crop improvement and intellectual property protection rights purposes.

Keywords*— *Finger millet, EST-SSR markers, Diversity analysis, Population structure.

I. INTRODUCTION

Finger millet (Eleusine coracana (L.) Gaertn) is a nutritious and important food crop, widely cultivated in the arid and semiarid regions in Africa and South Asia. In 2007, global millet production was estimated at 32 million tonnes (FAO, 2009). Finger Millet has a content of calcium (0.38%), dietary fiber (18%) and phenolic compounds (0.3–3%). Finger millets have health benefits, such as; anti-diabetic, anti-tumorigenic, atherosclerogenic effects, antioxidant and antimicrobial properties (Sarita, 2016). Millet is a good

source of micronutrients like, iron and zinc. Biofortification of staple crops is a sustainable and cost-effective approach for availability of micronutrients. Biofortified cultivars of finger millet for improved micronutrients are acceptable to consumers as their adoption does not call for change in dietary habits. Analysis of genetic diversity leading to molecular breeding is a major approach for development of bio fortified cultivars of finger millet. Finger Millet can also adapt to a wide range of ecological conditions with better productivity even in low nutrient input conditions (Kurma, 2018), (Sanjay et al, and 2017). Finger millet is an allotetraploid (2n = 4× = 36, AABB) annual cereal millet crop that includes two distinct subspecies: subsp. Coracana (cultivated finger millet) and subsp. africana (wild finger millet) The size of the assembled genome of Finger millet was about 1.2 GB, while the genome size measured by flow cytometry was 1.5 GB, with 62,348 predicted genes which is the double of genes identified in rice (Hatakeyama, Aluri*et al.* 2017). Analysis of genetic diversity, population structure and molecular characterization using molecular markers are a prerequisite for genetic improvement of any crop including finger millet for effective germplasm conservation. Plant genetics and breeding has changed since the development of molecular markers, like; Random Amplified Polymorphic DNA (RAPD Amplified Fragment Length Polymorphisms (AFLPs) and Simple Sequence Repeats (SSRs) Expressed Sequence Tags (EST) - SSRs and Single Nucleotide Polymorphisms (SNPs), (Gimode D, et al., 2013) (Kumar 2016). Simple sequence repeat (SSR) markers have been widely used to characterize the genetic diversity of germplasm because of their high polymorphism and also their wide distribution throughout the genome. However, few studies have reported the genetic diversity analysis of finger millet genotypes using simple sequence repeats (SSR) markers (Sood et al., 2016) reported the identification of 13 polymorphic SSR markers to analyze the genetic diversity of 103 finger millet accessions. Other study identified 56 new genic SSR markers developed from publicly available drought related ESTs (Pandian et al. 2018). EST-SSR markers have also been used in the assessment of genetic diversity of little millet germplasm (Lee MC *et al.*, 2017). In general terms, use of molecular markers such as SSRs to study the genetic diversity in millets, is one the most appropriate technique providing useful molecular data (Lee JK *et al*., 2017). The EST- SSR markers can serve as important information for better and efficient management of genetic resources of finger millet for; conservation, crop improvement and intellectual property protection rights purposes. The present study aimed to analyze the genetic diversity of 24 accessions of finger millet originating from Africa and Asia using the EST-SSR markers for the purpose of ongoing research and breeding programs.

II. MATERIALS AND METHODS

Twenty four accessions of finger millet for this research, was obtained from Zambia-Africa (south of the equator and Kenya-along the equator) and South Asia (India). Accessions 1-10, 23-24 were from India (12 in total from India), while accessions, 11-20 were from Kenya (Africa) and 21-23 were from Zambia (12 in total from Africa). The National Agrobiodiversity Centre of the Institute of Agriculture Sciences (Republic of Korea) provided the green houses, laboratory and software analysis of the scientific investigation. The whole research was conducted and supervised in South Korea in 2018.

DNA extraction and PCR amplification.

Genomic DNA was isolated from young leaves from each of the twenty four accessions using NucleoSpin Plant II Kit protocol (Macherey-Nagel (MN), Germany). DNA concentration was then estimated using a UV-Vis spectrophotometer microplate reader (Biotech instrument, Korea Ltd). Suitable dilutions was made for amplification in a protocol of a total volume of 20µl, containing 1 µl of genomic DNA (40 ng/µl, 2.0µl of 10x PCR buffer, 0.40µl dNTPS, enhancer of 1 µl, 14.1 µl of water, 0.5 µl of *Taq* polymerase and 0.5 µl of each the forward and the reverse primers. The PCR was then subjected to the following conditions: Initial denaturation at 95°C for 5 minutes followed by 35 cycles of denaturing at 95°C for 50 minutes, annealing at 52°C to 59°C for 40 seconds final extension at 72°C for 30 minutes. PCR was then done using lifeECO Thermocycler (BiortechHangzhar, China). Fragments were analyzed using the Fragment Analyzer Prosize 2.0 version from (Advanced Analytical, USA)

Data analysis

Analysis of different parameters of variability such as; number of alleles (N_A), expected heterozygocity (H_E), Observed heterozygosity (H_O) and polymorphic information content (PIC) were determined using Cervus 3.0.7. DARWin 6.0 was used to create a dendrogram using Unweighted Neighbor joining method. A principle Coordinate Analysis (PcoA) was done, using Gen Al Ex. The population structure analysis of finger millet accessions, were performed using STRUCTURE version 2.3.1, over 12

runs and for a number (*K*) of expected clusters ranging from 1 to 10 and Delta K values as a function of *K*. As indicated in figure 1, *k* value for 3 was optimal.

III. RESULTS AND DISCUSSION

The results for the genetic relationship and population structure analysis in 24 finger millet accessions, were as follows; three major groups were identified;11 accessions were in group 1, 7 in group 2, 5 in group 3 and 1 did not belong to any group. See figure1. The constructed Unweighted Neighbor-joining tree which was based on the genetic dissimilarity matrix data of SSR markers alleles, showed also the same three major grouping. See figure 2.

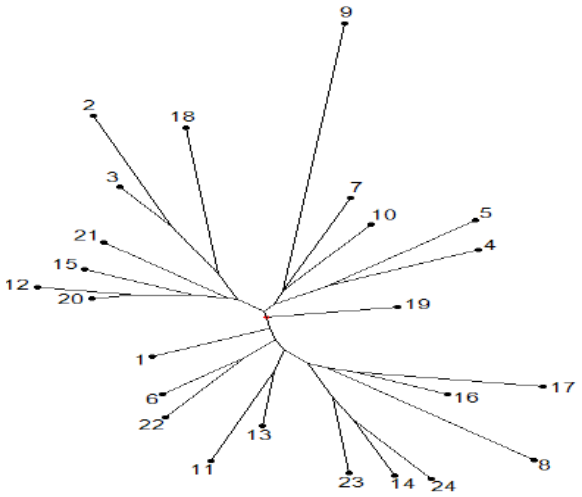

Fig.1: Unweighted neighbor-joining tree

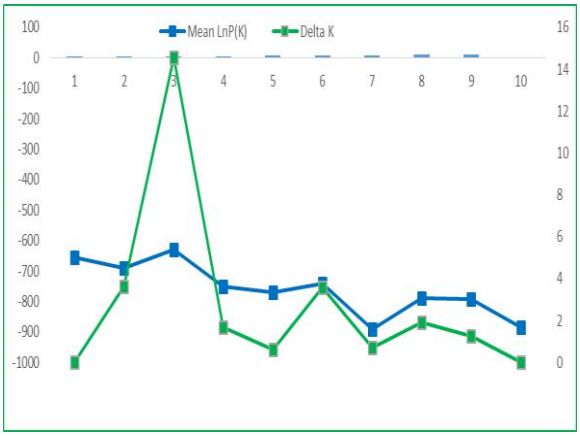

Fig. 2: Population structure analysis

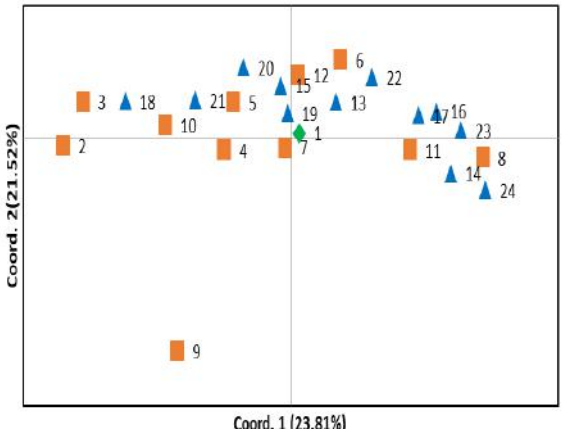

Fig.3: Principle Coordinate analysis

The allele frequency across all 3 groups was as follows: the expected heterozygosity was 0.018 for group 1, 0.32 for group 2 and 0.263 for group 3. Group 1 had no private allele, while group 2 and 3 had 0.32 and 0.263 respectively. Group 1 had 1.03 of effective alleles while group 2 and 3 had 1.557 and 0.107 number of effective alleles respectively. The allele number frequency greater or equal to 5% was 1.036 for group 1 and 2.036 for group 2, while group 3 had 1.821. See table 1.

Table 1. Allele frequency across all three groups.

	Group1	**Group2**	**Group3**
No. alleles (Na)	1.036	2.036	1.821
Na Freq. >= 5%	1.036	2.036	1.821
No. Effective alleles (Ne)	1.036	1.557	1.453
No. private alleles	0.000	0.321	0.107
Exp. H	0.018	0.320	0.263

The Principle Coordinate Analysis (PCoA) of 24 finger millets, which is based on a genetic distance estimation, showed that; the first two coordinates accounted for 23.81% and 21.52% for the total variation respectively. See figure 3. The presented genetic relationships analysis based on the respective 24 African (Zambia, Kenya) and Asian (India) genotypes using 27 genomic SSR markers, showed that African accessions had a high level of polymorphism and unique population structure compared to the Asian ones. This is a good cause for further investigation using more markers

and highly representative number of finger millet accession from respective regions.

IV. CONCLUSION AND RECOMMENDATIONS

In light of the foregoing results and discussion, the conclusion and recommendations are as follows; although the results are clear about the unique genetic diversity and population structure of the accessions in question, more analysis, discussions and recommendations needs to be done and published to a wider scientific community. A similar research needs to be done with several other finger millet germplasm in good proportionate samples from; Zambia, Uganda, Kenya and India respectively, using many EST-SSR markers. Results will be useful for the global scientific community and society at large. The Zambian National Plant Genetic Resources Centre has a unique diversity of more than 300 finger millet accessions collected from all the three agro ecological regions which is yet to be evaluated at the molecular level. This ongoing research on Zambian finger millet molecular evaluation will serve as important information for better and efficient management of genetic resources for; conservation, crop improvement and intellectual property rights protection purposes.

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