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RESEARCH ARTICLE

GENETIC DIVERSITY OF GHANAIAN PEARL MILLET LANDRACES

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ABSTRACT

One Hundred and fifty-one pearl millet accessions collected from their production zones of Upper East, Upper West and Northern Regions were genotyped using Thirty-Six (36) Pearl millet SSR markers (loci) in 20111. This study used the Simple Sequence Repeats (SSR) markers since these have demonstrated to be very informative in studying relationships in closely related plant species as well as readily detecting co-dominant inheritance and exhibiting a high level of polymorphism per loci. The results revealed a total of 316 alleles for all accessions using the 36 markers. A maximum of 20 alleles were observed by loci Xpsmp2270 and Xpsmp2068, and a minimum of 3 alleles were revealed by loci XPsmp2246 and Xpsmp2201 with 8.8 alleles per locus as average. The sizes of alleles ranged from 98bp at locus Xpsmp2068 to 377bp at locus M13_Xpsmp2203. The results have demonstrated the potential genetic variability inherent of the collected pearl millet accessions which can be explored for millet improvement.

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INTRODUCTION

Pearl millet [*Pennisetumglaucum* (L.) R. Br.] is a widely cultivated drought- and high-temperature tolerant C4 cereal grown in the tropics and sub-tropics of Africa, South Asia and the Americas. It is considered an orphan crop with relatively few genomic and genetic resources. In Ghana, it is mostly grown in the Guinea and Sudan Savanna zones comprising the Upper West, Upper East and Northern regions. Germplasm collections represent the store of genetic information available for crop breeding and improvement. Germplasm characterisation can be carried out by means of morphological, biochemical, or molecular analysis. Molecular analysis using genomic DNA is reliable since it can be carried out at any developmental stage of the plant and offers the opportunity to efficiently compare all accessions. It is also very fast and cuts down on the length of time that may be needed to release a variety using the normal conventional methods of breeding. In many cases, SSR containing sequences are part (in exons or introns) of, or linked to, some important genes of agronomic interest (Yu *et al.,* 2000). SSRs occur universally and amply in eukaryotic genomes and are often multi-allelic (Saghai-Maroof *et al.,* 1994). In addition to high levels of polymorphism, SSR sequences possess most of the desirable attributes of molecular markers. Whiles Priolli *et al.* (2002) reported that SSRs are useful for cultivar identification, pedigree analysis and the evaluation of genetic distance between organisms, Yu *et al*. (2000) asserts that they useful for genetic mapping. It was in the light of the effectiveness reported by microsatellites that this study was carried out with the objective of genotyping all the assembled landraces using Simple Sequence Repeats (SSR) markers

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MATERIALS AND METHODS

DNA Extraction

Seeds of 151 millet genotypes obtained from the germplasm collection of the CSIR-SARI were grown in the greenhouse. A maximum of 200mg healthy young leave was sampled from the sprouted seeds and genomic DNA isolated following the Egnin *et al.,* 1998 protocol modified with a boiling step to replace the use of phenol chloroform. Isolated genomic DNA was quantified using spectrophotometer at wavelength 260 and 280. Agarose gel at 0.8% was also used to verify the quality of the DNA and the approximate DNA quantity was 100ng/µl.

PCR and Electrophoresis

There are several molecular analysis systems available for genotyping including Random Amplified Polymorphic DNA (RAPD), Restriction fragment length polymorphism (RFLP), Amplified fragment length polymorphism **(**AFLP), Simple Sequence Repeat (SSR) and DArT. The SSR system is based on microsatellites, and this has demonstrated to be very informative to study relationship in closely related plant species (Bowcock *et al.,* 1994). Microsatellites can readily detect co dominant inheritance. They exhibit a high level of polymorphism per loci as it has been reported in barley with 37 alleles at an individual locus (Saghai-Maroof *et al.*, 1994) and also 26 alleles per loci in soybean (Rongwen *et al.*, 1995). The protocol used as described below was one developed by Tegelstrom (1992). PCRs were carried out in a10µl reaction mixture containing 10–15 g of genomic DNA, 2pmolofeachprimer, 1mMMgCl_2, 0.1mMofeachdNTP, 1×reaction buffer, and 0.2U*Taq* polymerase (Bioline). After one denaturing step of 3min at 94°C, a touch down amplification program was performed on Gene Amp 9700 thermal cycler (Applied Biosystems, USA). This profile consisted of a denaturing step of 25 sec at 94°C and an extension step of 30sec at 72°C. The initial annealing step was 20sec at 64°C for one cycle and subsequently the temperature was reduced by 1°C for every cycle untila final temperature of 55°C was reached. The annealing temperature of 55°C was maintained for the last 35 cycles of amplification, followed by final extension of 72°C for 7min. PCR products were size-separated on native polyacrylamide gels (6%) run on 0.5×TBE buffer at 600V for 3 hours using a Bio-Rad® sequencing gel apparatus. After electrophoresis, the banding patterns of PCR product on PAGE gels were visualized by silver-staining. The genomic DNA samples of millet were analyzed using 36 stipulated SSR markers labelled with a fluorescent dye (Fam/Vic/Ned/Pet) through capillary electrophoresis (ABI3730xl).

Data Analysis

The raw data obtained from the sequencer was analysed using Genemapper software of Applied Biosystems Inc., and the fragment sizes in base pairs (bp) were obtained. The Genemapper data was translated and scored as a present (1) or absent (0) on a spread sheet and subjected to clustering and dissimilarity analysis. Molecular (genotyping) data was subjected to analysis using Power marker version 3.25 (Paul and Demitri, 2012) to generate Polymorphic Information Content (PIC), Gene diversity (Expected Heterozygosity – Hexp) and Observed Heterozygosity (Hob).

Table 1. Polymorphic Microsatellite Primers and their Sequence (Forward and Reverse)

	Primer	Forward Sequence (5′ – 3′)	**Reverse Sequence** (5′ – 3′)
1	XPsmp2246_F	**5′**-CGGATGCTAAATTAACCGAAGC-**3′**	**5′**-CCAGCTTGCTTCTGTTGCGTTC-**3′**
2	M13_Xpsmp2077_V	GCCAATATTATTCCCAAGTGAACA	CTCTTGGTTGCATATCTTTCTTTT
3	Xpsmp2276_N	TGTGGCAATTACGGTCGAGC	CTACCTCTATCTTACTTCACC
4	Xctm12_F	GTTGCAAGCAGGAGTAGATCGA	CGCTCTGTAGGTTGAACTCCTT
5	Xicmp3027_V	ACACCATCACCGACAACAAA	AGTGACCTGGGGTACAGACG
6	Xpsmp2233_N	TGTTTTCTCCTCTTAGGCTTCGTTC	TGTTTTCTCCTCTTAGGCTTCGTTC
7	Xpsmp2085_P	GCACATCATCTCTATAGTATGCAG	AAAAGCATCCTCAAATACCCAT
8	Xpsmp2248_F	TCTGTTTGTTTGGGTCAGGTCCTTC	CGAATACGTATGGAGAACTGCGCATC
9	Xicmp3050_V	ATGTCCAGTGTTGACGGTGA	CGGGGAAGAGACAGGCTACT
10	Xicmp3032_N	AGGTAGCCGAGGAAGGTGAG	CAACAGCATCAAGCAGGAGA
11	Xpsmp2232_F	TGTTGTTGGGAGAGGGTATGAG	CTCTCGCCATTCTTCAAGTTCA
12	Xpsmp2249_V	CAGTCTCTAACAAACAAACACGGC	GACAGCAACCAACTCCAAACTCCA
13	Xpsmp2261_N	AATGAAAATCCATCCCATTTCGCC	CGAGGACGAGGAGGGCGATT
14	M13_Xpsmp2206_P	AGAAGAAGAGGGGGTAAGAAGGAG	AGCAACATCCGTAGAGGTAGAAG
15	Xpsmp2210_V	CAATGATGACCGTAATCTGGGTG	GGGCAAGATATGTGAAATCAAG
16	Xpsmp2270_N	AACCAGAGAAGTACATGGCCCG	CGACGAACAAATTAAGGCTCTC
17	Xpsmp2275_V	CCAGTGCCTGCATTCTTGGC3	GCATCGAATACTTCATCTCA
18	Xpsmp2277_N	GGAATGCTCATCCAATACCCTCC	CCAGGACTGATGAGGTGTGGC
19	Xpsmp2224_P	GGCGAAATTGGAATTCAGATTG	CGTAATCGTAGCGTCTCGTCTAA
20	Xicmp3002_F	CGAGCCGCCATAGTTGAC	TACACACACATTGCCACACG
21	Xpsmp2219_P	ACTGATGGAATCTGCTGTGGAA	GCCCGAAGAAAAGAGAACATAGAA
22	M13_Xpsmp2086_V	CGCTTGTTTTCCTTTCTTGCTGTT	CCTTCTCAGATCCTGTGCTTTCTT
23	M13_Xpsmp2030_F	ACCAGAGCTTGGAAATCAGCAC	CATAATGCTTCAAATCTGCCACAC
24	Xpsmp2045_V	TCATCTTCCCCTATCCGAAAC	ACTTGCCAATGCTATCTTCAC
25	Xpsmp2080_P	CAGAATCCCCACATCTGCAT	TGCAACTGAGCGAACATCAA
26	M13_Xpsmp2203_F	GAACTTGATGAGTGCCACTAGC	TTGTGTAGGGAGCAACCTTGAT
27	Xpsmp2222_V	TGGCTTCCAGACTAATCATCAC	TTATTTTAGCGGCGAGATTGAC
28	Xpsmp2068_P	CAATAACCAAACAAGCAGGCAG	CTTCACTCCCACCCTTTCTAATTC
29	Xpsmp2266_P	CAAGGATGGCTGAAGGGCTATG	TTTCCAGCCCACACCAGTAATC
30	Xpsmp2201_V	CCCGACGTTATGCGTTAAGTT	TCCATCCATCCATTAATCCACA
31	Xpsmp2208_P	GAAAGAGCAAACTGAACAATCCC	ACTTTGCCCTGGATGATCCTC
32	Xpsmp2227_P	ACACCAAACACCAACCATAAAG	TCGTCAGCAATCACTAATGACC
33	M13_Xctm10	GAGGCAAAAGTGGAAGACAG	TTGATTCCCGGTTCTATCGA
34	M13_Xpsmp2087	GGAACAGACTCCATACCTGAAA	TACCTGCCTGTGCTGTTAGT
35	M13_Xpsmp2090	AGCAGCCCAGTAATACCTCAGCTC	AGCCCTAGCGCACAACACAAACTC
36	M13_Xpsmp2273	AACCCCACCAGTAAGTTGTGCTGC	GATGACGACAAGACCTTCTCTCC

Some forward primers are labelled with M13 tail which is 19 nucleotide sequence given below.
M13-CACGACGTTGTAAAACGAC

The Polymorphism Information Content expressed as (PIC_i)

$$= 1 - \sum_{u=1}^{k} \boldsymbol{P}lu^2 - \sum_{u=1}^{k-1} \sum_{v=u+1}^{k} 2\,\boldsymbol{P}lu^2\boldsymbol{P}lv^2$$

Expected Heterozygosity as (HExp):

$$= (1 - \sum_{u=1}^{k} \boldsymbol{P}lu^2) / \{1 - (1+ f)/n\}$$

Observed Heterozygosity as (Hobs): $= 1 - \sum_{u=1}^{k} \boldsymbol{P}luu$

where;

Plu or Pu: *Allele population frequency*

Puv or Pluv: *Genotype population frequency*

f: Inbreeding coefficient

RESULTS

The analysis of presence or absence of an allele by Genstat 5.7 version generated a dendogram and a rooted tree (Fig: 1&2). Both dendgram and rooted tree revealed a distant relationship between sample 82 (M088) and the rest of the collections. However on the Principal Coordinate Analysis (Fig. 3), sample 82 (M088) grouped with samples 18 (M019) and 85 (M091) to which it was most closely related. Three major groupings were generated by the rooted tree and dendogram while the Principal Coordinate Analysis revealed 13 major clustering groups.

Gene Diversity Analysis

The millet 36 SSR markers (Table 1) used to genotype the 151 millet germplasm collection revealed a total of 316 alleles. A maximum of 20 alleles was revealed by locus Xpsmp2068_P, and a minimum of 3 alleles was revealed at loci XPsmp2246_F and Xpsmp2201_V. The average number of alleles was 8.78/locus. The sizes of alleles ranged from 98bp at locus Xpsmp2068_P to 377bp at locus M13_Xpsmp2203_F (Table 2). Fifteen microsatellites (41.67%) had allele numbers ranging from 10 to 20. Polymorphism Information Content (PIC) is a closely related diversity measure of a population. The results indicated that the PIC ranged from 0.142 and 0.927 in M13-Xpsmp2077 and Xpsmp2068 respectively with mean value of 0.613. Expected heterozygosity (H_e) is defined as theprobabilitythattworandomlychosenallelesfromthepopulation aredifferent and gene diversity is defined as 'the proportion of heterozygous individuals in the population'. M13-Xpsmp2077 had the lowest expected heterozygosity and observed heterozygosity (H_o) values of 0.145 and 0.108 respectively. Xpsmp2068 and Xpsmp2266 recorded the highest values of 0.932 and 0.579 respectively for expected and observed heterozygosity.

DISCUSSION

Germplasm collections represent the store of genetic information available for crop breeding and improvement. Germplasm characterisation can be carried out by means of morphological, biochemical, or molecular analysis. Molecular analysis using genomic DNA is reliable since it can be carried out at any developmental state of plant and offers the opportunity to efficiently compare all accessions within a short time.

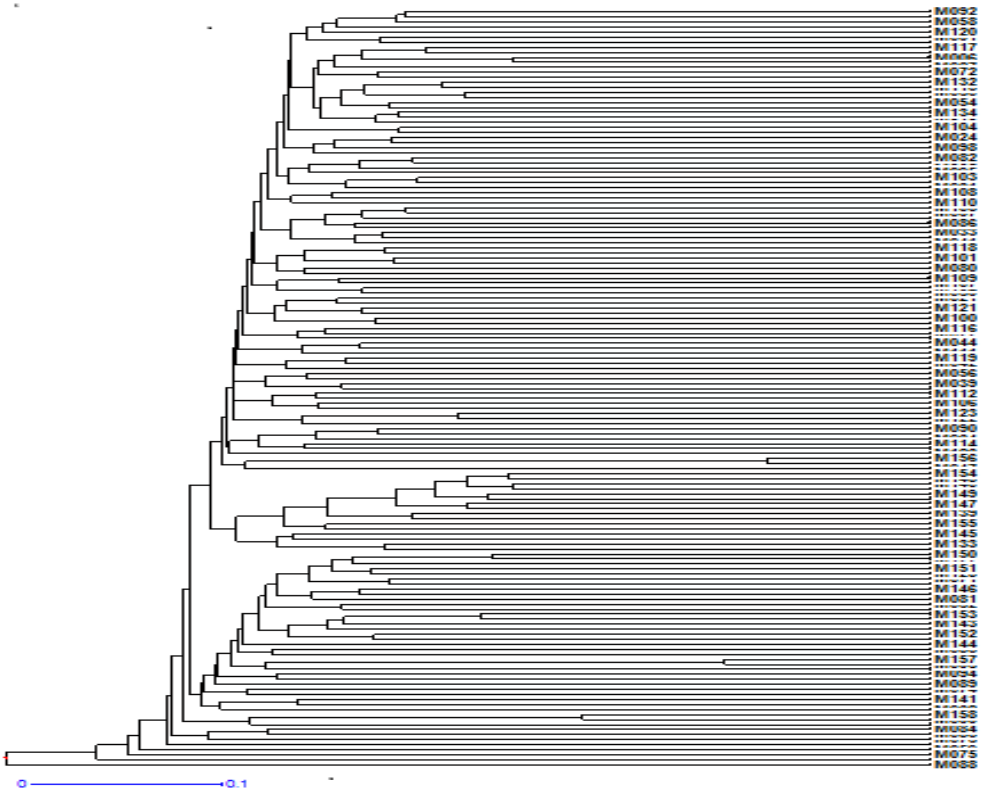

Figure 1. Cluster analysis of 151 Pearl millet accessions

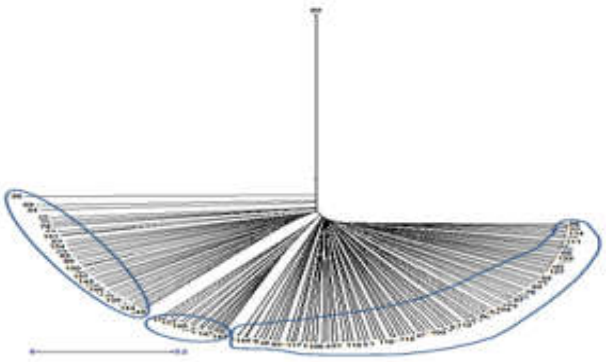

Fig 2: Rooted tree generated for the 151 pearl millet accessions

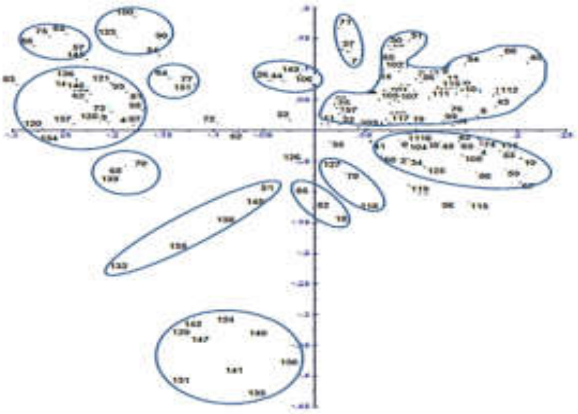

Fig 3: Principal Coordinate Analysis generated for the 151 pearl millet accessions

Table 2. Genetic Diversity Analysis using 36 Polymorphic microsatellite primers in 151 Pearl millet Accessions

Marker	Major Allele Frequency	NA	H_e	H_o	PIC	Min bp	Max bp
XPsmp2246_F	0.577	3	0.555	0.333	0.477	260	264
M13_Xpsmp2077_V	0.923	6	0.145	0.108	0.142	154	198
Xpsmp2276_N	0.455	8	0.712	0.402	0.675	266	286
Xctm12_F	0.409	10	0.761	0.261	0.733	319	335
Xicmp3027_V	0.288	5	0.755	0.542	0.711	198	208
Xpsmp2233_N	0.310	8	0.774	0.195	0.739	254	268
Xpsmp2085_P	0.509	5	0.630	0.410	0.570	166	174
Xpsmp2248_F	0.680	5	0.488	0.386	0.440	158	172
Xicmp3050_V	0.652	6	0.494	0.393	0.425	213	227
Xicmp3032_N	0.496	7	0.654	0.398	0.600	184	202
Xpsmp2232_F	0.366	7	0.738	0.361	0.694	223	241
Xpsmp2249_V	0.835	5	0.292	0.206	0.278	128	154
Xpsmp2261_N	0.280	15	0.832	0.505	0.812	175	199
M13_Xpsmp2206_P	0.528	7	0.663	0.449	0.629	217	231
Xpsmp2210_V	0.233	17	0.877	0.466	0.866	298	344
Xpsmp2270_N	0.250	20	0.889	0.388	0.881	138	188
Xpsmp2275_V	0.697	6	0.445	0.294	0.378	270	282
Xpsmp2277_N	0.453	10	0.721	0.402	0.688	228	258
Xpsmp2224_P	0.557	6	0.597	0.470	0.536	150	162
Xicmp3002_F	0.733	4	0.418	0.271	0.369	198	208
Xpsmp2219_P	0.466	7	0.661	0.458	0.604	229	291
M13_Xpsmp2086_V	0.449	12	0.730	0.188	0.700	108	148
M13_Xpsmp2030_F	0.688	10	0.508	0.286	0.490	127	153
Xpsmp2045_V	0.332	12	0.767	0.445	0.732	193	217
Xpsmp2080_P	0.318	12	0.823	0.365	0.804	144	196
M13_Xpsmp2203_F	0.245	10	0.814	0.545	0.788	353	377
Xpsmp2222_V	0.513	4	0.633	0.440	0.573	153	159
Xpsmp2068_P	0.119	20	0.932	0.496	0.927	98	138
Xpsmp2266_P	0.254	10	0.810	0.579	0.783	172	192
Xpsmp2201_V	0.856	3	0.255	0.127	0.237	346	366
Xpsmp2208_P	0.496	11	0.700	0.431	0.672	245	317
Xpsmp2227_P	0.809	4	0.326	0.280	0.302	195	211
M13_Xctm10	0.189	15	0.900	0.351	0.892	188	216
M13_Xpsmp2087	0.398	6	0.662	0.519	0.595	137	151
M13_Xpsmp2090	0.382	13	0.798	0.325	0.779	193	213
M13_Xpsmp2273	0.604	7	0.576	0.167	0.532	185	213
Mean	0.482	8.8	0.648	0.368	0.613		

NA: allele number, He: expected heterozygosity, Ho: observed heterozygosity, PIC: polymorphic information content, Min bp: minimum base pair, Max bp: Maximum base pair

Molecular marker diversity within the accessions

According to Rai *et al*. (1999), West Africa and India are the primary and secondary centres of diversity for pearl millet respectively. Broadening the genetic base of any crop is very important since inbreeding often leads to a reduction in genetic diversity, more so with open pollinated crop like pearl millet. Key to pearl millet germplasm preservation in Ghana is an understanding of the genetic relationship and diversity among available gene pool. Genetic diversity estimates relies very

much on the number of alleles within the population and this helps in comparing two or more accessions or landraces. From the 151accessions genotyped, a total of 316 alleles were recorded with 36 microsatellites and an average of 8.8 alleles per locus. These results indicated a very high level of polymorphism at the loci and thus a very high level of genetic diversity among the pearl millet accessions. Heng-Sheng *et al*. (2012), working on foxtail millet and using 40 microsatellites, had 2.4 alleles per locus which was considered to be high. Therefore the observed 8.8 alleles per locus for pearl millet is thus significantly higher than 2.5 and 6.16 alleles per locus reported by Jia *et al.* (2007; 2009).Using SSR markers on foxtail millet, 4.91 alleles per locus in broomcorn was reported by Cho *et al*. (2000), and 3.83 alleles per locus for rice in work done by Hu *et al*. (2009). The presence of significant genetic variation within the accessions is an indication that useful traits of many kinds are present in the collection and hence has the potentials for trait improvement through breeding. According to Deb (2009), agricultural productivity and food security can be maintained through sustained crop diversity and by default genetic diversity. This will hold true for pearl millet considering the erratic nature of rains in the last decade resulting from climate change effects as well as land use change. In genetic analysis, the PIC value is used to measure the discriminatory power of a marker and there is evidence to show that there is a positive correlation between PIC and number of alleles per locus (Jia *et al*., 2009). A PIC value greater than 0.5 (PIC > 0.5) is considered high, and for any marker, indicates that such marker can be effectively used in genetic diversity studies, especially in foxtail (Heng-Sheng *et al*., 2012). In the current work therefore, and taking the entire accessions as a whole, 26 (72.22%) microsatellites loci had PIC values above 0.5. The 151 accessions with the 36 microsatellites showed that eight (22.22%) and two (5.56%) microsatellites had medium (0.25 ≤ PIC < 0.5) and low (PIC < 0.25) number of polymorphism respectively.

Generally the mean PIC value (0.613) observed in this study compared favourably with those reported by Kapila *et al*. (2007), who reported an average PIC value of 0.58 for pearl millet. The results revealed that the expected heterozygosity (H_e) ranged from 0.145 to 0.932 with an average value of 0.648 and the Observed heterozygosity (H_o) ranged from 0.108 to 0.579 with average of 0.368. The average H_e values in this study is almost twice higher than the average value of 0.354 reported by Heng-Sheng *et al*. (2012) working on 324 foxtail millet and Cho *et al*. (2010), whose work on proso millet recorded average H_e value of 0.37, but less than the average value of 0.67 reported by De Campos *et al*. (2008) whose work was on 126 wild rice.. Also the mean H_o value of 0.368 for pearl millet was observed to be higher than the 0.045 for Indian foxtail millet (Gupta *et al*., 2012). The higher values obtained for H_e and H_o could be attributed to the fact that pearl millet is an open-pollinated crop and that most of the farmers resorted to natural selection methods in preserving seeds for regeneration year after year. Agro-morphological characterisation studies conducted on 119 of these accessions (Asungre *et al*., 2015) showed a wide range of trait variations across the collection regions. These results have also demonstrated the potential genetic variability inherent of the collected pearl millet accessions.

Conclusion

The relatively high estimates of observed and expected heterozygosity as well as the PIC in this studies could be effectively manipulated with appropriate breeding methods and programs to develop improved varieties and hybrids for use by farmers using these accessions. The variations in the allele size (from 98bp at locus Xpsmp2068_P to 377bp at locus M13_Xpsmp2203_F) further buttress the resolve that these accessions have a rich resources that can be explored. The evidence available for pearl millet genetic and agro-morphological variations associated with the Ghanaian pearl millet is a further boost for breeding work towards addressing climate smart issues. This information can be useful and accessed by both local and the international scientific community.

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