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Assessment of genetic diversity among cassava landraces using single nucleotide polymorphic markers

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This study seeks to determine the genetic diversity among cassava landraces using single nucleotide polymorphic (SNP) markers. One hundred and five cassava landraces were assayed with 195 SNP markers. Major allele frequency varied from 0.500 to 0.942 with an average of 0.728. Average gene diversity, heterozygosity and polymorphic information content (PIC) were 0.359, 0.314 and 0.286 respectively. These values were generally high considering the bi-allelic nature of SNPs, hence the cassava landraces studied showed moderate to high genetic diversity. This suggests availability of unique and useful alleles that could be exploited for breeding purposes. Inclusion of these landraces in our crop improvement activities will enhance the development of farmer preferred cassava varieties. SNP markers used for the study were highly informative, polymorphic and revealed good estimates of genetic diversity among the landraces. Higher level of genetic variation was observed within population based on analysis of molecular variance (AMOVA). Principal component analysis (PCA) and cluster analysis also grouped landraces into three distinct clusters; however, they did not group in accordance to geographical origin. This could be due to high frequency of germplasm exchange between farmers and subsequent change of the name of the same cultivar. Results from this study may contribute significantly to cassava breeding and germplasm conservation programs.

Key words: Genetic diversity, single nucleotide polymorphisms (SNPs), polymorphic information content (PIC), polymorphic, alleles, heterozygosity, germplasm.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz), member of the family Euphorbiaceae is a major root crop cultivated in most countries in sub-Saharan Africa. It is a staple for millions of households (Rabbi et al., 2014) and a subsistence crop due to its flexibility in planting and harvesting times. Cassava serves as a food security crop because of its high source of carbohydrate, ability to

thrive under different climatic conditions (Tumuhimbise et al., 2014) and multipurpose uses for human consumption, animal feed and industrial applications (Rabbi et al., 2012). In Ghana, cassava is a major root crop and it is cultivated in all regions in the country. The livelihoods of about 70% of smallholder farmers depend on the crop. Over the years, a lot of improved cassava varieties

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Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> have been developed and disseminated in Ghana. However, farmers still keep diverse sets of landraces on their farms, although they may be low yielding and susceptible to some biotic and abiotic stresses. These landraces may have increased genetic diversity which could promote gene flow through hybridization (Turyagyenda et al., 2012). Thus, landraces are important genetic resources (Mtunguja et al., 2014) for breeding and other crop improvement activities. They have different attributes which may present favorable characteristics that can contribute to food and nutritional security since it gives species the ability to adapt to changing environments including new pests, new diseases and new climatic conditions. They also provide opportunity for plant breeders to develop new and improved cultivars with desirable characteristics, for in situ conservation and studies on genetic diversity and evolution. To guarantee the security of these genetic resources. landraces must be maintained to avoid losses. However, they need to be characterized before they are maintained or conserved. This is necessary since there could be a mix up in the landraces where the same accession may have different names or different accessions may have the same name in different places. In addition, continuous exchange of planting materials between different farmers through both formal and informal distribution systems, make pedigree information limited and unreliable. Therefore, characterization of landraces is necessary as it will facilitate the removal of duplicates and creation of core collection for utilization by plant breeders. A prerequisite for any breeding programme is knowledge of the extent of genetic variability among cultivars. Such information guides the breeder to select distant parents to broaden the genetic base and to produce superior progenies.

Recent advances in molecular techniques have provided useful tools for characterization. Thus. molecular markers are widely used in plant genetic research and breeding. These markers permit the detection of genetic differences among closely related landraces, highly polymorphic, more stable and less influenced by the environment (Tiago et al., 2016). Several molecular markers have been used to characterize cassava germplasm to identify genetic variability. These include Random Amplified Polymorphic DNA (Rimoldi et al., 2010), Amplified Fragment Length Polymorphism (Benesi et al., 2010), microsatellites (Gonçalves et al., 2017) and more recently, Single nucleotide polymorphic (SNP) markers (Mtunguja et al., 2017).

With recent advances in high-throughput genotyping technologies, SNPs are increasingly becoming markers of choice for plant genetic studies and breeding. This is because of its cost effectiveness, locus specificity and codominant nature (Ren et al., 2013). Expressed sequence tags (ESTs) are fragments of mRNA sequences derived through single sequencing reactions performed on randomly selected clones from cDNA libraries (Parkinson and Blaxter, 2009). EST collections have been used to detect SNPs in crops such as maize (Ching et al., 2002) and soybean (Zhu et al., 2003). These SNPs have proved to be effective in the characterization of these crops. Similar studies in cassava has also led to the detection of SNPs from ESTs (Lopez et al., 2005; Ferguson et al., 2012), of which 1,190 have been validated and could be used in characterizing cassava. The aim of this study was to assess the genetic diversity among cassava landraces in Ghana using SNP markers. Understanding the extent of genetic diversity among cassava landraces may enhance efficient utilization by breeders for improving the crop.

MATERIALS AND METHODS

Source of germplasm

A total of 105 cassava landraces were collected from farmers' field in four major cassava growing zones (the rain forest, deciduous forest, transition and coastal savannah zones) of Ghana.

Screen house establishment

The planting materials were sprouted at the experimental station of the CSIR-Crops Research Institute, Kumasi, Ghana (6°41'N, 1°28'W).

Molecular characterization

DNA extraction

Total genomic DNA was extracted using standard procedures according to Egnin et al. (1998). About 200 mg of freshly harvested apical leaves of each accession were ground in liquid nitrogen into fine powder. Eight hundred microliters of extraction buffer (50 mM Tris HCL, pH 8.0, 300 mM NaCl, 20 mM EDTA, 20% PVP, 1.5% Sarcosine and 0.1 g/L $Na_2S_2O_2$) were used to lyse nuclear membranes.

Proteins and polysaccharides were precipitated by adding 400 µl of 5 M potassium acetate (instead of 800 µl of phenol chloroform isomyl alcohol) as used by Egnin et al. (1998) and the samples centrifuged at 13,000 rpm for 10 min. RNA was removed by adding 4 µl RNase A (10 mg/ml) and incubated at 37°C for thirty minutes. DNA was precipitated using 700 µl of ice-cold isopropanol and centrifuged at 13,000 rpm for ten minutes. Eighty percent ethanol was used to wash DNA and centrifuged at 13,000 rpm for five minutes. Ethanol was discarded and DNA pellets were air-dried at room temperature. DNA pellets were resuspended in 200 µl 1X, TE (Tris-ethylenediaminetetracetic acid) buffer after which quality of DNA was determined on 0.8% (w/v) ethidium bromide stained agarose gel. The purity and quantification of DNA was determined by measuring the absorbance at 260 nm (A₂₆₀) and 280 nm (A₂₈₀) with a spectrophotometer (Biochrom Libra S12).

SNP genotyping

KASPar technology was employed for SNP genotyping at the KBiosciences laboratories (United Kingdom). One hundred and ninety-five SNP markers developed from the Generation Challenge Programme (GCP) were used for the genotyping.

Table 1. Constituent reagent volumes for KASP genotyping mix.

Component	Volume (µl)
DNA	5.00
Mastermix	5.00
Primermix	0.14
Total reaction volume	10.00

 Table 2. The KASP thermal cycling program.

Temperature/Time	Number of cycles		
94°C for 20 s	Hot-start activation		
94°C for 20 s			
61-55°C for 60 s	TO cycles		
(dropping 0.6°C per cycle)			
94°C for 20 s			
55°C for 60 s	26 cycles		

The genotyping assay for KASPar consisted of two reagent components (KASP Primer mix and KASP Master mix) plus the DNA sample. The KASP Primer mix was made up of two allelespecific forward primers and one common reverse primer. The KASP Master mix contained the FAM and HEX specific FRET cassette system, Taq polymerase, dNTP's, 5-carboxy-X-rhodamine, succinimidyl ester (ROX) and MgCl₂ in an optimized buffer solution. The KASP Primer mix was combined with the KASP Master mix and added to the DNA samples (5 - 50 ng) to be genotyped (Table 1) in a 96-well plate and sealed using the Kube[™] heat-based sealer. The reaction was carried out in a standard thermal cycler with conditions comprising two temperatures (Table 2). After completion of the PCR run, the scan results were read, and allele call was generated from the KlusterCaller software. SNP markers that were monomorphic were considered non informative and were removed from further analysis. A total of 187 SNP markers were therefore retained for genetic diversity analysis.

Data analysis

The genetic analysis package PowerMarker version 3.2 (Liu and Muse, 2005) was used to generate the following genetic diversity parameters: gene diversity, heterozygosity and polymorphic information content (PIC) (Bostein and White, 1980). PIC values were calculated with the equation: $PIC=1-\Sigma P_i^2 - \Sigma 2P_i^2 P_J^2$ Where: $\Sigma P_i^2 = sum$ of each squared ith haplotype frequency. The software was used to calculate genetic distances among genotypes using the Euclidean method and neighbour-joining (NJ) algorithm (Nei, 1973) to construct a dendrogram from the distance matrix using MEGA 5.2 software (Tamura et al., 2007) embedded in PowerMarker. Analysis of molecular variance (AMOVA) and principal component analysis were performed using GenAIEx 6.4 software (Peakall and Smouse, 2006).

RESULTS AND DISCUSSION

Genetic diversity

Genetic variation among genotypes is important for

sustainable use of genetic resources to meet the demand for future food security as well as conservation strategies. This study was conducted to establish the genetic diversity among cassava landraces. Polymorphism frequencies are an important criterion that can be used to assess the value of molecular markers for germplasm characterization (Singh et al., 2013). From a total of 195 SNPs markers used for the study, 187 (96%) were polymorphic and 8 (4%) were monomorphic. The polymorphism observed in this study can be attributed to the fact that cassava landraces used for the study were diverse. In this regard 187 out of 195 SNPs used provided adequate informative polymorphism to evaluate genetic diversity of the cassava landraces. The high number of polymorphic SNPs is consistent with the mode of reproduction, genetic breeding system, and level of genetic variation in cassava. The results of this study were in close agreement with findings by Oliveira et al. (2014) who reported 1.5% of monomorphic SNPs when 1,280 cassava accessions were analyzed with 402 SNP markers.

PIC is a measure of the informativeness of a marker. The higher the PIC value the more informative the marker. PIC values varied from 0.049 to 0.375 with an average of 0.286 (Table 3), and approximately 56% of SNPs had estimates over 0.30. This suggests that the SNPs were informative and could discriminate among genotypes, hence could be candidate markers for genetic variability studies. Although the SNPs were informative, PIC values obtained with SNPs are generally lower compared to other molecular markers such as Simple Sequence Repeat (SSR) markers. In a recent study, PIC value varied between 0.030 to 0.780 when 89 cassava accessions were assayed with 35 SSRs (Adjebeng-Danquah et al., 2020). The low PIC value observed in this study is due to the bi-allelic nature of SNPs, hence

Marker	Major allele frequency	Gene diversity	Heterozygosity	PIC
327-SNP	0.9	0.18	0.2	0.164
379-SNP	0.665	0.446	0.479	0.346
958-SNP	0.847	0.259	0.2	0.225
1920-SNP	0.512	0.5	0.476	0.375
2216-SNP	0.894	0.19	0.17	0.172
2257-SNP	0.756	0.369	0.274	0.301
2300-SNP	0.542	0.496	0.516	0.373
2496-SNP	0.973	0.052	0.053	0.05
6331-SNP	0.831	0.28	0.267	0.241
6453-SNP	0.709	0.413	0.363	0.328
6464-SNP	0.954	0.088	0.051	0.084
6630-SNP	0.869	0.227	0.216	0.201
6780-SNP	0.594	0.482	0.322	0.366
6889-SNP	0.556	0.494	0.444	0.372
6912-SNP	0.882	0.208	0.236	0.186
6922-SNP	0.95	0.095	0.056	0.09
7138-SNP	0.833	0.278	0.313	0.239
7239-SNP	0.807	0.311	0.26	0.263
7259-SNP	0.925	0.139	0.129	0.13
7434-SNP	0.825	0.289	0.35	0.247
Me v4 MEF c 3242	0.506	0.5	0.354	0.375
 Me_v4_MEF_c_1018	0.511	0.5	0.348	0.375
Me v4 MEF c 1175	0.787	0.335	0.319	0.279
 Me_v4_MEF_c_1183	0.527	0.499	0.598	0.374
Me v4 MEF c 1220	0.876	0.217	0.135	0.193
Me v4 MEF c 1246	0.669	0.443	0.413	0.345
 Me_v4_MEF_c_1278	0.702	0.418	0.333	0.331
Me_v4_MEF_c_1320	0.75	0.375	0.364	0.305
Me_v4_MEF_c_2980	0.579	0.487	0.305	0.369
Me_v4_MEF_c_2990	0.604	0.478	0.659	0.364
Me_v4_MEF_c_2337	0.648	0.456	0.432	0.352
Me_v4_MEF_c_2363	0.546	0.496	0.517	0.373
Me_v4_MEF_c_2366	0.523	0.499	0.609	0.374
Me_v4_MEF_c_2851	0.689	0.428	0.432	0.337
Me_v4_MEF_c_2873	0.75	0.375	0.221	0.305
Me_v4_MEF_c_1387	0.882	0.208	0.191	0.186
Me_v4_MEF_c_2286	0.601	0.48	0.606	0.365
Me_v4_MEF_c_2283	0.733	0.392	0.395	0.315
Me_v4_MEF_c_3057	0.963	0.071	0.074	0.069
Me_v4_MEF_c_1447	0.506	0.5	0.41	0.375
Me_v4_MEF_c_1527	0.516	0.499	0.293	0.375
Me_v4_MEF_c_1566	0.705	0.416	0.35	0.329
Me_v4_MEF_c_1617	0.795	0.326	0.289	0.273
Me_v4_MEF_c_1637	0.876	0.217	0.135	0.193
Me_v4_MEF_c_1645	0.81	0.307	0.287	0.26
Me_v4_MEF_c_1679	0.689	0.429	0.444	0.337
Me_v4_MEF_c_1892	0.974	0.05	0.031	0.049
Me_v4_MEF_c_1919	0.849	0.256	0.215	0.223
Me_v4_MEF_c_1940	0.565	0.492	0.588	0.371
Me v4 MEF c 1945	0.747	0.378	0.11	0.306

 Table 3. Summary statistics of 105 cassava landraces assayed with 187 SNP markers.

Table 3. Contd.

Me, v4, MEF, c. 1986 0.864 0.236 0.152 0.028 Me, v4, MEF, c. 2034 0.545 0.416 0.235 0.33 Me, v4, MEF, c. 2043 0.89 0.196 0.1198 0.176 Me, v4, MEF, c. 2120 0.88 0.211 0.109 0.188 Me, v4, MEF, c. 2120 0.88 0.211 0.109 0.188 Me, v4, MEF, c. 2124 0.759 0.366 0.235 0.229 Me, v4, MEF, c. 2195 0.776 0.348 0.269 0.287 Me, v4, MEF, c. 2236 0.724 0.4 0.3668 0.32 Me, v4, MEF, c. 2238 0.714 0.408 0.327 0.325 Me, v4, MEF, c. 2409 0.565 0.491 0.248 0.371 Me, v4, MEF, c. 2419 0.565 0.491 0.232 0.239 0.249 Me, v4, MEF, c. 2437 0.823 0.291 0.293 0.249 Me, v4, MEF, c. 2447 0.775 0.345 0.239 0.246 Me, v4, MEF, c. 2448 0.523 0.499 </th <th>Me_v4_MEF_c_1947</th> <th>0.833</th> <th>0.278</th> <th>0.204</th> <th>0.239</th>	Me_v4_MEF_c_1947	0.833	0.278	0.204	0.239
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Me_v4_MEF_c_2189 0.53 0.498 0.28 0.374 Me_v4_MEF_c_2195 0.776 0.348 0.269 0.287 Me_v4_MEF_c_2236 0.724 0.4 0.368 0.32 Me_v4_MEF_c_2384 0.714 0.408 0.327 0.325 Me_v4_MEF_c_2402 0.672 0.441 0.355 0.344 Me_v4_MEF_c_2409 0.565 0.491 0.848 0.371 Me_v4_MEF_c_2419 0.569 0.491 0.238 0.37 Me_v4_MEF_c_2447 0.778 0.345 0.239 0.249 Me_v4_MEF_c_2447 0.778 0.345 0.239 0.249 Me_v4_MEF_c_2448 0.523 0.499 0.465 0.374 Me_v4_MEF_c_2466 0.741 0.384 0.42 0.31 Me_v4_MEF_c_2510 0.841 0.268 0.273 0.232 Me_v4_MEF_c_2552 0.683 0.433 0.366 0.333 Me_v4_MEF_c_2552 0.683 0.433 0.366 0.337 M	Me_v4_MEF_c_2124	0.759	0.366	0.235	0.299
Me_v4_MEF_c_2195 0.776 0.348 0.269 0.287 Me_v4_MEF_c_2226 0.724 0.4 0.368 0.32 Me_v4_MEF_c_2236 0.724 0.4 0.368 0.32 Me_v4_MEF_c_2384 0.714 0.408 0.327 0.325 Me_v4_MEF_c_2409 0.565 0.491 0.238 0.371 Me_v4_MEF_c_2419 0.569 0.491 0.238 0.371 Me_v4_MEF_c_2447 0.778 0.345 0.239 0.2285 Me_v4_MEF_c_2448 0.523 0.499 0.465 0.374 Me_v4_MEF_c_2466 0.827 0.237 0.246 Me_v4_MEF_c_2510 0.841 0.268 0.273 0.232 Me_v4_MEF_c_2562 0.683 0.433 0.366 0.339 Me_v4_MEF_c_2562 0.683 0.433 0.366 0.339 Me_v4_MEF_c_2562 0.663 0.477 0.511 0.363 Me_v4_MEF_c_2788 0.660 0.477 0.511 0.363 Me_v4_MEF_c_2780	Me_v4_MEF_c_2189	0.53	0.498	0.28	0.374
Me_v4_MEF_c_2286 0.823 0.292 0.312 0.249 Me_v4_MEF_c_2384 0.714 0.44 0.368 0.32 Me_v4_MEF_c_2402 0.672 0.441 0.355 0.344 Me_v4_MEF_c_2402 0.672 0.441 0.355 0.344 Me_v4_MEF_c_2409 0.565 0.491 0.238 0.371 Me_v4_MEF_c_2447 0.783 0.231 0.293 0.249 Me_v4_MEF_c_2447 0.778 0.345 0.239 0.285 Me_v4_MEF_c_2448 0.523 0.499 0.465 0.374 Me_v4_MEF_c_2486 0.741 0.384 0.42 0.31 Me_v4_MEF_c_2486 0.827 0.287 0.232 0.246 Me_v4_MEF_c_2552 0.683 0.433 0.366 0.339 Me_v4_MEF_c_2562 0.5 0.5 0.407 0.375 Me_v4_MEF_c_2768 0.660 0.477 0.511 0.363 Me_v4_MEF_c_2768 0.545 0.496 0.477 0.373 M	Me_v4_MEF_c_2195	0.776	0.348	0.269	0.287
Me_v4_MEF_c_2336 0.724 0.4 0.386 0.327 Me_v4_MEF_c_2384 0.714 0.408 0.327 0.325 Me_v4_MEF_c_2402 0.672 0.441 0.355 0.344 Me_v4_MEF_c_2419 0.569 0.491 0.283 0.37 Me_v4_MEF_c_2437 0.623 0.293 0.285 Me_v4_MEF_c_2444 0.523 0.499 0.465 0.374 Me_v4_MEF_c_2456 0.741 0.385 0.327 0.246 Me_v4_MEF_c_2456 0.741 0.288 0.327 0.246 Me_v4_MEF_c_2456 0.741 0.288 0.327 0.246 Me_v4_MEF_c_2510 0.841 0.268 0.327 0.246 Me_v4_MEF_c_2524 0.753 0.372 0.385 0.303 Me_v4_MEF_c_2562 0.683 0.433 0.366 0.339 Me_v4_MEF_c_2653 0.606 0.477 0.511 0.363 Me_v4_MEF_c_2748 0.339 0.114 0.101 0.107 Me_v4_MEF_c_2758 <td>Me_v4_MEF_c_2226</td> <td>0.823</td> <td>0.292</td> <td>0.312</td> <td>0.249</td>	Me_v4_MEF_c_2226	0.823	0.292	0.312	0.249
Me_v4_MEF_c_2384 0.714 0.408 0.327 0.325 Me_v4_MEF_c_2402 0.672 0.441 0.365 0.344 Me_v4_MEF_c_2409 0.565 0.491 0.848 0.371 Me_v4_MEF_c_2419 0.569 0.491 0.238 0.37 Me_v4_MEF_c_2437 0.778 0.345 0.239 0.249 Me_v4_MEF_c_2448 0.523 0.499 0.465 0.374 Me_v4_MEF_c_2486 0.827 0.287 0.327 0.246 Me_v4_MEF_c_2510 0.841 0.268 0.273 0.232 Me_v4_MEF_c_2521 0.681 0.433 0.366 0.339 Me_v4_MEF_c_2562 0.5 0.5 0.407 0.375 Me_v4_MEF_c_2728 0.939 0.114 0.101 0.107 Me_v4_MEF_c_2748 0.399 0.114 0.101 0.107 Me_v4_MEF_c_2888 0.86 0.241 0.191 0.375 Me_v4_MEF_c_2809 0.571 0.49 0.19 0.371 M	Me_v4_MEF_c_2236	0.724	0.4	0.368	0.32
Me_v4_MEF_c_2402 0.672 0.441 0.355 0.344 Me_v4_MEF_c_2409 0.565 0.491 0.238 0.371 Me_v4_MEF_c_2419 0.569 0.491 0.238 0.371 Me_v4_MEF_c_2437 0.823 0.291 0.293 0.249 Me_v4_MEF_c_2447 0.778 0.345 0.239 0.285 Me_v4_MEF_c_2448 0.523 0.499 0.465 0.374 Me_v4_MEF_c_2456 0.741 0.384 0.42 0.31 Me_v4_MEF_c_2466 0.827 0.287 0.327 0.246 Me_v4_MEF_c_2552 0.681 0.433 0.365 0.303 Me_v4_MEF_c_2562 0.5 0.5 0.407 0.375 Me_v4_MEF_c_2726 0.606 0.477 0.511 0.363 Me_v4_MEF_c_278 0.545 0.496 0.477 0.375 Me_v4_MEF_c_2801 0.608 0.477 0.462 0.363 Me_v4_MEF_c_2801 0.608 0.477 0.462 0.363	Me_v4_MEF_c_2384	0.714	0.408	0.327	0.325
Me_v4_MEF_c_2409 0.565 0.491 0.848 0.371 Me_v4_MEF_c_2419 0.569 0.491 0.233 0.37 Me_v4_MEF_c_2447 0.823 0.291 0.293 0.249 Me_v4_MEF_c_2447 0.778 0.345 0.239 0.285 Me_v4_MEF_c_2448 0.523 0.499 0.465 0.374 Me_v4_MEF_c_2486 0.827 0.287 0.327 0.246 Me_v4_MEF_c_2510 0.841 0.268 0.273 0.232 Me_v4_MEF_c_2552 0.683 0.433 0.366 0.339 Me_v4_MEF_c_2552 0.683 0.433 0.366 0.339 Me_v4_MEF_c_2768 0.606 0.477 0.511 0.363 Me_v4_MEF_c_2778 0.601 0.48 0.245 0.363 Me_v4_MEF_c_2801 0.608 0.477 0.373 Me_v4_MEF_c_2905 0.947 0.1 0 0.095 Me_v4_MEF_c_23070 0.563 0.492 0.292 0.371 Me_v4_MEF_c_3071 0.563 0.39	Me_v4_MEF_c_2402	0.672	0.441	0.355	0.344
Me_v4_MEF_c_2419 0.569 0.491 0.238 0.37 Me_v4_MEF_c_2437 0.823 0.291 0.293 0.249 Me_v4_MEF_c_2447 0.778 0.345 0.239 0.285 Me_v4_MEF_c_2456 0.741 0.384 0.42 0.31 Me_v4_MEF_c_2456 0.741 0.384 0.42 0.31 Me_v4_MEF_c_2456 0.827 0.287 0.327 0.246 Me_v4_MEF_c_2510 0.841 0.268 0.273 0.232 Me_v4_MEF_c_2552 0.683 0.433 0.366 0.339 Me_v4_MEF_c_2562 0.5 0.5 0.407 0.375 Me_v4_MEF_c_2653 0.606 0.477 0.511 0.363 Me_v4_MEF_c_2778 0.545 0.496 0.477 0.373 Me_v4_MEF_c_2801 0.608 0.477 0.462 0.363 Me_v4_MEF_c_2905 0.947 0.1 0 0.995 Me_v4_MEF_c_3070 0.563 0.492 0.292 0.371 Me_v4_ME	Me_v4_MEF_c_2409	0.565	0.491	0.848	0.371
Me_v4_MEF_c_2437 0.823 0.291 0.293 0.249 Me_v4_MEF_c_2447 0.778 0.345 0.239 0.285 Me_v4_MEF_c_2448 0.523 0.499 0.465 0.374 Me_v4_MEF_c_2456 0.741 0.384 0.42 0.31 Me_v4_MEF_c_2456 0.741 0.384 0.42 0.31 Me_v4_MEF_c_2456 0.827 0.287 0.327 0.246 Me_v4_MEF_c_2552 0.683 0.433 0.366 0.339 Me_v4_MEF_c_2652 0.663 0.433 0.366 0.339 Me_v4_MEF_c_2653 0.606 0.477 0.511 0.363 Me_v4_MEF_c_2778 0.545 0.496 0.477 0.373 Me_v4_MEF_c_2801 0.608 0.477 0.462 0.363 Me_v4_MEF_c_2805 0.947 0.1 0 0.995 Me_v4_MEF_c_3070 0.563 0.492 0.292 0.371 Me_v4_MEF_c_3081 0.734 0.39 0.323 0.314 Me_v	Me_v4_MEF_c_2419	0.569	0.491	0.238	0.37
Me_v4_MEF_c_2447 0.778 0.345 0.239 0.285 Me_v4_MEF_c_2448 0.523 0.499 0.465 0.374 Me_v4_MEF_c_2456 0.741 0.384 0.42 0.31 Me_v4_MEF_c_2510 0.841 0.268 0.273 0.232 Me_v4_MEF_c_2524 0.753 0.372 0.385 0.303 Me_v4_MEF_c_2552 0.683 0.433 0.366 0.339 Me_v4_MEF_c_2562 0.5 0.5 0.407 0.375 Me_v4_MEF_c_2726 0.606 0.477 0.511 0.363 Me_v4_MEF_c_2748 0.939 0.114 0.101 0.107 Me_v4_MEF_c_2801 0.608 0.477 0.462 0.363 Me_v4_MEF_c_2801 0.608 0.477 0.462 0.363 Me_v4_MEF_c_2905 0.947 0.1 0 0.095 Me_v4_MEF_c_3070 0.563 0.492 0.292 0.371 Me_v4_MEF_c_3131 0.8 0.32 0.275 0.269 Me_v4_ME	Me_v4_MEF_c_2437	0.823	0.291	0.293	0.249
Me_v4_MEF_c_2448 0.523 0.499 0.465 0.374 Me_v4_MEF_c_2456 0.741 0.384 0.42 0.31 Me_v4_MEF_c_2486 0.827 0.287 0.327 0.246 Me_v4_MEF_c_2510 0.841 0.268 0.273 0.232 Me_v4_MEF_c_2552 0.683 0.433 0.366 0.339 Me_v4_MEF_c_2653 0.606 0.477 0.511 0.363 Me_v4_MEF_c_2726 0.601 0.48 0.245 0.365 Me_v4_MEF_c_2726 0.601 0.48 0.245 0.365 Me_v4_MEF_c_2768 0.545 0.496 0.477 0.373 Me_v4_MEF_c_2801 0.608 0.477 0.462 0.363 Me_v4_MEF_c_2801 0.608 0.477 0.462 0.363 Me_v4_MEF_c_2905 0.947 0.1 0 0.095 Me_v4_MEF_c_3070 0.563 0.492 0.292 0.371 Me_v4_MEF_c_3131 0.88 0.325 0.259 0.235 0.2269	Me_v4_MEF_c_2447	0.778	0.345	0.239	0.285
Me_v4_MEF_c_2456 0.741 0.384 0.42 0.31 Me_v4_MEF_c_2486 0.827 0.287 0.327 0.246 Me_v4_MEF_c_2510 0.841 0.268 0.273 0.232 Me_v4_MEF_c_2552 0.683 0.433 0.366 0.339 Me_v4_MEF_c_2562 0.5 0.5 0.407 0.375 Me_v4_MEF_c_2653 0.606 0.477 0.511 0.363 Me_v4_MEF_c_2728 0.601 0.48 0.245 0.365 Me_v4_MEF_c_2788 0.608 0.477 0.511 0.363 Me_v4_MEF_c_2801 0.608 0.477 0.462 0.363 Me_v4_MEF_c_2801 0.608 0.477 0.462 0.363 Me_v4_MEF_c_2808 0.866 0.241 0.191 0.212 Me_v4_MEF_c_2909 0.571 0.49 0.19 0.37 Me_v4_MEF_c_3070 0.563 0.492 0.292 0.371 Me_v4_MEF_c_3120 0.634 0.464 0.588 0.356 Me_	Me_v4_MEF_c_2448	0.523	0.499	0.465	0.374
Me_v4_MEF_c_2486 0.827 0.287 0.327 0.246 Me_v4_MEF_c_2510 0.841 0.268 0.273 0.232 Me_v4_MEF_c_2524 0.753 0.372 0.385 0.303 Me_v4_MEF_c_2552 0.683 0.433 0.366 0.339 Me_v4_MEF_c_2562 0.5 0.5 0.407 0.375 Me_v4_MEF_c_2768 0.606 0.477 0.511 0.363 Me_v4_MEF_c_2748 0.939 0.114 0.101 0.107 Me_v4_MEF_c_2758 0.545 0.496 0.477 0.373 Me_v4_MEF_c_2801 0.608 0.477 0.462 0.363 Me_v4_MEF_c_2805 0.947 0.1 0 0.095 Me_v4_MEF_c_3070 0.563 0.492 0.292 0.371 Me_v4_MEF_c_3070 0.563 0.492 0.292 0.371 Me_v4_MEF_c_3131 0.8 0.322 0.275 0.269 Me_v4_MEF_c_3131 0.84 0.366 0.325 0.259 Me_v4_	Me_v4_MEF_c_2456	0.741	0.384	0.42	0.31
Me_v4_MEF_c_2510 0.841 0.268 0.273 0.232 Me_v4_MEF_c_2524 0.753 0.372 0.385 0.303 Me_v4_MEF_c_2552 0.683 0.433 0.366 0.339 Me_v4_MEF_c_2562 0.5 0.5 0.407 0.375 Me_v4_MEF_c_2653 0.606 0.477 0.511 0.363 Me_v4_MEF_c_2726 0.601 0.48 0.245 0.365 Me_v4_MEF_c_2778 0.545 0.496 0.477 0.373 Me_v4_MEF_c_2801 0.608 0.477 0.462 0.363 Me_v4_MEF_c_2801 0.608 0.477 0.462 0.363 Me_v4_MEF_c_2801 0.608 0.477 0.462 0.363 Me_v4_MEF_c_2905 0.947 0.1 0 0.095 Me_v4_MEF_c_3070 0.563 0.492 0.292 0.371 Me_v4_MEF_c_3081 0.734 0.39 0.323 0.314 Me_v4_MEF_c_3130 0.634 0.464 0.588 0.356 Me_v4	Me_v4_MEF_c_2486	0.827	0.287	0.327	0.246
Me_v4_MEF_c_2524 0.753 0.372 0.385 0.303 Me_v4_MEF_c_2552 0.683 0.433 0.366 0.339 Me_v4_MEF_c_2562 0.5 0.5 0.407 0.375 Me_v4_MEF_c_2653 0.606 0.477 0.511 0.363 Me_v4_MEF_c_2726 0.601 0.48 0.245 0.365 Me_v4_MEF_c_2778 0.545 0.496 0.477 0.373 Me_v4_MEF_c_2801 0.608 0.477 0.363 Me_v4_MEF_c_2801 0.608 0.477 0.462 0.363 Me_v4_MEF_c_2805 0.947 0.1 0 0.095 Me_v4_MEF_c_2905 0.947 0.1 0 0.095 Me_v4_MEF_c_3070 0.563 0.492 0.292 0.371 Me_v4_MEF_c_3084 0.771 0.353 0.394 0.291 Me_v4_MEF_c_3120 0.634 0.464 0.588 0.356 Me_v4_MEF_c_3131 0.8 0.32 0.275 0.269 Me_v4_MEF_c_3137 0.	Me_v4_MEF_c_2510	0.841	0.268	0.273	0.232
Me_v4_MEF_c_2552 0.683 0.433 0.366 0.339 Me_v4_MEF_c_2562 0.5 0.5 0.407 0.375 Me_v4_MEF_c_2653 0.606 0.477 0.511 0.363 Me_v4_MEF_c_2726 0.601 0.48 0.245 0.365 Me_v4_MEF_c_2748 0.399 0.114 0.101 0.107 Me_v4_MEF_c_2758 0.545 0.496 0.477 0.373 Me_v4_MEF_c_2801 0.608 0.477 0.363 Me_v4_MEF_c_2805 0.947 0.1 0 0.095 Me_v4_MEF_c_3070 0.563 0.492 0.292 0.371 Me_v4_MEF_c_3070 0.563 0.492 0.292 0.371 Me_v4_MEF_c_3081 0.734 0.39 0.323 0.314 Me_v4_MEF_c_3137 0.812 0.366 0.325 0.259 Me_v4_MEF_c_3137 0.812 0.306 0.325 0.259 Me_v4_MEF_c_3137 0.812 0.306 0.325 0.259 Me_v4_MEF_c_3137	Me_v4_MEF_c_2524	0.753	0.372	0.385	0.303
Me_v4_MEF_c_2562 0.5 0.5 0.407 0.375 Me_v4_MEF_c_2653 0.606 0.477 0.511 0.363 Me_v4_MEF_c_2726 0.601 0.48 0.245 0.365 Me_v4_MEF_c_2748 0.939 0.114 0.101 0.107 Me_v4_MEF_c_2758 0.545 0.496 0.477 0.373 Me_v4_MEF_c_2801 0.608 0.477 0.462 0.363 Me_v4_MEF_c_2805 0.947 0.1 0 0.095 Me_v4_MEF_c_2909 0.571 0.492 0.292 0.371 Me_v4_MEF_c_3070 0.563 0.492 0.292 0.371 Me_v4_MEF_c_3081 0.734 0.39 0.323 0.314 Me_v4_MEF_c_3137 0.812 0.306 0.325 0.259 Me_v4_MEF_c_3137 0.812 0.306 0.325 0.259 Me_v4_MEF_c_3142 0.847 0.259 0.235 0.226 Me_v4_MEF_c_3137 0.812 0.306 0.325 0.259 Me_v4	Me_v4_MEF_c_2552	0.683	0.433	0.366	0.339
Me_v4_MEF_c_2653 0.606 0.477 0.511 0.363 Me_v4_MEF_c_2726 0.601 0.48 0.245 0.365 Me_v4_MEF_c_2778 0.545 0.496 0.477 0.373 Me_v4_MEF_c_2801 0.608 0.477 0.462 0.363 Me_v4_MEF_c_2808 0.86 0.241 0.191 0.212 Me_v4_MEF_c_2905 0.947 0.1 0 0.095 Me_v4_MEF_c_2909 0.571 0.499 0.19 0.37 Me_v4_MEF_c_3070 0.563 0.492 0.292 0.371 Me_v4_MEF_c_3081 0.774 0.353 0.394 0.291 Me_v4_MEF_c_3120 0.634 0.464 0.588 0.356 Me_v4_MEF_c_3131 0.8 0.32 0.275 0.269 Me_v4_MEF_c_3131 0.812 0.306 0.325 0.259 Me_v4_MEF_c_3137 0.812 0.306 0.325 0.2269 Me_v4_MEF_c_3197 0.554 0.494 0.096 0.372 Me_v4	Me_v4_MEF_c_2562	0.5	0.5	0.407	0.375
Me_v4_MEF_c_2726 0.601 0.48 0.245 0.365 Me_v4_MEF_c_2748 0.939 0.114 0.101 0.107 Me_v4_MEF_c_2758 0.545 0.496 0.477 0.373 Me_v4_MEF_c_2801 0.608 0.477 0.462 0.363 Me_v4_MEF_c_2888 0.86 0.241 0.191 0.212 Me_v4_MEF_c_2905 0.947 0.1 0 0.095 Me_v4_MEF_c_2909 0.571 0.49 0.19 0.37 Me_v4_MEF_c_3070 0.563 0.492 0.292 0.371 Me_v4_MEF_c_3081 0.734 0.39 0.323 0.314 Me_v4_MEF_c_3094 0.771 0.353 0.394 0.291 Me_v4_MEF_c_3131 0.8 0.32 0.275 0.269 Me_v4_MEF_c_3137 0.812 0.306 0.325 0.259 Me_v4_MEF_c_3155 0.727 0.397 0.381 0.318 Me_v4_MEF_c_3197 0.554 0.494 0.096 0.372 Me_v4_ME	Me_v4_MEF_c_2653	0.606	0.477	0.511	0.363
Me_v4_MEF_c_2748 0.939 0.114 0.101 0.107 Me_v4_MEF_c_2758 0.545 0.496 0.477 0.373 Me_v4_MEF_c_2801 0.608 0.477 0.462 0.363 Me_v4_MEF_c_2888 0.86 0.241 0.191 0.212 Me_v4_MEF_c_2905 0.947 0.1 0 0.095 Me_v4_MEF_c_2090 0.571 0.492 0.292 0.371 Me_v4_MEF_c_3070 0.563 0.492 0.292 0.371 Me_v4_MEF_c_3081 0.734 0.39 0.323 0.314 Me_v4_MEF_c_3120 0.634 0.464 0.588 0.356 Me_v4_MEF_c_3131 0.8 0.32 0.275 0.269 Me_v4_MEF_c_3137 0.812 0.306 0.325 0.259 Me_v4_MEF_c_3142 0.847 0.259 0.235 0.226 Me_v4_MEF_c_3195 0.727 0.397 0.381 0.318 Me_v4_MEF_c_3190 0.672 0.441 0.323 0.344 Me_v	Me_v4_MEF_c_2726	0.601	0.48	0.245	0.365
Me_v4_MEF_c_2758 0.545 0.496 0.477 0.373 Me_v4_MEF_c_2801 0.608 0.477 0.462 0.363 Me_v4_MEF_c_2888 0.86 0.241 0.191 0.212 Me_v4_MEF_c_2905 0.947 0.1 0 0.095 Me_v4_MEF_c_2909 0.571 0.49 0.19 0.37 Me_v4_MEF_c_3070 0.563 0.492 0.292 0.371 Me_v4_MEF_c_3081 0.734 0.39 0.323 0.314 Me_v4_MEF_c_3084 0.771 0.353 0.394 0.291 Me_v4_MEF_c_3120 0.634 0.464 0.588 0.356 Me_v4_MEF_c_3131 0.8 0.32 0.275 0.269 Me_v4_MEF_c_3137 0.812 0.306 0.325 0.259 Me_v4_MEF_c_3137 0.812 0.306 0.325 0.226 Me_v4_MEF_c_3142 0.847 0.259 0.235 0.226 Me_v4_MEF_c_3195 0.859 0.243 0.152 0.213 Me_v4_M	Me_v4_MEF_c_2748	0.939	0.114	0.101	0.107
Me_v4_MEF_c_2801 0.608 0.477 0.462 0.363 Me_v4_MEF_c_2888 0.86 0.241 0.191 0.212 Me_v4_MEF_c_2905 0.947 0.1 0 0.095 Me_v4_MEF_c_209 0.571 0.49 0.19 0.37 Me_v4_MEF_c_3070 0.563 0.492 0.292 0.371 Me_v4_MEF_c_3081 0.734 0.39 0.323 0.314 Me_v4_MEF_c_3081 0.771 0.353 0.394 0.291 Me_v4_MEF_c_3120 0.634 0.464 0.588 0.356 Me_v4_MEF_c_3131 0.8 0.32 0.275 0.269 Me_v4_MEF_c_3137 0.812 0.306 0.325 0.226 Me_v4_MEF_c_3142 0.847 0.259 0.235 0.226 Me_v4_MEF_c_3155 0.727 0.397 0.381 0.318 Me_v4_MEF_c_3310 0.672 0.441 0.323 0.344 Me_v4_MEF_c_3338 0.72 0.404 0.244 0.322 Me_v4_MEF	Me_v4_MEF_c_2758	0.545	0.496	0.477	0.373
Me_v4_MEF_c2888 0.86 0.241 0.191 0.212 Me_v4_MEF_c2905 0.947 0.1 0 0.095 Me_v4_MEF_c2909 0.571 0.49 0.19 0.37 Me_v4_MEF_c3070 0.563 0.492 0.292 0.371 Me_v4_MEF_c3081 0.734 0.39 0.323 0.314 Me_v4_MEF_c3094 0.771 0.353 0.394 0.291 Me_v4_MEF_c3120 0.634 0.464 0.588 0.356 Me_v4_MEF_c3131 0.8 0.32 0.275 0.269 Me_v4_MEF_c3137 0.812 0.306 0.325 0.259 Me_v4_MEF_c3142 0.847 0.259 0.235 0.226 Me_v4_MEF_c3195 0.727 0.397 0.381 0.318 Me_v4_MEF_c3195 0.859 0.243 0.152 0.213 Me_v4_MEF_c3310 0.672 0.441 0.323 0.344 Me_v4_MEF_c3336 0.89 0.196 0.176 0.176 Me_v4_MEF_c336	Me_v4_MEF_c_2801	0.608	0.477	0.462	0.363
Me_v4_MEF_c_2905 0.947 0.1 0 0.095 Me_v4_MEF_c_2909 0.571 0.49 0.19 0.37 Me_v4_MEF_c_3070 0.563 0.492 0.292 0.371 Me_v4_MEF_c_3081 0.734 0.39 0.323 0.314 Me_v4_MEF_c_3094 0.771 0.353 0.394 0.291 Me_v4_MEF_c_3120 0.634 0.464 0.588 0.356 Me_v4_MEF_c_3131 0.8 0.32 0.275 0.269 Me_v4_MEF_c_3137 0.812 0.306 0.325 0.259 Me_v4_MEF_c_3142 0.847 0.259 0.235 0.226 Me_v4_MEF_c_3155 0.727 0.397 0.381 0.318 Me_v4_MEF_c_3195 0.859 0.243 0.152 0.213 Me_v4_MEF_c_3310 0.672 0.441 0.323 0.344 Me_v4_MEF_c_3336 0.89 0.196 0.176 0.176 Me_v4_MEF_c_336 0.556 0.494 0.238 0.372 Me_v4_ME	Me_v4_MEF_c_2888	0.86	0.241	0.191	0.212
Me_v4_MEF_c_29090.5710.490.190.37Me_v4_MEF_c_30700.5630.4920.2920.371Me_v4_MEF_c_30810.7340.390.3230.314Me_v4_MEF_c_30940.7710.3530.3940.291Me_v4_MEF_c_31200.6340.4640.5880.356Me_v4_MEF_c_31310.80.320.2750.269Me_v4_MEF_c_31370.8120.3060.3250.259Me_v4_MEF_c_31420.8470.2590.2350.226Me_v4_MEF_c_31550.7270.3970.3810.318Me_v4_MEF_c_31950.8590.2430.1520.213Me_v4_MEF_c_33100.6720.4410.3230.344Me_v4_MEF_c_33360.890.1960.1760.176Me_v4_MEF_c_33380.720.4040.2440.322Me_v4_MEF_c_33610.8240.2890.2450.248Me_v4_MEF_c_33760.8090.3090.3370.261Minimum0.50.0500.049Maximum0.940.50.8480.375	Me_v4_MEF_c_2905	0.947	0.1	0	0.095
Me_v4_MEF_c_3070 0.563 0.492 0.292 0.371 Me_v4_MEF_c_3081 0.734 0.39 0.323 0.314 Me_v4_MEF_c_3094 0.771 0.353 0.394 0.291 Me_v4_MEF_c_3120 0.634 0.464 0.588 0.356 Me_v4_MEF_c_3131 0.8 0.32 0.275 0.269 Me_v4_MEF_c_3137 0.812 0.306 0.325 0.259 Me_v4_MEF_c_3142 0.847 0.259 0.235 0.226 Me_v4_MEF_c_3155 0.727 0.397 0.381 0.318 Me_v4_MEF_c_3195 0.859 0.243 0.152 0.213 Me_v4_MEF_c_3197 0.554 0.494 0.096 0.372 Me_v4_MEF_c_3336 0.89 0.196 0.176 0.176 Me_v4_MEF_c_3338 0.72 0.404 0.244 0.322 Me_v4_MEF_c_3343 0.606 0.478 0.465 0.363 Me_v4_MEF_c_3361 0.824 0.289 0.245 0.248 <td< td=""><td>Me_v4_MEF_c_2909</td><td>0.571</td><td>0.49</td><td>0.19</td><td>0.37</td></td<>	Me_v4_MEF_c_2909	0.571	0.49	0.19	0.37
Me_v4_MEF_c_30810.7340.390.3230.314Me_v4_MEF_c_30940.7710.3530.3940.291Me_v4_MEF_c_31200.6340.4640.5880.356Me_v4_MEF_c_31310.80.320.2750.269Me_v4_MEF_c_31370.8120.3060.3250.259Me_v4_MEF_c_31420.8470.2590.2350.226Me_v4_MEF_c_31550.7270.3970.3810.318Me_v4_MEF_c_31950.8590.2430.1520.213Me_v4_MEF_c_31970.5540.4940.0960.372Me_v4_MEF_c_33100.6720.4410.3230.344Me_v4_MEF_c_33380.720.4040.2440.322Me_v4_MEF_c_33660.5560.4940.2380.372Me_v4_MEF_c_33610.8240.2890.2450.248Me_v4_MEF_c_33760.8090.3090.3370.261Minimum0.50.0500.049Maximum0.940.50.8480.375	Me_v4_MEF_c_3070	0.563	0.492	0.292	0.371
Me_v4_MEF_c_30940.7710.3530.3940.291Me_v4_MEF_c_31200.6340.4640.5880.356Me_v4_MEF_c_31310.80.320.2750.269Me_v4_MEF_c_31370.8120.3060.3250.259Me_v4_MEF_c_31420.8470.2590.2350.226Me_v4_MEF_c_31550.7270.3970.3810.318Me_v4_MEF_c_31950.8590.2430.1520.213Me_v4_MEF_c_31970.5540.4940.0960.372Me_v4_MEF_c_33360.890.1960.1760.176Me_v4_MEF_c_33380.720.4040.2440.322Me_v4_MEF_c_33560.5560.4940.2380.372Me_v4_MEF_c_33610.8240.2890.2450.248Me_v4_MEF_c_33760.8090.3090.3370.261Minimum0.50.0500.049Maximum0.940.50.8480.375	Me_v4_MEF_c_3081	0.734	0.39	0.323	0.314
Me_v4_MEF_c_31200.6340.4640.5880.356Me_v4_MEF_c_31310.80.320.2750.269Me_v4_MEF_c_31370.8120.3060.3250.259Me_v4_MEF_c_31420.8470.2590.2350.226Me_v4_MEF_c_31550.7270.3970.3810.318Me_v4_MEF_c_31950.8590.2430.1520.213Me_v4_MEF_c_31970.5540.4940.0960.372Me_v4_MEF_c_33100.6720.4410.3230.344Me_v4_MEF_c_33360.890.1960.1760.176Me_v4_MEF_c_33360.5560.4940.2380.372Me_v4_MEF_c_33610.8240.2890.2450.248Me_v4_MEF_c_33610.8240.2890.2450.248Me_v4_MEF_c_33760.8090.3090.3370.261Minimum0.50.0500.049Maximum0.940.50.8480.375	Me_v4_MEF_c_3094	0.771	0.353	0.394	0.291
Me_v4_MEF_c_31310.80.320.2750.269Me_v4_MEF_c_31370.8120.3060.3250.259Me_v4_MEF_c_31420.8470.2590.2350.226Me_v4_MEF_c_31550.7270.3970.3810.318Me_v4_MEF_c_31950.8590.2430.1520.213Me_v4_MEF_c_31970.5540.4940.0960.372Me_v4_MEF_c_33100.6720.4410.3230.344Me_v4_MEF_c_33360.890.1960.1760.176Me_v4_MEF_c_33380.720.4040.2440.322Me_v4_MEF_c_33610.8240.2890.2450.248Me_v4_MEF_c_33610.8240.2890.2450.248Me_v4_MEF_c_33760.8090.3090.3370.261Minimum0.50.0500.049Maximum0.940.50.8480.375	Me_v4_MEF_c_3120	0.634	0.464	0.588	0.356
Me_v4_MEF_c_31370.8120.3060.3250.259Me_v4_MEF_c_31420.8470.2590.2350.226Me_v4_MEF_c_31550.7270.3970.3810.318Me_v4_MEF_c_31950.8590.2430.1520.213Me_v4_MEF_c_31970.5540.4940.0960.372Me_v4_MEF_c_33100.6720.4410.3230.344Me_v4_MEF_c_33360.890.1960.1760.176Me_v4_MEF_c_33380.720.4040.2440.322Me_v4_MEF_c_33430.6060.4780.4650.363Me_v4_MEF_c_33560.5560.4940.2380.372Me_v4_MEF_c_33610.8240.2890.2450.248Me_v4_MEF_c_33760.8090.3090.3370.261Minimum0.50.0500.049Maximum0.940.50.8480.375	Me_v4_MEF_c_3131	0.8	0.32	0.275	0.269
Me_v4_MEF_c_31420.8470.2590.2350.226Me_v4_MEF_c_31550.7270.3970.3810.318Me_v4_MEF_c_31950.8590.2430.1520.213Me_v4_MEF_c_31970.5540.4940.0960.372Me_v4_MEF_c_33100.6720.4410.3230.344Me_v4_MEF_c_33360.890.1960.1760.176Me_v4_MEF_c_33380.720.4040.2440.322Me_v4_MEF_c_33430.6060.4780.4650.363Me_v4_MEF_c_33560.5560.4940.2380.372Me_v4_MEF_c_33610.8240.2890.2450.248Me_v4_MEF_c_33760.8090.3090.3370.261Minimum0.50.05500.049Maximum0.940.50.8480.375	Me_v4_MEF_c_3137	0.812	0.306	0.325	0.259
Me_v4_MEF_c_31550.7270.3970.3810.318Me_v4_MEF_c_31950.8590.2430.1520.213Me_v4_MEF_c_31970.5540.4940.0960.372Me_v4_MEF_c_33100.6720.4410.3230.344Me_v4_MEF_c_33360.890.1960.1760.176Me_v4_MEF_c_33380.720.4040.2440.322Me_v4_MEF_c_33430.6060.4780.4650.363Me_v4_MEF_c_33560.5560.4940.2380.372Me_v4_MEF_c_33610.8240.2890.2450.248Me_v4_MEF_c_33760.8090.3090.3370.261Minimum0.50.05500.049Maximum0.940.50.8480.375	Me_v4_MEF_c_3142	0.847	0.259	0.235	0.226
Me_v4_MEF_c_31950.8590.2430.1520.213Me_v4_MEF_c_31970.5540.4940.0960.372Me_v4_MEF_c_33100.6720.4410.3230.344Me_v4_MEF_c_33360.890.1960.1760.176Me_v4_MEF_c_33380.720.4040.2440.322Me_v4_MEF_c_33430.6060.4780.4650.363Me_v4_MEF_c_33560.5560.4940.2380.372Me_v4_MEF_c_33610.8240.2890.2450.248Me_v4_MEF_c_33760.8090.3090.3370.261Minimum0.50.055000.049Maximum0.940.50.8480.375	Me_v4_MEF_c_3155	0.727	0.397	0.381	0.318
Me_v4_MEF_c_31970.5540.4940.0960.372Me_v4_MEF_c_33100.6720.4410.3230.344Me_v4_MEF_c_33360.890.1960.1760.176Me_v4_MEF_c_33380.720.4040.2440.322Me_v4_MEF_c_33430.6060.4780.4650.363Me_v4_MEF_c_33560.5560.4940.2380.372Me_v4_MEF_c_33610.8240.2890.2450.248Me_v4_MEF_c_33760.8090.3090.3370.261Minimum0.50.05500.049Maximum0.940.50.8480.375	Me_v4_MEF_c_3195	0.859	0.243	0.152	0.213
Me_v4_MEF_c_33100.6720.4410.3230.344Me_v4_MEF_c_33360.890.1960.1760.176Me_v4_MEF_c_33380.720.4040.2440.322Me_v4_MEF_c_33430.6060.4780.4650.363Me_v4_MEF_c_33560.5560.4940.2380.372Me_v4_MEF_c_33610.8240.2890.2450.248Me_v4_MEF_c_33760.8090.3090.3370.261Minimum0.50.05500.049Maximum0.940.50.8480.375	Me_v4_MEF_c_3197	0.554	0.494	0.096	0.372
Me_v4_MEF_c_33360.890.1960.1760.176Me_v4_MEF_c_33380.720.4040.2440.322Me_v4_MEF_c_33430.6060.4780.4650.363Me_v4_MEF_c_33560.5560.4940.2380.372Me_v4_MEF_c_33610.8240.2890.2450.248Me_v4_MEF_c_33760.8090.3090.3370.261Minimum0.50.05500.049Maximum0.940.50.8480.375	Me_v4_MEF_c_3310	0.672	0.441	0.323	0.344
Me_v4_MEF_c_33380.720.4040.2440.322Me_v4_MEF_c_33430.6060.4780.4650.363Me_v4_MEF_c_33560.5560.4940.2380.372Me_v4_MEF_c_33610.8240.2890.2450.248Me_v4_MEF_c_33760.8090.3090.3370.261Minimum0.50.0500.049Maximum0.940.50.8480.375	Me_v4_MEF_c_3336	0.89	0.196	0.176	0.176
Me_v4_MEF_c_33430.6060.4780.4650.363Me_v4_MEF_c_33560.5560.4940.2380.372Me_v4_MEF_c_33610.8240.2890.2450.248Me_v4_MEF_c_33760.8090.3090.3370.261Minimum0.50.0500.049Maximum0.940.50.8480.375	Me_v4_MEF_c_3338	0.72	0.404	0.244	0.322
Me_v4_MEF_c_33560.5560.4940.2380.372Me_v4_MEF_c_33610.8240.2890.2450.248Me_v4_MEF_c_33760.8090.3090.3370.261Minimum0.50.0500.049Maximum0.940.50.8480.375	Me_v4_MEF_c_3343	0.606	0.478	0.465	0.363
Me_v4_MEF_c_33610.8240.2890.2450.248Me_v4_MEF_c_33760.8090.3090.3370.261Minimum0.50.0500.049Maximum0.940.50.8480.375	Me_v4_MEF_c_3356	0.556	0.494	0.238	0.372
Me_v4_MEF_c_3376 0.809 0.309 0.337 0.261 Minimum 0.5 0.05 0 0.049 Maximum 0.94 0.5 0.848 0.375	Me_v4_MEF_c_3361	0.824	0.289	0.245	0.248
Minimum 0.5 0.05 0 0.049 Maximum 0.94 0.5 0.848 0.375	Me_v4_MEF_c_3376	0.809	0.309	0.337	0.261
Maximum 0.94 0.5 0.848 0.375	Minimum	0.5	0.05	0	0.049
	Maximum	0.94	0.5	0.848	0.375

Table	3.	Contd.
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Mean	0.73	0.359	0.314	0.286
STD ^a	0.132	0.116	0.135	0.077
95% CI	0.021	0.019	0.022	0.012

PIC: Polymorphic information content; STD^a: Standard deviation with 95% confidence interval.

PIC values can range from 0.000 to 0.500, compared with SSRs which are multiallelic and can have PIC values above 0.500 and up to 1.000. Consequently, results from this present study demonstrate that the set of SNPs used were sufficiently informative and can be used for cassava genotyping to establish the relatedness between genotypes. PIC values however, observed in this present study was higher than that observed (PIC=0.170) by Karim et al. (2020) where cassava collections were analyzed with 5600 SNPs. The discrepancy could be attributed to differences in composition of experimental material, number and selection of SNP markers used for the studies.

Major allele frequency (MaF) for all the markers was generally high. It ranged from 0.500 to 0.942 with an average of 0.728 (Table 3). More than 50% of the polymorphic loci showed a major allele frequency higher than 0.700 and 9 loci showed more than 0.900. This is an indication that all the markers were polymorphic.

Heterozygosity, which is a measure of allelic diversity at a locus varied from 0.000 to 0.848 with an average of 0.314 (Table 3). Approximately 91% of the estimates were lower than 0.500. In contrast gene diversity (GD) varied from 0.50 to 0.500 with an average of 0.359, and only 29% of GD estimates were greater than 0.450. As heterozygosity is a measure of genetic variation among genotypes, the average heterozygosity observed could be expected to correlate with moderate to high genetic diversity. Average heterozygosity values however, observed in this present study was lower than that observed by Ferguson et al. (2019), who used 1,536 SNPs to genotype 522 cassava accessions (0.366). This is expected since most of the accessions used were collected from South America which encompasses the presumed centre of domestication and diversity of the crop. Average gene diversity value observed in this present study was higher than that observed by Kamanda et al. (2020), where 5,634 SNPs were used to assay 183 provitamin-A cassava accessions (GD=0.190). The differences could be attributed to the experimental materials and selection of SNP markers used. The average gene diversity was however higher than the heterozygosity in this study, suggesting inbreeding or heterozygote deficit in relation to that expected under the Hardy Weinberg equilibrium in the case of natural populations. A similar trend (GD=0.300; heterozygosity=0.230) was also observed by de Albuquerque et al. (2018). The high allelic richness coupled with estimates of gene diversity suggests a moderate to high genetic diversity among the cassava landraces. With SNPs, diversity values are generally low, and this could be explained by the bi-allelic nature, hence, the maximum gene diversity for a SNP marker is 0.5. In addition, cassava is allogamous and there is the possibility of identifying several alleles per locus.

According to several reports, to get the same level of information as SSRs for genetic diversity studies, a larger number of SNPs (7 to 11 times or more as compared to SSRs) must be used (Filippi et al., 2015). It is therefore important that the number and selection of SNPs, as well as the use of a large panel of accessions is considered to prevent bias and enhance the accuracy of diversity studies (Emmanuelli et al., 2013). However, Kawuki et al. (2009) have also reported that the number of SNPs required for a diversity studies is also dependent on the nature of the genetic resources. Hence, the more diverse the genetic resources, the fewer the markers required. In this study, 187 polymorphic SNPs were able to detect the genetic diversity in the cassava landraces which suggests that the number of SNPs used is within a suitable range. This suggests that the cassava landraces are diverse, and it supports the assertion by Kawuki et al. (2009). A similar trend was observed by Oliveira et al. (2014) who used 402 polymorphic SNPs to assess 1280 cassava accessions (Table 3).

Analysis of molecular variance (AMOVA)

AMOVA based on the SNP marker data suggests higher within group variation which accounted for 99% of the total variation compared with variation between groups which accounted for only 1% of the total variation (Table 4). The results suggest that there is a higher genetic diversity within population than as seen between populations. This result is similar to studies by Pedri et al. (2019) where analysis of molecular variance revealed a within group variation of 92%.

Principal coordinates analysis (PCoA)

The principal coordinates analysis generated is graphically presented in Figure 1. The coordinates were calculated for axes 2 and 3 with positive eigen values. The two axes accounted for 50.81% of the total variation with the second axis (PCoA2) accounting for 22.16% and third axis (PCoA3) accounting for 28.65%. The PCoA showed loose clustering and cassava landraces were

Source of variation	df	Sum of squares	Mean square	Estimated variance	% variance
Between populations	1	42.331	42.331	0.295	1
Within population	103	3083.640	29.938	29.938	99
Total	105	3125.971	72.269	30.233	100

Table 4. AMOVA of cassava landraces populations.





dispersed in all four quadrants. However, those which clustered together could suggest that they share some common alleles. The PCA analyses, however, showed a similar result to the clustering supporting the presence of four populations.

Cluster analysis

The dendrogram was constructed using Nei's genetic distance (1973) and this separated the 105 cassava landraces into three main Clusters (I, II and III) and five distinct sub-clusters (Figure 2). Clustering did not reflect the zones from which these landraces were collected and subsequently no duplicates were found. Cluster I composed of landraces from the coastal savanna zone while all other landraces from the rain forest, deciduous forest and transition grouped across the other two Clusters (II and III).

Grouping of landraces from different agro-ecological zones across the different clusters could be attributed to the high frequency of germpalsm exchange among farmers. Within cluster II, three sub-clusters (A, B and C) were formed, while two sub-clusters (D and E) were formed in cluster III. Sub-cluster size varied from four to 65. The largest sub-clusters, C and D had 33% each of the cassava landraces, while sub-clusters A and E had 6% each and sub-cluster B had 22%. Cluster analysis with the SNP markers was useful in revealing three distinct groups which may have diverse morphological, agronomical, physiological and molecular characteristics that may provide valuable genetic resources. This may enable breeders select diverse parents for targeted crosses to develop superior progenies.

Conclusion

This study concluded that 187 out of 195 SNP markers were highly informative and polymorphic to distinguish among the cassava landraces. These SNPs could therefore be used for future genetic diversity studies in cassava. In addition, SNP markers were able to separate the landraces into different clusters. However, they were not grouped in accordance to their geographical origin. Furthermore, this study has confirmed that fewer SNPs could be used to assess the genetic diversity if the genetic resources are diverse. The cassava landraces used for this study showed moderate to high genetic diversity, suggesting the availability of useful and unique alleles for desirable traits that could be exploited for breeding purposes. Inclusion of such landraces in cassava improvement activities would enhance the development of end-user varieties that will be easily adopted by farmers and other stakeholders along the value chain. The information generated will contribute significantly to manage conserved germplasm, develop core collections from which parental lines could be selected to improve existing cultivars.



Figure 2. Dendrogram of 105 cassava landraces using 187 SNPs based on Nei's genetic distance (1973) generated by PowerMarker 3.0 software.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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