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Bulk genetic characterization of Ghanaian maize landraces using microsatellite markers

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Abstract

Maize (*Zea mays* L) was first introduced into Ghana over five centuries ago and remains the most important cereal staple, grown in all agro-ecologies across the country. Yield from farmers' fields are low, which is attributed in part to farmer's preferences and/or reliance on local landraces for cultivation. Efforts are underway to improve some of these landraces for improved productivity. Seeds of maize landraces cultivated in all agro-ecologies were collected for genetic characterization using a bulked fingerprinting technique and 20 SSR markers. In all, 20 populations of 15 plants each from Ghana and 4 control populations from Latin America were characterized. The cluster analysis grouped the 20 landraces into two major groups corresponding to the vegetation/climatic conditions of the north and south of the country. Genotypes from Ashanti, which is centrally located, fell into both major clusters, which suggest its importance in maize seed distribution in Ghana and also the diverse climate/vegetation. A Structure analyses grouped the genotypes into two major clusters similar to the UPGMA cluster, and populations were not fully distinct according to F statistics. The results suggest that breeders should make performance data available to seed dealers for better productivity.

Keywords: maize, bulked fingerprinting, SSR, landraces

Introduction

The introduction of maize from the centre of origin in Mexico into different growing conditions in the tropical, subtropical, and temperate regions has resulted in the existence of hundreds of diverse landraces (Damsteegt and Igwegbe, 2005; Dubreuil et al, 2006; Rebourg et al, 2003).

Maize was first introduced into Africa via Ghana and the Sao Tome islands by Portuguese traders in the 16th century (Shephered et al, 2010), and also from the Caribbean, Central and South America (Damsteegt and Igwegbe, 2005). Subsequent introductions have been made from Europe and Asia, (McCann, 2001). In Ghana, the phenotypic diversity of the crop is quite impressive and it includes ears with dark purplish-black, dark-red, yellow/orange, variegated or white kernels, in floury, flint and dent consistencies. The local landraces vary for maturity and plant height, and are usually low yielding, with some being susceptible to diseases including streak, rust, blight, etc, thus reducing yields further still. Cultivated maize is dominated by open pollinated varieties (OPVs) and landraces with few hybrids (USAID/EAT, 2012), compared to the situation in developed countries. Yields in farmers fields average only 1.6 ton ha^{-1} (Agriculture-in-Ghana, 2010). Most farmers recycle seeds after initial acquisition from family members, friends, NGOs or seed companies (Morris et al, 1999).

Landraces and wild relatives represent an extraordinary genetic resource of the crop with significant allelic diversity, much of which has not been incorporated into improved varieties (Sharma et al, 2010; Warburton et al, 2008). Landraces are genetically diverse, heterogeneous populations that are typically selected by farmers for their adaptation to specific local environments and needs (Prasanna and Sharma, 2005). High levels of genetic diversity in maize are caused by active transposable elements, meiotic recombination following outcrossing, new introgressions from exotic germplasm of this highly traded crop species, genetic drift following new introductions, and natural and artificial selection by farmers as the crop adapts to new environments (Doebley, 2004). In maize these factors have produced numerous open-pollinated landraces, which constitute a possible source of diversity that can be exploited to widen the improved gene pool from which breeders can harness useful genes and alleles to meet the challenges of climate change and other objectives of

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modern breeding programmes.

Characterization of genetically heterogeneous populations using molecular markers has until recently been very expensive and time consuming (Prasanna, 2012). A method for microsatellite or simple sequence repeat (SSR) analysis of pools of individuals from a population has proven to be cost effective than genotyping multiple individuals per population, and much more accurate than genotyping only one individual per population (Dubreuil et al, 2006; Warburton et al, 2002). DNA fingerprinting that distinguishes between improved open-pollinated varieties (OPVs) is possible using SSR markers based on a population bulk DNA fingerprinting technique (Warburton et al, 2010).This method has enabled better resolution of diversity in genetically heterogenous populations/landraces and estimation of allele frequencies (Dubreuil et al, 1999; Dubreuil et al, 2006). Studies using the bulking method and SSR markers have allowed the elucidation of the origin of European landraces (Dubreuil et al, 2006) and relationships between Latin American landraces (Warburton et al, 2008). However, publications related to molecular diversity and characterization of population structure of maize landraces of African countries are few. Considering the importance of maize cultivation in Ghana, which is also the original introduction point of maize into the African continent, this knowledge gap is a crucial limiting factor. The understanding of relationships among maize landraces can provide clues as to why Ghanaian landraces are so low yielding, and hasten cultivar improvement and adoption. Therefore, the objective of the present study was to use the bulked population fingerprinting technique and SSR markers to characterize the molecular diversity and relationships among maize landraces collected throughout Ghana.

Materials and Methods

Over 500 maize populations were collected from farmers across Ghana who continues to cultivate landraces, in September through November, 2007 (data not shown). Not more than three cobs were collected from each farmer in the localities visited. These materials were initially stored at the CRI genebank and sub samples were sent to CIMMYT for storage and genetic characterization. A subset of 20 populations was randomly formed to represent the geographical/local diversity within the country (Table 1, Figure 1). Seeds from all cobs within each locality were mixed and 15 seeds were chosen randomly from each of the 20 populations. In addition, four other inbredlines (CML051, CML292 from CIMMYT, ANC 393 from Peru and GUAT606 from Guatemala) were used as controls. The bulked fingerprinting technique was used for the characterization, which was done at the ABC laboratories at CIMMYT, Mexico. The 15 seeds from each of the populations were germinated in the greenhouse. Leaf fragments of 10-15 cm were harvested from individuals and bulked to form

Table 1 - Summary of the 20 maize landraces collected from various locations in Ghana and used in the present study.

Population	Major town/Locality	Longitude	Latitude	No. of cobs collected
AshantiA	Bekwai	6°45N	1°5833W	38
AshantiB	Lake Bosomtwe	6°45N	1°4W	30
AshantiC	Ejura/Sekyedumase	7°3667N	1°35W	19
AshantiD	Kwaso	6°8333N	1°.5W	22
BrongAhafoA	Wenchi	7°733N	2°1W	15
BrongAhafoB	Goaso	6°7833N	2°5W	21
Central	Mankesim	5°4N	1°15W	18
EasternA	Anum	6°2167N	0°0667E	24
EasternB	Akuapim ridge	5°85N	0°333W	20
EasternC/AshE	Adawso/Essumeja	5°9333N	0°2W	29
NorthA	Nyankpala	9°3833N	0°9833W	36
NorthB	Walewale	9°4167N	0°0333E	31
NorthC/BA	Tamale/kintampo	9°4N	0°85W	24
Upper EastA	Navrongo/Paga	10°9833N	1°1W	25
Upper EastBNorth	Bawku/Kpana	11°144N	0°224W	27
VoltaA	Kpeve	6°6667N	0°7833E	30
VoltaB	Ohawu	6°1167 N	0°7833E	28
VoltaC/GAR	Golokwati/Ayimensa	5°8397N	0°174W	25
WesternA	Babiani	6°45N	2°3167W	27
WesternB/Volta	Axim/Ho	4°8667N	2°2333W	27

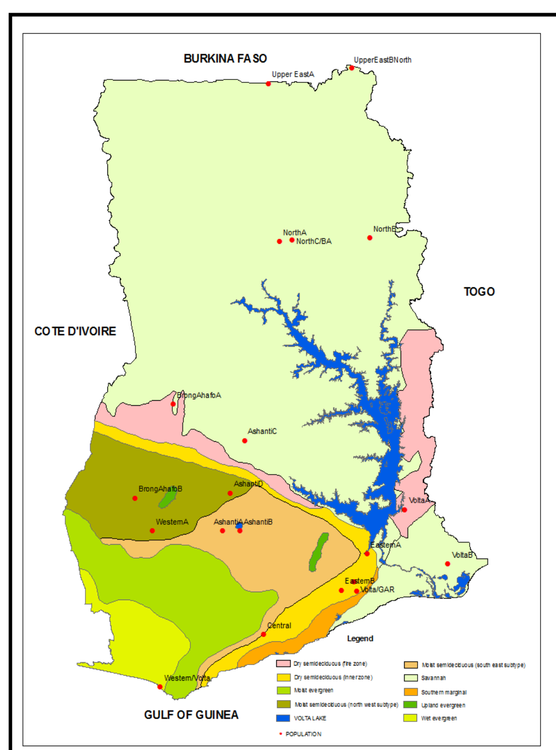

Figure 1 - Map of Ghana showing sampling centres and vegetation.

a composite sample. DNA was extracted from bulked freeze-dried tissues according to CIMMYT protocols (http://www.cimmyt.org/english/docs/manual/protocols/abc_amgl.pdf). DNA was quantified using the NanoDrop spectrophotometer (Thermo Scientific, Wilmington DE) and adjusted to 20 ng μl^{-1} for genotyping.

Genotyping

Twenty microsatellites covering 9 of the 10 chromosomes of maize were selected for genetic analysis (Table 2). The selected SSR markers have been optimized previously to work in pooled DNA samples (Warburton et al, 2002; Xia et al, 2005b). Capillary electrophoresis was carried out following PCR amplification with fluorescently labelled primers, using an automatic sequencer ABI 3100 (Applied Biosystems, Foster City, CA) to separate and size the fragments generated via PCR according to CIMMYT standard protocols (http://www.cimmyt.org/english/docs/manual/protocols/abc_amgl.pdf, page 48). The programs GeneScan ® 3.1 (PerkinElmer / Applied Biosystems, Foster City, CA) and Genotyper ® 2.1 (PerkinElmer / Applied Biosystems, Foster City, CA) were used to analyse the amplified fragments for size in base pairs, peak height (corresponding to intensity of the amplified fragment) and quality score.

Data analysis

Based on the peak heights which indicates the number of base pairs, the frequencies of all alleles found in every bulk were calculated using the Freqs-R program (Franco et al, 2005). This program can remove noise caused by PCR stuttering or preferential amplification, reducing the probability of finding false alleles. Next, each of the allelic frequencies were expressed in 15 simulated diallelic individuals through a simulation process that satisfies allele frequencies and expected heterozygosity under Hardy-Weinberg equilibrium conditions using the FtoL-R program (Franco et al, 2007). This was done because most population diversity analysis programs do not accept allele frequency data, but instead use data from individuals as input data. Numbers of alleles, genetic diversity, polymorphic information content (PIC), Major allele frequency and heterozygosity were calculated using Powermarker (Liu and Muse, 2005). The same program was used to calculate Modified Roger's genetic distances among populations, and phylogenetic analysis was done using UPGMA method.

Structure, a model-based (Bayesian) clustering method for using multilocus genotype data to infer population structure and assign individuals to populations (Pritchard et al, 2000) was also used to analyse population structure. This was done to see if all landrace individuals fall into the predetermined groups defined by the 20 populations. The analysis was run with the admixture model to assign each individual from the landraces to a population with similar allele frequencies at each locus. The method of (Evanno et al, 2005) was used to calculate the most suitable number of clusters or subpopulations (K). Probabilities for K were calculated from 1 to 8, using 1,000,000 replications after a burn in period of 500,000 iterations, and the procedure was repeated 5 times for each K value. K=5 was chosen as the most likely, and populations were assigned to one of each of the five groups for which they had an ancestry proportion greater than 51.0% (Reif et al, 2006); if an individual did not show an ancestry proportion higher than this value, it was assigned to the mixed group.

Table 2 - List of the 20 SSR markers used to study maize landraces collected in Ghana.

SSR	Bin	Repeat unit	Size
phi109275	1.00	AGCT	108-134
umc2047	1.09	GACT	119-133
phi127	2.08	AGAC	110-127
phi374118	3.03	ACC	214-238
umc1266	3.06	CAG	120-147
phi076	4.11	GAGCGG	156-177
phi079	4.05	CATCT	177-195
phi109188	5.00	AAAG	145-181
phi008	5.03	GGC	100-104
umc1332	5.04	CTA	126-147
phi075	6.00	CT	203-235
phi102228	3.04	AAGC	122 / 131
phi299852	6.08	AGC	102-147
phi034	7.00	3bp	125-143
umc1545	7.00	AAGA	64-80
phi114	7.02	GCCT	129-171
umc1304	8.02	TCGA	121-141
umc1161	8.06	GCTGGG	128-149
umc1196	10.07	CACACG	137-173
umc1266	3.06	CAG	120-147

Table 3 - Summary statistics of the markers used in the study including the frequency of the most common allele, number of alleles per marker, heterozygosity, Polymorphic Information Content (PIC) and F-statistics.

Locus	Major allele frequency	Number of alleles	Heterozygosity	PIC	Theta = F_{IS}	F = F_{IT}	f= F_{ST}
phi008	0.7181	6	0.381	0.366	-0.0091	0.1082	0.1163
phi034	0.6101	5	0.493	0.530	-0.0130	0.1401	0.1512
phi075	0.3736	13	0.603	0.698	-0.0075	0.1857	0.1918
phi076	0.3583	8	0.576	0.718	-0.0129	0.2401	0.2498
phi079	0.3848	6	0.724	0.664	0.0011	-0.0118	-0.0129
phi102228	0.7986	2	0.258	0.270	0.0055	0.1985	0.1940
phi109188	0.5014	6	0.539	0.644	-0.0078	0.2091	0.2152
phi109275	0.3306	9	0.672	0.753	-0.0059	0.1424	0.1474
phi114	0.4870	7	0.603	0.617	-0.0085	0.0972	0.1049
phi127	0.5397	9	0.375	0.463	-0.0243	0.3241	0.3401
phi299852	0.3942	18	0.736	0.756	-0.0070	0.0561	0.0627
phi374118	0.4971	10	0.612	0.610	-0.0068	0.0744	0.0806
umc1161	0.8594	7	0.200	0.249	0.0006	0.2210	0.2205
umc1196	0.4486	8	0.525	0.691	-0.0098	0.2765	0.2836
umc1266	1	1	0.000	0.000	-0.0044	-0.0384	-0.0338
umc1304	0.8275	4	0.316	0.288	-0.0006	0.0257	0.0263
umc1332	0.2591	10	0.776	0.765	-0.0067	0.0855	0.0916
umc1367	0.9611	7	0.069	0.075	-0.0089	0.0979	0.1059
umc1545	0.6116	5	0.493	0.485	-0.0029	0.1995	0.2018
umc2047	0.5917	5	0.425	0.446	-0.0072	0.1409	0.1470
Mean	0.5776	7.3	0.469	0.504			

F_{IS} = amount of inbreeding-like effects or correlation of alleles within individuals within subpopulations (level 2); F_{IT} = amount of correlation of alleles (identity by descent, or inbreeding-like effects) within the entire population (all individuals from all populations combined); F_{ST} = amount of correlation of alleles (identity by descent, or inbreeding-like effects) among (between) subpopulations (level 2).

Results and Discussion

Marker umc1266 was monomorphic and therefore was not included in the analyses. Missing data levels per marker ranged from 0% to 16.7% with an overall mean of 4%. This compares favorably with previous SSR characterization studies of maize landraces (Reif et al, 2006). Allelic frequencies and other summary statistics for each marker and averaged over all populations can be found in Table 3. Marker phi299852 had the highest number of alleles (18) followed by phi075 (13), and the lowest (other than umc1266) was phi102228 with only two alleles. The mean number of alleles per SSR locus was 7.3, and in total, 145 alleles were detected. This compares well with previously reported studies using SSRs with the same repeat type (Dubreuil et al, 2006; Qi-Lun et al, 2008). Gene diversity ranged from 0.795 (umc1332) to 0.076 (umc1367), with a mean of 0.544, sufficiently high to be informative for diversity and characterization studies. Reif et al (2006), reported a total gene diversity of 0.61 when they examined 25 accessions of 24 races of maize from Mexico, and 0.53, when they analyzed 150 European maize cultivars (Reif et al, 2005). Polymorphic information content (PIC) followed similar trends for the markers as did gene diversity (Table 3).

The F statistic values (Table 3) indicate that individual maize plants are not inbred, and that each population is not well differentiated. In fact, overall FST (correlation of alleles among populations) is only slightly higher than FIT (correlation of alleles within the entire study, all individuals from all populations combined), and thus each population probably contains a great deal of mixed germplasm. However, enough of the variation is partitioned within populations in order to differentiate each population in the Structure analysis. This is not an uncommon circumstance of maize landrace populations, which are outcrossing by nature, and farmers often exchange seeds of their best populations. Mixing thus occurs via pollen and seed flow.

Modified Rogers' genetic distance (MRD) is a modification of Euclidean distance and is well-suited for estimation of genetic distances when no information is available on the evolutionary forces influencing the genotypes under consideration and no specific mutation model can be attributed to the allelic variation observed at the SSR loci (Reif et al, 2005). Lower average MRD suggests a higher average degree of relatedness among the accessions. In this study, lowest MRD was 0.3551, which was found between Central and EasternA accessions; the highest MRD was 0.7190, between Volta/GAR and the control CML292. The relatively low MRD obtained in this study (excluding the control, unrelated populations) suggests the mixed constitution of the Ghanaian germplasm, compared with higher MRD reported by Xia et al (2005), who obtained average MRD between pairs of inbred lines with a range from 0.45 to 0.93. This agrees with the F statistics and with observations that seed traders in Ghana often sell seed of landraces not collected locally (USAID/EAT, 2012).

Cluster analysis using shared allele distances and the UPGMA algorithm grouped the Ghanaian maize landraces into two main groups, and the control populations were seen to be outliers as expected because they were from different climatic or agro-ecological regions (Figure 2). The Ghanaian clusters follow agro-ecological/climatic origins (Figure 2), with the northern group (Guinea savannah zone) and the southern group (forest and coastal savannah) being

well differentiated. Ashanti germplasm, named after the central region of Ghana, occurs in both clusters. Climatically/agro-ecologically distinct results are expected, as farmer and natural selection favor certain genotypes in certain areas, and similar findings have been reported by Sharma et al (2010), when they analyzed 48 landrace accessions selected from diverse agro-ecologies in India using 42 SSR markers.

The Baysian clustering performed with Structure (Pritchard et al, 2000) is shown in Figure 3. Five clusters were assumed. Six populations did not fall cleanly into one of the clusters, but were mixtures of more than one; however, frequently, nearly half of the individuals from these mixed populations did fall into one of the clusters according to Figure 3. The red group of Figure 3 consisted mainly of populations from WesternA and BrongAhafoB, EasternB, AshantiB and EasternA. The green group consisted of populations mainly from BrongAhafoA, AshantiC, NorthB and UppereastA. The blue group consisted of populations from Central, Western/Volta, VoltaA, and VoltaB. The yellow group consisted mainly of populations from AshantiA, Uppereast/North and NorthC/BA. The pink group consisted mainly of VoltaGAR population. One landrace (BrongAhafoA) fell into a population by itself (Supplementary Table 1). The yellow and green groups (plus the mixed populations with nearly half their individuals in the yellow or green

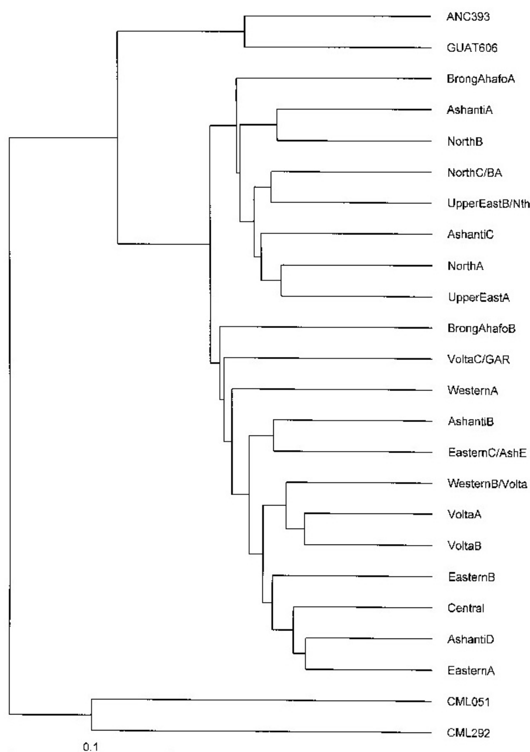

Figure 2 - Dendrogram showing relationships between 20 Ghanaian maize landraces and four controls as created using the shared allele distances between all pairs of populations in Powermarker

clusters) corresponded to the Northern cluster in the UPGMA dendrogram (Figure 2), with the exception of BrongAhafoB. This population, and the population VoltaC/GAR from the pink group, fell between the two UPGMA clusters, indicating mixtures between them. The red and blue Structure groups (and the mixed populations containing many red and blue individuals) corresponded exactly with the Southern UPGMA cluster. This shows the more non-discrete nature of the Ghanaian landraces (more in accordance with the analysis of F statistics). Populations from EasternB, EasternC/AshE, AshantiD and NorthA were highly mixed. Because the Structure analysis tends to break the large UPGMA clusters into subgroups, additional information was provided by Baysean analysis.

Some landraces with very similar names (AshantiA, B, BrongAhafo A and B, etc.) were split into different clusters; however, most landraces with similar names clustered well together. This will reflect their origins, but also the mixing that has obviously been occurring in Ghana since introduction or creation of each landrace. Ashanti is centrally located in Ghana and it is one of the main centres of the maize business. Farmers from all over the country converge here for trade and this probably is one of the reasons why germplasm collected in Ashanti fall within all the major groups. Similarly, the vegetation in Ashanti is both forests with some portions being forest/savannah or transition type, which may also contribute to the mixing of populations from here within the dendrogram.

Both clustering analyses show that the genotypes analyzed fall between the major vegetation/climatic conditions found in Ghana. The northern part of Ghana is drier with a savannah ecotype and a short, unimodal rainfall (May/June-September). The southern forest belt receives larger amounts of rainfall in two seasons; the long major season (March/April-June/July) and the shorter minor season (Aug/September-November). Farmers' choice of which maize genotype to grow is influenced by this fact and this could also be responsible for the differentiation seen in the northern and southern clusters. On the other hand, a factor working against geographic portioning may be attributed to trade within Ghana by women traders and distributors who dominate the maize seed supply chain. These small scale seed distributors buy seed from all sections of the country and sell them wherever they can, and the speculative nature of their trade brings about movement of germplasm into areas where the seed are not necessarily adapted. This is probably the cause of most of the population mixing seen in this study, and possibly much of the low yield in Ghanaian maize production. Since most farmers are small landholders and often unable to save their own seeds, they buy from these traders in the open market for planting; if they are able, they will then select genotypes they prefer for cultivation in subsequent seasons.

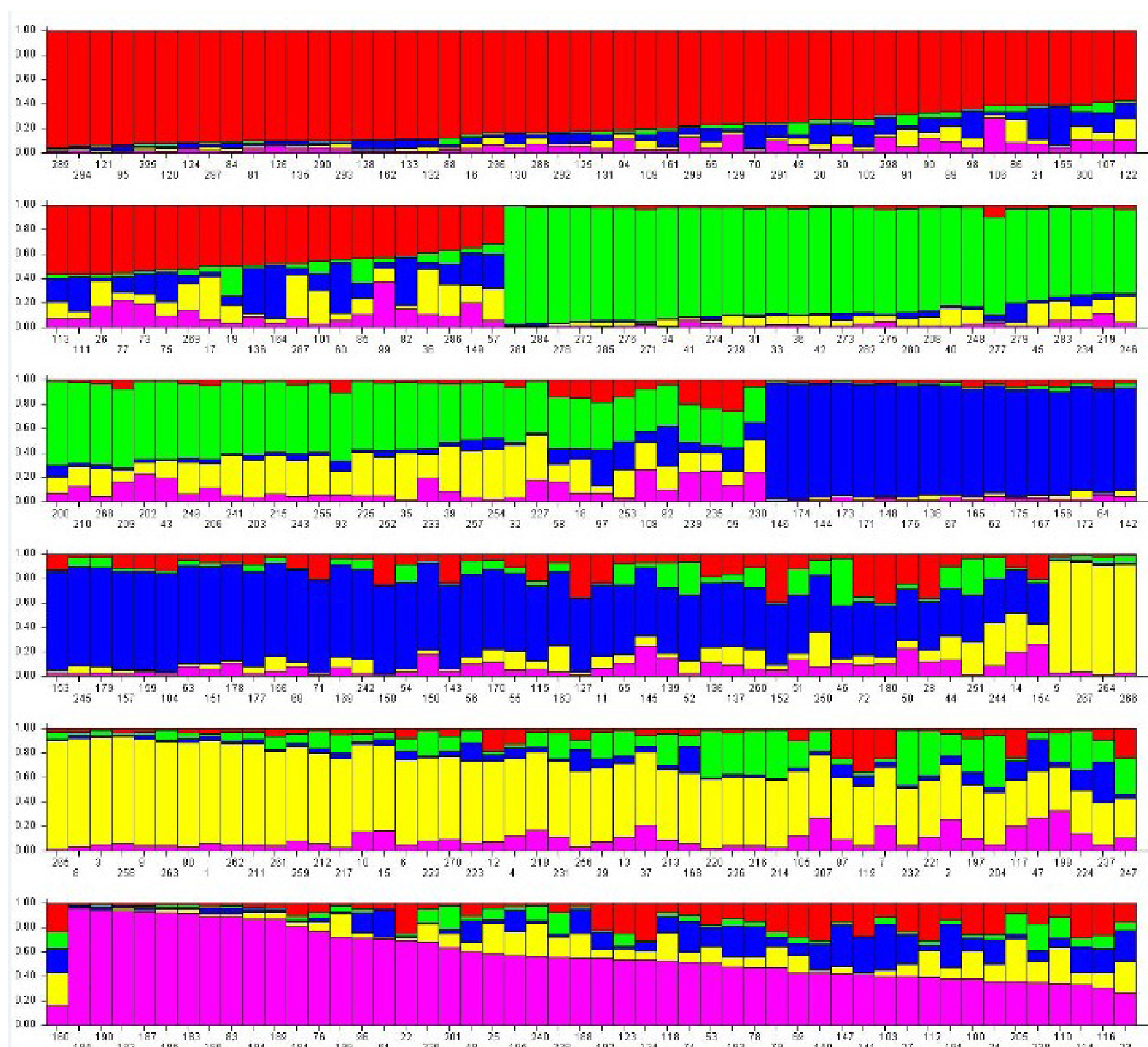

Figure 3 - Clusters of Ghanaian maize landraces created by the program STRUCTURE. Each column corresponds to one individual from one of 20 populations as explained in Supplementary Table 1. The different colours represent the various clusters, and each individual's probability of sharing ancestry to that cluster. Each individual may be composed of variation from more than one cluster. The percentage of each individual belonging to each cluster is seen in the amount of color each column contains. Highly mixed individuals (assigned to the "mixed" group in Supplementary Table 1) do not have a majority of any one color (and thus, no major probability of belonging to any one cluster.

The results of this study clearly show that although different maize germplasm is found in the north and the south of Ghana, much of it has apparently been mixed. Efforts to breed new maize genotypes should improve targeting specific climatic conditions for higher productivity. Unfortunately, this study also demonstrates that new germplasm may find their way to places where they are not intended for optimum yield and productivity. To ensure that the most adapted (and highest yielding) germplasm is grown in each area, extension officers and farmers should be well educated in the choice of suitable cultivars for specific agro-ecologies. The small scale seed traders who constitute an integral part of the maize seed business could be recruited and educated on improved seed choices suited for the different agro-ecologies. If they understand the yield potential of different varieties meant for targeted ecologies, they could help to increase yields across Ghana; on the contrary, if they are not included in the effort to boost maize productivity, they can slow the entire process by their seed mixing.

The results of this study are now being used to guide demonstration farm trials, which are to be set up by the Ministry of Food and Agriculture (MOFA) in Ghana. These demonstration trials would include landraces, high yielding OPVs, and eventually hybrids, planted in each region or agro-ecology. They would include both adapted and unadapted germplasm, allowing the farmers and seed traders to see

the difference in growth and yield of each type of germplasm. This would ensure that the farmers demand (and the traders provide) the best seed for each region, and could also be used as a tool to eventually convince the farmers of the benefits of hybrid cultivars. These demonstration trials should be underway soon, and the first landraces planted will be those that are relatively unmixed and found predominantly in each region, according to the results of this study. The development of new OPVs and hybrids by the universities and research institutions who collaborated in this study, in conjunction with the MOFA and, hopefully, private companies, will also be guided by these results, the results of the demonstration farm trials, and ongoing phenotypic characterization trials.

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