Tracing the Pattern of Maize Introduction and Spread in Africa using Chloroplast DNA Markers

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Abstract

A genetic assessment of maize landraces from 14 African countries was conducted in order to determine relationships among the landraces so as to infer the pattern of spread after introduction. Seeds were planted and DNA was extracted from ten randomly selected seedlings per accession. Eight published chloroplast SSR primers were used in PCR and purified products were directly sequenced. Allele size data from the chloroplast microsatellites were organized in MS Excel spreadsheets and sorted into haplotypes. Mantel's test and Principal Component Analysis were used in determination of haplotype diversity and biogeography of alleles. Results indicated that there was considerable variation in the geographic range of alleles. However, Mantel tests performed to examine the correlation between genetic and geographical distances likewise showed no association between genetic and geographic distances ($r = -0.0138$, $p = 0.5600$, 1000 permutations). Furthermore, principal component analysis provided little evidence for population differentiation based on geographical region; projection of the populations over the first two planes revealed two outlier accessions whilst the rest appear to be sub-accessions associated with South Africa. Hence, results from this study support a hypothesis of

successive introductions of landraces in Africa with South Africa as one of the likely entry points.

Key Words: *Maize Landraces, SSR Markers, Haplotype, Genetic Diversity, Africa.*

1 INTRODUCTION

There are no reports of maize cultivation in Africa before the 16th century but Spanish and Portuguese traders are credited with its introduction from the Americas along the western and eastern coasts (Miracle, 1966; Aci et al., 2015). What happened next is largely a matter of conjecture although it seems most likely that a combination of local trade and exploration of Africa's interior led to 'secondary' maize introduction across the continent by missionaries, merchants and slave traders (McCann, 2001; Smale & Jayne, 2004). Whereas maize was incorporated in existing farming structures in West Africa as a cash crop, it displaced indigenous cereals (sorghum and millet) in southern Africa and became the main food crop because of high yields and early maturity (McCann, 2001; Smale & Jayne, 2004). Nowadays, maize is by far the most important staple food in southern Africa accounting for over 50% of calories in local diets (Ranum et. Al., 2014). Of the twenty-three countries in the world with the highest percentage of maize consumed in national diets, the top three are all in Africa (Zambia, Malawi, and Lesotho), and surpass even Guatemala and Mexico, maize's homelands (Smale, 1995).

Genetic diversity within local landraces is important input for crop breeding programs and in the preservation of their genetic potential (Yusuf et al., 2018). Molecular studies in maize genetic diversity have reported a reasonably close genetic affinity between American varieties of maize and those found in southern Africa (Warburton, 2006). This would seem to imply an initial introduction into southern Africa and perhaps lends support to the compromise theory of multiple independent introductions of maize into Africa. Thus, the history of the introduction and subsequent movement of maize within Africa appears both complex and unclear. Work described in this paper aims to provide an initial attempt to address this problem by examining variation in chloroplast DNA (cpDNA) diversity among maize landraces across the continent, with a view to inferring the pattern of introduction and spread of maize across Africa. Such knowledge can be useful in tracing original or closely related sources of germplasm for breeding new varieties which can adapt to climate change effects. Thus, breeders can utilize the vast pool of useful alleles from the founding populations if the origin of introduction is known.

2 MATERIALS AND METHODS

2.1 Seed source

Collectively, 42 maize landrace accessions were donated by Centro Internacional de Mejoramiento de Maizy Trigo (CIMMYT) genebank in Mexico and the National Plant Genetic Resources of South Africa. A total of 12 countries were represented namely Angola, Congo, Egypt, Ethiopia, Kenya, Malawi, Mali, Morocco, Nigeria, Uganda, South Africa and Zimbabwe The number of samples sent was naturally limited by the stock held in the national collections i.e. there were more than five accessions from South Africa, Malawi, Morocco and Mali and single accessions from Egypt, Congo and Nigeria (Table 1).

Table 1 : *A summary of maize accessions used in the study*

SN	Number of accessions	Country of origin	Supplied by	status
1	4	Malawi	CIMMYT	FAOTRUST
2	1	Nigeria	CIMMYT	FAOTRUST
3	2	Uganda	CIMMYT	FAOTRUST
4	1	Egypt	CIMMYT	FAOTRUST
5	3	Angola	CIMMYT	FAOTRUST
6	1	Congo	CIMMYT	FAOTRUST
7	3	Ethiopia	CIMMYT	FAOTRUST
8	5	Mali	CIMMYT	FAOTRUST
9	4	Morocco	CIMMYT	FAOTRUST
10	2	Kenya	CIMMYT	FAOTRUST
11	1	Zimbabwe	CIMMYT	FAOTRUST
12	9	South Africa	South Africa	NGPR

For analysis purposes, ten seeds from each accession were randomly selected and grown in 25cm square pots containing peat-based compost (Levington F2) in a

heated glasshouse (minimum temperature 14ºC). Some accessions had mixed grain colours and seeds representing each of these colours were planted separately and recorded.

2.2 DNA extraction and Polymerase Chain Reaction (PCR)

Ten separate leaf samples per accession were collected from the ten seedlings and DNA was extracted using GenElute plant kit using the manufacturer's protocol. Eight published primers containing simple sequence repeat regions of different complexity from the maize chloroplast genome were used (Provan et al., 1998). Chloroplast microsatellites were chosen in this study because there is maternal inheritance of plastids in maize and this would facilitate the study of population structure and genetic relationship among the maize populations. In addition, microsatellites are abundant, highly polymorphic species-specific and co-dominant compared with other genetic markers. These markers can also be analyzed by a rapid, technically simple, specific and inexpensive polymerase chain reaction (PCR) based assay that requires only small amounts of DNA. A 10µl PCR final volume was assembled consisting of Master-mix: 5µl; genomic DNA: 2µl; nanopure water: 2µl; primer pair: 1µl. The reaction mixture was initially denatured at 94ºC for 5 min, followed by 35 cycles of denaturation at 94ºC for 30 s, annealing at 55ºC for 1 min and extension at 72ºC for 1 min 30 s and final extension at 72ºC for 10 min in a PTC-100 thermal cycler (MJ Research, Inc.). Thereafter, 5µl of the product was fractionated on 1.5% w/v agarose gel to assess the amplicon abundance and a 100 base pair ladder was used to estimate size of products. PCR products were cleaned using the PCR clean p Nucleofast (ABgene) and the purified PCR products were used directly for sequencing in an ABI 3700 sequencer.

2.3 Data analysis

Electropherograms were checked manually to annotate the identity of peaks and the output trace data files were classified to specific alleles by Genescan, Genotyper (version 3.7 NT) and GeneMapper software programmes (Applied Biosystems). Allele size data were organized in MS Excel spreadsheets and alleles of similar composition at all loci were grouped into haplotypes. The correlation between genetic distance and geographic distance was analysed using Mantel' test (Mantel, 1967) employing 1000 randomisations (MS Excel, PopTools). Principal Component Analysis (PCA) based on abundance and presence of alleles was used to analyse the distribution of variation in the collection of maize landrace accessions and allele frequencies among haplotypes were calculated using Minitab15 software (Minitab version 15.1.20.0, 2007).

3 RESULTS

3.1 Haplotype variation within accessions

Results for haplotype variation within accessions are presented in Table 2. There was a total of 42 identified haplotypes none of which was common to all countries even though there were a number of unique haplotypes present. Thirteen haplotypes i.e. numbers 7, 8, 13, 21, 23, 25, 27, 30, 32, 34, 35, 37 and 40 were found in two or more countries whilst haplotypes 25 and 34 were more common and occurred in five countries each.

Table 2: *Allele combinations of the identified 42 haplotypes*

	Primers and allele sizes							
Haplotype	**m3764**	**m6359**	**m8372**	**m16703**	**m17192**	**m18704**	**m20597**	**m20824**
1	159	199	139	138	117	123	204	134
2	159	199	139	138	116	123	204	134
3	159	200	140	139	119	123	204	134
4	160	199	140	138	118	123	204	134
5	159	199	140	139	118	123	204	134
6	159	199	140	138	118	123	204	134
7	160	199	140	139	117	123	213	134
8	159	199	140	139	117	123	213	136
9	159	199	140	138	117	123	213	136
10	159	199	140	139	116	123	213	136
11	159	199	140	138	116	123	213	134
12	159	199	140	138	117	123	213	134
13	159	199	140	139	117	123	213	134
14	161	199	140	138	116	123	213	134
15	161	199	140	139	116	123	213	134
16	159	199	140	139	116	123	213	134
17	160	199	140	139	116	123	213	134
18	161	199	140	139	117	123	213	134
19	161	199	140	138	117	123	213	134
20	161	199	139	139	117	123	213	134
21	159	199	139	138	117	123	213	136
22	159	199	140	139	118	123	213	135
23	159	199	140	139	116	123	213	135
24	159	200	140	139	117	123	213	135
25	159	199	140	139	117	123	213	135
26	159	199	139	138	118	123	213	137
27	159	199	139	138	117	123	213	137
28	159	199	139	138	116	123	213	137
29	159	199	139	138	118	123	213	136
30	159	199	140	139	117	123	213	137
31	159	200	140	139	118	122	204	135
32	159	200	140	139	118	123	204	135
33	160	199	140	139	117	123	204	134
34	160	199	140	139	117	123	213	135
35	160	199	140	139	117	123	204	135
36	160	199	140	139	118	123	204	135
37	161	199	140	139	117	123	213	135
38	160	200	140	139	119	123	204	135
39	159	199	139	138	117	123	213	135
40	159	200	140	139	117	123	204	135
41	159	200	140	139	119	123	204	135
42	159	199	139	138	116	123	204	135

Some haplotypes were apparently unique to a single country, with considerable variation between countries in the abundance of unique haplotypes. For instance, there were 22 haplotypes specific to South Africa, three to Malawi and a single haplotype unique to Angola, Ethiopia and Uganda.

One notable feature across all samples was the extent of haplotype variability present in multi coloured grain maize accessions. Of these, accessions 'Sa2' and 'Sa6' from South Africa displayed the highest variation in haplotype distribution. For instance, 'Sa2' had seeds that were white, yellow and violet in colour, with violet coloured grains containing three haplotypes, white coloured grains containing two haplotypes whilst the yellow coloured grains contained a single haplotype. Similarly, 'Sa6' had caryopses that were yellow and white which were partitioned into eight and four haplotypes respectively. There was similar infra-accession variability among some accessions with uniform grain colours. For instance, five haplotypes were observed for individuals of accession 'C26', a white variety from Malawi, four haplotypes for individuals in accession 'Sa10', a pink variety from South Africa and two haplotypes for accessions 'C44' and 'C36', yellow varieties from Morocco and Mali respectively. Once again, countries from southern Africa (South Africa, Malawi and Angola) and eastern Africa (Ethiopia) displayed greatest haplotype diversity when compared to other regions. Apart from two accessions from Morocco and Mali, the remaining accessions were represented by a single haplotype.

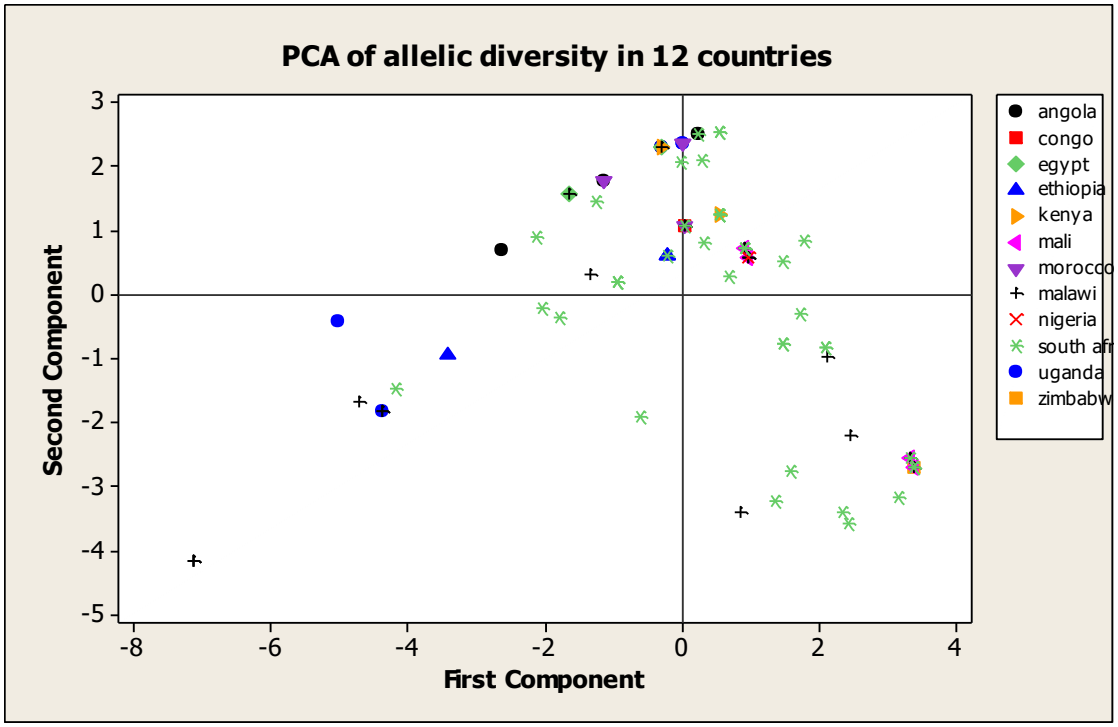
3.2 Biogeography of haplotypes

No one haplotype predominated overall, with the abundance of individual haplotypes usually being low and typically ranging from 0.192% ± 0.00192 for haplotypes that corresponded to single seedlings to 10.6% ± 0.0135 for haplotype 30 (present in 55 seedlings from six accessions). There was no obvious link between the number of seedlings exhibiting a particular haplotype and the number of countries in which it was found. For instance, the highest number of individuals (55) was found in haplotype 30 which was present in only three countries (Malawi, Mali and Nigeria). In contrast, haplotype 25 was found in 23 individuals but was recorded from five countries (South Africa, Congo, Ethiopia, Malawi and Morocco). Likewise, haplotype 34 found in 33 individuals was also recorded in five countries (Uganda, Egypt, Angola, Morocco and Kenya). Nevertheless, when viewed in a geographic context, there was a clear and obvious concentration of haplotypes in southern Africa; the largest number of haplotypes being recorded from South Africa (29 haplotypes) and Malawi (13 haplotypes). There was also a marked drop in the number of haplotypes recorded elsewhere, with all remaining countries each containing fewer than five haplotypes.

3.3 Biogeography of allele spread

There was considerable variation in the geographic range of alleles, with a general trend for the alleles that featured in many haplotypes tending also to be widespread. This trend is perhaps epitomized by the most common alleles (199 of locus m6359, allele 117 of locus m17192, allele 123 of locus m18704 and 213 of locus m20597) which perhaps unsurprisingly appeared in every country sampled. The converse was true for the alleles that featured in relatively few haplotypes since none of these exhibited a widespread distribution. There were nevertheless some notable exceptions to this tendency. For instance, some of the seemingly more common alleles in terms of haplotype representation appeared to exhibit a rather limited geographic distribution. For example, alleles 116, 118 and 119 of locus m17192 were restricted to countries from southern, south-west and eastern Africa (South Africa, Malawi, Angola, Uganda and Ethiopia) but were absent from western and northern maize samples from Mali, Morocco, Nigeria and Egypt.

Mantel tests performed to examine the correlation between genetic and geographical distances likewise showed no association between genetic and geographic distances ($r = -.0138$, $p = 0.5600$, 1000 permutations), indicating that there was no clear isolation-by distance effect on genetic diversity in the maize landraces. Similarly, Mantel tests for arbitrary 'northern' ($r = 0.242$, 1000 permutations) and 'southern' ($r = 0.048$, 1000 permutations) regions in Africa suggested only weak correlations between geographical and genetic distances. Principal Component Analysis (PCA) based on abundance and presence of alleles was used to analyse the distribution of variation in the collection of maize landrace accessions. The analysis provided little evidence for population differentiation based on geographical region; nearly all variation being represented by South African accessions (Figure 1). Projection of the populations over the first two planes reveals two outlier accessions from Malawi and Uganda whilst the rest appear to be sub-accessions associated with South Africa.



* south afr (South Africa)

Figure 1: *Principal Component Analysis of allele distribution across 12 countries.**

4 DISCUSSIONS

In this study, almost two thirds of the variation determined at molecular level was distributed within the landraces with little variation between groups and nearly all haplotypes were subsets of South African accessions. Considering that in evolutionary terms maize is a recent introduction in Africa and that there is a strong cultural practice of exchanging seeds throughout Africa, it seems the most plausible explanation for the differences in the amount of variation present within and (to a lesser extent) between accessions is founder effects which leads to the loss of genetic variation when a new colony (seed stock) is established from a very small number of individuals from a larger population. Such effects can apply across extremes of scale.

At the large scale, it could be founder effects associated with the introductions from South America, an intermediate scale applies for trade between seed merchants and on a small scale, on the basis of multiple minor founder effects as farmers acquire seed material from each other as the crop spread across the continent. In each

instance, the new populations created would contain a subset of alleles from the original parental source population and so could diverge from it genetically and phenotypically. Thus, if genetic erosion via repeated founder effects was the only process that was operating as maize spread across Africa, then the expectation would be for little variation within accessions and for strong genetic divergence between regions (depending on the original source material and the isolation of the population under study. Results from this study revealed that whilst accessions varied in allelic diversity and haplotype diversity (Table 2), there was no obvious geographic structure to the variation and most variability resided within rather than between accessions. Clearly then, the expected outcome of recurrent founder effects is completely incongruent with the observations made here and this warrants explanation.

Founder effects associated with maize spread in Africa and wind mediated gene flow do not adequately explain the pattern of genetic variability observed in this study. Even though selective adaptation does contribute to genetic diversity, the most plausible explanation appears to be seed mixing between and among farmers over short and long geographical distances. The practice of seed exchange is common in Africa because farmers usually select seed for planting and acquire supplementary seed from neighbours or markets. Different landraces are mixed in the same field and it is common to find hybrid maize growing side by side with local maize (Smale & Jayne, 2004). Such proximity of plants leads to exchange of pollen because maize is an outcrossing crop. Hence, the observed homogeneity between and high variability within accessions is consistent with effects of seed mixing. Landraces that are cultivated are not genetically diverse due to similar founding populations but rather differ due to differences in local environmental conditions. Regional seed exchanges between farmers maintain this balance. This observation is similar to what was reported by Belalia et al. (2018) and Prasana (2010).

The fact that the chloroplast haplotypes were found in several countries might suggest similar ancestry and seed exchange and mixing over wide distances. Correspondingly, several studies on the genetic structure have also reported, in line with these results, that different populations can be genetically identical rather than dissimilar. For instance, Pressoir & Berthaud (2004) studied a sample of local landraces cultivated by farmers in six villages located in Central valleys of Oaxaca. Their results showed little among-population differentiation and no isolation by distance and small among-village differentiation. All populations, even those separated by up to 100 km, were found to share chloroplast DNA haplotypes. Thus the authors concluded that the practice of saving seed from one season to the next coupled with acquisition of seed from both local and distant farmers or markets and growing the mixture contributed to the overlap in landrace distribution.

Another explanation for the commonality of haplotypes could be due to migration of people across the continent thereby leading to mixing of germplasm. Preliminary results by Charcosset et al. (2006) on a worldwide overview of maize diversity indicated that groupings among maize landraces from Americas, Africa and Europe were not perfect based on geography thereby suggesting mixing of germplasm due to migration. The landraces used in this study were from a much wider geographical region covering west, north, east, central and southern Africa. Isolation-by-distance was tested by correlating genetic and geographic distances between populations using the Mantel test and the results indicate that the pattern of genetic structure among the maize landraces is not correlated to geographic distance. A negative correlation obtained from Mantel test results ($r = -0.038$, $p = 0.5600$, 1000 permutations) indicates that the landraces share similar alleles regardless of geographic distance and hence similar allelic frequencies, translated into the low allelic richness (1.33 alleles/locus) observed. PCA results also indicated that the haplotypes were not genetically diverse but were subsets of landrace accessions from South Africa.

Similar pattern of association was reported by Nyaligwa et al. (2015) after assessing maize lines from CIMMYT collections. It was observed that CIMMYT/Zimbabwe accessions displayed unique clustering pattern than the rest of the collections, although they could possibly share common genetic background being sourced from CIMMYT. It was stated that an exchange of genetic materials between CIMMYT and collaborating countries for germplasm evaluation, breeding and release contributed to a high level of gene flow thereby leading to genetic homogeneity. In the present study, all collections were supplied by CIMMYT except those from South Africa.

No clinal variation was observed likely because the sampling was not carried out along a latitudinal gradient. The private unique haplotypes observed in this study might be a reflection of the high mutation rate in SSR loci, similar to what was reported by Belalia et al. (2018) in genetic diversity analysis of Algerian maize populations. Another study also reported that mutations introduced novel variation between Tuxpeño Sequía and Tuxpeño Crema, two different sub-populations derived from a Mexican landrace (Tuxpeño) which resulted in significant variation in maturity and other agronomic traits (Prasana, 2010). The average number of alleles and the number of SSRs used in this study were lower than those reported in previous studies on maize. For example, including

One hypothesis in this study was that there were multiple introductions of germplasm into Africa and that countries along the coast would possess higher genetic diversities in comparison to accessions from countries inland. The results seem to suggest that this is the case. However, Oppong et al. (2014) have also

reported that genotypes from Ashanti, which is centrally located and not along the coast was important in maize seed distribution in Ghana. Results from the present study indicate that haplotype diversity is extensive in South Africa compared to the rest of the countries likely because it had the highest number of accessions, some of which were multi coloured. It is also evident that there has been mixing of landraces due to multiple introductions in different areas because most haplotypes are common in more than one country. The likely explanation for this pattern of haplotype distribution is the mixing of seed as people move from one region to the other because seed is the common form of dispersal for many crops. Thus, the genetic structure within the landraces could have been maintained by a balance between dispersal (movement of people) and selection by farmers to suit local conditions.

5 CONCLUSION

This study has assessed landraces from a wider region in Africa (12 countries) than previously reported thus providing a broader picture of the genetic diversity available. Overall, the results indicate that microsatellite polymorphism is not related to geographic origin of the maize landrace accessions. The low level of chloroplast diversity could be a reflection of the small number of chloroplast markers that were assessed. However, the grouping pattern of accessions exhibited by PCA would be consistent with a hypothesis of successive introductions of landraces in Africa with South Africa as one of the likely entry points. The lack of samples from other coastal countries such as Mozambique limits pinpointing the exact number of entry points. The emergence of new pests and diseases as well as the adverse effects of climate change pose serious challenges to breeders, who would like to increase production by introducing resistance to multiple biotic and abiotic stresses. Knowledge about genetic diversity and relationships among diverse germplasm is thus useful for plant breeders because it supports decision making in selecting material for crossing as one way of adapting to ***climate-mediated crop failure***.

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REFERENCES

Aci, M., Lupini, A., Mauceri, A., Morsli, A., Khelifi, L. & Sunseri, F. (2018). Genetic variation and structure of maize populations from Saoura and Gourara oasis in Algerian Sahara. BMC Genetics 19:51

Belalia, N., Lupini, A., Djemel, A., Morsli, A., Mauceri, A., Lotti, C., Khelifi-Slaoui, M., Khelifi, L. & Sunseri, F. (2019). Analysis of Genetic Diversity and Population Structure in Saharan Maize (Zea Mays L.) Populations Using Phenotypic Traits and SSR Markers. Genetic Resources and Crop Evolution. https://doi.org/10.1007/s10722-018-0709-3

Charcosset, A., Mir, C., Warburton, M., George, L., Taba, S., Dreisgacker, S., Bedoya, C., Franco, J., Mahalakshmi, V., Menkir, A., Hearne, S., Dumet, D., Kolesnikova-Allen, M., Muthamia, Z., Zhang, S. H., Xie, C., Sutrisno, Yunus, M., Grudloyma, P., Chaba Jampatong, C., Xuan Hao, P. & Prasanna, B. M. (2006). A Worldwide View of Maize Diversity. CIMMYT.

Mantel, N. (1967). The Detection of Disease Clustering and a Generalized Regression Approach. Cancer Research **27:** 209-220.

McCann, J. (2001). Maize and Grace: History, Corn, and Africa's New Landscapes, 1500–1991. Society for Comparative study of Society and History. Boston University: 246-272.

Miracle, M. (1966). Maize in Tropical Africa. Madison: University of Wisconsin Press. 327pp

Nei, M. (1978). Estimation of Average Heterozygosity and Genetic Distance from a Small Number of Individuals. Genetics **89:** 583-590.

Nyaligwa, L., Hussein, S., Amelework, B. & Ghebrehiwot, H. (2015). Genetic Diversity Analysis of Elite Maize Inbred Lines of Diverse Sources Using SSR Markers. Maydica **60**:1-8.

Oppong, A., Bedoya, C.A., Ewool, M.B., Asante, M.D., Thompson, R.N., Adu-Dapaah, H., Lamptey, J.N.L., Ofori, K., Offei, S.K. & Warburton, M.L. (2014). Bulk Genetic Characterization of Ghanaian Maize Landraces Using Microsatellite Markers. Maydica **59:**1-8.

Prasanna, B. M. (2012). Diversity in Global Maize Germplasm: Characterization and Utilization. Journal of Biosci. **37**(5).

Pressoir, G. & Berthaud, J. (2004). Patterns of Population Structure in Maize Landraces from the Central Valleys of Oaxaca in Mexico. Heredity **92**: 88–94.

Provan, J., Lawrence, P., Young, G., Wright, F., Bird, R., Paglia, G. P., Cattonaro, F., Morgante, M. & Powell, W. (1999). Analysis Of The Genus z*ea* (Poaceae) Using Polymorphic Chloroplast Simple Sequence Repeats. Plant Sytematics and Evolution **218(3-4)**: 245-256.

Ranum, P., Pena-Rosas, J. & Garcia-Casal, M. (2014). Global maize production, utilization, and consumption. Annals of the New York Academy of Sciences. ISSN 0077-8923

Reif, J. C., Warburton, M. L., Xia, X. C., Hoisington, D. A., Crossa, J., Taba, S., Muminović, J., Bohn, M., Frisch, M. & Melchinger, A. E. (2006). Grouping Of Accessions of Mexican Races of Maize Revisited with SSR Markers. Theoretical and Applied Genetics **113**: 177-185.

Smale, M. (1995). Maize is Life: Malawi's Delayed Green Revolution. World Development **23**(5): 820.

Smale, M. & Jayne, J. T. (2004). Maize Breeding in Eastern and Southern Africa, 1960-2000. In Maize in Eastern and Southern Africa: "Seeds" of Success in Retrospect. NEPAD/IGAD Regional Conference on Agricultural Successes in the Greater Horn of Africa. Conference Paper No. **9**

Warburton, M. (2006). Tracing History's Maize. In Fredenburg, P. & Nelson, J. (Eds). Generation Challenge Programme Partner and Product Highlights 2006: 16-18. Generation Challenge Programme, CIMMYT, Mexico D.F.

Yusuf, Z, Petros, Y. & Ararsa, K. (2018). Genetic Diversity of Sorghum (Sorghum bicolor L. Moench) from East and West Hararghe Zones of Oromia Regional State, Ethiopia, Based on SSR Markers. World Research Journal of Agricultural Sciences **5(1)**: 132-140.