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Full Length Research Paper

Molecular analysis of quality protein (QPM) and normal maize varieties from the DR-Congo breeding program

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In recent years, the use of quality protein maize (QPM) with the objective of improving the nutritional value of maize grain protein has been the focus of several African breeding programs. Knowledge of genetic variation among QPM and normal maize varieties is important for an efficient selection and development of new varieties.The main objective of this study was to assess the level of genetic variation and relatedness among and within QPM and normal maize varieties selected in the DR-Congo breeding program using inter-simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) markers. For ISSR analysis, the mean level of polymorphism within each accession analyzed was 69%. Nei's gene diversity (h) was 0.26 on the average. The genetic distance among the accessions varied from 0.098 to 0.460. More than 80% of genetic distances were below 0.35. The mean level of polymorphic loci among accessions obtained with RAPD markers was higher (79%) compared to ISSR. The genetic distance values were also larger than ISSR data ranging from 0.16 to 0.61 with 50% of values smaller than 0.35. The mean level of polymorphisms within the accessions analyzed was 65%. The Nei's gene diversity (h) was 0.23. Overall, the genetic variation among and within QPM and normal varieties were high, but the genetic distance among them was small. QPM accessions clustered together, but the genetic closeness among QPM and normal maize varieties studied was not always related to their origin.

Key words: Quality protein maize, normal maize, genetic variation, genetic relatedness, inter-simple sequence repeat (ISSR), random amplified polymorphic DNA (RAPD), DR-Congo.

INTRODUCTION

Maize is the primarily food source for both humans and livestock in many regions around the globe. The outstanding diversity has enabled this crop to be adapted to vastly different growing regions and purposes. This has been the raw genetic material for its radical transformation into the world's highest yielding grain crop. Knowledge of patterns of diversity and genetic distance is of great importance in maize breeding to maintain diversity of breeding lines and to select appropriate parental materials for germplasm enhancement and variety development.

In recent years, the use of quality protein maize has been the focus of several African breeding programs (Krivanek et al., 2007; Sofi et al., 2009; Mbuya et al., 2010). Quality protein maize (QPM) varieties developed by the international maize and wheat improvement centre (CIMMYT) in the late 1990's produces 70 to 100% more lysine and tryptophan and yields more grain than the most modern varieties of tropical maize (Bjarnason and Vasal, 1992). Recent reviews have described the latest information on breeding methods of QPM in Africa, and revealed the lack of studies on genetic diversity of QPM varieties and their genetic relationships with normal maize varieties (Krivanek et al., 2007; Sofi et al., 2009). Senior et al. (1998) reported the utility of simple sequence repeats (SSR) for determining genetic similarities in maize. Li et al. (2006) analyzed genetic relationships among CIMMYT subtropical QPM and

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Chinese maize inbred lines based on SSRs. They concluded that Chinese normal maize lines and QPM lines were considered to have a narrower genetic diversity than the CIMMYT QPM lines.

In addition to SSRs, molecular markers such as Random Amplification of Polymorphic DNA (RAPD), Inter-Simple Sequence Repeats (ISSR), and Amplified Fragment Length Polymorphism (AFLP) have been successfully used to assess the genetic diversity in cultivars of many plant species (Pejic et al., 1998; Agarwal et al., 2008; Sharma et al., 2008). Each one of these marker systems offers a unique combination of advantages and disadvantages (Sharma et al., 2008). They differ in the type of sequence polymorphism detected (insertion/deletions vs. point mutation), the information content, the dominance relationships between alleles (dominant vs. co-dominant markers), the amount of DNA required, the need for DNA sequence information in the species under analysis, the development costs, the ease of use and the extent to which they can be automated.

The choice of marker systems is dictated by the specific application and there is probably not a single class of markers that can satisfy all the needs encountered by plant geneticists and breeders. ISSR and RAPD have been useful in genetic fingerprinting and genetic diversity analysis of many species of plants and animals. The former permits detection of polymorphism in inter-microsatellite loci, using a primer designed from dinucleotide or trinucleotide simple sequence repeats (Zietkiewicz et al., 1994; Semagn et al., 2006) and the latter detects nucleotide sequence polymorphisms using a single primer of arbitrary nucleotide sequence.

The main objective of this study was to assess the level of genetic variation and relatedness among and within quality protein and normal maize varieties selected in the DR-Congo breeding program using ISSR and RAPD markers.

MATERIALS AND METHODS

Genetic materials

Six QPM and seven normal maize varieties were used for this study. The QPM accessions included ECAQVE-3, ECAQVE-4, ECAQVE-6, QPM-SRSYNTH, Susuma, and QPM Longe-5. Salongo-2, Kasai-1, MUS-1, GPS-5 and Locale-1 from the DR-Congo and DMR-ESR-W along with AK9331-DMR-ESR-Y from IITA, Ibadan (Nigeria), were the normal varieties studied. The source and year of introduction of each variety to DR-Congo are described in Table 1.

DNA extraction and amplification

Total genomic DNA from seedling samples was extracted using the hexadecyltrimethylammonium bromide (CTAB) protocol described by Nkongolo (2003) and Mehes et al. (2007) with some modifications. The modification involved addition of polyvinyl-pyrrolidone (PVP) and β-mercaptoethanol to the CTAB extraction buffer. The purity was determined using a spectrophotometer (Varian Cary 100 UV-VIS spectrophotometer) and the concentration of each sample was determined using DNA quantification kit from Bio-Rad.

ISSR and RAPD analysis

Each DNA sample was individually primed with different oligonucleotide ISSR and RAPD primers described in Table 2. These primers were synthesized by applied Biosciences. The olymerase chain reaction (PCR) amplifications of RAPD and ISSR were carried out in accordance with the methods described by Vaillancourt et al. (2008) and Meheset et al. (2007), with some modifications. PCR amplification was performed in 2.1 µl of 10 x buffer (Applied Biosystems, Foster City, CA), 0.5 µl of 200 µM of each dNTP (dTTP, dATP, dCTP and dGTP) (Applied Biosystems, Foster City, CA), 2.5 µl of 2 mM $MgCl_2$, 0.5 µl of 0.5 µM primer, 20 ng of genomic DNA template and 4 µl of 0.625 units of *Taq* polymerase (Applied Biosystems, Foster City, CA) per 25 µl reaction. For each primer, a negative control reaction was included where ddH_2O was added instead of DNA. To each reaction tube, a drop of mineral oil was added to prevent evaporation. The amplification was carried out in a DNA thermal cycler (Perkin Elmer, Foster City, CA). The program was set to a hot start with an initial denaturation of 5 min at 95°C followed by 2 min at 85°C during which the *Taq* mix was added, and then 42 cycles of 1 min at 95°C, 2 min at 55°C and 1 min at 72°C, with a final extension of 7 min at 72°C and a subsequent cooling at 4°C. PCR products were analyzed on 2% agarose gels stained with ethidium bromide in 0.5 x TBE (Tris Borate EDTA) buffer and scored for band presence or absence.

PCR conditions were optimized to yield reproducible results and only fragments that amplified consistently and were reproducible in a minimum of two replicated reactions, were considered. ISSR and RAPD assays of each population were performed twice. Only reproducible amplified fragments were scored. The presence and absence of bands were scored as 1 or 0 respectively, in order to determine the variation within and among populations. The Quantity One software was used for the allele designation by comparing the alleles to the $1Kb^+$ ladder.

Data analysis

The resulting data matrix of the ISSR and RAPD phenotype was analyzed using POPGENE software (version 1.32) to estimate genetic diversity parameters (Yeh and Boyle, 1997). POPGENE is a computer software used for the analysis of genetic variation among and within populations using co-dominant and dominant markers and quantitative traits. The program was used to determine the intra and inter-population genetic diversity parameters such as percentage of polymorphic loci (P%), Nei's gene diversity (h), Shannon's information index (I), observed number of alleles (Na) and effective number of alleles (Ne). The genetic distances were calculated using Jaccard's similarity coefficient and estimated using the RAPDistance program version 1.04 (Armstrong et al., 1994) and Free Tree program (Pavlicek et al., 1999).

RESULTS

The DNA samples were amplified using several ISSR and RAPD primers. These primers were obtained from Applied Biosciences and they amplified reproducible bands. Figure 1 depicts the amplified products using a RAPD primer. The size and the intensity of the bands

Table 1. Origin, year of introduction and types of the 13 quality protein and normal maize varieties from the DR-Congo breeding program.

Variety	Origin/Provider	Year of introduction	Type	Category
ECAQVE-3	CIMMYT-Kenya	2008	QPM	OP*
ECAQVE-4	CIMMYT-Kenya	2008	QPM	OP
ECAQVE-6	CIMMYT-Kenya	2008	QPM	OP
DMR-ESR-W	IITA-Ibadan	1994	NORMAL	OP
AK9331-DMR-ESR-Y	IITA-Ibadan	1994	NORMAL	OP
QPM-SRSYNTH	CIMMYT-Kenya	2008	QPM	OP
SUSUMA	CIMMYT-Kenya	2008	QPM	OP
QPM-LONGE 5	NARI-Uganda	2008	QPM	OP
SALONGO-2	INERA-Gandajika	1976	NORMAL	OP
KASAI-1	INERA-Gandajika	1976	NORMAL	OP
MUS-1	INERA-Gandajika	1996	NORMAL	OP
GPS-5	INEAC-Gandajika	-	NORMAL	OP
LOCALE-1	Farmers-Gandajika	-	NORMAL	OP

*Open pollinated varieties; NARI, Namulonge Agriculture Research Institute; CIMMYT, International Maize and Wheat Improvement Centre; INERA, National Institute for Agronomic Studies and Research; INEAC, National Institute for Agronomic Studies in Belgium Congo; IITA, International Institute of Tropical Agriculture.

Table 2. The nucleotide sequences of ISSR and RAPD primers used to screen DNA samples of quality protein and normal maize varieties.

Primer identification	Nucleotide sequence (5'→3')	G + C content (%)
ISSR Primers		
Echt 5	AGAC AGAC GC	60.00
HB 13	GAG GAGGAG GC	72.70
HB 15	GTG GTGGTG GC	72.70
ISSR 1	AG AGAGAGAGAGAGAG RG	50.00
ISSR 5	ACG ACGACGACG AC	64.28
ISSR 9	GATC GATCGATC GC	57.14
UBC 825	AC ACACACACACACAC T	88.88
UBC 841	GA AG GA GAGAGAGAGA YC	45.00
17899A	CA CACACACACA AG	50.00
17898B	CA CACACACACA GT	50.00
RAPD Primers		
GRASSE 2	GTG GTC CGC A	70.00
GRASSE 6	CGT CGC CCA T	70.00
GRASSE 8	GGGTAACGCC	70.00
OPA 2	TGC CGA GCT G	70.00
OPA4	AAT CGG GCT G	60.00
OPA 8	GTG ACG TAG G	60.00
OPA 11	CAA TCG CCG T	60.00
P 23	CCC GCC TTC C	80.00
P 184	CAA ACG GCA C	60.00
UBC 186	GTG CGT CGC T	70.00
UBC 48	TTA ACG GGG A	50.00

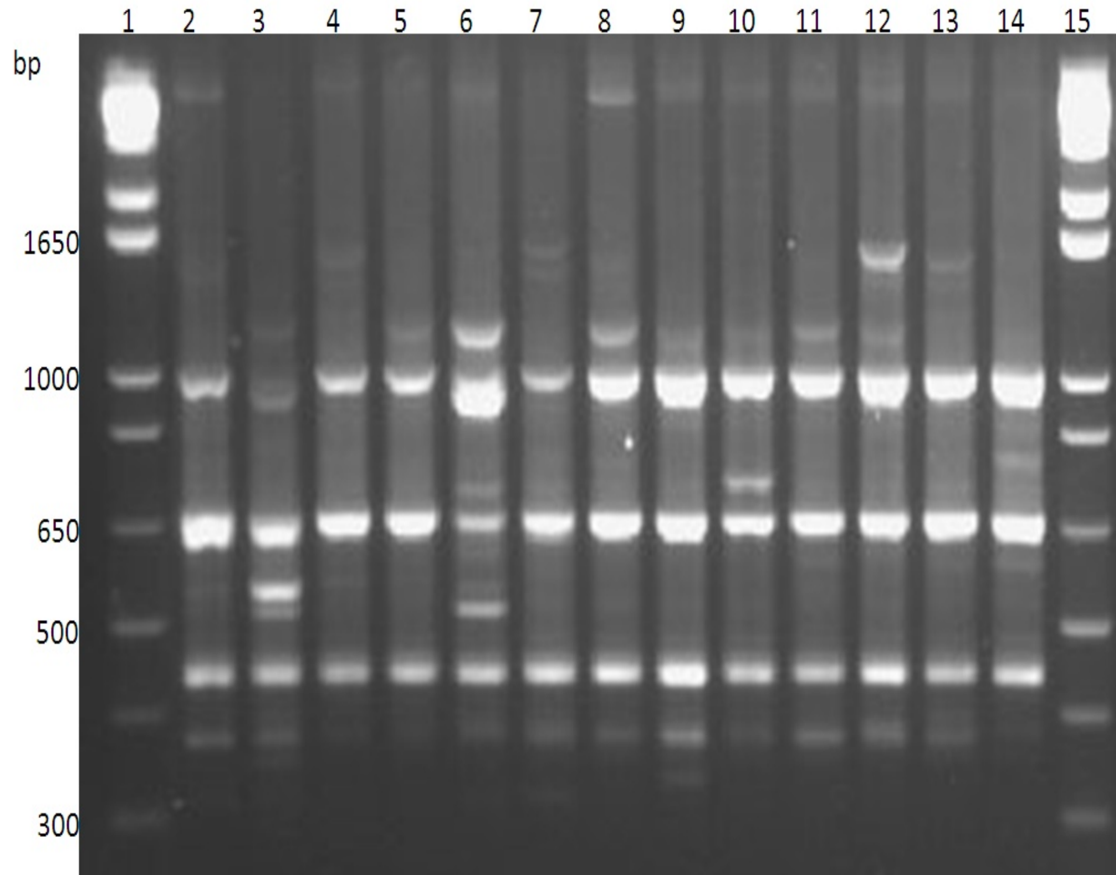

Figure 1. RAPD amplification of corn varieties with primer Grasse 8. Lanes 1 and 15 contain 1 kb+ ladder, lanes 2 to 14 contain corn varieties (lane 2: GPS5, lane 3: Salongo-2, lane 4: ECAQVE-6, lane 5: AK9133DMR-ESR-Y, lane 6: Mus-1, lane 7: Locale-1, lane 8: QPM Longe-5, lane 9: ECAQVE-3, lane 10: ECAQVE-4, lane 11: DMR-ESR-W, lane 12: Locale-2, lane 13: QPM-SRSYNTH, lane 14: Susuma).

were not associated with the marker systems used (ISSR and RAPD).

ISSR analysis

Overall, the level of polymorphism among accessions was 66%. The genetic relatedness among accessions was determined using Jaccard's similarity coefficients. The scale used for the genetic distance runs from 0 (meaning no genetic difference) to 1 (different for all conditions-criteria). The genetic distance among the 13 accessions from the DR-Congo breeding program varied from 0.098 to 0.460 (Table 3). In general, the genetic distances among accessions were small. More than 80% of genetic distances were below 0.35. QPMSRSYNTH and Susuma from CIMMYT – Kenya were the most genetically closely related accessions, while ECAQVE – 4 (QPM) and the normal maize ECAQVE-3 and Salongo-2 were the most distantly related. QPM-SRSYNTH (from CIMMYT-Kenya), and QPM Longe-5 (from Uganda) selected in DR-Congo in 2009 and 2010 based on high agronomic performance and nutritional qualities, were genetically closely related.

A dendrogram was constructed from the similarity coefficients (Figure 2). Three main clusters were identified with sub-groups. Some clusterings were consistent with the origin of the accessions while others were not. For example, accessions ECAQVE-3 and ECAQVE-4 from CIMMYT Kenya, clustered together, as well as QPM Susuma and QPM-SRSYNTH from the same origin (Kenya) (Figure 2). In general, none of the normal maize accessions formed a sub-group with any QPM. Normal maize varieties GPS-5, Locale-2, and Salongo-2 from INERA Gandajika (DR-Congo), belonged to the same sub-group and clustered together. In contrast, the normal varieties MUS-1 from INERA Gandajika (DR-Congo) and DMRESR-W from ITTA – Ibadan (Nigeria) clustered together. Similarly, the normal accession Locale-2 from farmers in Gandajika clusters with the normal accession AK – 913DMRESR-4 from ITTA – Ibadan (Nigeria) (Figure 2).

Three of the ISSR primers selected produced variety-diagnostic markers (Table 4). In fact, the amplification of

Table 3. Jaccard's similarity matrix of 13 corn varieties based on ISSR data.

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13
P1		0.460	0.262	0.295	0.302	0.290	0.267	0.288	0.288	0.295	0.339	0.306	0.270
P2			0.456	0.415	0.441	0.456	0.415	0.485	0.485	0.415	0.455	0.400	0.412
P3				0.222	0.231	0.299	0.222	0.242	0.242	0.222	0.266	0.206	0.200
P4					0.177	0.303	0.226	0.274	0.328	0.254	0.183	0.238	0.231
P5						0.309	0.234	0.281	0.308	0.177	0.277	0.246	0.185
P6							0.250	0.297	0.373	0.328	0.238	0.313	0.279
P7								0.217	0.217	0.167	0.183	0.210	0.203
P8									0.175	0.217	0.317	0.258	0.277
P9										0.274	0.344	0.200	0.277
P10											0.270	0.180	0.175
P11												0.281	0.246
P12													0.098
P13													

P1 = GPS-5; P2 = Salongo-2; P3 = DMR-ESR-W; P4 = AK9133-DMR-ESR-Y; P5 = MUS-1; P6 = Locale-1; P7 = QPM-LONGE-5; P8 = ECAQVE-3; P9 = ECAQVE -4; P10 = DMR –ESR-W; P11 = LOCALE -2; P12 = QPM- SRSYNTH; P13 = Susuma.

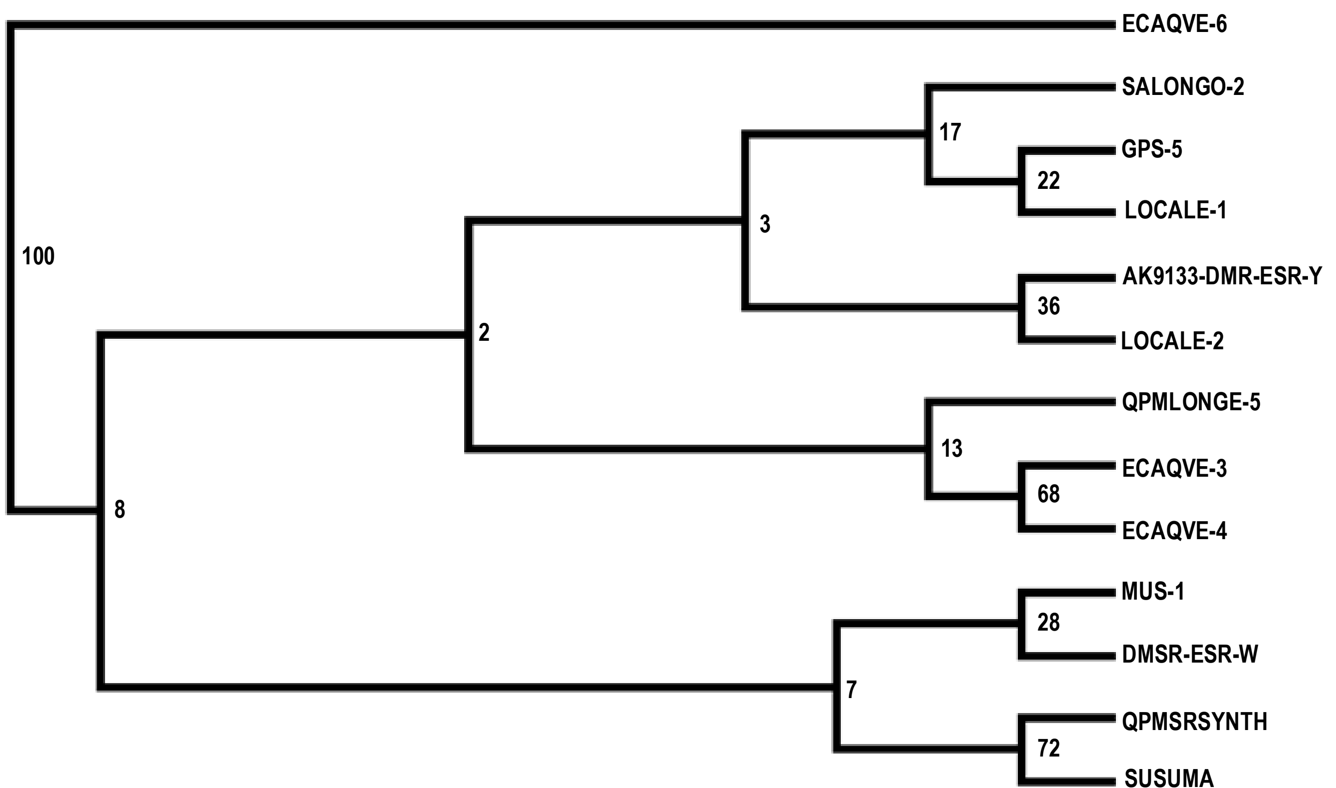

Figure 2. Dendrogram of the genetic relationships among quality protein and normal maize varieties using the data generated from the Jaccard's similarity matrix based on ISSR profiles. The values indicate the patristic distances based on the neighbor-joining (NJ) analysis.

DNA from all the corn varieties with the ISSR primer 9 produced one diagnostic marker at 475 bp for the QPM accession Susuma. Primer 17989B generated a diagnostic marker at 850 bp for ECAQVE-6. The ISSR primer HB 15 produced two diagnostic markers at 630 bp and 400 bp for the normal accession Salongo-2 (Table 4). The level of polymorphism within each of the five accessions analyzed in details was high (69% on average) varying from 62 in Salongo-2 to 77% in MUS-1 (Table 5). Nei's gene diversity (h) were not significantly

Table 4. Primers used and variety-diagnostic markers identified in quality protein and normal maize varieties from the DR-Congo breeding program.

Primer name	Variety-diagnostic marker	
	Size	Source variety
ISSR		
17989 B	850 bp	ECAQVE-6
HB15	630 bp	Salongo-2
	400 bp	Salongo-2
ISSR 9	475 bp	Susuma
RAPD		
Grasse 8	800 bp	Susuma
	340 bp	ECAQVE-3
	350 bp	Salongo -2
	500 bp	MUS-1
	700 bp	ECAQVE-4
UBC 186	390 bp	Salongo-2
	840 bp	Salongo-2
	430 bp	Locale-2

Table 5. Genetic diversity parameters of QPM and normal maize based on ISSR data.

Accession	Polymorphism	h	I	Na	Ne
Mus-1	76.92	0.2810	0.4157	1.7692	1.4893
Susuma	67.31	0.2568	0.3797	1.6731	1.4440
Locale 1	67.31	0.2361	0.3533	1.6731	1.4049
DMR- ESR-W	67.31	0.2571	0.3784	1.6731	1.4495
Salongo-2	61.54	0.2536	0.3666	1.6154	1.4602
QPM Longe-5	73.08	0.2754	0.4072	1.7308	1.4785

h = Nei's gene diversity; I = Shannon's information index; Na = observed number of alleles; Ne = effective number of alleles.

different and ranged from 0.24 (locale 1) to 0.28 (MUS-1) with a mean of 0.26. A similar pattern was observed for the Shannon's information index (I), with the highest value of 0.42 observed in MUS-1 and the lowest value of 0.35 observed in Locale-1. The observed number of alleles (Na) and the effective number of alleles (Ne) ranged from 1.62 to 1.77 and 1.40 to 1.49, respectively (Table 5).

RAPD analysis

The overall genetic variation among accessions generated with RAPD primers was significantly higher compared to ISSR data. In fact, the mean level of polymorphic loci was 79% based on RAPD primers used. The genetic relatedness among accessions was also determined using the Jaccard's similarity coefficients. The data revealed that the distance values were larger compared to ISSR ranging from 0.16 to 0.61 with 50% of genetic distance values over 0.35 (Table 6). QPM accessions ECAQVE-3 and ECAQVE-4 were the most genetically closely related, while the normal maize varieties GPS-5 and Salongo-2 from INERA Gandajika (DR-Congo) were the most distantly related (Table 6).

A dendrogram was constructed from the similarity coefficients (Figure 3). As with ISSR, three main clusters were identified. These were the QPM ECAQVE-3 and QPM ECAQVE-4 from CIMMYT Kenya, which formed a sub-group; the selected QPM accessions (QPM Longe-5 from Uganda and QPM-SRSYNTH from CIMMYT Kenya) based on agronomic trials and the normal maize accessions (Salongo-2, MUS-1, and GPS-5) from INERA Gandajika (DR-Congo). Surprisingly, the two normal

Table 6. Jaccard's similarity matrix of 13 corn varieties based on RAPD data.

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13
P1		0.606	0.343	0.385	0.313	0.333	0.388	0.317	0.359	0.369	0.451	0.429	0.385
P2			0.543	0.567	0.514	0.545	0.522	0.594	0.609	0.552	0.577	0.577	0.588
P3				0.286	0.366	0.313	0.343	0.373	0.412	0.323	0.338	0.313	0.388
P4					0.308	0.381	0.306	0.254	0.355	0.224	0.302	0.403	0.300
P5						0.429	0.408	0.414	0.472	0.343	0.425	0.425	0.451
P6							0.359	0.254	0.300	0.339	0.302	0.379	0.328
P7								0.262	0.333	0.290	0.308	0.281	0.306
P8									0.161	0.237	0.286	0.338	0.193
P9										0.283	0.328	0.328	0.300
P10											0.286	0.258	0.193
P11												0.303	0.354
P12													0.302
P13													

P1 = GPS-5; P2 = Salongo-2; P3 = DMR-ESR-W; P4 = AK9133-DMR-ESR-Y; P5 = MUS-1; P6 = Locale-1; P7 = QPM-LONGE-5; P8 = ECAQVE-3; P9 = ECAQVE -4; P10 = DMR–ESR-W; P11 = Locale-2; P12 = QPM- SRSYNTH; P13 = Susuma.

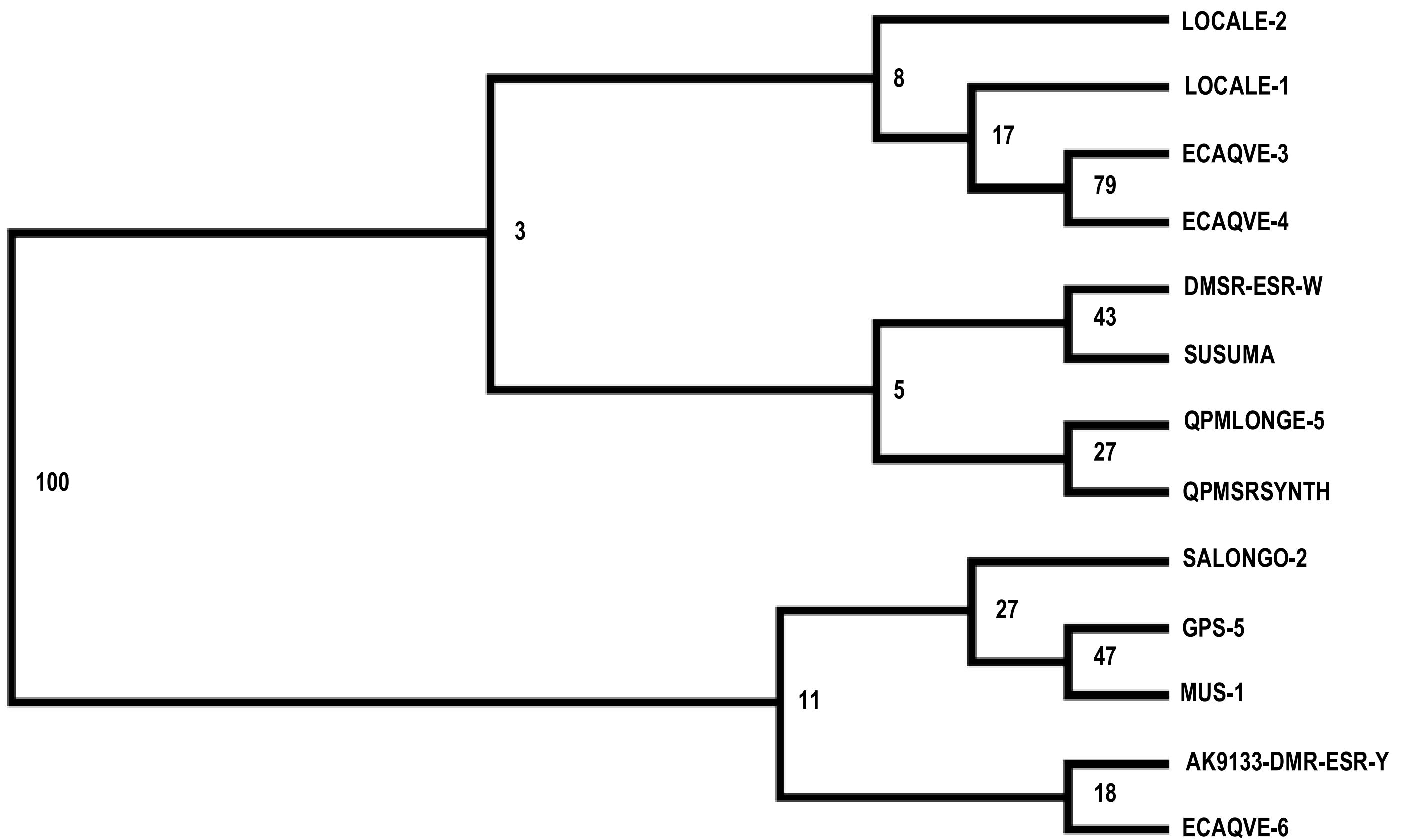

Figure 3. Dendrogram of the genetic relationships among the 13 quality protein and normal maize varieties using the data generated from the Jaccard's similarity matrix based on RAPD profiles. The values indicate the patristic distances based on the neighbor-joining (NJ) analysis.

accessions from IITA (DMR-ESR-W and AK9331-DMR-ESR-Y) did not cluster together, but each formed a sub-group with a QPM from CIMMYT, Kenya.

Diagnostic RAPD markers were identified (Table 4). They included a 800 bp diagnostic for QPM Susuma, a 340 bp diagnostic for ECAQVE-3, a 350 bp diagnostic for

Table 7. Genetic diversity parameters of QPM and normal maize accessions based on RAPD data.

Accession	Polymorphism	h	I	Na	Ne
Mus-1	70.21	0.2599	0.3834	1.7021	1.4533
Susuma	59.57	0.2180	0.3207	1.5957	1.3856
Locale 1	65.96	0.2318	0.3452	1.6596	1.4006
DMR- ESR- W	57.45	0.2289	0.3348	1.5745	1.4042
Salongo-2	72.34	0.2384	0.3600	1.7234	1.4014
QPM Longe-5	65.96	0.2228	0.3364	1.6596	1.3687

h = Nei's gene diversity; I = Shannon's information index; Na = observed number of alleles; Ne = effective number of alleles.

Salongo-2, a 500 bp diagnostic for MUS-1, and a 700 bp for ECAQVE-4; all generated with the primer Grasse-8. The RAPD primer UBC 186 produced a diagnostic marker for locale-2 at 430 bp, and two diagnostic bands for Salongo-2 at 390 bp and 840 bp, respectively (Table 4).

As with ISSR markers, the polymorphisms within the accessions analyzed were similar varying from 57 to 72%, with a mean of 65 % (Table 7). Nei's gene diversity (h) ranged from 0.22 (Susuma) to 0.26 (MUS-1), with a mean of 0.23. A similar pattern was observed for the Shannon's information index (I), with the low value of 0.32 observed in Susuma and a high value of 0.38 observed in MUS-1. The observed number of alleles (Na) and the effective number of alleles (Ne) ranged from 1.60 to 1.72 and 1.37 to 1.45 respectively (Table 7).

DISCUSSION

In general, the level of genetic variation among accessions was much higher with RAPD primers compared to the ISSR primers. There were no significant differences among the QPM and normal maize varieties analyzed for genetic variation within the accessions studied using ISSR and RAPD. The two marker system generated similar level of Nei's gene diversity index, Shannon's information index, observed and effective number of alleles. The high level of polymorphism (79%) among the accessions observed with RAPD marker is comparable to reports in the literature. Bruel et al. (2007) obtained 84.4% polymorphic loci in inbred maize lines and Lanza et al. (1997) reported 80.6% polymorphic loci using RAPD markers. For ISSR analysis, this study represents the first detailed report of application of ISSR markers in maize.

Genetic distance values were calculated according to the Jaccard's similarity coefficient. Both ISSR and RAPD analyses revealed great variation in regard to the genetic relatedness among the accessions analyzed. In general, the genetic distance values revealed that the different maize accessions were genetically closely related. The relative small genetic distance values are indicative of narrow genetic basis in African maize collections. The small genetic distances among accessions suggest the effect of bottlenecks or selection. In fact, Vigouroux et al. (2005) using SSR markers reported that the reduction in genetic diversity was explained largely by a bottleneck effect with a smaller effect from selection. Li et al. (2002, 2004, 2006) reported small genetic distances among Chinese inbred based on SSRs and AFLP markers. In this study, normal maize varieties from the DR-Congo clustered together, although they do not share any parent in their pedigree. These reduced genetic distance values among accessions from the same source can be ascribed in part to gene flow. In low-input farming systems such as DR-Congo, gene flow can be considered a function of pollen flow and seed exchange, which in different ways, are influenced by natural and human selection pressure (Nkongolo, 2003). Both seed exchange and pollen flow contribute significantly to the level of genetic make-up and variability among and within maize accessions grown within the region, and the country. Pollen flows among maize fields during seed multiplications are difficult to control when the seeds are grown outside the research station plots. In additions to seed exchange among relatives and friends, seed multiplications continue to increase seed contamination from other maize accessions during the seed handling process. This reduces over several generations the genetic distance among accessions within the same geographic areas. To address this problem, seed purification is conducted from time to time by breeders to restore to a certain degree, the identity of each accession.

In this study, RAPD markers detected more inter-accession polymorphisms compared to ISSR. Both markers target different regions within the genome and have different distribution. ISSR repeats are regions lying within the microsatellite repeats, have a high capacity to reveal polymorphism and offer great potential to determine intra and inter-specific variation compared to other arbitrary primers like RAPDs (Zietkiewicz et al., 1994; Agarwal et al., 2008). ISSR tends to be found in

the high copy repeat regions and detects polymorphism in inter-microsatellite loci using primer designed from di- or tri- nucleotide simple sequence repeats.

Conclusion

Diagnostic RAPD and ISSR markers are important steps toward an efficient molecular breeding in the DR-Congo maize program. These markers will be useful in tracking the DNA of targeted accessions in synthetic maize populations being developed. Additional diagnostic markers for QPM accessions being used in the DR-Congo breeding program need to be identified and characterized to make this molecular breeding more robust. Overall, the results indicate that incorporating genes from QPM lines into DR-Congo maize accessions would enhance the genetic basis of the latter in addition to boosting the nutritional value of the existing maize gene pool.

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