Original Paper Open Access

Estimation of genetic diversity of germplasm used to develop insect-pest resistant maize

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Abstract

Genetic diversity is important in ensuring viability of germplasm for a breeding program. The objective of this study was to determine the genetic diversity of 130 S_4 families from two populations; stemborers' resistant and storage pests' resistant populations, each with 65 lines; using 30 simple sequence repeats (SNP) molecular markers. The markers were found to be polymorphic with 0.46 - 0.48 polymorphism for both populations, except locus umc1367, which was monomorphic for storage pests' resistant population. A total of 109 alleles were recorded from stemborers' resistant population. Allele's scores ranged from 2 to 6 alleles per locus, with a mean of 3.6, and product length ranging from 47 bp to 362 bp. The storage pests' resistant population had a total of 103 alleles, with scores of 1 to 6 per locus, and a mean of 3.4 alleles; and product length of 47 - 320 bp. Observed gene diversity was 0.27, with expected gene diversity of 0.45 for stem borers' resistant population and 0.48 storage pests' resistant population. Mean polymorphism information content values ranged from 0.46 to 0.48, while unbiased expected heterozygosity ranged from 0.4 to 0.45 for both populations. Cluster analysis revealed three major clusters in each population; with cluster 1 comprising 34 - 40% of the genotypes in both populations. Cluster 2 had 55% for stem borers' resistant genotypes, and 53% of storage pests' resistant genotypes. The study indicates that there is ample genetic diversity in both populations which can be exploited in extracting new inbred lines for use in breeding insect resistant maize hybrids.

Keywords: genetic diversity, insect resistance, SSR markers, dissimilarity matrices

Introduction

In insect-pests resistance breeding, genetic diversity studies are important to start with an adequate genetic base. Host-plant resistance (HPR) is a quantitative trait, which is polygenic and controlled by multiple genes, and therefore, influenced by environmental conditions. Genetic diversity studies are an important tool that enables breeders to make good selection of parents to ensure genetic variability. Heterosis between genotypes is often enhanced when the two parents are genetically diverse (Makumbi et al, 2011). Again, conservation and estimation of alleles in the gene pool of the breeding program is important for future follow up (Prasanna, 2012). Other uses of genetic studies include enriching and enhancement of germplasm in breeding programs (Dagne, 2008). Genetic diversity studies are, therefore, a critical component for a given breeding program (Jarvis and Hodgkin, 2005; Dhliwayo and Pixley, 2003; Hari et al, 2004).

Diversity studies can be achieved at the phenotypic, biochemical or molecular levels (Beyene et al, 2014). The most reliable of these however, is the use of molecular markers (Cholastova et al, 2011). various types of molecular markers have been used for diversity studies including restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs), single nucleotide polymorphisms (SNP), and diversity array technology markers (DATm) (Yuan et al, 2000; Perumal et al, 2007; Bouchet et al, 2012).

Locally adapted maize (*Zea mays* L) varieties are known to have good genetic diversity, with alleles that are adapted to the constraints within a given region (Warburton et al, 2008). The Kenya Agricultural and Livestock Research Organization (KALRO) in collaboration with the International Maize and Wheat Improvement Centre (CIMMYT) have developed maize varieties which have insect pests' resistance traits (Mugo et al, 2001). Unfortunately, the resistance to field pests' trait is in separate germplasm from the resistance to storage pests. To compound the problem, most of the already developed insect-pests resistant maize hybrids are late maturing (Mugo et al, 2007). This makes them unsuitable for production in the dry mid-altitude ecologies of eastern Kenya which experience random drought and high insect pests' infestation rates in the field and in storage. Moreover, the

 RECEIVED 5/11/2016

warm temperatures and climatic conditions prevalent in these agro-ecologies are conducive for rapid pests' multiplication.

A breeding program was started in the year 2010 to develop early-maturing stem borer and storage pests' resistant maize inbred lines. This was done with the aim of developing early-maturing maize germplasm with combined resistance to *Chilo partellus* and *Sitophilus zeamais*, the two most important maize insect-pests in Africa. This required use of divergent germplasm in order to generate superior progeny from which pedigree breeding could be initiated. In order to avoid use of parentage stock with narrow genetic base, the potential parental germplasm was subjected to molecular analysis using microsatellite markers to determine the divergence of the germplasm. Use of Katumani composite as a parent for earliness is also important in exploiting the genetic base as a good source of desired alleles in breeding.

The objectives of this study were to determine; i) The genetic diversity between the stem borers' resistant S4 maize lines maize, ii) The genetic diversity of storage pests' resistant S_4 maize lines; and iii) Quantification of the genetic diversity through use of SSR markers.

Materials and Methods

Maize germplasm and sampling procedure

Seeds of 130 S_4 lines; 65 from stem-borer resistance (SBR) population, and 65 S_4 lines from a storage pests' resistant (SPR) population were selected at random. The populations were developed by crossing elite CIMMT inbred lines with insect-pests resistance to the target pests with a locally adapted maize variety, Katumani composite. The seeds were sent to Biosciences Eastern and Central Africa (BecA) laboratories in Nairobi, Kenya during the long rainy season (2012) for genotyping. They were planted in pots at a greenhouse at BecA, leaf tissue was harvested three weeks after germination and placed in tubes of a 96-well tube containing one stainless steel ball in each tube. The 96-well tube box was then placed in a bucket containing liquid nitrogen to chill the tubes. The chilled samples were placed in a genogrinder machine set at 500 strokes per minute and ground for two minutes.

DNA extraction and genotyping

The plates were weighed and placed in a centrifuge machine set at 3,500 rpm for three minutes. Using a multichannel pipette, 450 µl of 65°C pre-heated extraction buffer was added to each tube and caped. The samples were then placed in a water bath set at 65°C, and incubated for 40 minutes. Deoxyribonucleic acid (DNA) was extracted by solvent modi-

Table 1 - Microsatellite markers, their motif and coloading information.

Marker Entry	Name	Repeat Sequence	Repeat number	Bin Number	Coloading
1	nc130	AGC	Tri	5	6
2	nc133	GTGTC	Penta	2.05	19
3	Phi227562	ACC	Tri	1.11	
4	phi029	AG/AGCG	Compound	3.04	10
5	phi031	GTAC	Tetra	6.04	2
6	phi041	AGCC	Tetra	10	3
7	phi046	ACGC	Tetra	3.08	
8	phi056	CCG	Tri	1	3
9	phi062	ACG	Tri	10.04	23
10	phi065	CACTT	Penta	9.03	17
11	phi072	AAAC	Tetra	4	3
12	phi075	CT	Di	6	4
13	phi076	AGCGGG	Hexa	4.11	7
14	phi079	AGATG	Penta	4.05	1
15	phi084	GAA	Tri	10.05	6
16	phi102228	AAGC	Tetra	3.06	2
17	Phi112	AG	Di	7.01	1
18	Phi114	GCCT	Tetra	7.03	23
19	phi123	AAAG	Tetra	6.07	21
20	phi299852	AGC	Tri	6.07	23
21	Phi308707	AGC	Tri	1.1	1
22	Phi331888	AAG	Tri	5.04	4
23	Phi374118	ACC	Tri	3.02	5
24	Phi96100	ACCT	Tetra	2.01	5
25	umc1161	(GCTGGG)5	Hexa	8.06	22
26	umc1304	(TCGA)4	Tetra	8.02	10
27	umc1367	(CGA)6	Tri	10.03	5
28	umc1545	(AAGA)4	Tetra	7	18
29	umc1971		Tri		
30	umc2250	(ACG)4	Tri	2.04	7

Table 2 - Product length defences as observed from 30 SSR markers.

	Stem bore resistant			Storage pests resistant		
Locus	Min	Max	Difference	Min	Max	Difference
nc130	136	145	9	136	145	9
nc133	107	112	5	107	112	5
phi029	147	159	12	147	159	12
phi031	186	222	36	185	222	37
phi041	197	213	16	60	64	4
phi046	52	252	200	237	255	18
phi056	237	255	18	159	162	3
phi062	159	363	204	131	152	21
phi065	131	151	20	131	162	31
phi072	141	353	212	150	162	12
phi075	225	239	14	225	239	14
phi076	160	178	18	160	178	18
phi079	180	190	10	180	189	9
phi084	157	160	3	157	160	3
Phi112	136	152	16	136	160	24
Phi114	132	168	36	134	170	36
phi123	141	145	4	141	145	4
Phi96100	274	298	24	274	296	22
phi102228	121	129	8	121	129	8
Phi227562	305	308	3	305	320	15
phi299852	110	149	39	110	149	39
Phi308707	114	132	18	114	132	18
Phi331888	128	137	9	128	137	9
Phi374118	213	225	12	213	226	13
umc1161	131	155	24	130	153	23
umc1304	123	139	16	123	138	15
umc1367	150	159	9	159	159	0
umc1545	65	81	16	66	80	14
umc1971	131	143	12	131	143	12
umc2250	47	50	3	47	51	4

fied method as described by Dellaporta et al (1983) and Semagn et al (2012). A set of 30 microsatellites (SSR) markers, were used to genotype the samples. The PCR products were run and detected on capillary system ABI-3730 using the LIZ500 as internal size standard.

Data collection and analysis

The data was then captured and done using the Genscan® software (Applied Biosytems) with reference dyes used as; Ned(Y) Pet R) 6-FAM (B) and Vic (G). The resulting fragments were analyzed and the alleles scored using the Genemapper® software ver4.1 (Applied Biosystems), and then compiled into a spreadsheet as a standard Genemapper output file.

Genetic diversity analysis

The genotypic data was subjected to analysis using DarWin version 6.0.10, and GENALEX version 6.5 (Peakall and Smouse, 2007). Data analysis was done using the protocol of Nei and Li (1979) to determine differences in allelic frequencies among the SSR markers. Polymorphic information content (PIC), number of effective alleles per locus, total number of alleles per locus (Na), allelic richness observed heterozygosity (Ho), average gene diversity (He), and total gene diversity (Ht), was generated. This information was calculated using the following formulae:

PIC = 1- i =1 -n ($\sum fi^2$) – (fi^2)^2;

Na = n° of Different Alleles;

Ne = n° of Effective Alleles = 1 / (Sum pi^2);

I = Shannon's Information Index = -1 Sum (pi * Ln (pi));*

Ho = Observed Heterozygosity = No. of Hets / N;

He = Expected Heterozygosity = 1 - Sum pi^2;

Ht = Ho + He

*uHe = Unbiased Expected Heterozygosity = (2N / (2N-1)) * He;*

F = Fixation Index = (He - Ho) / He = 1 - (Ho / He);

where, pi is the frequency of the i^{th} allele for the population and Sum pi^2 is the sum of the squared population allele frequencies.

Background Information on 30 Microsatellite Markers

Microsatellite analysis revealed that 6.7% of the SSR motifs represented are dinucleotide, 40% are trinucleotide, and 53.3% are compound nucleotide (Table 1).

Cluster analysis

Cluster analysis was done for both stem borer and storage pests resistant populations with neighbor-joining algorithm. The unweighted pair group method of DARwin 6.0 software was used, with Bootsrap values set at 10,000 iterations. Genetic dissimilarity dendograms were generated for each population, and graphical representation of populations done (Perrier and Jacquemoud-Collet, 2006).

Table 3 - Genetic diversity information generated from analysis of 65 S_4 Stem borer resistant population using 30 microsatellite markers.

Locus	Na	Ne	I	Ht	Ho	He	uHe	F	PIC
nc130	4	1.54	0.73	0.51	0.16	0.35	0.35	0.55	0.35
nc133	2	1.35	0.43	0.48	0.22	0.26	0.26	0.17	0.26
phi029	4	2.37	1.00	0.94	0.37	0.58	0.58	0.37	0.58
phi031	5	2.81	1.21	1.45	0.81	0.64	0.65	-0.25	0.64
phi041	5	3.23	1.37	0.84	0.15	0.69	0.70	0.79	0.69
phi046	6	2.12	1.00	0.67	0.14	0.53	0.53	0.74	0.53
phi056	5	3.63	1.44	1.00	0.28	0.72	0.73	0.62	0.72
phi062	4	1.98	0.79	0.71	0.22	0.49	0.50	0.56	0.49
phi065	2	1.30	0.40	0.34	0.11	0.23	0.24	0.52	0.23
phi072	6	2.40	1.09	0.81	0.23	0.58	0.59	0.60	0.58
phi075	4	2.61	1.08	0.88	0.26	0.62	0.62	0.58	0.62
phi076	4	3.26	1.26	1.28	0.59	0.69	0.70	0.15	0.69
phi079	3	2.39	0.96	0.98	0.40	0.58	0.59	0.32	0.58
phi084	2	1.76	0.62	0.54	0.11	0.43	0.44	0.75	0.43
phi102228	2	1.10	0.19	0.15	0.06	0.09	0.09	0.30	0.09
Phi112	2	1.13	0.23	0.15	0.03	0.12	0.12	0.73	0.12
Phi114	4	2.65	1.16	1.00	0.38	0.62	0.63	0.40	0.62
phi123	2	1.43	0.48	0.45	0.15	0.30	0.30	0.49	0.30
Phi227562	2	1.45	0.49	0.34	0.03	0.31	0.31	0.90	0.31
phi299852	6	4.74	1.66	1.19	0.40	0.79	0.80	0.49	0.79
Phi308707	4	3.97	1.38	1.05	0.30	0.75	0.75	0.60	0.75
Phi331888	4	2.56	1.04	1.01	0.40	0.61	0.61	0.34	0.61
Phi374118	4	2.94	1.17	1.16	0.50	0.66	0.66	0.24	0.66
Phi96100	4	1.88	0.85	0.62	0.15	0.47	0.47	0.67	0.47
umc1161	4	2.40	1.08	0.75	0.17	0.58	0.59	0.71	0.58
umc1304	3	1.08	0.18	0.12	0.05	0.07	0.08	0.38	0.07
umc1367	3	1.06	0.15	0.12	0.06	0.06	0.06	-0.03	0.06
umc1545	4	3.12	1.23	1.01	0.33	0.68	0.68	0.52	0.68
umc1971	3	1.42	0.55	0.47	0.17	0.30	0.30	0.42	0.30
umc2250	2	2.00	0.69	1.48	0.98	0.50	0.50	-0.97	0.50
Mean	3.63	2.26	0.86	0.75	0.27	0.48	0.48	0.42	0.48
SE	0.23	0.17	0.08	0.07	0.04	0.04	0.04	0.07	0.04

‡Data: Na = total number of alleles per locus; Ne = number of effective alleles per locus; I = Shannon's Information Index; Ht = total gene diversity; Ho = observed gene diversity within genotypes; He = average gene diversity within genotypes; uHe = Unbiased Expected Heterozygosity; F_{IS} = inbreeding coefficient; PIC = polymorphic information content.

Results

Microsatellite markers characterization

A total of 3,859 data points were achieved out of the expected 3 Statistic summary of phenotypic diversity in 290 inbred lines for 7 traits scored over two years 900 data points giving an overall success rate of 99%, from the analyzed marker data (Table 2).

The shortest product size in both SBR and SPR population was observed in locus umc2250 with 47 base pairs (bp), while the longest for SBR was observed in locus phi062 with 363 bp, and, the longest for SPR was observed in locus Phi227562 with 320 bp. The highest difference in variation from the same locus was 212 bp, observed in phi072 (Table 2).

Allelic content of stem borer resistant population

Analysis using 30 SSR markers identified total of 109 alleles from SBR population. The number of polymorphic alleles scored ranged from 2 to 6 per loci, with a mean of 3.6 alleles. Out of the 30 markers used, eight markers, (nc133, phi065, phi084, phi102228, Phi112, phi123, Phi227562, and umc2250), amplified two alleles each; four markers (phi079, umc1304, umc1367, and umc1971), amplified 3 alleles each; 12 markers (nc130, phi029, phi062, phi075, phi076, Phi114, Phi308707, Phi331888, Phi374118, Phi96100, umc1161, and umc1545) amplified 4 alleles each, and three markers each (phi031, phi041, phi056, and phi046, phi072, phi299852) amplified 5 and 6 alleles, respectively. Conversely, 43% of the loci have Ne value less than 2, 36-37% have Ne values of 2 - 3, and 20% have Ne value greater than 3. The PIC values for SBR population ranged from 0.06 observed in locus umc1367 to 0.79, observed in phi299852, with mean of 0.48. Observed uHe indicated gene diversity of 0.06 (umc1367) -0.80 (phi299852) in SBR population, and a mean of 0.48 (Table 3).

Fifty seven (57%) of the markers had a PIC value greater than 0.5, and Ht values ranged from 0.12 observed in locus umc1304 and umc1367, to 1.48 observed in locus umc2250, with a mean of 0.75 (Table 3). The locus differentiation, FIS, for SBR population was extreme from -0.97 (umc2250) to 0.9 (Phi227562), having a mean of 0.42 (Table 3).

Cluster analysis of stem borer resistant population

Table 4 - Genetic diversity information generated from analysis of 65 S_4 storage pests resistant population using 30 microsatellite markers.

Locus	Na	Ne	I	Ht	Ho	He	uHe	Fis	PIC
nc130	4	2.07	0.91	1.02	0.50	0.52	0.52	0.03	0.52
nc133	3	1.73	0.76	0.70	0.28	0.42	0.42	0.34	0.42
phi029	4	2.22	0.90	0.81	0.26	0.55	0.55	0.52	0.55
phi031	6	5.34	1.73	1.78	0.97	0.81	0.82	-0.19	0.81
phi041	4	3.20	1.26	0.81	0.13	0.69	0.69	0.82	0.69
phi046	2	1.69	0.60	0.61	0.20	0.41	0.41	0.51	0.41
phi056	5	3.00	1.28	0.96	0.30	0.67	0.67	0.55	0.67
phi062	2	1.20	0.31	0.23	0.06	0.17	0.17	0.63	0.17
phi065	3	1.27	0.43	0.38	0.17	0.21	0.21	0.19	0.21
phi072	3	1.64	0.63	0.59	0.20	0.39	0.39	0.48	0.39
phi075	4	2.70	1.12	0.83	0.20	0.63	0.63	0.68	0.63
phi076	4	2.69	1.10	1.14	0.51	0.63	0.63	0.19	0.63
phi079	3	2.52	1.01	0.87	0.27	0.60	0.61	0.56	0.85
phi084	2	2.00	0.69	0.70	0.20	0.50	0.50	0.60	0.50
phi112	3	1.37	0.54	0.36	0.09	0.27	0.27	0.66	0.27
phi114	5	2.46	1.03	0.93	0.34	0.59	0.60	0.43	0.59
phi123	2	1.47	0.50	0.50	0.18	0.32	0.32	0.42	0.32
phi96100	4	3.25	1.27	1.06	0.37	0.69	0.70	0.47	0.69
phi102228	2	1.10	0.19	0.15	0.06	0.09	0.09	0.30	0.09
phi227562	4	1.75	0.80	0.53	0.10	0.43	0.43	0.78	0.43
phi299852	6	4.64	1.64	1.22	0.43	0.78	0.79	0.45	0.78
phi308707	3	2.15	0.91	0.75	0.22	0.53	0.54	0.60	0.53
phi331888	3	2.18	0.85	0.74	0.20	0.54	0.55	0.63	0.54
phi374118	3	1.97	0.72	0.80	0.31	0.49	0.50	0.37	0.49
umc1161	5	2.17	1.07	0.71	0.17	0.54	0.54	0.69	0.54
umc1304	3	1.08	0.18	0.12	0.05	0.07	0.08	0.38	0.07
umc1367	1	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
umc1545	4	1.56	0.68	0.57	0.22	0.36	0.36	0.40	0.36
umc1971	3	1.08	0.18	0.12	0.05	0.08	0.08	0.38	0.08
umc2250	3	2.03	0.73	1.48	0.97	0.51	0.51	-0.91	0.51
Mean	3.43	2.15	0.80	0.72	0.27	0.45	0.45	0.41	0.46
SE	0.22	0.18	0.08	0.08	0.04	0.04	0.04	0.06	0.04

‡Data: Na = total number of alleles per locus; Ne = number of effective alleles per locus; I = Shannon's Information Index; Ht = total gene diversity; Ho = observed gene diversity within genotypes; He = average gene diversity within genotypes; uHe = Unbiased Expected Heterozygosity; F_{IS} = inbreeding coefficient; PIC = polymorphic information content.

Three major cluster groups (C1, C2, and C3) were observed in the SBR population from cluster analysis of the SBR population using SSR markers. Cluster C1, comprised 40% of the genotypes, and was further classified into sub-clusters. The second cluster, C2, was by far the largest comprising of 55.4% of genotypes. It had two major sub-clusters (SC1 and SC2). The third grouping, C3, was the smallest of the clusters with comprising three genotypes (6, 16, and 64), and constituting only 4.6% of genotypes in SBR population. The pairwise dissimilarity values for SBR generated ranged from a minimum of 0.18 to a maximum of 0.71, with a mean value of 0.45.

Allelic content of storage pests' resistant population

Similar analysis with the same 30 SSR markers on SPR population identified a total of 103 alleles. The allele's scores ranged from 1 to 6 alleles per loci, with a mean of 3.4 alleles (Table 4). Only one marker (umc1367) was monomorphic out of the 30 SSR markers used. Five markers, (phi046, phi062, phi084, phi123, phi102228), amplified two alleles each; 11 markers (nc133, phi065, phi072, phi079, phi112, phi308707, phi331888, phi374118, umc1304, umc1971, umc2250), amplified 3 alleles each; eight markers (nc130, phi029, phi041, phi075, phi076, phi96100, phi227562, umc1545) amplified 4 alleles each, and, three markers (phi056, phi114, umc1161), amplified 5 alleles each, and two markers (phi031, phi299852) amplified 6 alleles (Table 4).

The Ne values for the SPR population ranged between 1.0 and 5.3, with 47% of loci having Ne values less than 2, 43% of loci had between 2 - 3, and, 13% had Ne values greater than 3. Apart from umc1367, which had a PIC value of 0, the PIC values for SPR population ranged from 0.07 (umc1304) to 0.85 (phi079), with a mean of 0.46. Fifty three (53%) of the loci had PIC values greater of 0.5 or above (Table 4).

Similar to SBR population, the locus differentiation, FIS, for SPR population was also extreme ranging from -0.91 (umc2250) to 0.82 (Phi041) and having a mean of 0.41. Observed uHe indicated gene diversity of 0.08-0.82 in SPR population, with a mean of 0.45 (Table 4).

Cluster analysis of storage pests' resistant population

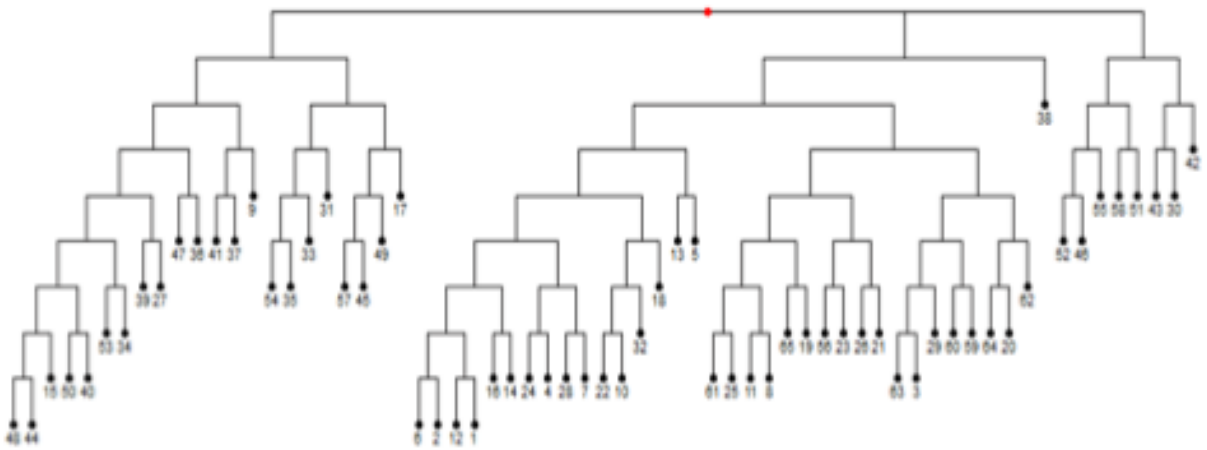

Figure 2 - Dissimilarity matrixes dendogram of SPR population using Neighbour Joining algorithm, indicating genetic relationships of genotypes.

The SPR population revealed three major cluster groupings (C1, C2, and C3) after analysis using SSR markers. Cluster C1, comprised 33.8% of the genotypes, and was further classified into two major sub-clusters (SC1 and SC2). The second cluster (C2) comprised of 53.8% of the genotypes, and was further sub-divided into two major clusters, one of which is composed of only one genotype, entry 38. The other sub-cluster had two major sub-sub-clusters with different number of genotypes in each.

The third grouping (C3) was the smallest of the clusters comprising eight genotypes, constituting only 12.3% of the genotypes in SPR population (Figure 2). The pairwise dissimilarity values for SPR generated ranged from a minimum of 0.17 to a maximum of 0.64, with a mean value of 0.4.

Discussion

Information obtained about the SSR markers used in the current study indicates that these markers were polymorphic. It is only one of the markers that was monomorphic for the SPR population. High success rate was achieved when these markers were used and therefore, it can be deduced that they had the ability to differenciate genotypes within the populations. Legesse et al (2007) and Wende et al (2013) reported that dinucleotide SSR loci amplified the largest number of alleles as well as high PIC values. However, there was no observed correlation between marker nucleotide repeats and number of alleles amplified in the current study.

The avearge alleles obtained from the current study of 3.4 for SPR and 3.6 for SBR populations, are comparable to those reported by (Choukan et al, 2006). However, Xia et al (2004) reported that the total number of alleles in diversity studies is proportional to the sample size. Given that the sample size for the current study was 65 genotypes per population, this may be linked to the number of alleles observed which is 109 alleles for SBR and 103 alleles for SPR. There were also differences in allele number and effective alleles which can be attributed to the variation of major allele frequencies in the genotypes (Beyene et al, 2014). These results of 3.4 -3.6 alleles per locus are higher than those reported by Kostova et al (2006), who reported much lower mean value of 1.9 alleles per locus; but below the mean allele value of 9.6 in 143 maize genotypes reported by Zhi-zhai et al (2010) in China.

The variation of fragment sizes within locus in the current study may be attributed to a phenomenon called slip-strand mispairing which occurs during DNA replication and can lead to great variation in allele size, as observed in loci phi062 with 362 bp and Phi227562 with 320 bp (Levinson and Gutman, 1987; Beyene et al, 2014). The same phenomenon can be due to potential mutations occuring on the binding site of primers leading to low primer binding (Dillon et al, 2005).

The levels of diversity obtained using these 30 SSR microsatellite markers in the two populations was 0.45 – 0.48. These are slightly lower than diversity values reported from previous studies (Legesse et al, 2007) with average diversity of 0.59-0.65. Other authors have reported both lower values of diversity of 0.2 (Akinwale et al, 2014; Yu et al, 2007) as well as high values by 0.69-0.82 (Van Inghlelandt et al, 2010; Liu et al, 2003) possibly due to the source of the maize germplasm or the pedigrees.

The discriminatory power of loci was further estimated using PIC values considering the number of alleles and their relative frequencies (Smith et al, 2000). In the current study, PIC values were high, with 57% of loci having PIC values greater than 0.5, and two of the loci (phi308707 and phi299852), with values above 0.75 in SBR population, and two (phi031 and phi079) with values greater than 0.8. This is an indication that these markers were able to effectively discriminate among all test genotypes and the results

demonstrate their informative nature when detecting differences among genotypes. These findings are consistent with studies done by Elçi and Hançer (2014) which reported high PIC values of 0.69. Conversely, 43% of the loci in the current study yielded PIC values of less than 0.5 for the two populations, which is higher than PIC value of 0.33 reported by Legesse et al (2007). It is noteworthy to mention that even moderate PIC values of 0.26 can be useful for classification of lines (Dao et al, 2014). It is therefore, important to note that these findings agree with studies by Smith et al (2000) and Geleta et al (2006), who reported that even moderate PIC values can be useful for classification of lines.

The presence of high values of uHe confirms that these lines were not yet pure inbred lines because the lines were at S_4 and still in the process of being selfed after selection based on the breeding goals. The results further indicate that there are high levels of polymorphism in the test populations, supporting other studies on utility of SSR markers in maize (Smith et al, 1997; Senior et al, 1998). Genetic distances revealed the relatedness of the S_4 lines used for this study, with clarity of markers being able to distinguish closely related lines with minimum genetic distances (Smith et al, 1997).

Cluster analysis of the two populations, SBR and SPR, showed a good fit to the data with the dendrograms showing clear distinction of the different clusters. The three major clusters observed in the two populations could be an indication of pedigree relatedness of the S_4 lines. This agrees with previous finding (Reif et al, 2006), when he used SSR markers for heterotic groupings of maize. Similar findings were reported by (Senior et al, 1998), when investigating the genetic similarity and relatedness in maize. Further, the clustering could be due to insect resistance levels in the test genotypes. This can be confirmed through other studies incorporating phenotypic data after screening the genotypes for resistance to *C. partellus* or *S. zeamais* insects. It is also important to note that clustering could be due to maturity grouping of the S_4 lines, since most of lines used were of early to medium maturity regime. This data was however not reported in the current study.

Of great interest was entry 38 genotype, which fell in a sub-cluster of its own. This could be due to either genetic mix-up or incomplete pedigree records. Such observations have been reported by other researchers as occurrences due to effects of selection, some mutations, or genetic drift, as well as human error (Warburton et al, 2008). Hartl and Clark (1997) argued that the differentiation of genotypes provides reason for breeders to select their preferred germplasm and fix desired alleles in each population.

Conclusions

Overall, this study has shown that there is genetic variability in the genotypes, and that the chosen SSR markers differentiated the S_4 lines, indicating that there were robust for the current diversity studies. Further, the genetic variability could be exploited for further breeding, and act as a valuable source of alleles especially in insect resistance studies.

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