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Full Length Research Paper

A comparative study of molecular and morphological methods of describing genetic relationships in traditional Ethiopian highland maize

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The comparison of different methods of estimating the genetic diversity could define their usefulness in plant breeding and conservation programs. In this study, a total of 15 morphological traits, eight AFLP-primer combinations and 20 simple sequence repeat (SSR) loci were used (i) to study the morphological and genetic diversity among 62 selected highland maize accessions, (ii) to assess the level of correlation between phenotypic and genetic distances, and (iii) to classify the accessions into groups based on molecular profiles and morphological traits. The analysis of variance of the morphological data revealed significant differences among accessions for all measured traits. The mean morphological dissimilarity (0.3 with a range of 0.1-0.68) was low in comparison to dissimilarity calculated using SSR markers (0.49 with a range 0.27-0.63) and AFLP markers (0.57 with a range 0.32-0.69). The correlation between the morphological dissimilarity matrix and the matrices of genetic dissimilarity based on SSR and AFLP markers was 0.43 and 0.39, respectively (p = 0.001). The correlation between SSRs and AFLPs dissimilarity matrices was 0.67 (p = 0.001). This congruence indicates that both marker systems are equally suited for genetic diversity study of maize accessions. Cluster analysis of morphological and marker distances revealed three groups of maize accessions with distinctive genetic profiles and morphological traits. This information will be useful for collections, conservation and various breeding programs in the highlands of Ethiopia.

Key words: AFLP, correlation, phenotypic diversity, SSR.

INTRODUCTION

Knowledge of genetic variation and relationships between accessions or genotypes is important: (i) to understand the genetic variability available and its potential use in breeding programs, (ii) to estimate any possible loss of genetic diversity, (iii) to offer evidence of the evolutionary forces shaping the genotypic diversities, and (iv) to choose genotypes to be given priority for conservation (Thormann et al., 1994). Characterization of genetic resource collections has been greatly facilitated by the availability of a number of molecular marker systems. Morphological traits were among the earliest markers used in germplasm management, but they have a number of limitations, including low polymorphism, low heritability, late expression, and vulnerability to environmental influences (Smith and Smith, 1992).

On the other hand, DNA markers do not have such limitations. They can be used to detect variation at the DNA level and have proven to be effective tools for distinguishing between closely related genotypes. Different types of molecular markers have been used to assess the genetic diversity in crop species, but no single technique is universally ideal. Therefore, the choice of the technique depends on the objective of the study, financial constraints, skills and facilities available. Amplified fragment length polymorphisms (AFLPs; Vos et al., 1995) and microsatellites, or simple sequence repeats (SSRs;

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Tautz, 1989) are the most frequently used molecular markers in the analysis of genetic resources, because they can be automated and so have great potential in large-scale genetic diversity studies. The chromosomal locations of SSR markers are frequently known, thus providing additional information in genetic diversity studies and on the other hand, AFLPs has a high multiplex ratio, offering a distinctive advantage when genome coverage is a major issue (Pejic et al., 1998).

Powell et al. (1996) examined the utility of RFLP, RAPD, AFLP and SSR markers for soybean germplasm analysis by evaluating information content (expected heterozygosity), number of loci simultaneously analyzed per experiment (multiplex ratio) and effectiveness in assessing relationships between accessions. In this study SSR markers had the highest expected heterozygosity, while AFLP markers had the highest effective multiplex ratio.

The Highland Maize Germplasm Collection Mission was launched throughout the highlands of Ethiopia in 1998 in collaboration with CIMMYT (Twumasi-Afriyie et al., 2001). As part of this project, 287 maize accessions were collected from farmers' fields throughout the highland regions of Ethiopia. A recent field study of 180 of 287 maize accessions revealed that these accessions are highly variable for morphological and agronomic characteristics (Beyene et al., 2005) and thereby generated baseline data for future breeding and molecular studies. However, morphological variation does not always reflect real genetic variation because of genotype X environment interaction and the largely unknown genetic control of polygenic morphological and agronomic traits (Smith and Smith, 1992).

The objectives of this study were thus (i) to investigate genetic diversity and relationships among 62 selected highland maize accessions using morphological, AFLP and SSR markers, (ii) to assess the correlation between distance estimates based on morphological traits and molecular markers, and (iii) to classify the accessions into groups based on a combination of molecular profiles and morphological traits.

MATERIALS AND METHODS

Field evaluation and DNA extraction

A total of 62 maize accessions collected from the Northern, Southern and Western highlands of Ethiopia were used in this study (Table 1). The accessions were grown at Alemaya University in Ethiopia during the 2002 main cropping season in a randomized complete block design with three replications. Each accession was grown in two row plots. Each row had 25 plants, which constitute 44444 plants per hectare recommended for the testing site. From each accession, 20 plants were selected at random to record 15 morphological traits.

All 62 accessions were fingerprinted with AFLP and SSR markers. All plants used in this molecular analysis were generated from seed and grown in the greenhouse. As this study did not aim to estimate the degree of heterozygosity and heterogeneity within the accessions, 15-plant bulks were analyzed in order to represent the genotypic variability present within each maize accession. DNA was extracted using the QIAGEN DNeasy plant Mini Kit, (QIAGEN, GmbH, Hilden) and homogenization was performed using the FP-120 FastPrep instrument (QBiogene, Carlsbad, CA, USA; Myburg et al., 2001). DNA quantity and quality was determined on 0.8% (w/v) agarose gel electrophoresis using known quantities of lambda DNA as a concentration standard.

AFLP analysis

AFLP template preparation was performed using AFLP template preparation kits from LI-COR Biosciences (LI-COR, Lincoln, NE, USA) according to the manufacturers' instructions. Polymerase chain reactions (PCRs) were performed using a BIO-RAD iCycler (Version 3.021, BIO-RAD Laboratories, inc). The preselective amplification cycle profile was as follows: incubation for 10 s at 72°C, followed by 30 cycles of denaturation for 10 s at 94°C, annealing for 30 s at 56°C, and extension for 1 min at 72°C with a 1 s per cycle increasing extension time. Selective amplification was performed on 1:20 diluted (in SABAX water) preselective amplification products with the following cycling profile: 13 cycles of 2 min at 94°C, 30 s at 65°C (reduced by 0.7°C per cycle), and 1 min at 72°C; followed by 20 cycles of 10 s at 94°C, 30 s at 56°C, and 1 min (extended 1 s per cycle) at 72°C. The selective amplification primer pairs all had three-nucleotide extensions at the 3' end. In all reactions only the *Eco*RI primers were 5'labelled with infrared dyes (IRDye 700 or IRDye 800, LI-COR). Initially, eight accessions (which were expected to represent a high level of genetic diversity due to difference in collection sites and morphological traits) were chosen to test the amplification successes of different primer combinations. Eight primer combinations (Table 4) with the highest polymorphism rates and large numbers of clearly scorable fragments were selected to analyze the full set of 62 accessions.

An equal volume of loading solution (LI-COR) was added to each selective amplification reaction. Samples were denatured at 95°C for 3 min and placed on ice for 10 min before loading. A volume of 0.8 µl was loaded with an 8-channel syringe (Hamilton, Reno, Nevada) onto 25-cm 8% Long Ranger gels (BMA, Rockland, ME, USA). Electrophoresis and detection of AFLP fragments were performed on LI-COR IR^2 (model 4200S) automated DNA analyzers. The electrophoresis parameters were set to 1500V, 40 mA, 40 W, 50°C, and a scan speed of 3. The run-time was set to 4 h and gel images were saved as TIF files for further analysis. The gel images were scored using a binary scoring system that recorded the presence and absence of bands as 1 and 0, respectively. Semi-automated scoring was performed with $SAGA^{MX}$ (Version 3.2, LI-COR) and followed by manual editing to make adjustments to the automated score where necessary. A locus was scored as polymorphic when the frequency of the most common allele (band present or absent) was less than 0.97 (absent or present in at least two individuals). Bands with the same mobility were considered as identical products (Waugh et al., 1997), receiving equal values regardless of their fluorescence intensity.

SSR analysis

A total of 105 SSR primers were selected from previous studies (Senior et al., 1998; Matsuoka et al., 2002; Warburton et al., 2002) and from the public Maize DB (http://www.agron.missouri.edu/ssr_probes/ssr.htm) based on their high polymorphism information content and chromosome locations (at least 10 SSRs per chromosome, data not shown). The 105 SSRs were assayed in eight diverse highland maize accessions, which were expected to represent a high level of genetic diversity due to difference in

Table 1. Traditional Ethiopian highland maize accessions used in the study.

No	Accession	Collection site	Major agroecology	Altitude[a]
1	Ad-1-01	Gonder	North	2360
2	Ad-1-03	Armachew	North	2771
3	Ad-1-9-6	Adi Arkay	North	1837
4	Ad-1-9-8	Adi Arkay	North	1741
5	Ad-1-1-16	Armachew	North	2527
6	Ad-1-1-17	Armachew	North	1850
7	Ad-1-2-20	Armachew	North	1765
8	Ad-1-3-21	Armachew	North	2354
9	Ad-1-4-26	Dembia	North	2133
10	Ad-1-3-32	Dembia	North	2100
11	Ad-1-3-35	Chilga	North	1900
12	Ad-3-6-40	Gondar	North	2105
13	Ad-3-6-42	Fogera	North	1930
14	Ad-3-7-45	Farta	North	2400
15	Ad-3-7-46	Farta	North	2674
16	Ad-3-7-50	Este	North	2728
17	Ad-4-11-55	Sera	North	2544
18	Ad-5-13-59	Yilmana	North	2266
19	Ad-5-13-60	Yilmana	North	2300
20	Ad-5-13-61	Yilmana	North	2432
21	Ad-5-14-64	HuletEynes	North	1980
22	Ad-5-16-67	HuletEynes	North	2512
23	Ad-5-17-69	GoneraSiso	North	2654
24	Ad-5-17-68	GoneraSiso	North	2651
25	Ad-5-17-70	GoneraSiso	North	2668
26	Ad-5-18-71	Debrework	North	2598
27	Ad-5-18-72	Enemay	North	2474
28	Ad-5-19-76	Awabel	North	2554
29	Ad-5-21-79	Gozamin	North	2529
30	Ad-4-24-81	Gozamin	North	2383
31	Ad-6-28-89	Quarit	North	2000
32	Ad-6-28-92	Sekela	North	2500
33	Ad-6-28-94	Awi	North	1580
34	Ad-6-26-96	Awi	North	1714
35	Ad-1-31-101	Banja Awi	North	2200
36	Aw-03	Merka	South	1950
37	Aw-10	Agere Mariam	South	2180
38	Aw-13	Kofele	South	2500
39	Aw-17	Hitosa	South	2230
40	Aw-18	Boloso	South	1950
41	Aw-21	Arero	South	2160
42	Aw-25	Agere Mariam	South	2290
43	Aw-29	Agere Mariam	South	2200
44	Aw-33	Tiyo	South	2300
45	Aw-35	Tiyo	South	2515
46	Aw-41	Agere Mariam	South	2200
47	Aw-44	Ejere	South	2300
48	Aw-54	Merka	South	2145
49	Baw-01	Wolemra	West	2260
50	Baw-10	Wolemra	West	1800
51	Baw-11	Dendi	West	2290
52	Baw-12	Ambo	West	2270
53	Baw-13	Weliso	West	2300
54	Baw-14	Jeldu	West	2010
55	Baw-15	Becho	West	2225
56	Baw-17	Sululta	West	2350
57	Baw-18	Sululta	West	2350
58	Baw-20	Ambo	West	2280
59	Baw-22	Dega	West	2250
60	Baw-28	Bedele	West	1880
61	Baw-30	Ambo	West	2305
62	Baw-33	Limu	West	2080

[a] Meter above sea levels

represent a high level of genetic diversity due to difference in collection sites and morphological traits. A final set of 20 SSR primers (Table 4), which gave consistent and easily scorable bands across the eight accessions were chosen for further analyses.

Polymerase chain reactions (PCRs) were performed in 15 µl reaction mixes consisting of 50 ng template DNA, 0.4 mM dNTPs, 0.4 µM SSR primers (forward and reverse), 0.1 mM $MgCl_2$, 0.5 U Taq polymerase (Roche) and 1X reaction buffer (10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$). PCR reactions were performed in a BIO-RAD iCycler (Version 3.021, BIO-RAD Laboratories, Inc.) with the following touch-down PCR program: an initial denaturation at 94°C for 2 min, followed by 10 cycles of 30 s at 94°C, 45 s at 65°C (reduced by 1°C per cycle), and 1:30 min at 72°C; followed by 28 cycles of 30 s at 94°C, 30 s at 55°C, and 1:30 min at 72°C. A final extension step of 72°C for 15 min was performed. The SSR amplification products were resolved on 3% agarose gels (1:1 mixes of Molecular Screening, MS-8, agarose, LSS-Gibco and molecular grade agarose, Gibco-BRL, a cheaper alternative with similar resolution; Pinto et al., 2003) in 0.5X TBE buffer. Gels were run in a large format (23 x 40 cm) horizontal gel system (Model A3-1, Owl Separation Systems, Portsmouth, NH, USA) at 150 V for 3.5 h and were photographed under UV light (Geldoc, BIO-RAD Laboratories, Inc) after ethidium bromide staining.

Statistical analysis

Analysis of variance was performed for all measured traits in order to test the significance of variation among accessions. The standardized traits mean values (mean of each trait was subtracted from the data values and the result divided by the standard deviation) were used to perform principal component and cluster analyses using NCSS 2000 software (Jerry, 2000). To group the accessions based on morphological dissimilarity, cluster analysis was conducted on the Euclidean distance matrix with the

Table 2. Summary statistics of the agro-morphological traits measured in 62 traditional Ethiopian highland maize accessions.

Traits	Mean	St. dev.	Minimum	Maximum
Days to tasseling	65.1	3.2	51.5	76.0
Days to silking	71.5	3.0	58.0	80.5
Plant height (cm)	217.8	14.4	161.0	288.0
Ear height (cm)	125.9	26.3	74.0	227.5
Leaf length (cm)	71.3	9.1	51.8	100.8
Leaf width (cm)	9.1	1.0	6.4	12.7
Numbers of leaf	6.1	0.3	5.2	6.6
Foliage rating	6.2	0.9	4	7.0
Days to maturity	143.8	7.8	108	167.5
Ear diameter (cm)	3.9	0.2	3.3	4.6
Ear length (cm)	18.1	2.2	14.5	22.7
Rows per ear	10.7	1.5	7	13.9
Kernels per row	27.42	3.6	18	36.9
1000 seed weight (g)	295.8	41.3	229	410.0
Yield (kg ha $^{-1}$)	2645.4	195.4	1305.2	42.82.3

Table 3. Eigenvectors, eigenvalues, individual and cumulative percentage of variation explained by the first four principal components (PC) after assessing morphological traits in 62 traditional Ethiopian highland maize accessions.

Traits	PC1	PC2	PC 3	PC 4
Days to tasseling	-0.32*	-0.20	0.03	-0.23
Days to silking	-0.32	-0.19	0.00	-0.21
Plant height (cm)	-0.37	-0.09	-0.06	0.08
Ear height (cm)	-0.36	-0.09	-0.08	0.12
Leaf length (cm)	-0.34	-0.06	-0.01	-0.13
Leaf width (cm)	-0.24	0.10	-0.13	-0.40
Number of leaves	-0.06	0.03	0.59	-0.31
Foliage rating	-0.15	0.49	0.17	-0.22
Days to maturity	-0.15	0.02	0.29	0.67
Ear diameter (cm)	-0.21	0.39	-0.37	0.11
Ear length (cm)	-0.22	0.39	-0.34	0.07
Rows per ear	-0.33	-0.10	0.14	0.14
Kernels per row	-0.14	0.35	0.21	0.23
1000 seed weight (g)	-0.28	-0.14	0.27	0.13
Yield (kg ha -1)	0.09	0.44	0.36	-0.12
Eigenvalue	6.31	1.88	1.55	1.00
Individual percent	42.05	12.57	10.51	6.67
Accumulated variation %	42.05	54.61	65.12	71.79

*Traits that are corresponding to underlined numbers are the most significant traits that contribute much of the variation in each PC

unweighted pair group method based on arithmetic averages (UPGMA).

For molecular diversity analysis, matrices of binary data were constructed with rows equal to accessions, and columns equal to distinct molecular marker fragments (bands in the case of AFLP and alleles for SSR). For the 62 maize accessions, the body matrix contained zeros and ones, corresponding to the absence or presence of marker band/alleles, respectively. Dissimilarity matrices were constructed from the binary data with Nei and Li (1979) similarity coefficients. From these matrices of dissimilarity coefficients, we calculated the mean genetic distances, standard deviations and distribution of dissimilarity values. Finally, to determine the efficiency of each marker type in detecting polymorphisms, the assay efficiency index, AEI (Pejic et al., 1998; AEI = BP/T, where BP is the total number of polymorphic fragments detected and T is the total number of marker assays performed), and the proportion of polymorphic fragments (total number of polymorphic fragments detected/total number of fragments detected) were calculated. The average Polymorphism Information Content (PIC) for AFLP markers was calculated according to Riek et al. (2001), while for SSR markers it was calculated according to Powell et al. (1996).

The relationships between the Euclidean distance matrix based on morphology and the Nei and Li distance matrices obtained with AFLP and SSR markers were analyzed using the approach developed by Mantel (1967). All the data analyses were performed using the software package NCSS-2000 (Jerry, 2000) and Statistical for Windows (1995).

RESULTS

Morphological variability

The analysis of variance revealed highly significant differences among accessions for all of the traits suggesting that there was a high degree of phenotypic diversity among the accessions (Table 2). Grain yield, plant and ear height and days to maturity showed wide variation, while number of leaves, leaf width and ear diameter showed a narrower range of phenotypic variation (Table 2).

The first four principal components (PCs), which had eigenvalue higher than one, explained a total of 71.8% of the phenotypic variation (Table 3). In the first PC, which explained 42.1% of the total variation, the most important traits were plant and ear height, leaf length and days to tasseling and silking. Number of rows per ear also appeared to be important in the first PC. In the second PC, which explained 12.6% of the total variation, predominant traits were ear traits (yield, ear length, ear diameter and kernels/row) and foliage rating. The third principal component, which accounted for 10.5% of the total variation, was dominated by traits such as number of leaves, ear diameter, yield and ear length, while days to maturity, leaf width and number of leaves were important delineating traits associated with the fourth principal component, which accounted for 6.7% of the total variation.

Variation in molecular markers

The 62 traditional Ethiopian highland maize accessions were fingerprinted with AFLP and SSR markers. The levels of polymorphism detected with both marker systems and polymorphism information content are reported in Table 4. The total number of bands was 650 based on eight AFLP primer combinations, and 98 alleles were detected for 20 SSR loci. All 20 SSR loci and 89.5% of AFLP bands were polymorphic (Table 4). The average number of scored bands was 81.3 for AFLP primer combinations and ranged from 69 (E-AAC/M-CCG) to 109 (E-ACA/M-CGA). The mean number of alleles per SSR locus was 4.9, ranging from 3-10 (Table 4). The PIC values for primer enzyme combinations of AFLP ranged from 0.279 to 0.370, with an overall mean of 0.325. For SSR analysis this value ranged from 0.06 (*umc1357*) to 0.76 (*nc003*) with a mean of 0.61. The assay efficiency index of AFLPs was far superior to that of SSRs (AEI = 72.6 *vs*. 4.9), but the proportion of polymorphic fragments was higher for SSRs (Table 4).

Distribution of dissimilarity coefficients

A histogram of pairwise dissimilarity for the 62 traditional Ethiopian highland maize accessions generated from markers and morphological data is presented in Figure 1 and a comparison of dissimilarity coefficients (range, mean and standard deviation) is presented in Table 5. The dissimilarity coefficients based on morphology ranged from 0.1 to 0.68 with an average of 0.3. Based on SSR, these values ranged from 0.27 to 0.63 with an overall mean of 0.49. For AFLP, it ranged from 0.32 to 0.69 with an overall mean of 0.57. More than 71% of AFLP- based pairwise comparisons exhibited genetic dissimilarity higher than 0.5.

Correlations between dissimilarity matrices

In order to compare the extent of agreement between dendrograms derived from morphology, SSRs and AFLPs, a distance matrix was constructed for each assay and compared using the Mantel matrix correspondence test (Table 6). A highly significant positive correlation was found between the two molecular data sets ($r = 0.67$; $p = 0.001$). The AFLP data was significantly correlated with the morphological data ($r = 0.39$, $p = 0.001$), and the SSR data was also correlated with morphological data ($r = 0.43$; $p = 0.001$). The significant correlations indicate that these three independent sets of data likely reflect the same pattern of genetic diversity and validate the use of these data to calculate the different diversity statistics for Ethiopian highland maize accessions**.**

A dendrogram generated from the standardized morphological data is presented Figure 2. The UPGMA cluster analysis revealed four clusters at the mean genetic dissimilarity of 0.3. The first cluster contained 36 accessions, with most collected from the Northern agroecology. Short plants and early maturity characterized accessions in this group. The second cluster contained 12 accessions, of which 11 were collected from the Western and one from the Southern agroecology. Accessions in this group had tall plants and ear heights. This group also had the maximum yield per ha. The third cluster contained only two accessions with dissimilarity values of 0.4. The fourth cluster contained 11 accessions collected from all three agroecologies, and there was one outlier (AD-1-9-8) that did not fall into any cluster (Figure 2).

The dendrogram generated based on a combined SSR and AFLP data set showed three major clusters (Figure 3). Cluster I consisted of 20 accessions, all collected from the Northern agroecology. Accessions in this cluster had short plant height (average 178.5 cm) and matured earlier (average 123 days) than any of the other clusters. Cluster II consisted of 19 accessions collected from three agroecologies. Accessions in this cluster were tall plants (on average 220 cm) and they needed more than 150 days to reach maturity. The group also had the highest mean values for ear traits (18.2 cm in ear length, 30 kernels per row, 11 rows per ear and 3884 kg ha $^{-1}$ in grain yield). Cluster III contained 23 accessions,

Table 4. The 20 SSR loci (bin number, motif, number of alleles and PIC) and 8 AFLP primers pairs (total and polymorpic bands, % of ploymorphic band and PIC) used to the study the 62 traditional Ethiopian highland maize accessions.

	General information for SSR				General information for AFLP				
Microsatellite	**Bin number**	**Motif**	**Number of alleles**	**PIC**	**Primer combination**	**Total number of bands**	**Number of polymorphic bands**	**% Of polymorphic band**	**PIC**
Bnlg1276	1.03		7	0.72	E-AGG/ M-CAG	73	64	87.7	0.370
Phi037	1.08	AG	3	0.66	E-ACG/ M-CCG	72	66	91.6	0.320
Nc003	2.06	AG	8	0.76	E-ACA/ M-CGA	109	98	89.9	0.279
Umc2129	2.07	(CGC)5	5	0.59	E-ACA/ M-CCC	86	76	88.0	0.321
Phi453121	3	ACC	6	0.71	E-AAC/ M-CAC	72	68	94.5	0.359
Umc2152	3.09	(TG)8	3	0.66	E-ACG/ M-CGG	74	68	91.8	0.321
Phi021	4.03	AG	5	0.66	E-AAC/ M-CCG	69	56	81	0.327
Phi026	4.05	CT	6	0.75	E-AAC/ M-CGG	95	86	90.5	0.300
Phi079	4.05	AGATG	3	0.59					
Umc1537	5.07	(TCG)4	3	0.60					
Umc1153	5.09	(TCA)4	5	0.60					
Umc2040	6.05	(CGC)4	6	0.74					
Umc1632	7.01	(AGC)5	3	0.56					
Phi034	7.02	CCT	4	0.75					
Umc2190	7.06	(CCT)4	3	0.33					
Phi015	8.08	AAAC	5	0.68					
Phi042	9.04	CATA	6	0.59					
Umc1357	9.05	(CTG)8	3	0.06					
Phi054	10.03	AG	4	0.66					
Bnlg2190	10.06	AG(31)	10	0.59					
Total			**98**	**na**		**650**	**582**	**na**	**na**
Mean			**4.9**	**0.61**		**81.25**	**72.8**	**89.5**	**0.325**
AEI	**72.6**					**4.9**			

characterized by tall and late maturing plants that had broad and long leaves. This cluster also had the lowest mean values for all of the ear traits.

DISCUSSION

In this study, we used AFLP and SSR markers and morphological traits to characterize a set of 62 traditional Ethiopian highland maize accessions collected throughout the highlands of Ethiopia. There was high and significant correlation between the SSR and AFLP data. This congruence indicates that the two techniques are equally suited for the analysis of genetic diversity in maize. This study allowed us to distinguish three groups of maize accessions with distinctive genetic profiles and morphological traits, which will be useful for breeding, collection and conservation strategies in the highlands of Ethiopia.

The 62 accessions represent genetic diversity in a much larger set of 287 accessions collected from different highland regions of Ethiopia. The broad range in the means of accessions for the various traits implies great potential for the development of improved open-pollinated varieties, inbred lines and hybrids for these regions. The existence of broad morphological and agronomical diversity among the highland maize accessions is further substantiated by principal component analysis (Table 3), which indicated that the total variation was fairly distributed across all of the

Table 5. Mean, standard deviation and range of Nei and Li dissimilarity coefficients (calculated using AFLP and SSR markers) and Euclidean distance (calculated using morphological traits). The total sample of all accessions in this study is shown followed by accessions collected from the three agroecologies.

Parameters	Accessions	Morphological	AFLPs	SSRs
Mean	Entire collection	0.30	0.57	0.49
	Northern	0.28	0.58	0.51
	Southern	0.23	0.54	0.43
	Western	0.29	0.57	0.46
Standard deviation	Entire collection	0.09	0.05	0.05
	Northern	0.08	0.06	0.06
	Southern	0.09	0.04	0.05
	Western	0.10	0.04	0.05
Range	Entire collection	0.1-0.68	0.32-0.69	0.27-0.63
	Northern	0.12-0.57	0.32-0.66	0.27-0.65
	Southern	0.12-0.54	0.44-0.64	0.34-0.51
	Western	0.10-0.37	0.47-0.65	0.32-0.59

Table 6. Correlation between dissimilarity matrices obtained with different marker types.

	Morphology	SSR	AFLP
Morphology		0.43	0.39
SSR			0.67

Mantel test, $p = 0.001$

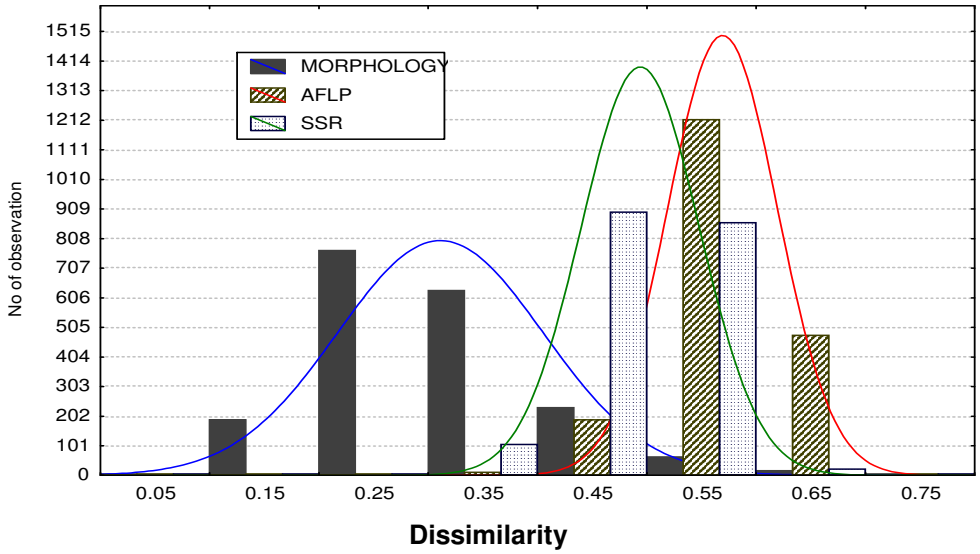

Figure 1. Frequency distribution of genetic dissimilarity among pairwise combinations of 62 traditional Ethiopian highland maize accessions based on morphology, AFLP and SSR data.

morphological and agronomical traits.

In this study, SSR marker polymorphism was screened on agarose gel system, which is less costly and more widely available (Senior and Heun, 1993; Senior et al., 1998). In our study, higher genetic diversity values were obtained for AFLP than for SSR (Table 4). The reason might be the difference in marker screening systems (agarose gels for SSR and polyacrylamide gels for AFLP)

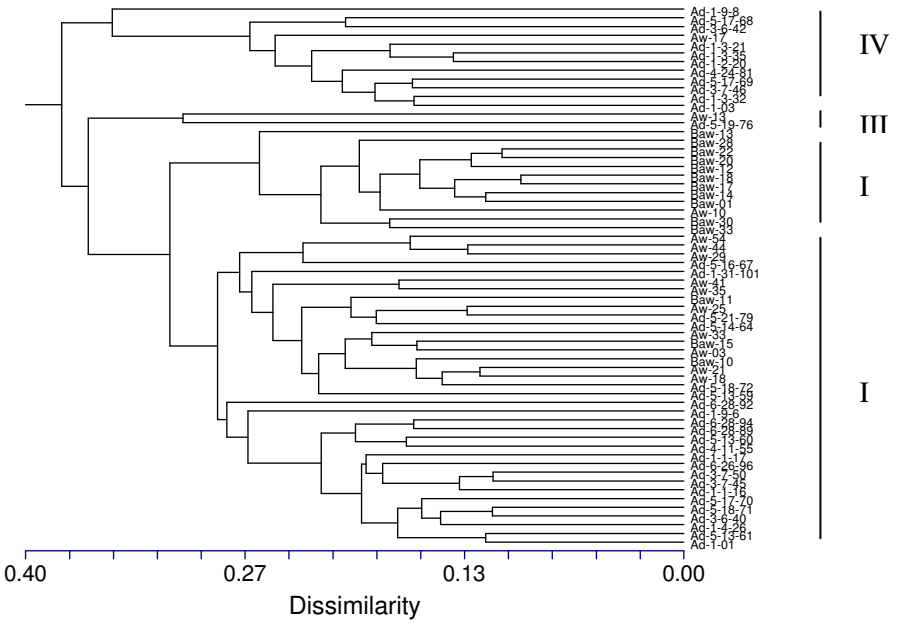

Figure 2. Dendrogram of traditional Ethiopian highland maize accessions derived by UPGMA from the dissimilarity matrix of the morphological data.

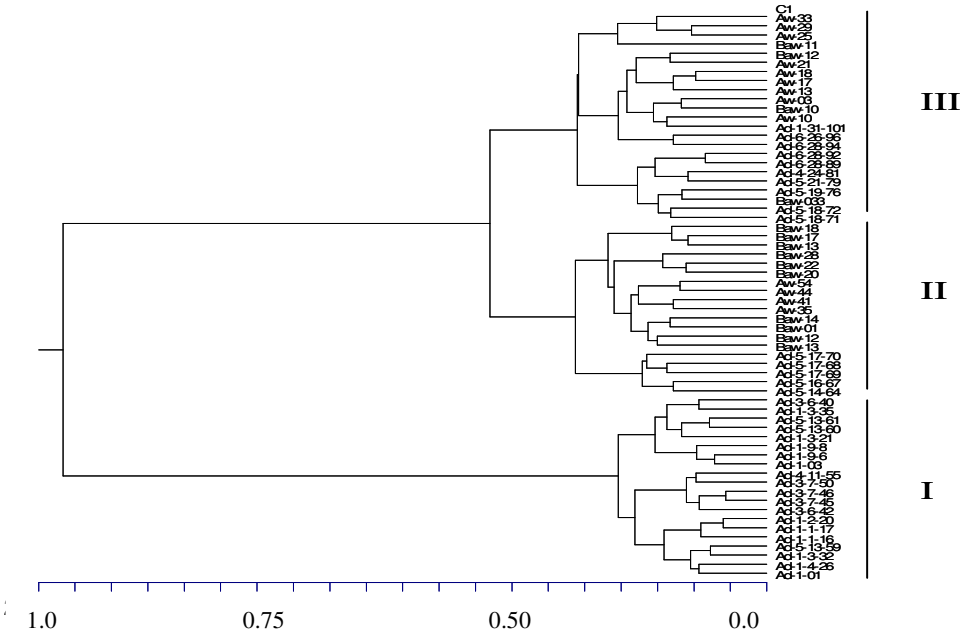

Figure 3. Dendrogram of the 62 traditional Ethiopian highland maize accession based on the Ward minimum variance method applied to the dissimilarity matrix generated by Nei and Li dissimilarity coefficients of the pooled AFLP and SSR data.

and data collection procedures (automated for AFLP, manual scoring of alleles for SSR). Acrylamide gels have greater resolving power than agarose gels. This is especially true for dinucleotide repeats whose amplification products are difficult to resolve on agarose gels and inaccuracies in scoring due to the production of

stutter bands. Unlike the present study, lower mean value of genetic dissimilarity for AFLP than SSR were reported by Pejic et al. (1998) for maize and by Uptmoor et al. (2003) for sorghum genotyped by automatic DNA sequencers in polyacrylamide gels. As in other studies, AFLP analysis in Ethiopian traditional maize accessions detected many polymorphic bands and is an efficient method for diversity study. With a single combination of selective primers, the average number of bands detected was 81.3 per accession, of which 89.5% were polymorphic. As expected, the assay efficiency index of AFLPs was far superior to that of SSRs (72.6 *vs*. 4.9, Table 4). However, the proportion of polymorphic fragments was 10% higher for SSRs (Table 4). The high AEI of the AFLP markers is due to the large number of loci detected per AFLP primer combination. The low AEI of SSR can, however, be increased by using multiplexing techniques, whether in PCR reaction or at the time of loading (Mitchell et al., 1997). In addition, more than 2000 SSRs have already been mapped onto maize chromosomes so that the genome can be uniformly sampled, which increases the precision of genetic diversity estimates and is useful for locating quantitative trait loci (QTLs).

The distribution of values for morphological dissimilarity and genetic dissimilarity (calculated with SSRs and AFLPs) differed substantially (Table 5). The morphological dissimilarity covered a greater range, but was significantly skewed towards small values (Figure 1). Comparing the two marker types, although there was little difference in the range, SSRs had the lowest dissimilarity values while the AFLPs data were skewed towards higher values (71% of the values were higher than 0.5). This data suggest that SSR marker can better differentiate pairs of accessions than AFLPs that show a low level of genetic variation between them. The subsets of the sample show that accessions collected from the Northern agroecology were on average more dissimilar than accessions collected from the Western and Southern agroecologies as measured by SSRs and AFLPs (Table 5). This partly reflects the frequent introduction of high yielding and uniform varieties in the surrounding intermediate altitudes of the Western and Southern regions might have replaced some of the traditional varieties.

To provide an objective comparison, we examined correlations between distance matrices calculated on the basis of AFLP, SSR and morphological data using a Mantel test (Table 6). As shown in Table 6, the correlation between SSR and morphological data was higher than between AFLP and morphological data. The results suggest that SSR markers may be a better choice for marker-traits association genetic studies of open-pollinated maize accessions than AFLP. Working with 16 ryegrass varieties Roldan-Ruiz et al. (2001) reported a correlation value of $r = -0.06$ between AFLP and 15 morphological characters. In comparison with ryegrass, traditional Ethiopian highland maize accessions appear to be environmentally more stable, as suggested by the higher agreement between phenotypic and molecular distances, and indicates that the observed phenotypic variation was at least partly caused by genetic factors. The correlation between the two molecular markers was higher than the morphology (Table 6). Therefore, when compared with DNA fingerprinting techniques, morphological traits are relatively less reliable and efficient for precise discrimination of closely related accessions and analysis of their genetic relationships. Despite this limitation, morphological traits are useful for preliminary evaluation because it is fast, simple, and can be used as a general approach for assessing genetic diversity among morphologically distinguishable accessions.

This study allowed us to distinguish three groups of maize accessions, with distinctive genetic profiles and morphological traits. The first group constitutes the early maturing, short-statured accessions (Figure 3, cluster I), which were collected from the Northern agroecology from which they probably acquired earliness. The second group includes the tall, high yielding varieties (Figure 3, cluster II), which are currently the most important landraces grown in the Southern and Western parts of Ethiopia. The third group includes tall, late maturing and low yielding accessions (Figure 3, cluster III), which are being cultivated in some parts of the Northern, Western and Southern highlands of Ethiopia. Therefore, accessions from the Northern agroecology may be used as base materials for the development of improved varieties for the drier parts in the highlands of Ethiopia, because these accessions are able to grow and produce under very harsh environmental conditions (drought, poor soils, excessive radiation, etc), and have adaptation traits (e.g. short flowering, short ear and plant height narrow leaf), while accessions from the Western and Southern agroecologies can be used for the development of high yielding varieties suitable for high potential maize growing regions of Ethiopia.

From a conservation perspective, sampling many accessions from all agroecologies would be an effective way of capturing genetic variation for future collections. Moreover, seeds should be collected from the Western and Southern agroecologies before the existing diversity is lost as result of the introduction of high yielding and uniform varieties in the neighboring areas.

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