CHAPTER 4

BULKED-AFLP ANALYSIS OF GENETIC DIVERSITY AMONG TRADITIONAL ETHIOPIAN HIGHLAND MAIZE ACCESSIONS

Journal of Biodiversity and Conservation 2005 (in press)

4.1 ABSTRACT

In the highland regions of Ethiopia the heterogeneity of the land, climate, and soil favors the presence of a large number of landraces. A representative sample of 62 traditional Ethiopian highland maize accessions was analyzed using amplified fragment length polymorphism (AFLP) markers, to determine the degree of genetic diversity and relationships among these accessions and to study agroecological variation. Eight *Eco*RI/*Mse*I primer combinations were used. Of a total of 650 AFLP markers that were scored, 89.5% were polymorphic. Pair-wise genetic dissimilarity estimates revealed dissimilarity coefficients ranging from 0.32 to 0.69, with a mean of 0.57. Cluster analysis grouped most accessions collected from the Northern highlands into one major cluster while the Western and Southern accessions clustered together. In addition, variation partitioning revealed that only 9% of the total genetic variation was found between agroecologies, whereas 91% was found within agroecologies in Ethiopia. This finding may be explained by long distance seed exchange, continuous seed introduction and gene flow between the two agroecologies. The consistency of the results between clustering method and variation partitioning showed that AFLP markers accurately revealed genetic structure among maize accessions. Overall, the AFLP marker analysis indicated the existence of ample genetic diversity in highland maize accessions, which can be exploited by hybridization and selection.

Keywords: AFLP markers, bulked analysis, genetic resources, highland maize

4.2 INTRODUCTION

Maize was first introduced in Ethiopia in the 16th or 17th century (Hafnagel, 1961). Since its introduction, it has gained importance as a food and feed crop. Currently, it is the second most important crop, exceeded only by teff [*Eragrostis tef* (Zucc) Trotter] in terms of production area. However, it exceeds all other cereals in terms of annual production and yield ha $^{-1}$ (EARO, 2000). Maize is one of the cereals that provide most of the calorie requirements in the traditional Ethiopian diet. It is prepared and used as unleavened bread, roasted and boiled green ears, parched mature grain porridge and in local drinks like 'tella', 'borde' and 'areke' (Mulatu *et al*., 1992). Apart from these uses, maize leaves are fed to animals, while dry stalks are used as fuel and for the construction of fences and huts.

Ethiopia is a diverse country in terms of altitude, temperature, rainfall and soil types. Such diversity is apparent within even a short distance in a given locality. Diverse environments result in the presence of diverse vegetation, crop species and varieties in farmers' fields in most parts of the country (Vavilov, 1951). Maize varieties that are used in the highland regions of Ethiopia are well adapted, but are generally low-yielding open-pollinated varieties developed by local farmers. Many of these varieties resulted from centuries of planting, harvesting and selection. The highland maize varieties may be grouped into a number of completely or partially isolated populations, which may each, be adapted to different highland conditions (Chapter 3). Unfortunately, formal breeding programs have had little success in developing improved varieties for the diverse agroecological conditions in the highlands of

Ethiopia. In the past decade, only two improved open-pollinated maize varieties were developed for the highland zone (Twumasi-Afriyie *et al*., 2001).

Effective plant breeding and crop improvement programs depend on the availability of genetic diversity. Landraces are the original source of variation for breeding programs and are still the major source for new breeding programs in many developing countries. To assess the genetic diversity present in Ethiopian highland maize accessions, seed samples were collected from 287 highland locations of Ethiopia (Twumasi-Afriyie *et al*., 2001). A recent field study revealed that these accessions are highly variable for morphological and agronomic characteristics (Chapter 3). 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).

Molecular markers can be used to study the genetic diversity and genetic relationships among maize accessions directly at the DNA level. Amplified Fragment Length Polymorphism (AFLP) markers (Vos *et al*., 1995) have gained importance in crop genetic analyses, mainly due to the high multiplex ratio of this marker system. AFLP markers have been extensively used to study genetic diversity in maize inbred lines (Lubberstedt *et al*., 2000; Vuylsteke *et al*., 2000b). Although the technique is relatively simple and rapid, the large number of individual plants that need to be processed may limit AFLP analysis of cross-pollinated species like maize. One approach to overcome this limitation is to analyze one, or several, bulked samples per

accession, rather than individual plants. Kolliker *et al*. (2001) demonstrated that bulking equal amounts of leaf material before DNA extraction is an effective approach to produce representative AFLP marker profiles in white clover.

In this study the results of a genetic diversity analysis of Ethiopian highland maize accessions using bulked AFLP markers analysis will be reported. The objectives of this study were: (i) to assess the amount of genetic diversity and relationships among the highland maize accessions and (ii) to understand the distribution of genetic variation within and among agroecologies. The suitability of bulking leaf samples for genetic diversity of maize accessions has been investigated using both individual plants and their bulked samples.

4.3 MATERIALS AND METHODS

4.3.1 Plant materials and DNA extraction

A total of 62 traditional Ethiopian highland maize accessions were used for this study (Table 4.1). Previously, a representative subset of 180 of the 287 maize accessions collected from different highland regions in Ethiopia was analyzed for 15 morphological and agronomic traits (Chapter 3). Principal component and cluster analyses grouped these 180 accessions into four main clusters. The 62 accessions were chosen from the four clusters to represent the different agroecologies of Ethiopia and the range of morphological and agronomic variation observed in the field. For each of the 62 accessions, genomic DNA was extracted from leaf discs, harvested from 15 three-week

old plants (one 10-mm leaf disc per plant). For two accessions, individual DNA samples were also isolated from the 15 plants used for bulked sampling. 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.

Table 4.1 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

4.3.2 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, except that 10 µl diluted R/L mix, 2.0 mM $MgCl_2$ and 1.0

U Taq polymerase were used in the preamplification step. 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 preselective and selective amplification primer pairs all had two and three-nucleotide extensions at the 3' end, respectively. In all reactions only the *Eco*RI primers were 5'labelled with infrared dyes (IRDye 700 or IRDye 800, LI-COR). Initially, eight accessions were chosen to test the amplification successes of different primer combinations. The polymorphism rates and the total number of scorable fragments were evaluated in these eight accessions with 32 primer combinations. Eight primer combinations (Table 4.3) with the highest polymorphism rates and large numbers of clearly scorable fragments were selected to analyze the full set of 62 accessions.

4.3.3 Gel electrophoresis and scoring

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.

4.3.4 Data analysis

The binary data were exported into Microsoft Excel and formatted for use in the NCSS statistical software package. The average Polymorphic Information Content (PIC) for dominant markers was calculated according to Riek *et al*. (2001) by the following formula:

$PIC = 1 - \left[f^2 + (1 - f^2)^2 \right]$; where f is the frequency of the marker in the data set. Agroecology-specific alleles (private alleles) were recorded, if present for all primer pair combinations. Chi-squared test was used to test the significance difference in band frequency among agroecologies. Genetic similarity between accessions was

calculated according to Nei and Li (1979) using the formula:

$s_{ij} = \frac{2a}{(2a+b+c)}$, where s_{ij} is the similarity between two accessions i and j, a is the number of bands present in both i and j, b is the number of bands present in i and absent in j and c is the number of bands present in j and absent i. This formula excludes bands absent in both individuals, which cannot be necessarily being attributed to a common cause (Kolliker *et al*., 2001). Genetic dissimilarity was calculated as $1 - s_{ij}$. Cluster analysis was performed on the genetic dissimilarity matrix using Ward's minimum variance method (Ward, 1963). The Ward method was found to be a more suitable clustering technique than UPGMA as it avoided chaining effects that are often observed with UPGMA (Dubreuil *et al*., 1996). Genetic dissimilarity between accessions was also calculated based on Euclidean distances (Sneath and Sokal, 1973), which allowed us to estimate genetic variance within and among agroecologies according to Van Eeuwijk and Baril (2001) with the following formula:

$$d^2_{gi;g'i'} = \sum_{m=1}^{M} \left(x_{mgi} - x_{mg'i'}\right)^2,$$

Let d^2 is the distance between an individual *i* in group *g* and an individual *i'* in group *g'* for marker (from 1 to *M)*. Accordingly, the squared distance is the sum of the squared differences between individual accessions over all variables. The total variation (V_T) can be shown to be equal to the sum of all squared pair-wise distances between individual accessions (over all groups), divided by the total number of accessions (overall groups). The within group variation (V_W) is the sum over groups of the sum of squared pair-wise distances within a group divided by the group size. The between group variation (V_B) can be obtained by subtraction, $V_B = V_T - V_W$.

4.4 RESULTS

4.4.1 Detection limit of bulked AFLP analysis

The detection threshold of AFLP bands was tested in two 15-plant bulks by analyzing individuals and their bulked leaf samples separately. A total of 120 polymorphic markers were scored in the two bulked samples (15 individual/accession) and 30 individual samples using three selective primer combinations. The analysis of the AFLP profiles generated from bulked leaf samples demonstrated that most of the bands present in individual plants were present in AFLP profiles from bulked leaf samples (Figure 4.1).

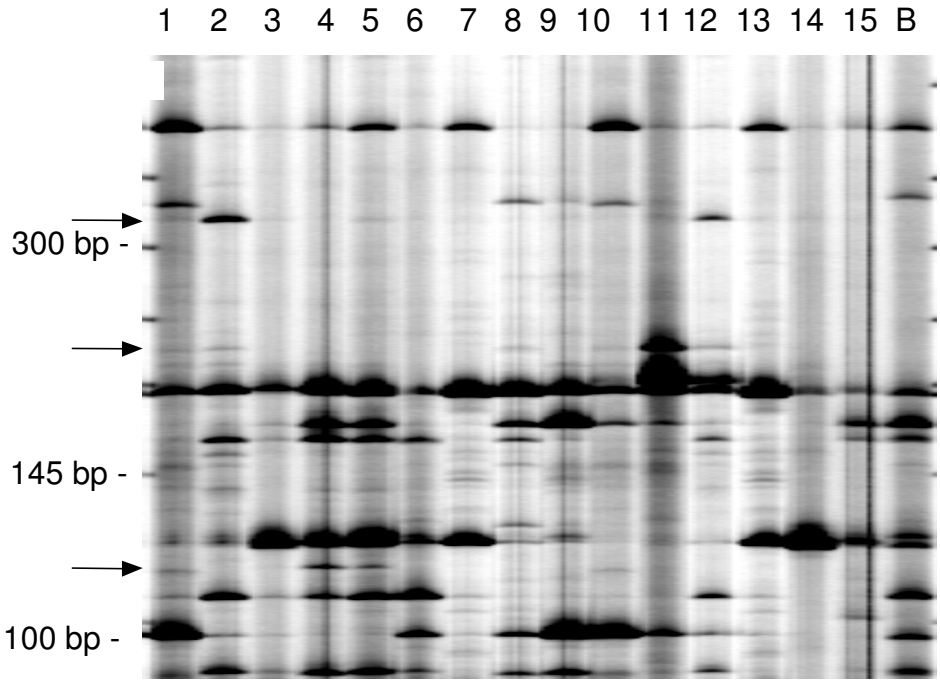

Figure 4.1 An example of the LI-COR AFLP image generated using E-AAC/M-CGG primer combinations showing banding patterns of 15 individuals (1-15) and their

bulked leaf samples (B). A molecular marker is indicated at the beginning and at the end of the gel. Arrows show examples of bands present in individuals and absent in bulked samples

The relationships between band frequency and presence and absence in the bulks of 15 plants of the two accessions (average of three primer pairs) is presented in Table 4.2 Bands which are only present in single plant (7%), are present in bulks of accession 'Baw-01' (58%), and 'Ad-1-03' (46%). High proportion (83%) of bands which are only present in less than 20% (3 out of 15) of the individual plants were present in bulks across the two accessions. However, all bands that are shared by more than 50% of the individual plants were represented in bulked samples in both accessions across the three primer pair combinations (Table 4.2).

Table 4.2 Relationships between AFLP band frequencies in individual plants and representation in bulks of 15 individuals based on the average of three primer pair combinations

		Accession		
	Band frequency in individual plants	'Baw-01'	'Ad-1-03'	Average of the two accessions
% Bands represented in bulk	Upto7%	0.58	0.46	0.52
	7-20%	0.82	0.83	0.83
	21-50%	0.85	0.92	0.89
	50-75%	100	100	100
	75-100%	100	100	100
% Total bands in individuals present in bulks		90	89	89.5

4.4.2 Marker polymorphism

To assess the genetic diversity of the 62 highland maize accessions, a total of 650 AFLP bands with fragment sizes ranging from 52 to 720 bp were generated using eight selective AFLP primer pair combinations. Of these, 89.5% were polymorphic among the 62 accessions (Table 4.3). The number of polymorphic bands per primer combination ranged from 56 to 98 with an average number of 72.8 (Table 4.3). PIC values for primer enzyme combinations ranged from 0.279 to 0.370, with an overall mean of 0.325. A typical LI-COR AFLP image generated using E-ACG/M-CGG primer combination across 62 maize accessions is presented in Figure 4.2.

Table 4.3 Degree of polymorphism and average polymorphism information content for the eight AFLP primer combinations used to analyze the 62 Ethiopian maize accessions

No	Primer combination [a]	Total number of bands	Number of polymorphic bands	% of polymorphic bands	PIC
1	E-AGG/ M-CAG	73	64	87.7	0.370
2	E-ACG/ M-CCG	72	66	91.6	0.320
3	E-ACA/ M-CGA	109	98	89.9	0.279
4	E-ACA/ M-CCC	86	76	88.0	0.321
5	E-AAC/ M-CAC	72	68	94.5	0.359
6	E-ACG/ M-CGG	74	68	91.8	0.321
7	E-AAC/ M-CCG	69	56	81	0.327
8	E-AAC/ M-CGG	95	86	90.5	0.300
	Total	650	582	Na.	Na.
	Mean	81.3	72.8	89.5	0.325

[a] E, *Eco*RI & M, *Mse*I

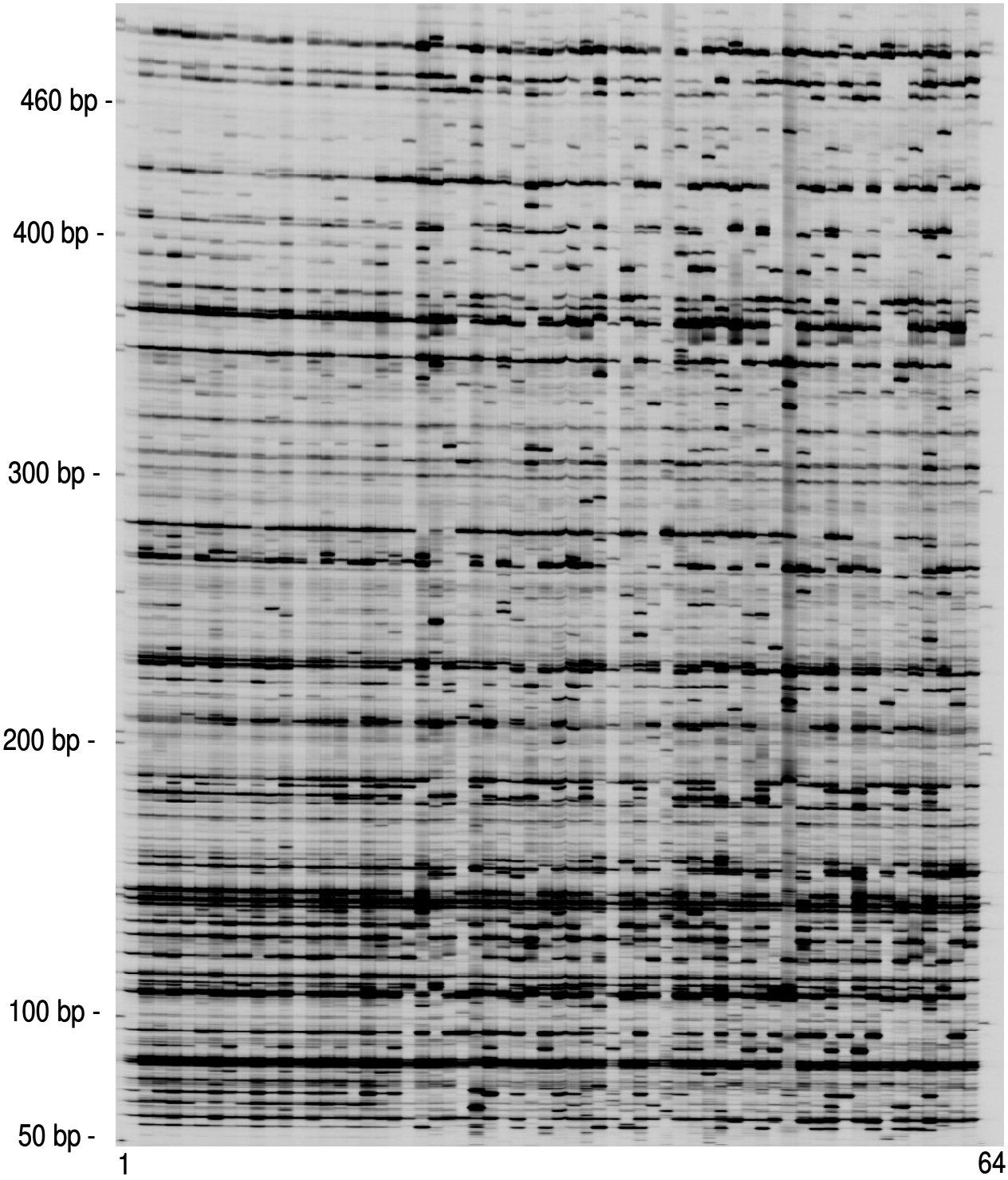

Figure 4.2 Typical a LI-COR AFLP image produced by selective amplification using the E-ACG/M-CGG primer combination in 62 maize accessions. Lanes 1 and 64 are IRDye 700 molecular weight standards (LI-COR Biosciences)

4.4.3 Distribution of bands across the three agroecologies

Table 4.4 shows the total number of bands and their frequency for each agroecology. According to Chi-square tests, accessions collected from the Northern agroecology were significantly (p = 0.01) different from the Southern and Western agroecologies with respect ot rare bands, which present up to 25% of the accessions. There were significant difference (p = 0.01) between the Northern and Western agroecologies with respect to bands that present up to 50% of the accessions. However, there was no significant difference between the Western and Southern agroecologies in any of the bands frequencies. Comparing the three agroecologies simultaneously, they differ significantly in all band frequencies except bands that occurred at 50-70% frequencies. Among 650 markers 73 bands were unique to the Northern agroecology. However, the Western and Southern agroecologies had 6, and 5 unique bands, respectively.

Table 4.4 Distribution of AFLP bands expressed as the percentage of accessions that carry a particular band in each agroecology

	Northern agroecology		Southern agroecology		Western agroecology	
Frequency of bands	No. of bands	% of bands from the total	No. of bands	% of bands from the total	No. of bands	% of bands from the total
< 5	73	0.11	6	0.01	5	0.01
5-15	152	0.24	84	0.15	73	0.13
15-25	59	0.09	122	0.21	135	0.24
25-50	114	0.18	131	0.23	163	0.29
50-75	121	0.19	110	0.19	106	0.19
75-100	120	0.19	119	0.21	89	0.16
Total	639		572		571	

4.4.4 Genetic dissimilarity of maize accessions

In order to evaluate the molecular diversity of the 62 accessions, the pair-wise genetic dissimilarity was calculated for the 1891 pairs of Ethiopian highland maize accessions (Figure 4.3). Pair-wise dissimilarity ranged from 0.32 (accession no. 1 and accession no. 13 from the Northern agroecology) to 0.69 (accession no. 1 and accession no. 40 from the Northern and Southern agroecologies, respectively) with an overall mean of 0.57. More than 71% of the pair-wise comparisons exhibited genetic dissimilarity higher than 0.5.

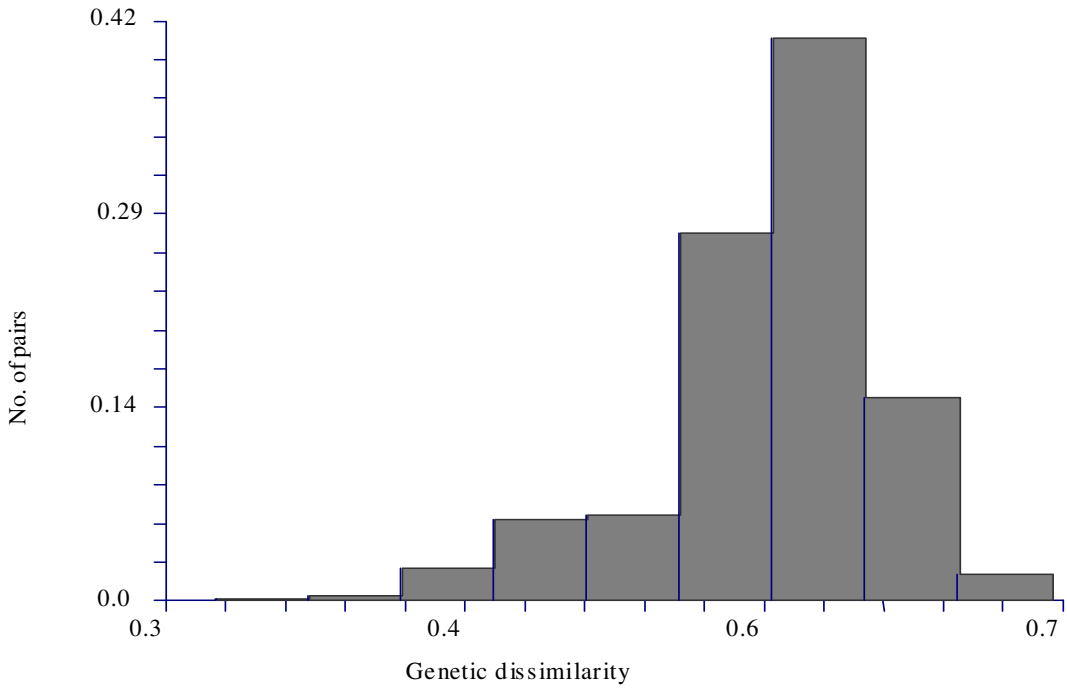

Figure 4.3 Frequency distribution of the genetic dissimilarity of pairs of 62 Ethiopian highland maize accessions

To visualize the relationships among the accessions, a dendrogram was generated

from the dissimilarity matrix using Ward minimum variance as clustering method (Figure 4.4). The Ward method combines the two clusters in each step whose fusion leads to the smallest increase in the Euclidean sum of squares within groups, thus leading to a maximized variance within groups and a minimized variance within groups. This was particularly important for the accessions, since the variance within accessions was already very high. The dendrogram showed three major clusters. Cluster I consisted of 20 accessions, all collected from the Northern agroecology. Cluster II consisted of 21 accessions and 85.7% were collected from Southern and Western agroecologies. Cluster III contained 21 accessions, collected from the three agroecologies.

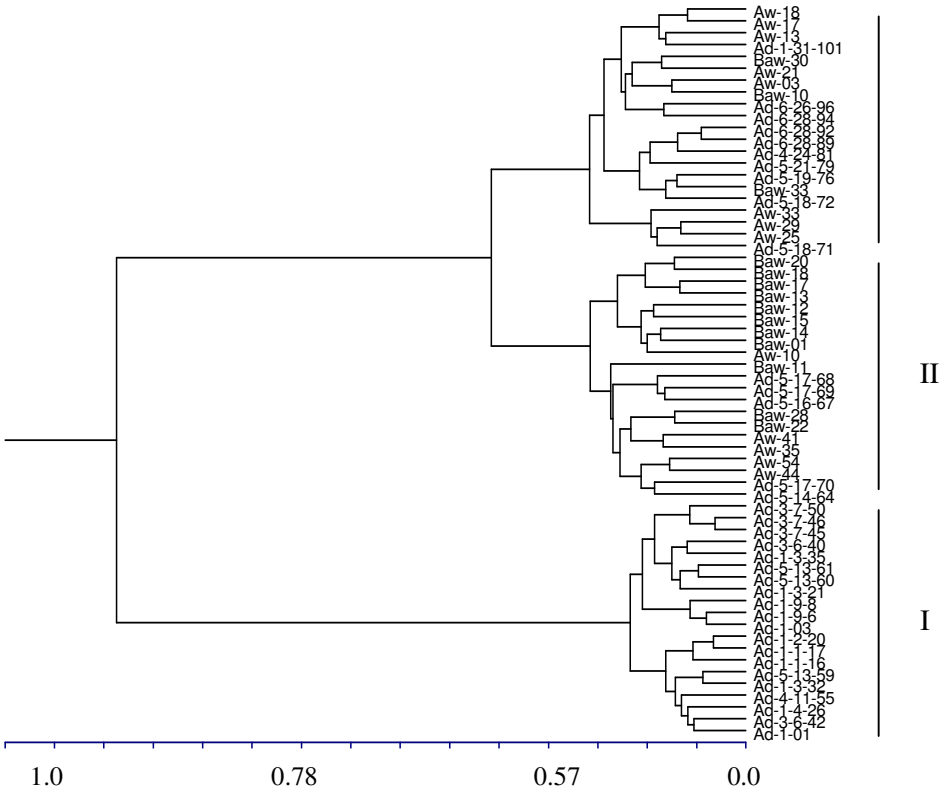

Figure 4.4 Dendrogram of traditional Ethiopian highland maize accessions derived by

Ward' minimum variance method from the dissimilarity matrix of AFLP data

4.4.5 Partitioning of genetic variation

Partitioning the total genetic variability using the analysis of distance method (van-Eeuwijk and Baril, 2001) revealed that 91% of the total variation was found within and the remaining 9% among agroecologies (Table 4.5) suggesting limited genetic differentiation among the agroecologies. Furthermore, partitioning of the within agroecology variation into different agroecologies, indicated that accessions collected from the Northern agroecology contributed 54.3% of the total variation. The Southern and the Western agroecologies contributed only 21.7 and 24% to the total variability, respectively (Table 4.5).

Table 4.5 Partitioning of the total genetic variation of traditional Ethiopian highland maize accessions into within and between agroecologies variation

Sources of variation	Number of accession	Variance component	Percentage of variation	Mean genetic distance
Total	62	10		0.57
Within	-	9.1	91.0	
Northern	35	4.9	54.3	0.57
Southern	13	1.0	21.7	0.43
Western	14	2.2	24.0	0.45
Among	-	0.9	9.0	

4.5 DISCUSSION

Knowledge about genetic diversity and relationships among diverse germplasm is

useful for plant breeders. It supports their decisions on the selection of parents for crossing and is helpful to widen the genetic basis of breeding programs. A genetic improvement program has been initiated at Ambo, Ethiopia in collaboration with CIMMYT with the goal of producing improved maize cultivars for the highlands of Eastern African countries. To initiate the improvement effort, a basic understanding of the genetic diversity and relationships among the highland maize accessions was considered essential. In this chapter, the results of a genetic diversity analysis of 62 traditional Ethiopian highland maize accessions collected from different highlands of Ethiopia using bulk-AFLP markers analysis will be reported.

Molecular markers are a very efficient approach to rapidly attain genetic diversity estimates to be used in various breeding programs and policies. However, the cost of DNA extraction and subsequent analysis is a major consideration that limits the use of molecular markers for large-scale genetic diversity study. This is particularity true for comparisons of open-pollinated crops where many individuals per population should be sampled (to capture the genetic variability within a single population) and cannot be used for the routine characterization of large germplasm collections. One approach to overcome this problem is the use of bulking leaf/DNA samples per population rather than several individual per population. Bulking strategies provide a means of large-scale diversity analysis in cross-pollinated crop species (Kolliker *et al*., 2001; Rebourg *et al*., 2001).

To determine the detection limits and suitability of bulked AFLP analysis for the measurement of genetic diversity in maize accessions, the results of AFLP gel profiles

from 15 individual plants and their bulked leaf samples was compared. The AFLP patterns obtained from bulked leaf samples were highly representative of the AFLP patterns obtained from individual plants of the same accession (Figure 4.2). The detection limit found in this study varied somewhat among primer combinations. Some bands that appeared to be of low frequency (less than 3 out of 15) were detectable in bulked samples, while other present in higher frequency (up to 6 out of 15) were not detected in bulked samples (Table 4.2). This observation was in agreement with the work of Kolliker *et al.* (2001), who reported that some bands that were present in individual white clover plants at a higher frequency (20-50%) were absent in bulked samples, whereas some bands present in individual plants at low frequency (less than 5%) were represented in bulked samples. The authors speculated that complex competition processes during PCR amplification in AFLP technique could be the reason for this. In a previous study two bulk of 15 individuals/population has been used in study of European maize populations using RFLP markers (Rebourg *et al*., 2001). In this study, on average 89.5% of the bands, which were scored in individual plants, were present in bulked samples suggesting that pooling leaf samples before DNA extraction is an effective means of producing representative profiles of individual plants. This allowed higher sample throughput during DNA extraction and minimized reagent cost for genetic diversity estimation among Ethiopian highland maize accessions. Similarly, Kolliker *et al.* (2001) have demonstrated that bulking at leaf stage is effective in producing representative profiles in white clovers using AFLP analysis.

The proportion of polymorphic bands (89.5%, Table 4.3) obtained in the present study is high compared to the work of Lubberstedt *et al.* (2000) who reported an average

polymorphism rate of 84% in early European maize inbred lines selected from different heterotic groups. 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. Considering the technical simplicity and sensitivity to DNA polymorphism of this technique, it is advantageous for studies of open-pollinated species than other similar techniques. The average PIC value (0.325, Table 4.3) in this study was also close to the high end of the range (0.29 to 0.33) previously reported for maize (Lubberstedt *et al*., 2000; Vuylsteke *et al*., 2000b). The high PIC values may be due to the fact that we prescreened AFLP primer combinations and selected the eight primer combinations (Table 4.3) with the highest polymorphism rates and largest numbers of clearly scorable fragments.

The range and average dissimilarity based on AFLP data (range: 0.32 to 0.69, mean: 0.57) observed in the present study were similar to that reported by Rebourg *et al*. (2001; range: 0.106 to 0.793, average of 0.55) for European maize populations. The average genetic diversity among Ethiopian highland maize accessions is therefore as diverse as observed among maize populations collected from the whole of Europe. The high genetic diversity observed among the traditional Ethiopian highland maize accessions suggests ample opportunity for the development of improved varieties for different highland parts of Ethiopia. This might be due to the nature of the materials used in the study. The highland accessions are open-pollinated varieties developed by local farmers over centuries and there has been a continuous introduction of seed into these regions. Another factor might be the sampling strategy employed in this study,

which maximized the geographical and morphological range among the 62 selected highland maize accessions.

The dendrogram revealed that the Northern accessions are more differentiated compared to the Western and Southern accessions (Figure 4.3). The separation of the Northern accessions from the rest of the accessions might be (a) due to strong selection by local farmers for adaptation to the drier growing conditions and as result they had large number of unique bands (73, Table 4.4), (b) due to the little introduction of high yielding and uniform varieties into this agroecology, and (c) due to the general restriction of seed movement into the Northern region from other agroecologies due to geographical isolation. There was, however, no differentiation between accessions collected from the Western and Southern agroecologies. This was also supported by Chi-square tests where there was no significant difference in band distribution between the Western and Southern accessions (Table 4.4). The reasons might be the result of several factors: Firstly, in these agroecologies, maize is the staple food (mainly as porridge) and hence local farmers have selected similar accessions suitable for food properties. Secondly, these two agroecologies are physically in close proximity and there might be gene flow between farmers' varieties. Finally, there have been continuous introductions of high yielding and uniform varieties released in the surrounding intermediate regions by government and non-government extension programs (Sasakawa-Global2000, 2002). The average yield of introduced varieties (5.6 t/ha) was three times higher than that obtained from traditional maize varieties (1.2 t/ha). All these activities together with the tradition of local framers to acquire seeds from distant places might be the reasons for the low

genetic differentiation between the Southern and the Western agroecologies.

To our knowledge no published data so far available on the use of bulked AFLP for genetic diversity study of maize accessions. These results indicate that bulked AFLP analysis can be successfully applied to study the genetic diversity and relationships among maize accessions/landraces.