Ethiop. J. Biol. Sci., 7(2): 95-110, 2008
 ISSN: 1819-8678

AFLP AND MICROSATELLITE FINGERPRINTING TO ESTIMATE GENETIC DIVERSITY IN ETHIOPIAN TETRAPLOID WHEAT (*TRITICUM TURGIDUM* L.) LANDRACES

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ABSTRACT: Genetic variation of 150 OTUs representing 26 Ethiopian tetraploid wheat (*Triticum turgidum* L.) landraces and 4 tetraploid wheat cultivars were investigated using the AFLP and microsatellite methods. Nine primer combinations (PCs) for AFLP and twelve wheat microsatellite (WMS) located on different A- and B-genome chromosome arms were used to amplify DNA segments from the genomic DNA. For AFLP, a total of 84 polymorphic bands were scored, with an average of 9.3 polymorphic bands per primer combination. For microsatellite, a total of 96 alleles were scored, with an average of 7.9 alleles per locus. AFLP genetic diversity revealed that coefficient of gene differentiation for the within accession component is high (0.66), indicating that the high accession variation is due to the within component rather than the between accessions component. UPGMA dendrograms that were constructed on the basis of AFLP band similarities demonstrated that the accessions in the dendrograms do not show a clear pattern of geographical clustering. Regarding microsatellite, the relative coefficient of gene differentiation for the within accessions component accounted for 45% while 55% of the total diversity was due to the inter-accessional component. According to microsatellite analysis, genetic diversity was much higher for the within than for the between class component of the sub-regions and altitude classes. Based on UPGMA denderogram, most of the accessions were loosely grouped according to altitude and to a lesser extent to region of collection.

Key words/phrases: AFLP, Ethiopian tetraploid wheat landraces, Genetic diversity, Microsatellite

INTRODUCTION

Twelve thousand wheat landrace accessions have been collected and conserved in the gene bank of Ethiopia (Mehari Zewdie, 1994). Accessions are often composed of various morph-types because farmers traditionally grow mixtures of various biotypes. This phenomenon together with the existing natural variation might cause to inflate the number of wheat accessions preserved in the Institute of Biodiversity Conservation (IBC). To allow a sustained and efficient (*ex-situ* and *in-situ*) conservation of these materials, a thorough knowledge of genetic diversity and relationships

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between the accessions is required. Until now, the characterization of wheat landraces preserved in IBC was based on only phenotypic traits. These characters are prone to environmental conditions and/or are controlled by multiple genes (Newbury and Ford-Lloyd, 1997). Although isozyme marker technology was included for the characterization of the landraces, the number of polymorphisms in isozyme markers is limited and their expression has been shown to be affected by environmental conditions as well (Tsegaye Seyfu *et al*., 1996).

To limit redundancy and to represent all genotypes within the collection, a diversity study using reproducible methods that are capable of discriminating genotypes on the basis of unique fingerprints is required. Such genomic bar codes for each distinct genotype thus provide an effective preservation strategy of the national wheat diversity in the gene bank.

Paetku and Strobeck (1994) suggested that the use of robust marker tools is required for a more reliable assessment of genetic diversity in wheat. Barrett and Kidwell (1998) and DeVries and Toenniessen (2001) confirmed that molecular markers have great value in the design of conservation programmes. Rapid progress in molecular biology has resulted in the development of a number of techniques for detecting variation at the DNA level. Such DNA marker systems are based on either hybridization with single copy probes of DNA digested with specific restriction fragments revealing restriction fragment length polymorphism (RFLP) or on the amplification of genome segments between arbitrary or specific oligonucleotide priming sets using PCR (polymerase chain reaction) (Karp *et al*., 1996).

For diversity study in cereals, molecular markers like amplified fragment length polymorphism (AFLP) are increasingly employed. AFLP is a molecular fingerprinting technique that is based on PCR amplification of short restriction endonuclease - digested genomic DNA fragments onto which adapters possessing 3' selective nucleotides of one to four bases are used in a selective amplification reaction. Subsequent to selective amplification by PCR, a separation of labeled amplified products is followed by denaturing polyacrylamide gel electrophoresis (Vos *et al*., 1995; Wang *et al*., 2000). The length polymorphisms of DNA fragments represent loci from all over the genome and can be used to measure within-species diversity. In comparison to other molecular markers, AFLP has the advantage of allowing simultaneous identification of a large number of amplification products (Qi and Lindhout, 1997; Byerlee and Fischer, 2001)

as well as detecting genetic diversity between and within closely related species. AFLP markers are mostly inherited dominantly and have successfully been used to study diversity in various cereals, including *Triticum* spp. Microsatellite is another DNA marker that is designed for assessing genetic diversity between and within species. Although the methods often display various co-dominant alleles per locus (Edwards *et al*., 1991, 1992; Plaschke *et al*., 1995) they have been used successfully for genotype identification (Chakraborty, 1984; Scribner *et al*., 1994; Allen *et al*., 1995) and for estimating genetic and geographic diversity (Bruford and Wayne, 1993; Paetku and Strobeck, 1994; Gottelli *et al*., 1994; Roy *et al*., 1994; Bowcock *et al*., 1994; Forbes *et al*., 1995).

The objective of this study was to assess genetic diversity in 26 randomly selected accessions of Ethiopian tetraploid wheat landraces and four cultivars using AFLP and microsatellite markers.

MATERIALS AND METHODS

Materials

For this study, twenty-six tetraploid Ethiopian wheat landraces *(T. turgidum* L.), and the Ethiopian tetraploid cultivars Boohai (Et1) and Arendato (Et2), the South-African tetraploid cultivar Golden Ball (Er1) and the Italian tetraploid cultivar Capeiti (Er2) (Table 1) were used. The non-Ethiopian tetraploid cultivars were obtained from the Centre of Genetic Resources, (CGR) Wageningen, the Netherlands. The 26 accessions for this study were selected based on phenotypic characterization. The twenty-six landraces came from the Institute of Biodiversity Conservation (IBC), Addis Ababa, and were originally collected from farmers' fields, threshing areas, farmers' stores and markets in the central region, Shewa. The study was conducted on the accessions collected from Shewa because most of the improvement work in tetraploid wheat was widely conducted in or around the region. Accessions were randomly grouped from four sub-regions (North, East, South and West) and four altitude classes of 1600-2000 m, 2001-2400 m, and 2401-2800 m and above 2801m (Table 1). Accessions were collected from the localities encompassing the altitude range (Table1). From each accession on average, 5 plants were randomly selected (Operational taxonomic unit hereafter known as OTU). Tetraploid wheat of each accession was a mixture of *T. durum* and *T. turgidum*, thus why it was considered and categorized under tetraploid wheat accession. It was difficult to find pure line *T. durum* or *T. turgidum* landraces. So, each of the 26 accessions for this study were composed of *T. durum* and *T. turgidum*,

although the composition for each accession varies.

Table1 List of altitude and regions of wheat accessions collection.

Altitude	No of accessions studied per altitude range	
1600 - 2000	7	
2001- 2400	7	
2401 - 2800	7	
≥2801	5	
Cultivars	**Donating Institution**	**Taxonomic grouping**
Boohai (Et1) Arendato Et2) Goldenball(Er1) Capeiti(Er2)	EIAR EIAR The Netherlands gene bank	All are tetraploid wheat

Accessions were collected from Yerer and Keryu (East), Gebat and Mecha (West), Tegulet and Bulga (North) and Kembata and Hadia (South)

Sample size selection was done based on earlier conducted phenotypic data. Some of the accessions were similar and thus why reduction of samples was done. The standards were given by the Ethiopian Institute of Agricultural Research (EIAR) and the Netherlands gene bank.

Methods

AFLP method

Geographic region from seeds for this study collected are illustrated in Fig 1. and Table 1. Per accession, five randomly selected plants were grown in green house (OTU).

For AFLP method, ten to eleven days old leaves of each OTU were collected and stored in 15 mL screw capped glass tubes. The leaves were used either directly or stored in liquid nitrogen.

DNA extraction and amplification

Genomic DNA was isolated from fresh leaves of growing plants using the CTAB method (Van der Beek *et al.*, 1992). The AFLP method followed essentially the protocol of Vos (1993), with adaptation for the number of selective nucleotides and pre amplification steps. The protocol includes: 1) restriction of genomic DNA with *Eco*RI and *Mse*I, and ligation of adapter sequences to the restriction fragments in order to generate the primary template; 2) selective pre-amplification of this primary template with AFLP primers with various additional 3' selective nucleotides (Table 2); 3) selective amplification with ^{33}P-labeled *Eco*RI primers having three selective nucleotides and *Mse*I primers with three 3' selective nucleotides

(Table 2), 4) separation of labeled amplification products on a denaturating polyacrylamide sequencing gel using an S2 sequencing gel electrophoresis apparatus (Gibco BRL, Life Technologies USA, Maryland, USA).

Table 2 AFLP adapters and primers used to detect diversity in tetraploid Ethiopian wheat landraces and cultivars.

Adapter	Extension	Sequence /Primers
E+1	A	5'-CTCGTAGACTGCGTACCCATCTGACGCATGGTTAA-5'
M+1	A	5'-GACGATGAGTCCTGAGTACTCAGGACTCAT-5'
Primers		
E35	ACA	5'-AGACTGCGTACCAATTCACA
E37	ACG	5'-AGACTGCGTACCAATTCACG
E42	AGT	5'-BGACTGCGTACCAATTCAGT
M36	ACC	5'-GATGAGTCCTGAGTAAACC
M37	ACG	5'-GATGAGTCCTGAGTAAACG
M38	ACT	5'-GATGAGTCCTGAGTAAACT
M40	AGC	5'-GATGAGTCCTGAGTAAAGC

The anodal buffer was supplemented with 1 x TBE to generate a salt gradient, which contributes to a better separation of the larger fragments. The gels were dried on Whatman 3MM paper, exposed to X-ray films (Konica, Tokyo, Japan) at -20^{0}C for 3-7 days depending on signal intensity. Finger printing patterns were analyzed and scored visually.

Microsatellite method

For the microsatellite method, ten to eleven days old leaves representing each OTU were collected and stored in 15 mL screw capped glass tubes. The leaves were either directly further processed or stored in liquid nitrogen until later use.

DNA extraction and amplification

DNA was extracted according to the CTAB protocol as in Van der Beek *et al*. (1992), with minor modifications. Two grams of frozen leaf tissue were homogenized in 3 mL 2% CTAB-buffer. The homogenate was incubated at 65 for 90 min, while gently mixing at a 10 minutes interval. After two chloroform extractions, the DNA was precipitated and dissolved in 0.5 mL TE buffer. The microsatellites were studied as sequence-tagged microsatellite markers by PCR amplification (Bredemeijer *et al*., 1998). Amplification reaction was performed as previously described (Plaschke *et al*., 1995; Röder *et al*., 1998; Bredemeijer *et al*., 1998). For each PCR reaction a volume of 5 µL wheat genomic DNA, 2.5 µL fluorescently labelled forward primer (Pharmacia) and 2.5µL unlabeled reverse primer (Isogen, the Netherlands), 2.5 µL each dNTP, 2.5 µL $MgCl_2$, 2.5 µL 10x

PCR buffer, 1 µL 0.5 U/L ampliTaq gold (Perkin Elmer) and 1.5 µL distilled water were used. The forward primer (P1) was labelled at the 5' end with Cy5 (Pharmacia). Amplification was done using Hybaid Omni Gene thermal cycler under the following temperature profiles: An initial denaturation step of one min at 94^0C, followed by annealing temperature (60^0C or 55^0C) for one min, and extension temperature at 72^0C for one min. After 45 cycles, the last cycle was followed by a final extension step of five min at 72^0C. Finally the amplified samples were stored at 4-25^0C. A 6 % acrylamide sequencing gel was used to separate the PCR products on automated laser fluorescence (ALF express) sequencer (Pharmacia, the Netherlands). Per gel 6 µL PCR products with 6 µL mix of loading buffer and size standard was combined. Then 2 to 4 µL of the mix was loaded to each gel lane in the presence of 1x TBE gel running buffer. Prior to loading, each DNA sample was denatured by heating the mix at 90^0C for 4 min followed by quenching on ice. DNA fragment sizes were analyzed with the computer program Fragment Manager by comparing with size standards and finally translated into a binary (presence 1 and absence 0) data matrix. Previous studies have assigned the microsatellite used in this study to the chromosome 1A, 2A (S), 3A, 4A, 6A, 7A, 1B (S), 2B, 3B, 4B, 6B and 7B (Röder *et al*., 1998).

Data analysis

Based on the dominant and co-dominant nature of the DNA markers AFLP and microsatellite, respectively, data analysis was done using Shannon-Weaver and Nei diversity index (Nei, 1973).

AFLP data

Genetic diversity per polymorphic band within accession, and the pattern of diversity within geographical regions were estimated according to the Shannon-Weaver index (Statistics department, Wagenen University, the Netherlands, 2000).

The Shannon-Weaver diversity index (H) for estimating the variation within and between accessions, and within and between altitude classes and within and between sub-regions was calculated as:

$$H = \sum_{i=1}^{n} pi \ln pi$$

Where n equals the number of phenotypic classes for a particular trait and pi is the proportion of total number of entries (n) in the i-th class.

Ht (total variation) was calculated for each trait and for all OTUs, by dividing H by the logarithm of the number of trait classes. Hs (the between component of variation) equal the average H for a specific group (accession or geographical group). The between component of variation (Dst) is then calculated as:

Dst = Ht –Hs

and subsequently (among variation) Gst = Dst/Ht

The presence or absence of AFLP fragments was scored on the autoradiogram and transferred into a 1 (present) and 0 (absent) matrix over all operational taxonomic units (OTUs). The phenetic analysis was performed with the Jaccard algorithm in an Unweighted Pair-Group Method with Arithmetic Averages (UPGMA) clustering analysis. Dendrograms were generated using the euclidean distance matrix.

Microsatellite

Nei's (1973) diversity index (H) was computed as follows:

$$H = 1 - \sum_{i}^{a} pi^2$$

Where variable pi is the frequency of variant; i is the number of variants and H is the Nei index based on all accessions of the OTUs.

The mean diversity index for groups (accession, cultivar groups, and altitude or sub-regions) was calculated as the average Nei index per group (Hs). Dst and the proportion of between components differences was computed as:

Dst = Ht – Hs,

which gives the relative contribution of the between group variation (Gst).

Gst = Ht–Hs/Ht

When a Gst value equals 1, groups are fixed for different alleles.

For the construction of the UPGMA dendrograms for the relations among and between OTUs and accessions that were used in this study, the SAS statistical software was used.

RESULTS

AFLP

Both AFLP and microsatellite markers showed that some of the OTUs from

the 26 accessions had the same bands, indicating the presence of duplicates within the accessions (Data not shown). Using nine AFLP PCs (primer combinations) the 26 landraces and four cultivars generated a total of 84 polymorphic bands. Since AFLP is a dominant marker, it was not possible to distinguish AFLP band intensity differences as an indication of heterozygosis. The number of different bands detected per PC over all accession ranged from 2 to 19. Of all the AFLP markers, PC E35M37 generated the highest number of polymorphic bands (19) while PC E42M37 had the lowest (2). Out of the 84 polymorphic bands, two were specific for eleven OTUs and the five for the two cultivars used as standards (Et1 and Er1). Table 3 shows Shannon-Weaver diversity index values calculated to estimate total (Ht) diversity, mean diversity within accession (Hs), mean among accession (Dst), and coefficient of gene differentiation (Gst). Mean total diversity (Ht) for all accessions and loci was 0.50 and ranged from 0.42 - 0.67 per locus. A far higher relative differentiation value for the within accession variation (Gst 0.34) was observed than for the between accession variation. A large difference between PCs was observed for Gst values indicating that some discriminate much more between accessions than the others. The accessions 5904 and 6028 showed relatively higher diversity values.

Table 3 Shannon -Weaver diversity for within and between accession variations.

SW	E35M37	E37M36	E37M38	E37M40	E35M40	E35M36	E42M37	E42M36	Mean
Ht	0.42	0.47	0.47	0.47	0.50	0.48	0.54	0.67	0.50
Hs	0.24	0.30	0.19	0.36	0.37	0.31	0.50	0.40	0.33
Dst	0.18	0.17	0.28	0.11	0.13	0.17	0.04	0.27	0.17
Gst	0.42	0.36	0.60	0.23	0.26	0.35	0.07	0.40	0.34

Means
SW – Shannon Weaver

To estimate the variation within and between *durum* and *turgidum*, the Shannon-Weaver diversity values for these cultivar groups were calculated. The mean total (Ht) genetic diversity value for the *durum* and *turgidum* were 0.48 and 0.16, respectively, showing that *durum* is three-fold higher in total (Ht) diversity than *turgidum*. The Gst value (0.36) showed differentiation between both groups. This difference was not reflected by a difference in the number of polymorphic bands between the *durum* and *turgidum* cultivar groups (Table 4).

Table 4 Shannon-Weaver diversity values for *durum* and *turgidum* and numbers of polymorphic bands per primer combination and per cultivar group.

Primer combinations	Shannon Weaver for accessions		Number of polymorphic bands AFLP marker	
	Durum	*Turgidum*	*Durum*	*Turgidum*
E35M37	0.40	0.17	14	13
E37M36	0.45	0.21	11	10
E37M38	0.45	0.19	9	8
E37M40	0.45	0.20	7	9
E35M40	0.48	0.12	3	5
E35M36	0.47	0.12	4	4
E42M37	0.53	0.19	5	5
E42M36	0.62	0.13	2	2
E35M38	0.46	0.11	6	5
Mean	0.48	0.16	7	7

AFLP

Diversity by geographical areas

The Gst values for altitude and sub-region classes revealed that almost 92-96% of the variation was because of the within component (Table 5). The means for the four altitude class Shannon-Weaver index compared by t-test showed no significant difference ($P<0.05$) between the classes (data not shown).

Table 5 Shannon-Weaver diversity in relation to altitudes and sub-region.

	Region classes					Altitude classes				
SW	I	II	III	IV	Mean	I	II	III	IV	Mean
Hs	0.47	0.52	0.53	0.50	0.50	0.40	0.51	0.42	0.52	0.46
Gst					0.04					0.08

SW – Shannon Weaver

Hs and Gst are the mean diversity index for groups and the proportion of between components differences, respectively.

Cluster analysis

The association among the 26 accession was shown by UPGMA cluster analysis. The UPGMA dendrogram showed two groups. The Ethiopian cv Boohai (Et1) and the South African (Er1) cv are separated from the rest of the accessions. The Italian cv Capeiti (Et2) together with Arendato are within the large clusters of the landraces. In the dendrogram for the 26 accessions, the similarity level ranged from 78 to 90%. Although accessions from different altitude and region groups often group together, the dendrogram did not show a clear pattern of clustering according to region or altitude (The dendrogram is not shown in this paper, because it is not

informative enough).

Microsatellite

The 150 OTUs representing 26 accessions and four cultivars showed, for twelve markers, a total of 96 polymorphic bands. The number of alleles per locus varied from 3 to 17. The highest number (17) of alleles was detected for WMS577 and the lowest (3) for WMS160.

The number of alleles per locus ranged 1-4 for the accessions and 1 or 2, incidentally 3 (WMS577) for the cultivars. For the loci WMS631, 18, 619, 493, 513 and 680, fifty percent or more of the accessions did not show polymorphism. The accessions 5441, 5588, 5725, 6038, 5180, and 5976 had nine or more polymorphic loci. Cultivars were less polymorphic than the accessions (Table 6) and four of them had identical alleles. In the cultivars, the B-genome was more differentiated (Dst 0.36) than the A-genome (Dst 0.12, Table 6). Heterozygocity (0.4 %) was observed for locus WMS577 in five OTUs (5588-184, 6038-210 - 213, 5180-277 - 285), for locus WMS155 in one OTU (7190-80), for locus WMS18 in one OTU (7190-80), for locus WMS631 in the cultivar Er1 and for the locus WMS513 in the cultivar ER2, in one OTU each.

Table 6 Nei's diversity index, number of polymorphic alleles per cultivar and per microsatellite marker.

Shannon Weaver per microsatellite primers					N° of polymorphic alleles for cultivars for accessions			
Primers	Ht	Hs	Dst	Gst	E1	E2	E3	E4
WMS357	0.32	0.08	0.24	0.25	1	1	1	2
WMS95	0.00	0.00	0.00	0.00	1	1	1	1
WMS155	0.32	0.08	0.24	0.25	2	1	1	1
WMS160	0.00	0.00	0.00	0.00	1	1	1	1
WMS169	0.32	0.08	0.24	0.25	2	1	1	1
WMS631	0.00	0.00	0.00	0.00	1	1	1	1
WMS18	0.00	0.00	0.00	0.00	1	1	1	1
WMS619	0.32	0.08	0.24	0.25	1	1	1	1
WMS493	0.96	0.24	0.72	0.25	2	2	2	1
WMS513	0.32	0.08	0.24	0.25	1	1	1	2
WMS680	0.32	0.08	0.24	0.25	1	1	1	1
WMS577	0.94	0.24	0.70	0.26	1	3	1	2
Mean	0.48	0.12	0.36	0.21	2	3	2	2
Total mean	0.32	0.08	0.24	0.17				

Table 6 shows Nei's diversity index values, which were calculated to estimate diversity for total accession (Ht), mean within accessions (Hs), mean among accessions (Dst) and coefficient of gene differentiation (Gst). The average total genetic diversity per marker (Ht) was 0.54, ranging from 0.08 to 0.84. Accessions like 5314 and 6028 had relatively high diversity values for all loci, except for the loci WMS619 and 631. Average diversity for all accessions and loci was 0.28. The differentiation within accessions

accounts for only slightly more than 50% of the variation (Gst 0.45). The landraces are more differentiated from each other (Dst 0.26) than the cultivars (0.24, Table 6). The highest differentiation coefficient (Gst 0.66) was observed for WMS160.

Assessing the diversity of 26 accessions, it was found that 40 of the alleles (53%) were common in *durum* and *turgidum*. Thirty-five alleles (47 %) were specific for *durum* and only one allele was specific for *turgidum.* The Nei's mean total diversity values (Ht) for the *durum* and *turgidum* cv. groups were 0.52 and 0.40, respectively (Tables 7). A comparison of the two tetraploid wheat cultivar groups indicated that variation was only slightly higher in the *durum* group than in the *turgidum* group (Table 7). The Gst value between the two groups (0.15) indicated that there was differentiation between groups.

Table 7 Comparisons of *durum* and *turgidum* cv groups, Nei's diversity index values and number of different alleles.

Microsatellite primers	Shannon Weaver		Polymorphic alleles		Total number of alleles
	Durum	*Turgidum*	*Durum*	*Turgidum*	
WMS357	0.66	0.51	9 (106)*	5 (22) *	9
WMS95	0.78	0.63	8 (107)	3 (22)	8
WMS155	0.66	0.48	7 (114)	3 (16)	7
WMS160	0.63	0.50	7 (102)	2 (22)	7
WMS169	0.91	0.55	5 (102)	4 (22)	5
WMS631	0.08	0.10	3 (108)	2 (22)	3
WMS18	0.60	0.17	6 (107)	3 (22)	6
WMS619	0.24	0.25	5 (108)	3 (22)	5
WMS493	0.15	0.38	3 (107)	3 (22)	4
WMS513	0.23	0.17	4 (108)	3 (22)	4
WMS680	0.50	0.25	4 (107)	3 (22)	4
WMS577	0.81	0.82	17 (105)	9 (21)	17
Mean for all markers	0.52	0.40	7	4	7

Mean all markers
*Number of OTUs used for the microsatellite analysis

Diversity by geographical areas

The microsatellite data calculated according to Nei's diversity index per altitude and sub-region class are shown in Table 8. The within-altitude component of diversity (Hs) was not very different from the total diversity (Ht), which indicates that the within altitude group variation is much larger than the between altitude group variation. The difference in H values for the different groups was relatively small (data not shown).

The total mean within sub-region variation was higher than the between sub-region variation, showing that the differentiation between sub-regions was limited (Table 8).

Table 8 Microsatellite diversity in relation to altitude and region according to Nei's indices.

Geographic features	Hs	Gst
Altitudes	0.44	0.23
Sub-region	0.27	0.43

Hs and Gst are the mean diversity index for groups and the proportion of between components differences, respectively.

The UPGMA dendrogram displays the relation between the 26 accessions and four cultivars, and their geographic areas. The dendrogram depicted two main groups in which group I contained all the accessions and the Et2 cultivar, whereas group II contained the remaining three cultivars (The figure was not included in this paper because it was not informative enough).

DISCUSSION

The nine AFLP primer combinations and twelve A- and B-genome wheat microsatellites were helpful in discriminating duplicate OTUs in the tetraploid wheat accessions and assessment of diversity in the remaining 150 OTUs. The techniques were sensitive and powerful in determining the extent of diversity in the landraces and cultivars, and between cv. groups of our *durum* and *turgidum* wheat in this study. The experimental reproducibility, coupled with the diagnostic ability of these techniques also has made AFLP and microsatellite techniques useful molecular tools for measuring the diversity of tetraploid wheat landraces. The reliability of AFLP and microsatellite techniques in detecting genetic variation was reported in several studies (Barrett and Kidwell, 1998; John *et al*., 1998; Roger *et al*., 1997). The observation that showed high accession variation (AFLP, 0.66 and microsatellite 0.84) and the presence of heterozygocity might be due to: firstly, mixtures of various forms are preserved in the farmers' fields (Harlan, 1971, 1992; Hailu Mekbib and Giorgis Habtemariam, 1990), which is traditional agricultural practice in Ethiopia. Secondly, traditionally produced crops grown nearby could allow occasional gene exchanges (Jana, 1993). Thirdly, natural cross pollination in wheat, which is very rare at high humidity, was reported to be frequent in dry regions at a frequency of 3 to 4% (Rao, 1974). Vavilov (1951) also observed that some of the Ethiopian tetraploid wheat open their glumes, the stamens lengthen outside the glumes and the anther hang out for some minutes enabling pollen shedding in the air and thus cross pollination. According to Vavilov (1951) and Moshe (1976) there is a possibility of high cross pollination and gene introgression and formation of new genotypes among the Ethiopian tetraploid wheat. Ethiopia's variable environmental conditions might have favored the conditions for natural cross-pollination, thus for

frequent gene exchanges and existence of high genetic variations. The farmers' exchange of seeds and market sampling may account for the maintenance of high variation in accessions. Moreover, primitive crop populations are known to have a high plasticity due to their genetic variability (Bennett, 1970) in spatially heterogeneous and often adverse environmental conditions. These phenomena could be some of the major reasons for the existence of high variation in Ethiopian tetraploid wheat landraces.

The comparison between Ethiopian *durum* and *turgidum* wheat landraces based on genetic diversity showed that *durum* was more diverse than *turgidum* (Tables 4 and 7). The observation that *durum* and *turgidum* shared common bands makes it clear that they are much related, which might be explained by sharing common ancestor, emmer wheat (*T. monococcum*) (Grubben and Soetjipto, 1996) and by frequent exchange of genetic materials in the Ethiopian agricultural practice. It is also possible that the *durum* cv groups have diverged from the *turgidum* by accumulation of specific alleles, especially alleles that control some morphological traits. The difference that was observed in allele number between *durum* (65%) and *turgidum* (35%) might be due to the smaller sample size (Tables 4 and 7).

The dendrogram based on similarities of the 150 OTUs (Figs. are not shown) showed a strong clustering of cultivars, with the exception of the Ethiopian cultivar Arendato (ET2), which displayed far higher similarity with the landraces than the other three cultivars. This indicates that the ET2 cultivar might originate from Ethiopian landraces. The dendrogram revealed some differentiation of the *durum* and *turgidum* OTUs. Clustering of either similar *turgidum* or *durum* plants was mainly observed in rather related genotypes within accessions. Assignment of the OTUs to either *durum* or *turgidum* cv groups was equivocal in a few cases when representative characteristics were intermediate. Such intermediates are common for Ethiopian wheat accessions and were previously described in Hailu Mekbib and Giorgis Habte Mariam (1990) and Tesfaye Tesmma *et al*. (1991). No obvious clustering was observed for altitude and sub-region groups.

The isozyme work on barley landraces suggests that extreme altitude greater than 3500 m are poor in terms of genetic diversity (Zemede Asfaw, 1989; Abebe Demissie and Asmud, 1997). However, in the present research no strong differentiation between altitude groups was observed.

CONCLUSION

AFLP was observed to have usually restricted number of bands from 50 to 100, while microsatellite had no such restriction. Moreover, unlike microsatellite marker, AFLP markers do not detect heterozygocity within species. Other than these differences, AFLP and microsatellite markers were suitable to assess genetic diversity among genotypes and within species level, respectively. Finally it can be concluded that AFLP and microsatellite markers have enabled us to detect genetic diversity in tetraploid wheat.

ACKNOWLEDGEMENT

The author would like to thank Prof. Carlos Alonso-Blanco, Dr. Ben Vosman, Dr. Hans de Jong and Prof. Maarten Koornneef for their valuable advice that helped to select versatile markers for wheat diversity assessment study and critically reading this manuscript. Many thanks to Dr.Gerard Bredemeyer for his technical advice during conducting the molecular laboratory work in microsatellite. Special thanks to Dr. Ronald van den Berg and Mr Gerrit Gort, for their statistical advice during data analysis and critically reading the manuscript.

REFERENCES

Abebe Demissie and Asmud, B. (1997). Geographical, altitude and agro-ecological differentiation of isozyme and hordein genotypes of landrace barleys from Ethiopia: implications to germplasm conservation. *Genet. Resour. Crop. Ev.* **44**: 43 – 55.

Allen, P.J., Amos, W., Pomeroy, P.P. and Twiss, S.D. (1995). Microsatellite variation in grey seals (*Halichoerus grypus*) shows evidence of genetic differentiation between two British breeding colonies. *Mol. Ecol.* **4**: 653 – 662.

Barrett, B.A. and Kidwell, K.K. (1998). AFLP-based genetic diversity assessment among wheat cultivars from the Pacific Northwest. *Crop Sci.* **38**: 1261 – 1271.

Bennett, E. (1970). Adaptation in wild and cultivated plant populations. In: **Genetic resources in plant-their exploration and conservation,** pp 115 – 130 **(**Frankel, O.H. and Bennett, eds.). Blackwell Scientific Publ., Oxford and Edinburgh,.

Bowcock, A.M., Ruiz, L.A., Tomfohrde, J., Minch, E., Kidd, J.R. and Cavalli, S.L.L. (1994). High resolution of human evolution with polymorphic microsatellites. *Nature* **368**: 455 – 457.

Bredemeijer, G.M.M., Arens, P. and Wouters, D. (1998). The use of semi-automated fluorescent microsatellite analysis for tomato cultivars identification. *Theor. Appl. Genet.* **97**: 584 – 590.

Bruford, M.W. and Wayne, R.K. (1993). Microsatellites and their application to population genetic studies. *Curr. Opin. Genet. Dev.* **3**: 939 – 943.

Byerlee, D. and Fischer, K. (2001). Accessing modern science: Policy and institutional option for agricultural biotechnology in developing countries. *IP Strategy Today* No.1, www.biodevelopments.org/ipstl.pdf.

Chakraborty, R. (1984). Detection of non-random association of alleles from the

distribution of the number of heterozygous loci in a sample. *Genetics* **108**: 719 – 731.

DeVries, J. and Toenniessen, G. (2001). Biotechnology: Expanded possibilities. In: **Securing the harvest, biotechnology, breeding and seed systems for African crops**, pp 59-74 (DeVries, J. and Toenniessen, G., eds.). CABI publishing, Rockefeller Foundation. New York, USA.

Edwards, A., Civitello, A., Hammond, H.A. and Caskey, C.T. (1991). DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. *Am. J. Hum. Genet.* **49**: 746 – 756.

Edwards, A., Hammond, H.A., Jin, L., Caskey, C.T. and Chakraborty, R. (1992). Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups. *Genomics* **12**: 241 – 253.

Forbes, S.H., Hogg, J.T., Buchanan, F.C., Crawford, A.M. and Allendorf, F.W. (1995). Microsatellite evolution in congeneric mammals: Domestic and bighorn sheep. *Mol. Biol. Evol.* **12**: 1106 – 1113.

Gottelli, D., Sillero, Z.C., Applebaum, G.D., Roy, M.S., Girman, D.J., Garcia-Moreno, J., Ostrander, E.A. and Wayne, R.K. (1994). Molecular genetics of the most endangered canid: the Ethiopian wolf *Canis simensis*. *Mol. Ecol.* **3**: 301 – 312.

Grubben, G.J.H. and Soetjipto, P. (1996). Cereals. In: **Plant resources of Southeast Asia No.10.** pp 136 – 143 (Grubben, G.J.H. and Soetjipto, P., eds.) Backhuys Publ., Leiden, the Netherlands.

Harlan, J.R. (1971). Agricultural origins: Centers and noncenters. *Science* **74**: 468-473.

Harlan, J. R. (1992). Crops and Man. American Society of Agronomy and Crop Science Society of America, Madison, WI: 295. Wisconsin, USA.

Hailu Mekbib and Giorgis Habtemariam, (1990). Evaluation and utilization of Ethiopian wheat germplasm. In: **Wheat genetic resources**, pp 178 – 185 (Srivastava, J.P. Damania, A.B., eds.). John Wiley and Sons, Chichester, UK.

Jana, S. (1993). Utilization of biodiversity from *in situ* reserves, with special references to wild wheat and barley. In: **Biodiversity and wheat improvement**, pp 311 – 323 (Srivastava, J.P. and Damania, A.B., eds.). ICARDA. John wiley and Sons, New York.

John, R., Law, Paolo, D., Robert, M.D., Koebner, J.C. and Robert, J.C. (1998). DNA profiling and plant variety registration III: The statistical assessment of distinctness in wheat using amplified fragment length polymorphisms. *Euphytica* **102**: 335 – 342.

Karp, A., Ole, S. and Marcello, B. (1996). Molecular techniques in the assessment of botanical diversity. *Ann. Bot-London* **78**: 143 – 149.

Mehari Zewdie (1994). Current status of germplasm holdings at PGRC/E. PGRC/E germplasm newsletter. Addis Ababa, Ethiopia, No 2:1-5

Moshe, F. (1976). Wheats. In: **Evolution of crop plants,** pp 120–128 (Simmonds, N.W., ed.). Longman London and New York.

Nei, M. (1973). Analysis of gene diversity in sub-divided populations. *Proc. Natl. Acad. Sci.* **70**: 3321 – 3323.

Newbury, H.J. and Ford-Lloyd, B.V. (1997). Estimation of genetic diversity. In: **Plant genetic conservation-the *in situ* approach**, pp 192–206 (Maxed, N., Ford-Lloyd, B.V. and Hawkes, J.G., eds.). Chapman, Hall, London.

Paetku, D. and Strobeck, C. (1994). Microsatellite analysis of genetic variation in black bear accessions. *Mol. Ecol.* **35**: 489 – 495.

Plaschke, J., Ganal, M.W. and Röder, M.S. (1995). Detection of genetic diversity in closely related bread wheat using microsatellite markers. *Theor. Appl. Genet.* **91**: 1001 – 1007.

Qi, X. and Lindhout, P. (1997). Development of AFLP markers in barley. *Mol. Genet.* **254**: 330 – 336.

Rao, M.V. (1974). Wheat. In: **Evolutionary studies in world crops, diversity and change in the Indian sub-continent**, pp 33-45 (Hutchinson, J., ed.).Cambridge University press.

Röder, M.S., Korzun, V., Wendehake, K., Plaschke, J., Tixier, M.H., Leroy, P. and Ganal, M.W. (1998). A microsatellite map of wheat. *Genetics* **149**: 2007 – 2023.

Roger, P.E., James, W., McNicol, Eileen, B., Allan, B., Pat, L., Bill, T. and Wayne, P. (1997). The use of AFLP to examine genetic relatedness in barley. *Mol. Breeding* **3**: 359 – 369.

Roy, M.S., Gffen, E., Smith, D., Ostrander, E.A. and Wayne, K. (1994). Patterns of differentiation and hybridisation in North American wolf like canids, revealed by analysis of microsatellite loci. *Mol. Bio. Evol.* **11**: 553 – 570.

SAS (1989). SAS statistical software. SAS Institute, Cary, NC, version 6.

Scribner, K.T., Arntzen, J.W. and Burke, T. (1994). Comparative analysis of intra- and inter population genetic diversity in *Bufo bufo*, using allozyme, single locus microsatellite, minisatellite, and multilocus minisatellite data. *Mol. Biol. Evol.* **11**: 737 – 748.

Tesfaye Tessma, Getachew Belay and Melaku Worede (1991). Morphological diversity in tetraploid wheat landrace accessions from the central highlands of Ethiopia. *Hereditas* **114**: 171 – 176.

Tsegaye Seyfu, Tesfaye Tesemma and Getachew Belay (1996). Relationships among tetraploid wheat landrace accessions revealed by isozyme markers and agronomic traits. *Theor. Appl. Genet.* **93**: 600 – 605.

Van der Beek, J.G., Verkerk, R., Zabel, P. and Lindhout, P. (1992). Mapping strategy for resistance genes in tomato based on RFLPs between cultivars: Cf9 (resistance to *Cladosporium fulvum*) on chromosome 1. *Theor. Appl. Genet.* **84**: 106 – 112.

Vavilov, N.I. (1951). The origin, variation, immunity and breeding of cultivated plants. *Chron. Bot.* **13**: 1 – 364.

Vos, P. (1993). Selective restriction fragment amplification: A general method for DNA fingerprinting. European patent Application number: 92402629.7, Publication number 0534 858.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van de Lee, T. and Hornes, M. (1995). AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Res.* **23**(21): 4407 – 4414.

Wang, R., Guegler, K., Labrie, S.T., and Crawford, N.M. (2000). Genomic analysis of nutrient responses in *Arabidopsis* reveals diverse expression patterns and novel metabolic and potential regulatory genes induced by nitrate. *Plant cell* **12**:1491-1510.

Zemede Asfaw (1989). Relationship between spike morphology, hordeins and altitude within Ethiopian barley, *Hordeum vulgare* L. (Poaceae). *Hereditas* **110**: 203 – 209.