# Genetic Variability in Barley (*Hordeum vulgare* l.) Landraces from Ethiopia as Measured by Morphological Characters and SDS-page of Seed Storage Proteins

**Alemayehu Assefa[1], M.T. Labuschagne[2] and H. Maartens[2]**

[1]*Ethiopian Institute Agricultural Research, Holetta Research Center;* [2]*Unicersity of the Free State, Department of Plant Sciences, P.O. Box 339, Bloemfontein 9300, South Africa*

## Abstract

*Data on 44 barley landraces comprising collections and farmers' cultivars from north Shewa, Ethiopia were studied for variability in morphological characters and Sodium Dodecyl Sulphate Polyacrylamide Gel Electophoresis (SDS-PAGE) of seed storage proteins. The phenotypic frequencies of morphological characters (qualitative and quantitative) were analysed by the Shanon Weaver diversity index (H') to estimate within landrace genetic variability for individual characters. Variability for seed protein banding patterns was assessed by SDS-PAGE. Both morphological and SDS-PAGE data demonstrated the variability existing in the landraces. H' values pooled over morphological characters ranged from 0.12 to 0.58. Among the qualitative characters, landraces showed higher levels of polymorphism for spike type than for kernel color, spike density and caryopsis type (covered or naked). Caryopsis type was the least diverse character observed. Diversity for quantitative characters pooled over landraces was generally very high especially for number of seeds spike[-1] and days to maturity with respective H' values of 0.90 and 0.98. SDS-PAGE data based on representative lines from each landrace showed very low to high within landrace variability for banding patterns. Lines from landraces differed from each other in number and migration distances of bands. Some landraces that looked uniform for spike morphology also showed differences in banding patterns. It was also observed, on the other hand, that some landraces displaying different spike characters and hence assumed to exhibit differences of comparable magnitude in storage protein variability did not reveal much differences. Variability between landraces was higher than within landraces and variability within farmers' cultivars was lower than within accessions. Clustering results of landraces from SDS-PAGE data were different in composition from those formed by morphological characters. Clustering from morphological data highlighted distinct grouping of landraces based on similarities in morphological characters whereas SDS-PAGE data did not depict such distinctness.*

## Introduction

Knowledge of genetic diversity in the crop gene pool is central to the development of effective *ex situ* and *in situ* germplasm conservation strategies. Evaluation of genetic diversity levels among adapted elite germplasm can also provide predictive estimates

of genetic variation among segregating progeny for pure line cultivar development (Manjarrz-Sandoval et al., 1997), for parental selection in breeding programs (Souza & Sorrels, 1991) and may estimate the degree of heterosis in progeny of some parental combinations (Barbosa-Neto et al., 1996; Cox & Murphy, 1990). Crosses made between genetically distant genotypes within major clusters of adapted cultivars are expected to produce higher variances for quantitatively inherited characters in segregating populations than crosses between closely related cultivars (Cox et al., 1985). Hence, information on levels and patterns of genetic diversity among adapted germplasm sources can be useful for identifying diverse parental combinations to create segregating progenies with maximum genetic variability for selection. Thus by selecting parents first on the basis of performance *per se* and subsequently, making crosses only between genetically divergent parents, breeders could focus their resources on the most promising populations. This is of great help not only for new breeders unfamiliar with the available germplasm, but also for the experienced breeders who need criteria for selecting well performing parents that have not been used in crossing programs.

Protein markers such as isozyme variants of esterases (Kahler and Allard, 1970; Havid and Nielson, 1977), allozymes (Bekele, 1983a), and hordeins (Doll & Brown, 1979; Shewry et al., 1978b, Shewry et al., 1978a) have been employed to assess genetic diversity in wild and cultivated barley populations. As genetic markers, these proteins are characterized by a high level of polymorphism, limited environmental influence on their electrophoretic pattern, simple genetic control, a complex molecular basis for genetic diversity, and homologies between storage proteins that extend across taxa (Gepts, 1990). Quantitative morphological characters, on the other hand, often do not portray genetic relationships because of environmental interactions, epistatic interactions and coding by an unknown number of genes (Smith & Smith, 1989). Attractive features of quantitative characters out weigh the disadvantages because germplasm collections or breeders crossing block entries can often be used for clustering (Jain et al., 1975; Spagnoletti & Qualset, 1987), information on quantitative characters adds to an understanding of ideotype-performance relations, and heterosis may show closer association with distance measures based on such characters (Cox & Murphy, 1990). Therefore the two measures of variability shall be used complementary in order to have full understanding of variability in germplasm materials.

In Ethiopian circumstances, the use of biochemical criteria such as esterase and acid phosphatase (Bekele, 1983a;b), flavonoids (Bekele, 1984), and hordeins (Asfaw, 1989a;b Demissie & Bjornstad, 1997) have been used for the estimation of diversity of barley landraces. Variability for spike type, seed color, spike density, and other phenological characters has been documented (Negassa, 1985; Asfaw 1988; Engels, 1991; Demissie & Bjornstad, 1996). Although the information generated increased knowledge on barley landraces of Ethiopia, it had limited practical implications from a breeding point of view since the evaluations were not systematic and region specific. Moreover, complementary use of morphological characters and biochemical or molecular markers to assess variability in landraces is lacking. The aim of this study was to assess variation within and between barley landraces from north Shewa in

Ethiopia with the help of morphological and SDS-PAGE data to generate information that facilitates the efficient utilization of landraces from specific adaptation domains. The study focused on farmers' cultivars and collections from areas where these cultivars are grown.

# Materials and Methods

## Greenhouse Experiment

Materials for this study are shown in Table 1. The 44 landraces were grown in 2000 at the University of Free State in pots in a greenhouse, each replicated three times. Eight seeds were planted per pot and were thinned to five later. Daytime temperature in the green house was 20°C throughout the growing period. Scoring for 10 quantitative and qualitative characters was done from five random main plants from each of the cultivars and accessions in each replication totaling 15 plants per accession or farmers' cultivars. Only two landraces were represented by 10 plants and eight by 14. The characters scored were kernel row number (two, six, or irregular), spike density (lax or dense), kernel color (white, black, purple or gray), kernel covering (covered or naked), days to heading, days to maturity, plant height (cm), spike length (cm), number of seeds spike$^{-1}$ and grain yield spike$^{-1}$(gm).

Table 1. List of farmers' cultivars and accessions from north Shewa for the variability study

| No. | Cultivar/accession | Locality | No. | Cultivar/accession | Locality |
|-----|--------------------|----------|-----|--------------------|----------|
| 1 | Acc.296 | Girar Jarso | 23 | Acc.3676 | Kuyu |
| 2 | Acc.653 | Girar Jarso | 24 | Acc.4319 | Kuyu |
| 3 | Acc.659 | Girar Jarso | 25 | Acc.4320 | Kuyu |
| 4 | Acc.1551 | Girar Jarso | 26 | Acc.4601 | Kuyu |
| 5 | Acc.1552 | Girar Jarso | 27 | Mage | Kimbibit |
| 6 | Acc.1570 | Girar Jarso | 28 | Kessele | Kimbibit |
| 7 | Acc.1814 | Girar Jarso | 29 | Tikur Gebs | Ankober-Mezezo |
| 8 | Acc.1822 | Girar Jarso | 30 | Feres Gama | Kimbibit |
| 9 | Acc.3679 | Girar Jarso | 31 | Bukura | Kimbibit |
| 10 | Acc.4959 | Girar Jarso | 32 | Feleme | Kimbibit |
| 11 | Acc.4964 | Girar Jarso | 33 | Netch Gebs | Kimbibit |
| 12 | Acc.4970 | Girar Jarso | 34 | Demoye | Kimbibit |
| 13 | Acc.973 | Wuchale | 35 | Key Ferke | Ankober-Mezezo |
| 14 | Acc.1182 | Wuchale | 36 | Acc.3395 | Kimbibit |
| 15 | Acc.976 | Wuchale | 37 | Acc.1017 | Kimbibit |
| 16 | Acc.2812 | Wuchale | 38 | Acc.144 | Kimbibit |
| 17 | Acc.984 | Debre Libanos | 39 | Acc.1609 | Kimbibit |
| 18 | Acc.987 | Debre Libanos | 40 | Netch Ferke | Ankober-Mezezo |
| 19 | Acc.4993 | Debre Libanos | 41 | Yeferenge Gebs | Ankober-Mezezo |
| 20 | Acc./1153 | Were Jarso | 42 | Tolese | Degem |
| 21 | Acc.1156 | Were Jarso | 43 | Baleme | Welmera |
| 22 | Acc.3151 | Kuyu | 44 | Haddo | Degem |

The phenotypic frequencies of the characters were analyzed by the Shannon-Weaver diversity index to estimate the diversity of each character. The Shannon-

Weaver diversity index (H') as described by Jain et al.(1975) and Negassa (1985) is given as $H' = -\Sigma Pi\ log_e Pi$ where n is the number of phenotypic classes for a character and Pi is the relative frequency or proportion of total number of genotypes in the ith category or class of the Kth character. H' was determined for individual characters. Each value of H' was divided by its maximum value, $log_e n$ in order to keep it in the range of 0-1. The average diversity ($\overline{H}$') over K traits was estimated as $\overline{H}' = \Sigma H'/K$. Landraces with different spike types were split into their component spikes and cluster analysis was performed on data for morphological characters to see genetic relationships between landraces.

## SDS-PAGE of Seed Storage Proteins

Single kernel extraction was employed throughout this study. Individual seeds were ground with mortar and pestle and the ground kernels were transferred to 1.5ml Eppendorf tubes. Extraction buffer (500 µl containing urea, 2 % v/v 2 β-mercaptoethanol as a reducing agent, and distilled water) was added to each tube for the extraction of storage protein of barley (hordein). The Eppendorf tubes were put in a hot water bath at 60°C for one hour. In the absence of 2-mercaptoethanol relatively less of the medium molecular weight hordein bands were extracted especially from seed containing a high level of nitrogen (Shewry et al., 1977; Shewry et al., 1978a). Samples of the clear supernatant, obtained after centrifugation at 10 000 rpm for two to three minutes, were diluted with sample buffer and centrifuged for two minutes prior to electrophoretic fractionation. The sample buffer was prepared from 1 g Tris and 90 ml 50 % n-propanol titrated to pH 8.0 with NaHCL to which 40 g glycerol, 2 g SDS and 0.02 g bromophenol blue were added.

The gel system adapted was that described by Singh et al. (1991) with some modifications. Acrylamide separating gel (pH 8.0) and acrylamide stacking gel (pH 6.8) were used. Fourty microliter Temed and 100 µl 10 % APS were used as catalysts. A twenty sample well former (0.75 mm perspex comb) was inserted into the stacking gel and left overnight to polymerize. Hordein extracts from individual kernels (40 µl) were loaded into each sample well with a micropipette. Twenty slot gels were run with standard cultivar Clipper applied in slots of the first two, the tenth, and the last two slots and the 15 samples from each landrace were placed in the remaining sample wells to ensure that no samples were run at the slots adjacent to the edge of the gels where edge effects may be observed. Two gels were run simultaneously on one Biorad vertical gel electrophoresis apparatus at a constant current of 66mA and 15 °C constant temperature of the cooling system. Each run took about 3:30 to 3:45 hours until the dye front reached the bottom of the gel. Upon completion of each run, gels were removed from the gel cassette and put in a plastic box containing a fixing solution (50 ml glacial acetic acid, 200 ml methanol and 250 ml distilled water) for more than an hour. Staining was done overnight with 0.1 g Coomassie Brilliant Blue solution containing 30g trichloroacetic acid and 200 ml distilled water.

## Statistical Analysis

Distances of the different polypeptide proteins of the 15 lines sampled from each landrace were pooled and presence (1) or absence (0) of a band was assigned to use as a binary data matrix to estimate genetic distances among the landraces. Presence or absence of bands was scored by spike types for landraces with different spike types for the purpose of comparison of clusters from morphological and electrophoresis data. Protein bands were scored as absence (0) and presence (1) and entered for each landrace separately as a binary data matrix for statistical analysis. Genetic distance was calculated using NCSS 2000 software applying pair-wise comparison using the formula *1-F=1-[2n$_{xy}$/(n$_x$+n$_y$)]* as described by Nei & Li (1979) where F is the ratio of shared bands between x and y, 2n$_{xy}$ is the number of shared bands, and n$_x$ and n$_y$ are the number of bands observed in individual x and y, respectively. The unweighted pair group method using arithmetic averages (UPGMA) cluster analysis was used to estimate genetic distances among components of each landrace and a dendrogram was constructed using the pair-wise genetic distance values to see genetic relationships between landraces.

# Results and Discussion

## Genetic Variability within Landraces

Variability for morphological characters and seed storage proteins within landraces are shown in Tables 2 and 3, respectively. Morphological characters differed in amount of variation between landraces. Mean diversity within landraces pooled over all characters (qualitative and quantitative) ranged from 0.12 to 0.58, and eight landraces had mean diversity larger than 0.50 (Table 2). Among the qualitative characters, variation for spike type (two-rowed, irregular or six-rowed) was high in many landraces. Of the landraces, 34 % showed diversity index values in the range of H′=0.55 to H′=0.92 for this character. The overall diversity of spike types pooled over all landraces was also very high (H′=0.89). Among the localities, landraces from Degem, Wuchale, Girar Jarso and Kuyu were highly diverse with H′=0.96, 0.93, 0.91 and 0.87, respectively. Diversity for spike types at Ankober-Mezezo was very low (H′=0.39). Within landraces variability for kernel color was generally low except in landraces from Kuyu. White kernel color was predominant over the others.

Assessment of variability by SDS-PAGE of seed storage proteins based on 15 samples from each landrace showed very low to high levels of genetic variability within landraces with mean genetic distance values ranging from 0.353 in Tikur Gebs to 0.678 in acc. 3676. The mean genetic distance value pooled over the 44 landraces was 0.63. Out of the 44 landraces six (Demoye, Feleme, Feres Gama, Kessele, tikur Gebs and acc. 1609) had very low mean genetic distance values in the range of 0. 353 in Tikur Gebs to 0.494 in Feleme while the rest of the landraces had values > 0.530. Some landraces, the farmers' cultivars Tikur Gebs, Demoye, Nech Gebs, Feres Gama, Feleme, and Kessele in particular, comprised a significant proportion of genetically identical lines where as high as 46 % of the pair-wise comparisons between individuals in the landraces were identical (Table 3).

Table 2. Estimates of diversity indices within 44 barley landraces from different localities of north    Shewa, Ethiopia, 2000

| Landraces | N | Spike type | Kernel color | Spike density | Caryopsis type | Spike length | PLH | DMA | NS/SP | $\overline{H'}$ ± SE |
|---|---|---|---|---|---|---|---|---|---|---|
| 144 | 14 | 0.00 | 0.00 | 0.46 | 0.00 | 0.98 | 0.68 | 0.63 | 0.71 | 0.43±0.14 |
| 1017 | 15 | 0.63 | 0.00 | 0.00 | 0.00 | 0.00 | 0.63 | 0.46 | 0.90 | 0.32±0.13 |
| 1609 | 15 | 0.45 | 0.00 | 0.53 | 0.00 | 0.84 | 0.23 | 0.61 | 0.35 | 0.38±0.10 |
| 3395 | 15 | 0.00 | 0.00 | 0.23 | 0.00 | 0.37 | 0.89 | 0.58 | 0.78 | 0.36±0.13 |
| Bukura | 10 | 0.55 | 0.00 | 0.55 | 0.00 | 0.00 | 0.56 | 0.87 | 0.55 | 0.39±0.11 |
| Demoye | 15 | 0.35 | 0.00 | 0.23 | 0.00 | 0.97 | 0.78 | 0.23 | 0.24 | 0.35±0.11 |
| Feres | 15 | 0.00 | 0.00 | 0.72 | 0.00 | 0.97 | 0.58 | 0.61 | 0.57 | 0.43±0.13 |
| Kessele | 12 | 0.25 | 0.00 | 0.00 | 0.00 | 0.81 | 0.51 | 0.58 | 0.51 | 0.34±0.11 |
| Mage | 15 | 0.23 | 0.00 | 0.00 | 0.00 | 0.99 | 0.63 | 0.58 | 0.23 | 0.33±0.13 |
| Nech Gebs | 15 | 0.23 | 0.00 | 0.38 | 0.00 | 0.78 | 0.61 | 0.61 | 0.78 | 0.43±0.11 |
| Feleme | 14 | 0.00 | 0.00 | 0.55 | 0.00 | 0.58 | 0.23 | 0.23 | 0.35 | 0.24±0.08 |
| Tikur Gebs | 15 | 0.35 | 0.18 | 0.00 | 0.00 | 0.97 | 0.91 | 0.92 | 0.89 | 0.53±0.53 |
| Key Ferke | 15 | 0.23 | 0.00 | 0.00 | 0.00 | 0.72 | 0.63 | 0.00 | 0.00 | 0.20±0.09 |
| Nech | 14 | 0.61 | 0.00 | 0.97 | 0.00 | 0.72 | 0.63 | 0.63 | 0.81 | 0.54±0.13 |
| Yeferenge | 15 | 0.00 | 0.00 | 0.23 | 0.00 | 0.91 | 0.55 | 0.00 | 0.84 | 0.35±0.14 |
| *296* | 15 | 0.35 | 0.00 | 0.58 | 0.00 | 0.84 | 0.95 | 0.84 | 0.78 | 0.54±0.14 |
| 653 | 14 | 0.47 | 0.00 | 0.05 | 0.00 | 0.87 | 0.82 | 0.72 | 0.75 | 0.46±0.14 |
| 659 | 15 | 0.23 | 0.00 | 0.00 | 0.00 | 0.56 | 0.46 | 0.58 | 0.00 | 0.23±0.09 |
| 1551 | 15 | 0.61 | 0.00 | 0.63 | 0.00 | 0.97 | 0.35 | 0.78 | 0.92 | 0.53±0.12 |
| 1552 | 15 | 0.00 | 0.00 | 0.35 | 0.00 | 0.56 | 0.00 | 0.53 | 0.00 | 0.18±0.09 |
| 1570 | 10 | 0.45 | 0.00 | 0.61 | 0.00 | 0.47 | 0.61 | 0.46 | 0.61 | 0.40±0.09 |
| 1814 | 14 | 0.47 | 0.00 | 0.62 | 0.00 | 0.00 | 0.96 | 0.64 | 0.70 | 0.43±0.43 |
| 1822 | 14 | 0.00 | 0.00 | 0.23 | 0.00 | 0.00 | 0.72 | 0.59 | 0.88 | 0.30±0.13 |
| 3679 | 15 | 0.92 | 0.47 | 0.00 | 0.00 | 0.91 | 0.67 | 0.61 | 0.77 | 0.55±0.13 |
| 4959 | 15 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.58 | 0.69 | 0.67 | 0.24±0.12 |
| 4964 | 15 | 0.00 | 0.36 | 0.00 | 0.00 | 0.37 | 0.35 | 0.58 | 0.73 | 0.30±0.10 |
| 4970 | 15 | 0.61 | 0.00 | 0.35 | 0.00 | 1.00 | 0.53 | 0.58 | 0.84 | 0.49±0.13 |
| *973* | 15 | 0.00 | 0.00 | 0.00 | 0.00 | 0.37 | 0.58 | 0.00 | 0.00 | 0.12±0.08 |
| 976 | 14 | 0.23 | 0.00 | 0.59 | 0.00 | 0.98 | 0.59 | 0.96 | 0.87 | 0.53±0.14 |
| 1182 | 15 | 0.68 | 0.00 | 0.35 | 0.00 | 0.56 | 0.57 | 0.58 | 0.90 | 0.46±0.11 |
| 2812 | 15 | 0.00 | 0.00 | 0.00 | 0.00 | 0.56 | 0.90 | 0.39 | 0.00 | 0.22±0.12 |
| 984 | 15 | 0.35 | 0.00 | 0.63 | 0.00 | 0.56 | 0.52 | 0.53 | 0.84 | 0.43±0.10 |
| 987 | 15 | 0.58 | 0.00 | 0.35 | 0.00 | 0.37 | 0.46 | 0.46 | 0.69 | 0.36±0.09 |
| 4993 | 15 | 0.61 | 0.00 | 0.00 | 0.00 | 0.37 | 0.58 | 0.58 | 0.61 | 0.34±0.10 |
| 3151 | 15 | 0.89 | 0.00 | 0.61 | 0.00 | 0.97 | 0.90 | 0.53 | 0.58 | 0.56±0.14 |
| 3676 | 14 | 0.47 | 0.36 | 0.87 | 0.00 | 0.94 | 0.74 | 0.23 | 0.56 | 0.52±0.11 |
| 4319 | 15 | 0.45 | 0.45 | 0.63 | 0.00 | 0.84 | 0.61 | 0.58 | 0.84 | 0.55±0.09 |
| 4320 | 15 | 0.58 | 0.62 | 0.97 | 0.00 | 0.72 | 0.23 | 0.58 | 0.88 | 0.57±0.11 |
| 4601 | 14 | 0.23 | 0.26 | 0.00 | 0.86 | 0.37 | 0.62 | 0.00 | 0.45 | 0.24±0.08 |
| 1153 | 14 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.81 | 0.34 | 0.00 | 0.27±0.15 |
| 1156 | 15 | 0.63 | 0.00 | 0.61 | 0.00 | 0.99 | 0.46 | 0.23 | 0.61 | 0.44±0.12 |
| Haddo | 11 | 0.95 | 0.00 | 0.43 | 0.00 | 0.62 | 0.53 | 0.43 | 0.78 | 0.47±0.12 |
| Tolese | 15 | 0.67 | 0.00 | 0.46 | 0.00 | 0.91 | 0.78 | 0.92 | 0.78 | 0.56±0.13 |
| Baleme | 11 | 0.59 | 0.00 | 0.00 | 0.00 | 0.94 | 0.86 | 0.63 | 0.25 | 0.41±0.14 |
| **North** | **630** | **0.89** | **0.76** | **0.88** | **0.33** | **0.73** | **0.89** | **0.84** | **0.96** | **0.76±0.09** |

Table 3. Mean and range of genetic distances between lines within barley landraces as revealed by SDS-PAGE of seed storage proteins

| No | Landraces | Genetic distances | | | | | Identical pairs (%) |
|---|---|---|---|---|---|---|---|
| | | Range | Mean | <0.50 (%) | 0.50-0.70 (%) | >0.70 (%) | |
| 1. | Acc.976 | 0.000-0.790 | 0.618 | 11 | 60 | 29 | 1 |
| 2. | Acc.1017 | 0.342-0.840 | 0.606 | 23 | 55 | 22 | - |
| 3. | Acc.1153 | 0.420-0.874 | 0.634 | 13 | 61 | 26 | - |
| 4. | Acc.1182 | 0.387-0.866 | 0.634 | 6 | 65 | 29 | - |
| 5. | Acc.144 | 0.242-0.845 | 0.622 | 15 | 62 | 23 | - |
| 6. | Acc.1552 | 0.229-0.888 | 0.625 | 13 | 56 | 31 | - |
| 7. | Acc.1822 | 0.218-0.845 | 0.635 | 8 | 72 | 20 | - |
| 8. | Acc.2812 | 0.00-0.919 | 0.608 | 26 | 46 | 28 | 1 |
| 9. | Acc.296 | 0.346-0.894 | 0.664 | 9 | 53 | 38 | - |
| 10. | Acc.3151 | 0.408-0.866 | 0.618 | 12 | 56 | 32 | - |
| 1.1. | Acc.3395 | 0.408-0.841 | 0.663 | 5 | 53 | 42 | - |
| 12. | Acc.3676 | 0.408-0.912 | 0.678 | 5 | 47 | 48 | - |
| 13. | Acc.3679 | 0.235-0.881 | 0.571 | 40 | 25 | 35 | - |
| 14. | Acc.4319 | 0.258-0.856 | 0.640 | 10 | 58 | 32 | - |
| 15. | Acc.4601 | 0.288-0.912 | 0.659 | 9 | 44 | 47 | - |
| 16. | Acc.4959 | 0.447-0.806 | 0.624 | 6 | 67 | 28 | - |
| 17. | Acc.4964 | 0.267-0.845 | 0.581 | 22 | 59 | 19 | - |
| 18. | Acc.4970 | 0.353-0.829 | 0.614 | 9 | 68 | 23 | - |
| 19. | Acc.4993 | 0.342-0.907 | 0.630 | 18 | 52 | 30 | - |
| 20. | Acc.987 | 0.365-0.856 | 0.646 | 6 | 63 | 31 | - |
| 21. | Baleme | 0.000-0.935 | 0.648 | 13 | 35 | 52 | 4 |
| 22. | Bukura | 0.000-0.881 | 0.611 | 30 | 21 | 49 | 9 |
| 23. | Demoye | 0.000-0.816 | 0.356 | 62 | 22 | 16 | 34 |
| 24. | Feleme | 0.000-0.894 | 0.494 | 42 | 26 | 32 | 24 |
| 25. | Feres Gama | 0.000-0.100 | 0.474 | 59 | 11 | 30 | 24 |
| 26. | Kessele | 0.000-0.816 | 0.394 | 63 | 22 | 15 | 24 |
| 27. | Mage | 0.000-0.935 | 0.565 | 17 | 50 | 33 | 8 |
| 28. | Nech Ferke | 0.000-0.845 | 0.547 | 32 | 45 | 23 | 7 |
| 29. | Nech Gebs | 0.000-0.866 | 0.565 | 28 | 7 | 65 | 28 |
| 30. | Tikur Gebs | 0.000-0.866 | 0.353 | 46 | 19 | 35 | 46 |
| 31. | Tolese | 0.000-0.942 | 0.575 | 37 | 22 | 41 | 1 |
| 32. | Acc.1551 | 0.000-0.886 | 0.564 | 29 | 51 | 20 | 1 |
| 33. | Haddo | 0.000-0.881 | 0.585 | 30 | 39 | 31 | 6 |
| 34. | Acc.653 | 0.235-0.881 | 0.642 | 8 | 64 | 28 | - |
| 35. | Acc.**1**156 | 0.229-0.888 | 0.641 | 18 | 52 | 30 | - |
| 36. | Acc.984 | 0.267-0.755 | 0.551 | 31 | 57 | 12 | - |
| 37. | Acc.973 | 0.000-0.894 | 0.530 | 45 | 32 | 23 | 2 |
| 38. | Acc.1609 | 0.000-0.894 | 0.373 | 63 | 31 | 6 | 34 |
| 39. | Acc.1570 | 0.267-0.886 | 0.615 | 19 | 51 | 30 | - |
| 40. | Acc.1814 | 0.267-0.886 | 0.632 | 12 | 50 | 38 | - |
| 41. | Y. Gebs | 0.000-1.000 | 0.561 | 43 | 19 | 38 | 16 |
| 42. | Key Ferke | 0.000-1.000 | 0.574 | 39 | 27 | 34 | 11 |
| 43. | Acc.659 | 0.272-0.838 | 0.610 | 17 | 58 | 25 | - |
| 44. | Acc.4320 | 0.288-0.912 | 0.670 | 9 | 40 | 51 | - |

Accordingly, mean genetic distance within most of the farmers' cultivars was lower and the frequency of genetic distance less than 0.50 was higher when compared to many of the accessions denoting less variation within farmers' cultivars than within the accessions. Baleme and Bukura were the exceptions, however.

It was observed that landraces displaying different spike types and that were highly variable for some of the qualitative characters (acc.1017, acc.1609, acc.3679, acc.4970, and acc.3151, for example) and hence were assumed to exhibit differences of comparable magnitude in protein variability did not reveal high differentiation among lines. On the other hand SDS-PAGE uncovered components within landraces different from each other which otherwise were difficult to distinguish phenotypically. Worth mentioning are some of the farmers' cultivars (Feres Gama, Nech Gebs, Feleme, and Kessele) which look uniform in spike morphology but showed slight differentiation. Among the localities, landraces from Kuyu displayed relatively higher genetic variability with a mean genetic distance of the components ranging from 0.618 in acc. 3151 to 0.678 in acc. 3676.

## Variability between Landraces from SDS-PAGE

The mean genetic distance estimate among all landraces was 0.640 with values ranging from 0.235 to 0.881. Only 12.24 % of the possible pair-wise comparisons had genetic distance values $\leq$ 0.500 and 29.33 % had values greater than 0.700. The rest (58.43 %) of the pair-wise comparisons had values in the range of 0.510 to 0.700 (Figure 1) demonstrating the existence of high genetic divergence among landraces.

Comparison of genetic distances of landraces between different localities showed a relatively closer genetic relationships between landraces of Degem vs Welmera (0.405$\pm$0.05), Were Jarso vs Kuyu (0.555$\pm$0.12) and Wuchale and Debre Libanose (0.568) (Table 5). On the other hand, distant genetic relationships were observed between landraces of Girar Jarso and Were Jarso (0.704$\pm$0.07), and that between landraces from Wuchale, Kimbibit, Ankober-Mezezo, and Kuyu with that of landraces from Degem/Welmera. Thirty five (53 %) out of the 66 pair-wise comparisons with the highest genetic distance were comprised of between landraces of Degem plus Welmera vs landraces from other localities mainly from Kimbibit and Ankober-Mezezo. Genetic distances among landraces within localities were generally lower than among localities (Table 5) and the lowest mean value (0.457$\pm$0.06) was observed among landraces at Debre Libanose. Only two landraces (Kessele and acc.1609(6R) from Kimbibit exhibited very high genetic distance. Distantly and closely related landraces sorted out of all possible pair-wise genetic distance comparisons are presented in Table 4. One can deduce from this result that apparent progress in genetic improvement may not be achieved from crosses involving parents that are genetically very close (No.1 to 34). Conversely, it would be possible to exploit variability from progenies involving parents that are genetically distant (No. 35 to 63) but adapted to similar environments provided that agronomically important lines are isolated for crossing. Genetic distance value merely provides information on the degree of relatedness of the landraces and in its own can not be a reflection of desirable                              agronomic                              traits.

Table 4. Pair-wise genetic distances (GD) among landraces with close or distant genetic relationships based on SDS-PAGE of storage proteins.

| | **Closely related landraces** | | | | **Distantly related landraces** | | |
|---|---|---|---|---|---|---|---|
| **No.** | **Landraces** | **GD** | **Loacality** | **No** | **Landraces** | **GD** | **Locality** |
| 1. | Acc.1551xacc.1552 | 0.235 | G/JarsoxG/Jarso | 35. | N.Frke(6R)xBaleme(2R) | 0.881 | ANK-MEZxWelmera |
| 2. | Acc.3679(IR)xacc.4970(IR) | 0.333 | G/JarsoxG/Jarso | 36. | Acc.1609(6R)xBaleme(2R) | 0.881 | KimbibitxWelmera |
| 3. | Acc.659xacc.1552 | 0.343 | G/JarsoxG/Jarso | 37. | K.FerkexBaleme(2R) | 0.881 | ANK-MezxWelmera |
| 4. | Acc.659xacc.3679(IR) | 0.343 | G/JarsoxG/Jarso | 38. | DemoyexHaddo(6R) | 0.881 | KimbibitXDegem |
| 5. | Acc.659xacc.4970(IR) | 0.343 | G/JarsoxG/Jarso | 39. | Acc.1156(6R)xacc.1017 | 0.881 | W/JarsoxKimbibit |
| 6. | Acc.1570xacc.1814 | 0.408 | G/JarsoxG/Jarso | 40. | Acc.1156(IR)xN.Ferke(6R) | 0.881 | W/JarsoANK-MEZ |
| 7. | Acc.1814x3679(IR) | 0.408 | G/JarsoxG/Jarso | 41. | Acc.4964xBaleme(IR) | 0.881 | G/JarsoxWelmera |
| 8. | Acc.1814xacc.4970(IR) | 0.408 | G/JarsoxG/Jarso | 42. | Acc.4959xacc.1156(IR) | 0.881 | G/JarsoxW/Jarso |
| 9. | Acc.3679xacc.3679(IR) | 0.408 | G/JarsoxG/Jarso | 43. | N.Ferke(IR)xBaleme(IR) | 0.849 | ANK-MEZxWelmera |
| 10. | Acc.3679xacc.4970(IR) | 0.408 | G/JarsoxG/Jarso | 44. | N.Ferke(6R)xBaleme(IR) | 0.849 | ANK-MEZxWelmera |
| 11. | Acc.4970(IR)xacc.4970(2R) | 0.408 | G/JarsoxG/Jarso | 45. | Acc.1609(6R)xHaddo(2R) | 0.849 | KimbibitxDegem |
| 12. | Acc.1570xacc.3679 | 0.408 | G/JarsoxG/Jarso | 46. | Acc.1609(6R)xBaleme(IR) | 0.849 | KimbibitxWelmera |
| 13. | Acc.4964xBukura | 0.235 | G/JarsoxKimbibit | 47. | Acc.1017xTolese | 0.849 | KimbibitxDegem |
| 14. | Acc.3679(IR)xacc.4601 | 0.333 | G/JarsoxKuyu | 48. | N.Ferke(IR)xBaleme(2R) | 0.816 | ANK-MEZxWelmera |
| 15. | Acc.973xN.Ferke(IR) | 0.333 | WuchalexANK-MEZ | 49. | Acc.1609(IR)xHaddo(6R) | 0.816 | KimbibitxDegem |
| 16. | Acc.976xacc.4319 | 0.333 | WuchalexKuyu | 50. | Acc.1609(IR)xTolese | 0.816 | KimbibitxDegem |
| 17. | Acc.984xacc.987(IR) | 0.333 | WuchalexD/Libanose | 51. | Acc.144xBaleme(IR) | 0.816 | KimbibitxWelmera |
| 18. | Acc.984xacc.987(6R) | 0.333 | WuchalexD/Libanose | 52. | K.FerkexHaddo(2R) | 0.849 | ANK-MEZxDegem |
| 19. | Acc.1552xFeleme | 0.408 | G/JarsoxKimbibit | 53. | K.FerkexHaddo(6R) | 0.849 | ANK-MEZxDegem |
| 20. | Acc.1570xTolese | 0.408 | G/JarsoxDegem | 54. | N.FerkexBaleme(IR) | 0.849 | ANK-MEZxWelmera |
| 21. | Acc.1570xHaddo(6R) | 0.408 | G/JarsoxDegem | 55. | BukuraxBaleme(IR) | 0.849 | KimbibitxWelmera |
| 22. | Baleme(2R)xBaleme(IR) | 0.235 | WelmeraxWelmera | 56. | Kesselexacc.1609(6R) | 0.849 | KimbibitxKimbibit |
| 23. | Acc.1156((IR)xacc.1156(6R) | 0.333 | W/JarsoxW/Jarso | 57. | Acc.4320xBaleme(IR) | 0.849 | KuyuxWelmera |
| 24. | Acc.1156(IR)xacc.3676 | 0.333 | W/JarsoxKuyu | 58. | Acc.3151(6R)xHaddo(2R) | 0.849 | KuyuxDegem |
| 25. | Acc.3679(IR)xacc.1153 | 0.408 | G/JarsoxD/Libanose | 59. | Acc.1156(6R)xacc.1609(IR) | 0.849 | W/JarsoxKimbibit |
| 26. | Acc.4959xacc.144 | 0.408 | G/JarsoxKimbibit | 60. | Acc.1156(6R)xacc.144 | 0.849 | W/JarsoxKimbibit |
| 27. | F.GamaxBukura | 0.333 | KimbibitxKimbibit | 61. | Acc.2812xHaddo(2R) | 0.849 | WuchalexDegem |
| 28. | BukuraxN.Ferke(IR) | 0.333 | KimbibitxANK-MEZ | 62. | Acc.4964xBaleme(2R) | 0.849 | G/JarsoxWelmera |
| 29. | Acc.1609(6R)xN.Ferke(6R) | 0.333 | KimbibitxANK-MEZ | 63. | Acc.973xBaleme(IR) | 0.849 | WuchalexWelmera |
| 30. | Baleme(IR)xHaddo(2R) | 0.333 | WelmeraxDegem | | | | |
| 31. | Acc.3679xacc.4601 | 0.408 | G/JarsoxKuyu | | | | |
| 32. | Acc.4970(IR)xacc.1153 | 0.408 | G/JarsoxD/Libanose | | | | |
| 33. | Acc.4970(IR)xHaddo(6R) | 0.408 | G/JarsoxDegem | | | | |
| 34. | Acc.4970(2R)xacc973 | 0.408 | G/JarsoxWuchale | | | | |

*Genetic Variability in Barley (Hordeum vulgare l.) Landraces from Ethiopia*

Table 5. Comparison of genetic distances (mean $\pm$SD) among landraces within and between localities

| | Within localities | Wuchale | D/Libanose | W/Jarso | Kimbibit | Ank-Mez | Kuyu | Degem | Welmera |
|---|---|---|---|---|---|---|---|---|---|
| G/Jarso | 0.543+0.10 | 0.612+0.07 | 0.564+0.07 | 0.650+0.11 | 0.623+0.09 | 0.631+0.09 | 0.653+0.09 | 0.599+0.11 | 0.683+0.09 |
| Wuchale | 0.561+0.08 | | 0.568+0.11 | 0.659+0.07 | 0.633+0.08 | 0.583+0.09 | 0.590+0.09 | 0.727+0.06 | 0.747+0.06 |
| D/Libanose | 0.457+0.06 | | | 0.576+0.08 | 0.647+0.09 | 0.616+0.07 | 0.592+0.08 | 0.665+0.05 | 0.656+0.09 |
| W/Jarso | 0.462+0.11 | | | | 0.709+0.11 | 0.675+0.10 | 0.559+0.11 | 0.636+0.04 | 0.579+0.06 |
| Kimbibit | 0.585+0.10 | | | | | 0.609+0.11 | 0.679+0.07 | 0.746+0.06 | 0.758+0.06 |
| Ank-Mez | 0.615+0.10 | | | | | | 0.648+0.08 | 0.747+0.09 | 0.757+0.09 |
| Kuyu | 0.605+0.11 | | | | | | | 0.703+0.07 | 0.714+0.06 |
| Degem | 0.571+0.09 | | | | | | | | 0.405+0.05 |

Ank-Mez = Ankober-Mezezo

Hence, evaluation for important agronomic traits and crossing among those distantly related landraces will help combine traits of economic significance and broad segregation of the characters concerned.
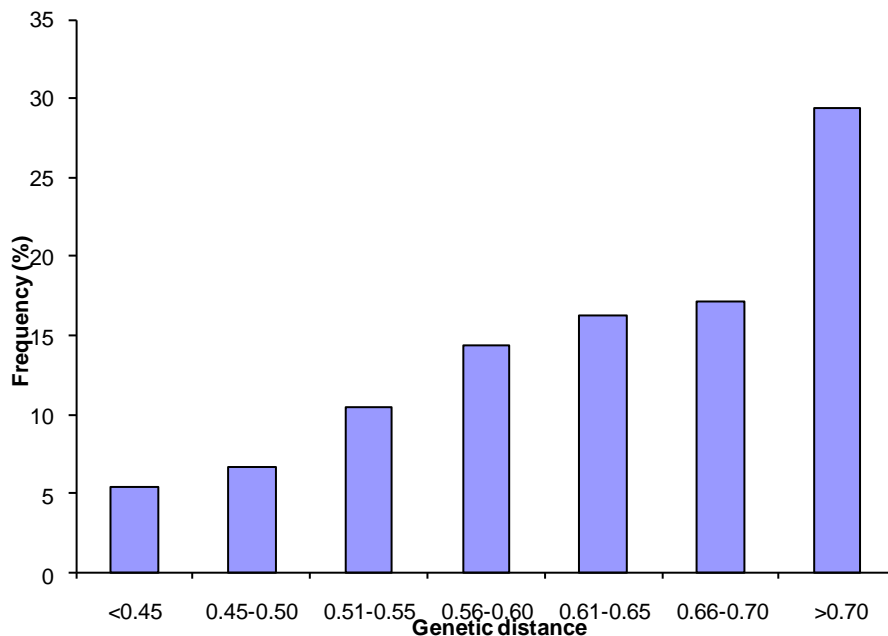


Figure 1. Frequency (%) distribution of genetic distances values among barley landraces from pair-wise comparisons resulting from SDS-PAGE of seed storage proteins.

The magnitude of genetic variability within some of the landraces (Table 3) was comparable to that of variability among landraces. The expectation, for self pollinated crops such as barley, would have been a higher level of genetic variability among landraces rather than within landraces because of restricted gene flow from plant to plant. However, landrace populations are connected by gene flow probably due to seed dispersal and this may bring low divergence among landraces. Papa et al. (2000) from RAPD and isozyme analyses, Nevo et al. (1983) from hordein data and Alemayehu & Parlevliet (1997) from a morphological diversity study found more variation within populations than among populations of barley. Low frequency of cross fertilization and rare mutation together with continued self fertilization, and incidental survival of volunteer plants from another landrace of a previous sowing could easily lead to the high level of within landraces variability (Alemayehu & Parlevliet, 1997). Tsegaye et al. (1996) from his study on durum wheat landraces also demonstrated that the inter-population diversity accounted for almost 15 % of the total diversity while 85 % of the total was due to the within landraces component. Hence, in this study, although genetic distance between landraces is predominantly larger than within landraces comparable variability observed within some of the landraces is not exceptional.

## Cluster Analysis

Clustering results of landraces from SDS-PAGE data were different in composition from those formed by morphological characters (Figures 2 & 3). Clustering from morphological data highlighted distinct grouping of landraces based on similarities in agronomic characters where as SDS-PAGE data did not depict such distinctness. Hierarchal cluster analysis on the basis of genetic distances from SDS-PAGE produced 10 main clusters each consisting two to 11 landraces and three landraces (acc.4320, Mage, and acc.1182) that appeared in their own in separate clusters (Figure 2). Most (82 %) of the landraces from Kimbibit locality appeared in clusters four and six, predominantly in the former and the four landraces from Welmera and Degem areas appeared in cluster nine. However, it is not possible to conclude that SDS-PAGE provided discrimination between landraces according to their origin because the clustering did not follow this trend throughout all the landraces which is true also for clusters resulting from morphological data (Figure 3). Demissie et al. (1998) from a RFLP study on barley landraces, Bekele (1984) from enzymatic (flavonoid pattern) and morphological data and Tsegaye et al. (1996) from isozyme and morphological study of durum wheat landraces also demonstrated no marked trend in clustering of landraces in relation to geographical distances implying that isolation by distance can not be a factor to bring about such differences in grouping.

## Association of Data from SDS-PAGE and Morphology

Some landraces that displayed high levels of phenotypic variability based on Shanon Weaver diversity index (H′) did not correspond with that of genetic variability at storage protein level. Correlation analysis between estimates of genetic distance based on SDS-PAGE data and morphology based distance values revealed a non significant association (r=0.03) at P> 0.05. Similarly, no significant association (r=0.21) was observed between mean genetic distance values within landraces from SDS-PAGE data and that of within landraces genetic variability based on the Shanoon Weaver mean diversity index values (H′). Clusters based on hordein and morphology data (Figures 2 & 3) produced different cluster groups with different components from each other although slight overlap existed. For instance Feres Gama, Feleme, and Nech Gebs appeared together in cluster I from morphological data and cluster VI from SDS-PAGE while Nech Ferke(6R), Nech Ferke(IR) and Demoye(IR) in cluster III and cluster I from morphological and SDS-PAGE data, respectively.

Similar studies in barley (Bekele, 1984; Asfaw, 1989b; Ruiz et al., 1997) and in durum wheat (Tsegaye et al., 1994; Tsegaye et al., 1996) showed very poor association between biochemical and/or molecular and morphological markers. This is because morphological traits, in particular the qualitative traits, are highly heritable and are controlled by a few genes with a major phenotypic effect, but they hardly represent all the genes in a plant (Gepts, 1990). For instance, two-rowed and six-rowed barley types are very distinct phenotypically and close genetic relationships may be assumed within six-rowed or two-rowed barley types rather than between two-rowed and six-rowed types. However, only a single recessive gene (*v*) is responsible for two-rowed barley becoming six-rowed. Hence, it is possible to find closer genetic relationships
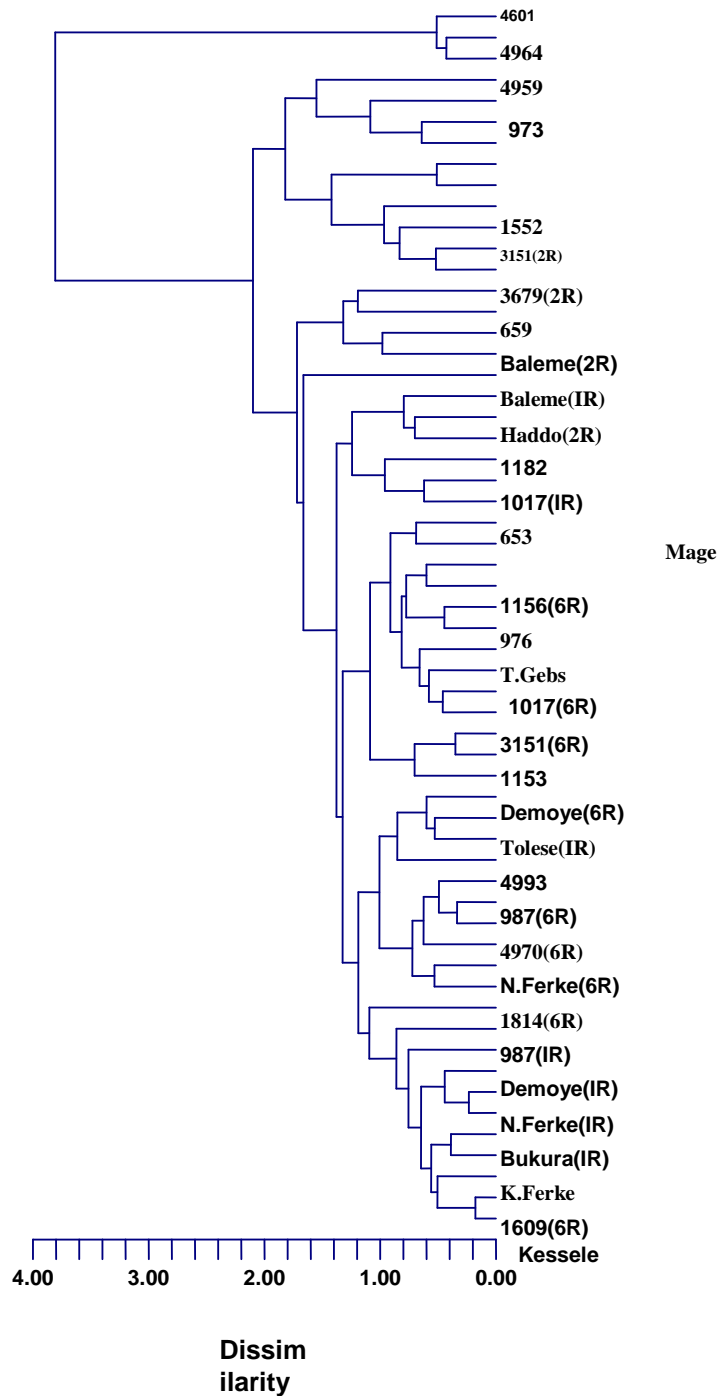
Figure 2. Dendrogram showing genetic relationships among landraces cluster analysis
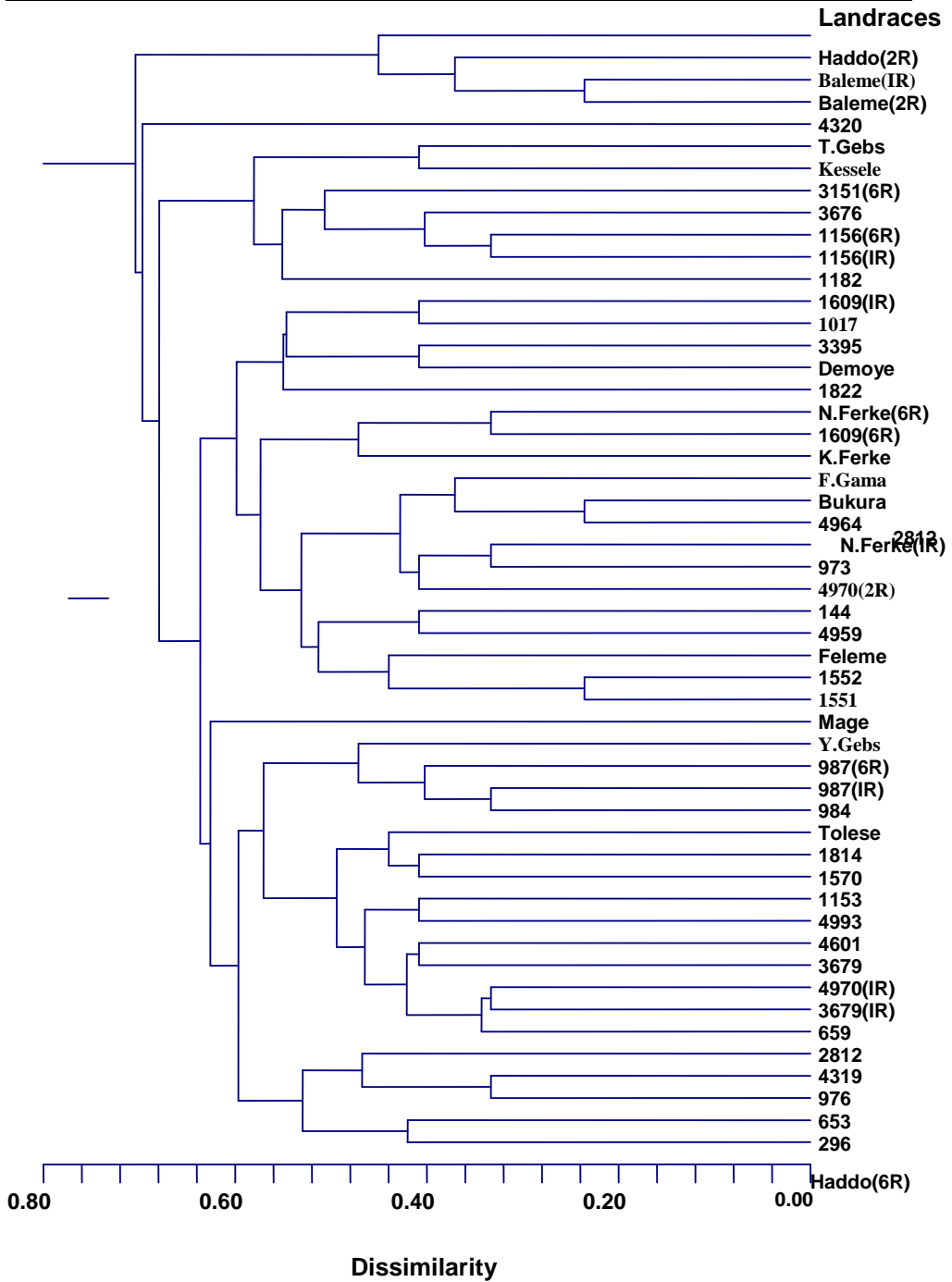of data from SDS-PAGE of seed storage proteins

Figure 3. Dendrogram depicting the cluster groups based on the morphological data set from 14 farmers' cultivars and 30 accessions.

between two-rowed and six-rowed barley types rather than between six-rowed by six-rowed or two-rowed by two-rowed types. Similarly, a single recessive gene (*n*) with a major phenotypic effect controls the naked character in barley grain and it is not surprising that a closer genetic relation ship was observed between the naked and covered types than vise versa. As a consequence, the variation not evident by morphological traits was revealed by hordein polypeptide banding patterns. Hence, clustering based on the data from the two measures of variability resulted in different groupings. Although it has been observed that certain hordein bands have association with morphological traits (Asfaw, 1989b; Ruiz et al., 1997), clustering using the morphological and the hordein data gave different groupings indicating the two categories of descriptors evolving along different evolutionary lines (Asfaw, 1989b; Tsegaye et al., 1996).

# Conclusions

Assessment of genetic variability has been done with the help of SDS-PAGE to supplement variability studies with morphological descriptors. SDS-PAGE revealed very low to high levels of genetic variability within landraces. Mean genetic distances within landraces ranged from 0.353 to 0.678 with an overall mean of 0.63 while that among landraces was in the range of 0.235 to 0.881 with overall mean of 64. Some of the landraces that looked uniform phenotypically (for example, Feres Gama, Feleme, and Kessele) have shown variation among their components although to a lesser extent demonstrating the presence of biotypes. Although there had been cases in which the variability within some of the landraces was comparable to that of among landraces, genetic divergence between landraces was larger than within landraces. Mean genetic distance between landraces within localities were generally lower (0.462$\pm$0.11 for Were Jarso to 0.615$\pm$0.10 for Ankober-Mezezo) than mean genetic distance between landraces of different localities whose values range from 0.405$\pm$0.05 for landraces of Degem vs Welmera to 0.758$\pm$0.06 for landraces of Kimbibit vs Welmera. No association has been observed between measures of genetic variability based on morphological characters and SDS-PAGE of seed storage proteins. Although some landraces appeared to be clustered according to their geographical origin, it was not possible to conclude that SDS-PAGE provided discrimination between landraces according to their origin because the clustering did not follow a similar trend through out all the landraces.

The information from this study can help make decisions on which landraces to select and make region specific crossings among the adapted landraces. Divergence for morphological traits *per se* may not reflect the true genetic distance between landraces. Hence, selection of parents for crossing will be supplemented based on data from variability in hordein banding patterns to get the expected progeny variance. Since morphological traits have great influence on the attitude and preference of farmers to a particular cultivar, their inclusion as selection criteria of parents for crossing will help to incorporate traits of farmers' interest.

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